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Karl-Erik Andersson
Martin C. Michel
Editors

Urinary Tract

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Urinary Tract

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This book is dedicated to the memory of the late Prof. Alison Brading who passed away while this book was being produced.

Preface

Functional disorders of the urinary tract are common and in many cases have major adverse effects on the quality of life of the afflicted patients. These include disorders of the ureters, the bladder, the urethra, the prostate and the pelvic floor. For a long time, only surgical approaches and conservative treatment could be offered for such disorders. The last two decades have seen an amazing proliferation of knowledge in the field of anatomy, physiology and pharmacology of the urinary tract. Several major new treatments have emerged from such research and are benefitting millions of patients. However, therapeutic needs remain and only a profound knowledge on the underlying tissue, cell and molecular processes is likely to provide novel treatments addressing such needs. Therefore, we are very happy that internationally leading experts have contributed to the writing of this book and we thank all of them for their efforts. Their combined insight provides a comprehensive overview of the state of knowledge regarding the pharmacology of the urinary tract. Therefore, we trust that this book will become a valuable source of information for basic and clinical researchers alike in a dynamically growing field.

Winston-Salem, NC, USA — Karl-Erik Andersson
Amsterdam, The Netherlands — Martin C. Michel
September 2010

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Overview on the Lower Urinary Tract

Christopher Chapple

Contents

Abstract This chapter overviews our current knowledge on the subject of the urinary tract, whose fundamental role is to transport urine from the kidneys and then store it at low pressure in the lower urinary tract until it can be voided at a socially convenient time. Current understanding of lower urinary tract function and dysfunction is summarized, with reference to anatomy, innervation, and function. The importance of the neurological system in the normal function of the lower urinary tract is emphasized, with a brief overview of the consequence of neural injury at different levels within the central nervous system. The role of urodynamics in the evaluation of lower urinary tract symptoms is discussed with particular reference to the currently recommended terminology advocated by the International Continence Society and The International Urogynaecological Association.

Keywords Bladder · Physiology · Pharmacology · Neuro-urology · Urodynamics

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1 Introduction

The urinary tract consists of two mutually dependent components:

- Upper tract (kidneys and ureters)
- Lower tract (bladder and urethra)

This provides a highly sophisticated system of conduits that converts the continuous involuntary production of urine by the kidneys into the intermittent, consciously controlled voiding of urine (micturition) in appropriate circumstances.

Both kidneys continuously produce greater than 0.5 ml of urine per kg of body weight per hour (i.e. >35 ml per hour in a 70 kg man) when functioning properly and adequately hydrated. This urine empties into the kidney's collecting systems that then drain via the ureters.

The ureters function as low-pressure distensible conduits with intrinsic peristalsis, which transport urine from the kidneys to the bladder. The urine drains into the bladder at the vesico-ureteric junction (VUJ) at the lower end of each ureter. Each junction, if correctly functioning only, allows the one way flow of urine and contains a mechanism to prevent retrograde transmission of urine back into the ureters from the bladder. This serves to protect the upper tract from the high pressures encountered within the bladder during voiding and to prevent infection entering the upper tracts.

The bladder has two main functions:

- Collection and low-pressure storage of urine
- Expulsion of urine at an appropriate time and in an appropriate place

Urinary continence during bladder filling, urine storage in the bladder, and the efficiency of subsequent voiding all depend upon accurate coordination of the opposing forces of:

- Detrusor contraction
- Urethral closure pressure

Symptomatic evaluation of urinary tract dysfunction is difficult because the bladder often proves to be an "unreliable witness", not only because of subjective bias but also because there is considerable overlap between the symptoms for different disorders.

1.1 Innervation

Before considering the clinical investigation and treatment of disorders of micturition, it is first essential to consider the neural mechanisms controlling urinary tract function. Although most contemporary knowledge is based on studies with experimental animals, it is difficult and often misleading to relate the findings from such animal models directly to man (Michel and Chapple 2009; Fry et al. 2010). However, data for

humans are limited as they can only be obtained from studying clearly defined clinical syndromes and isolated spinal cord lesions (Wyndaele et al. 2010).

The pioneering neurophysiologist, Barrington, initially described five reflexes associated with micturition in the cat, to which he added a further two after further study. Two of these reflexes had reflex centres in supraspinal sites (medulla and pons) and caused strong and sustained contractions. He considered that these were essential for normal micturition because bladder contraction and urethral relaxation are not coordinated after experimentally produced high spinal transection. The remaining five reflexes appeared to be confined to the spinal cord. More recently, it has been proposed that many interrelated reflexes act upon the sacral micturition centre, exerting both excitatory and inhibitory effects (Drake et al. 2010a, b; Fowler et al. 2008).

The detrusor muscle is controlled by the autonomic nervous system and is richly innervated by three groups of nerves:

- The principal population comprises presumptive cholinergic nerves (identified by their content of the enzyme acetylcholinesterase and demonstrated by the use of electron microscopy to lie in close apposition to muscle cells) – by releasing the neurotransmitter acetylcholine, they provide the major motor control of the detrusor muscle
- The sympathetic innervation comprises a sparse distribution of noradrenergic neurones, which occur in greatest concentration towards the bladder base and are thought to be of principal importance in contracting the outlet of the bladder to facilitate storage
- The third population of nonadrenergic noncholinergic (NANC) sensorimotor nerves contains a variety of putative neurotransmitters (principally peptides), which can be identified by immunofluorescent techniques

The close juxtaposition of these neural populations allows them to interact. To facilitate this, there are potential neural links via ganglia at every level from the spinal cord to the target organs (prostate, bladder, sphincters), particularly between the parasympathetic and sympathetic nervous systems.

The spinal segments S2–S4 act via efferent parasympathetic cholinergic neurones to initiate and maintain detrusor contraction. Damage to these spinal segments abolishes the micturition reflex.

After leaving the sacral foramina, the pelvic splanchnic nerves containing the parasympathetic innervation to the bladder pass lateral to the rectum to enter the inferior hypogastric or pelvic plexus. They are joined by the hypogastric nerve containing efferent sympathetic nerve fibres originating from the spinal cord segments T10–L2. When combined, they form a plexus at the base of the bladder.

It has been suggested that:

- The pelvic nerves provide the main afferent pathway of the micturition reflex – there is now increasing evidence to suggest that the urothelium and its associated afferent innervation has an important role in the normal control of micturition

- Sympathetic neuronal pathways in the hypogastric nerves (innervating the trigone) passing to the spinothalamic tracts (bladder and urethral sensation) provide additional afferent information

The sympathetic nerves provide the main motor control for urethral and prostatic smooth musculature. The somatic pudendal nerve contributes an additional component to the striated sphincter mechanism.

1.1.1 Disruption of Normal Peripheral or Central Nervous System Control Mechanisms

A neurological classification is invaluable for counselling and can be of useful prognostic significance. Certain characteristic patterns – peripheral denervation, suprasacral spinal cord lesions, and cerebral (suprapontine) lesions – can be identified (see below).

Peripheral Denervation

The clinical picture of peripheral denervation depends upon the extent of denervation. Complete lesions decentralize the lower urinary tract, and although ganglionic activity may persist, an acontractile bladder will result with an inactive urethra. Subsequent continence is governed by the functional competence of the bladder neck mechanism. The urethra has a fixed resistance, and bladder emptying depends upon abdominal straining or manual compression. Partial lesions often result in detrusor hyperreflexia.

Suprasacral Spinal Cord Lesions

If the spinal cord is transected above the fifth lumbar segment, a "cord bladder" develops. A principal feature of this lesion is loss of coordinated detrusor–sphincter behaviour, which results in simultaneous contraction of the detrusor and urethral sphincter (detrusor–sphincter dyssynergia). Sphincter contractions are not usually prolonged throughout the period of detrusor action, so there is intermittent voiding as well as urine retention. Voiding function can be particularly ineffective in people who have lesions of the thoracolumbar cord, and in these people, low compliance is an important feature.

Cerebral (Suprapontine) Lesions

Lesions of the midbrain rarely result in disturbances of continence and micturition. It is likely that this is due to

- The bilateral representation of nuclei at this level
- The poor prognosis of patients who have extensive lesions

Damage to the basal ganglia results in a reduced threshold for the transmission of impulses through the reticulospinal tracts controlling micturition. The typical picture is therefore of involuntary bladder contractions, which occur in people who have Parkinson's disease and following cerebrovascular thrombosis or haemorrhage.

Lesions of the cerebral cortex, particularly involving the inner surface of the cerebral hemispheres or the frontal cortex, can result in incontinence. It is felt that these patients lose the centrally mediated inhibition of the pontine voiding reflex, resulting in involuntary bladder contractions and urgency incontinence.

Many urinary disorders seen in clinical practice may have a neurological cause, but a classification based on specific abnormalities and particularly the site of a neurological lesion is not practical because

- The aetiology and pathogenesis of many disorders is at present unclear
- Lesions are often difficult to locate, and once located, can be difficult to relate to the neurological signs (e.g. multiple sclerosis)
- Different lesions can produce identical functional changes in the lower urinary tract

1.2 Sphincteric Mechanisms

Apart from the obvious anatomical differences (the longer urethra and presence of a prostate gland in men), there are important differences in the histological structure, innervation, and function of the outflow tract between males and females.

1.2.1 Males

In the male, there are two important sphincteric mechanisms:

- A proximal "bladder neck mechanism"
- A urethral mechanism lying at the apex of the prostate (the "distal sphincter mechanism")

The male bladder neck is a powerful sphincter subserving both the urinary and genital roles, the latter function being of primary importance in preventing retrograde ejaculation. Ultrastructurally, in males, the bladder neck consists of two muscular layers – a powerful inner layer of muscle bundles arranged in a circular orientation containing a rich adrenergic sympathetic nerve supply and an outer layer contiguous with the detrusor muscle and receiving both a cholinergic and adrenergic innervation.

The distal sphincteric mechanism is also extremely important as evidenced by its ability to maintain continence even when the bladder neck has been

rendered totally incompetent by bladder neck incision or prostatectomy. Conversely, in patients who have a damaged distal urethral sphincter (e.g. as in pelvic fracture-associated urethral disruption), continence is maintained by the bladder neck mechanism.

The prostate is made up of smooth muscle and glandular tissue, the proportion of smooth muscle being increased in benign prostatic hyperplasia. This muscle is controlled by the sympathetic nervous system, which acts by releasing noradrenaline onto α_{1A} adrenoceptors located on prostatic smooth muscle cells.

1.2.2 Females

The female bladder neck is a far weaker structure than the male bladder neck. It is poorly defined with the muscle fibres having a mainly longitudinal orientation and the predominant innervation being cholinergic and can be incompetent, even in nulliparous young women.

Urinary continence in women usually relies upon the integrity of the intrinsic urethral sphincteric mechanism. This is composed of intrinsic urethral smooth muscle and extrinsic striated muscle components and extends throughout the proximal two-thirds of the urethra, being most developed in the middle one-third of the urethra, particularly dorsally. The efferent innervation of the striated muscle of the extrinsic component of the urethral sphincter arises predominantly from cell bodies lying in a specific area of the sacral anterior horn known as Onuf's nucleus. Various aspects of the innervation of this sphincter are controversial – not only the neural pathways involved but also the relative contribution of somatic and autonomic nerves. The limited knowledge available suggests that the pudendal nerve transmits urethral mucosal sensation.

Damage to the innervation of the urethral sphincter (particularly the pudendal nerve) by obstetric trauma predisposes to urinary stress incontinence.

1.3 Lower Urinary Tract Symptoms

The term "the bladder is an unreliable witness" was first coined with the recognition that lower urinary tract symptoms (LUTS) were not disease- or gender-specific, could be reported inaccurately by the patient, or be poorly documented by the investigator (Chapple and Roehrborn 2006). In recent years, attempts have been made to quantify the symptoms by the use of disease-specific symptom scores and quality of life measures. Well-known examples include the International Prostate Symptom Score (IPSS) for suspected prostate and the King's Health Questionnaire for incontinence-related problems. Currently, internationally acceptable questionnaires are being evaluated for incontinence (http://www.iciq.net/).

LUTS are best subdivided into *storage* of urine (also "irritative"), *voiding* (also "obstructive"), and post-micturition symptom groups (Tables 1 and 2).

Urine storage and voiding are two interrelated yet distinct phases of lower urinary tract function. The bladder and urethra possess intrinsic tone produced by the muscle and connective tissue they contain. At rest, the urethral tone keeps the walls in apposition and aids continence. During filling, the walls of the bladder exhibit receptive relaxation (i.e. the vesical lumen expands without resulting in a concomitant rise in intravesical pressure). Once a threshold level of filling has been achieved (which will depend upon circumstances and vary between individuals), increasing afferent activity will start to impinge on consciousness, resulting in awareness that the bladder is filling up. Except during infancy, in health, there is complete volitional control over these reflex pathways, and voiding will be initiated in appropriate circumstances.

1.3.1 Storage Phase

During the storage phase, the bladder is filled with urine from the ureters. For the majority of the time (greater than 99%), the lower urinary tract will be in the storage phase, whilst less than 1% of time is spent voiding. The bladder needs to accommodate to the increase in volume without an appreciable rise in bladder (intravesical) pressure. The extent to which a change in volume (V) occurs in relation to a change in intravesical pressure (P) is known as the bladder compliance (V/P).

Factors that contribute to compliance are:

- The passive elastic properties of the tissues of the bladder wall
- The intrinsic ability of smooth muscle to maintain a constant tension over a wide range of stretch or "tonus"
- The neural reflexes, which control detrusor tension during bladder filling

During bladder filling, afferent activity from stretch receptors increases and passes via the posterior roots of the sacral cord and the lateral spinothalamic tracts to the brain, thereby mediating the desire to void. Activity within the striated component of the urethral sphincter is increased, and the local spinal reflex activity enhances the activity within striated muscles of the pelvic floor and sphincter to tighten up the bladder outlet mechanisms and so augment continence.

Table 1 LUTS

Storage	Voiding	Post-micturition
• Urgency	• Hesitancy	• Feeling of incomplete emptying
• Increased daytime frequency	• Intermittency	• Post-micturition dribble
• Nocturia	• Slow stream	
• Urinary Incontinence	• Splitting or spraying	
• Altered bladder sensations	• Straining	
	• Terminal Dribble	

Table 2 Lower urinary tract symptom terminology

	Terminology	Definition	Notes
Storage symptoms	*Increased daytime frequency*	The complaint by the patient who considers that he/she voids too often by day	Term is equivalent to pollakiuria used in many countries
	Nocturia	The complaint that the patient has to wake at night one or more times to void	
	Urgency	A sudden compelling desire to pass urine, which is difficult to defer	
	Urinary incontinence (*UI*)	Any involuntary leakage of urine	
	Stress urinary incontinence (*SUI*)	Involuntary leakage on effort or exertion, or on sneezing or coughing	
	Urge(ncy) urinary incontinence (*UUI*)	Involuntary leakage accompanied by or immediately preceded by urgency	Urge urinary incontinence is a misnomer since it is urgency that is associated with this incontinence and we therefore believe it should in fact be called "urgency incontinence" and not urge incontinence
	Mixed urinary incontinence (*MUI*)	Involuntary leakage associated with urgency and also with exertion, effort, sneezing or coughing	A mixture of urgency urinary incontinence and stress urinary incontinence symptoms
	Mixed urinary symptoms	Involuntary leakage associated with exertion, effort, sneezing or coughing, but not associated with urgency	
	Enuresis	Any involuntary loss of urine	Similar to definition of urinary incontinence
	Nocturnal enuresis	Loss of urine occurring during sleep	Involuntary symptom; as opposed to "nocturia", which is a voluntary symptom
	Continuous urinary incontinence	The complaint of continuous leakage	
	Other types of urinary incontinence	May be situational	For example, incontinence during sexual intercourse, or giggle incontinence
	Normal bladder sensation	Aware of bladder filling and increasing sensation up to a strong desire to void	

Bladder sensations during storage phase	*Increased bladder sensation*	Feels an early and persistent desire to void	
	Reduced bladder sensation	Aware of bladder filling but does not feel a definite desire to void	
	Absent bladder sensation	No sensation of bladder filling or desire to void	
	Non-specific bladder sensation	No specific bladder sensation but may perceive bladder filling as abdominal fullness, vegetative symptoms, or spasticity	These are most frequently seen in neurological patients, particularly those with spinal cord trauma or malformations of the spinal cord
Voiding symptoms	*Slow stream*	The perception of reduced urine flow, usually compared to previous performance or in comparison to others	
	Splitting or spraying	Description of the urine stream	
	Hesitancy	Difficulty in initiating micturition, resulting in a delay in the onset of voiding after the individual is ready to pass urine	
	Intermittent stream (Intermittency)	Urine flow, which stops and starts, on one or more occasions, during micturition	
	Straining	The muscular effort used to either initiate, maintain or improve the urinary stream	
	Terminal dribble	A prolonged final part of micturition, when the flow has slowed to a trickle/dribble	Compare to post-micturition dribble
Post-micturition symptoms	*Feeling of incomplete emptying*	A feeling experienced by the individual after passing urine	
	Post-micturition dribble	The involuntary loss of urine immediately after an individual has finished passing urine, usually after leaving the toilet in men, or after rising from the toilet in women	Compare to Terminal dribble
Other symptoms	*Symptoms associated with sexual intercourse*	E.g. dyspareunia, vaginal dryness and incontinence	Should be described as fully as possible. It is helpful to define urine leakage as: during penetration, during intercourse, or at orgasm
	Symptoms associated with pelvic organ prolapse	E.g. “something coming down”, low backache and dragging sensation	May need to digitally replace the prolapse in order to defaecate or micturate

(*continued*)

Table 2 (continued)

	Terminology	Definition	Notes
	Genital and lower urinary tract pain	Pain, discomfort, and pressure may be related to bladder filling or voiding or may be felt after micturition, or even be continuous	The terms "strangury", "bladder spasm", and "dysuria" are difficult to define and of uncertain meaning and should not be used, unless a precise meaning is stated Dysuria literally means "abnormal urination". However, it is often incorrectly used to describe the stinging/burning sensation characteristic of urinary infection (UTI)
Painful bladder syndrome symptoms	*Painful bladder syndrome/ interstitial cystitis (PBS/IC)*	Subrapubic pain associated with other LUTS, usually increased frequency (but not urgency)	Diagnosed only in the absence of UTI or other obvious pathology Interstitial Cystitis is a specific diagnosis usually confirmed by typical cystoscopic and histological features. If these features are not present, then the term PBS is preferable

Important local factors facilitating bladder filling include both receptive relaxation and the passive viscoelastic properties of the bladder wall. Conditions that contribute to poor bladder compliance and detrusor overactivity include:

- Abnormal bladder morphology resulting from collagenous infiltration, hypertrophy, or altered muscle structure (e.g. obstructed bladder)
- Abnormal detrusor smooth muscle behaviour, either primary or secondary to neural dysfunction

During the storage phase, the urethra and sphincteric mechanisms should be closed, thereby maintaining a high outlet resistance and continence. Storage symptoms (nocturia, frequency, urgency, and urge incontinence – the so-called frequency urgency syndrome/overactive bladder syndrome) may arise from failure of the bladder to store urine. This may be due to a reduced anatomical capacity (shrunken bladder after surgery/radiotherapy/infections such as tuberculosis) or a reduced functional capacity resulting from abnormally increased bladder sensation (e.g. interstitial cystitis/painful bladder syndrome – beware the need to exclude carcinoma in situ – or bladder overactivity). Non-urological conditions (e.g. diabetes mellitus, diabetes insipidus, polydypsia) can also present with frequency and nocturia.

Urgency is often considered to be a pivotal symptom in the genesis of overactive bladder syndrome and is defined as a sudden compelling desire to pass urine, which is difficult to defer. It may arise as a consequence of disordered peripheral afferent function or central interpretation of afferent symptoms (Griffiths and Tadic 2008; Birder 2010; Roosen et al. 2010).

Frequency is a very troublesome symptom and is the complaint by the patient who considers that he/she voids too often by day. A frequency of voiding of more than eight times per day is usually taken to be abnormal.

Nocturia (sleep-disturbing voiding) is an interesting symptom since it may result from changes in bladder function as well as a harbinger of other physiological disorders such as cardiac failure. By the age of 65, a nocturia rate of once a night is taken to be the norm. Indeed, in many elderly patients, a reversal of the normal diurnal voiding pattern is seen, with more than 30% of the 24-h urine volume being produced overnight. In these cases, a frequency–volume chart (measuring and timing fluid intake and output and incontinence episodes for a minimum of 3 days) is essential in both investigation and treatment.

Incontinence. Urinary incontinence is the involuntary loss of urine. This can be constant or intermittent, and with (urgency) or without (stress) a detrusor contraction.

Enuresis, which represents incontinence occurring at night, can be associated with severe detrusor overactivity, but is also a classical symptom seen in association with chronic retention. Overflow incontinence is the classical cause in elderly men presenting with enuresis. The bladder has become acontractile and overfills, and empties only when the volume exceeds the anatomical capacity, under the influence of the elastic forces in the bladder wall. These patients pass small volumes of urine, frequently without any control. Chronic retention is an important condition to consider in any patient as many will present with renal impairment.

1.3.2 Voiding Phase

The bladder must cease relaxing and instead contract to expel the urine, and the urethra and sphincteric mechanisms must "open" to decrease the outlet resistance and allow passage of urine. Voiding should be efficient, and there should be minimal or no urine remaining in the bladder at the end of the voiding phase.

Micturition initiated by the cerebral cortex is likely to involve a complex series of bladder–brain stem reflexes. During voiding, the following mechanisms occur:

1. The urethral relaxation precedes detrusor contraction.
2. Simultaneous relaxation of the pelvic floor muscles occurs.
3. "Funnelling" of the bladder neck occurs to facilitate flow of urine into the proximal urethra.
4. Detrusor contraction occurs to forcefully expel urine.

The underlying mechanism includes:

- Increased activity within parasympathetic neurones results in the removal of the central inhibitory influences acting on the sacral centres
- Voiding is initiated under the influence of pontine medullary centres

There is, therefore, a parasympathetically controlled detrusor contraction associated with a corresponding relaxation of the urethra/prostate/bladder neck complex resulting from reciprocal nerve-mediated inhibition of the sympathetic nerve-mediated outflow.

In addition to these primary actions, other important secondary events are:

- Contraction of the diaphragm and anterior abdominal wall muscles
- Relaxation of the pelvic floor
- Specific behavioural changes associated with voiding

At the end of voiding, the proximal urethra is closed in a retrograde fashion, the "milkback" seen at videocystometry. Once these events have been completed, the sacral centres are re-inhibited by the cortex and the next filling cycle starts.

During the voiding phase, the reverse activity to the storage phase must occur. Voiding symptoms (poor stream, hesitancy, interruption, and straining) are either due to the loss of detrusor power or progressive outflow obstruction, which, it is presumed, may progressively lead to detrusor failure and retention.

1.3.3 Return to Storage Phase

At the end of voiding, the proximal urethra is closed in a retrograde fashion, thus milking back the urine into the bladder. This "milkback" is seen during contrast studies of the lower urinary tract when the patient is asked to stop voiding. Following this, the bladder returns to a state of relaxation.

1.4 Urodynamic Parameters

1.4.1 Normal Function

Normal function of the human lower urinary tract depends upon integrated coordination of the neural control of the bladder and outflow tract, for which an intact spinal cord is essential.

Under normal circumstances:

- Bladder capacity is approximately 500 ml and the bladder empties, leaving no residual urine
- Males void at a pressure of 40–50 cm H_2O and a maximum flow rate of 30–40 ml/s
- Females void at a pressure of 30–40 cm H_2O and a maximum flow rate of 40–50 ml/s

The difference between males and females is a consequence of the higher outflow resistance exerted by the male urethra.

1.4.2 Abnormal Function

Disordered lower urinary tract function can result from:

- Disruption of the normal peripheral or central nervous system (CNS) control mechanisms
- Disordered bladder muscle function, either primary (of unknown aetiology) or secondary to an identifiable pathology such as prostatic-mediated bladder outflow obstruction

Patients who have disordered lower urinary tract function in routine clinical practice represent a heterogeneous collection for most of whom there is no identifiable neurological abnormality. Some of these patients will have a primary neural or muscular disorder (e.g. primary idiopathic detrusor overactivity) in contrast to postobstructive secondary detrusor overactivity where the major aetiological factor is likely to be peripheral disruption of local neuromuscular function.

It is essential to use well-calibrated equipment with the technique being performed in an appropriate fashion (Schäfer et al. 2002). It is essential that standardized terminology is used when discussing LUTS and the results of urodynamic investigations, to allow accurate exchange and comparison of information for clinical and research purposes. The official terminology is as suggested by the International Continence Society (ICS) in 2002 (http://www.icsoffice.org; Abrams et al. 2002) and there has been a recent update of this for women (Haylen et al. 2010). The management of lower urinary tract dysfunction lies outside the remit of this chapter, and the interested reader is directed to recent reviews on the subject (Abrams et al. 2008, 2009, 2010; Andersson et al. 2009).

References

Abrams P, Cardozo L, Fall M et al (2002) Standardisation Sub-committee of the International Continence Society. The standardisation of terminology of lower urinary tract function: report from the Standardisation Sub-committee of the International Continence Society. Neurourol Urodyn 21(2):167–178

Abrams P, Agarwal M, Drake M et al (2008) A proposed guideline for the urological management of patients with spinal cord injury. BJU Int 101(8):989–994

Abrams P, Chapple C, Khoury S et al (2009) International Scientific Committee. Evaluation and treatment of lower urinary tract symptoms in older men. J Urol 181(4):1779–1787

Abrams P, Andersson KE, Birder L et al (2010) Fourth international consultation on incontinence recommendations of the International Scientific Committee: evaluation and treatment of urinary incontinence, pelvic organ prolapse, and fecal incontinence. Neurourol Urodyn 29(1):213–240

Andersson KE, Chapple CR, Cardozo L et al (2009) Pharmacological treatment of overactive bladder: report from the International Consultation on Incontinence. Curr Opin Urol 19(4):380–394

Birder LA (2010) Urothelial signaling. Auton Neurosci 153(1–2):33–40

Chapple CR, Roehrborn CG (2006) A shifted paradigm for the further understanding, evaluation, and treatment of LUTS in men: focus on the bladder. Eur Urol 49(4):651–659

Drake MJ, Tannenbaum C, Kanai AJ (2010a) Potential insights into lower urinary function derived from CNS imaging. Neurourol Urodyn 29(4):629–633

Drake MJ, Fowler CJ, Griffiths D (2010b) Neural control of the lower urinary and gastrointestinal tracts: supraspinal CNS mechanisms. Neurourol Urodyn 29(1):119–127

Fowler CJ, Griffiths D, de Groat WC (2008) The neural control of micturition. Nat Rev Neurosci 9(6):453–466

Fry CH, Daneshgari F, Thor K et al (2010) Animal models and their use in understanding lower urinary tract dysfunction. Neurourol Urodyn 29(4):603–608

Griffiths D, Tadic SD (2008) Bladder control, urgency, and urge incontinence: evidence from functional brain imaging. Neurourol Urodyn 27(6):466–474

Haylen BT, de Ridder D, Freeman RM et al (2010) International Urogynecological Association; International Continence Society. An International Urogynecological Association (IUGA)/ International Continence Society (ICS) joint report on the terminology for female pelvic floor dysfunction. Neurourol Urodyn 29(1):4–20

Michel MC, Chapple CR (2009) Basic mechanisms of urgency: preclinical and clinical evidence. Eur Urol 56(2):298–307

Roosen A, Chapple CR, Dmochowski RR et al (2010) A refocus on the bladder as the originator of storage lower urinary tract symptoms: a systematic review of the latest literature. Eur Urol 2009, 56(5):810–819

Schäfer W, Abrams P, Liao L et al (2002) International Continence Society. Good urodynamic practices: uroflowmetry, filling cystometry, and pressure-flow studies. Neurourol Urodyn 21(3):261–274

Wyndaele JJ, Kovindha A, Madersbacher H et al (2010) Neurologic urinary incontinence. Neurourol Urodyn 29(1):159–164

Animal Models in Overactive Bladder Research

Brian A. Parsons and Marcus J. Drake

Contents

Abstract Overactive bladder syndrome (OAB) is a symptom-based diagnosis characterised by the presence of urinary urgency. It is highly prevalent and overlaps with the presence of bladder contractions during urine storage, which characterises the urodynamic diagnosis of detrusor overactivity. Animal models are needed to understand the pathophysiology of OAB, but the subjective nature of the symptom complex means that interpretation of the findings in animals requires caution. Because urinary urgency cannot be ascertained in animals, surrogate markers such as frequency, altered toileting areas, and non-micturition contractions have to be used instead. No model can recapitulate the subjective, objective, and related factors seen in the clinical setting. Models used include partial bladder outlet obstruction, the spontaneous

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hypertensive rat, the hyperlipidaemic rat, various neurological insults and some gene knock-outs. Strengths and weaknesses of these models are discussed in the context of the inherent difficulties of extrapolating subjective symptoms in animals.

Keywords Adenosine triphosphate · Animal models · Detrusor overactivity · Knock-out studies · Nitric oxide · Oestrogen · Overactive bladder syndrome · Prostaglandin · Transgenic · Urgency · Uroplakin

Abbreviations

ATP	Adenosine triphosphate
BOO	Bladder outlet obstruction
BPE	Benign prostatic enlargement
COX	Cyclooxygenase enzyme
DO	Detrusor overactivity
DSD	Detrusor sphincter dyssynergia
EAE	Experimental autoimmune encephalomyelitis
EP	Family of G-protein coupled receptors
ER	Oestrogen receptor
ICS	International Continence Society
MCA	Middle cerebral artery
MPTP	Neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MS	Multiple sclerosis
NANC	Non-cholinergic non-adrenergic
NMDA	N-methyl-D-aspartate
nNOS	Neuronal nitric oxide synthase
OAB	Overactive bladder syndrome
P_2X	Purinergic receptor
PD	Parkinson's disease
PGE_2	Prostaglandin E_2
SHR	Spontaneous hypertensive rat
VR-1	Vanilloid receptor type 1

1 Introduction

Overactive bladder syndrome (OAB) is a clinical diagnosis based on the presence of the symptom of urinary urgency. The subjective nature of the defining symptom is a crucial consideration since it is therefore not possible to ascertain the syndrome's presence or otherwise in an animal. The discussion below must be read with the

consideration of the inherent limitations of relying on surrogate markers to infer a situation akin to the clinical scenario. Seemingly more objective is the concept of detrusor overactivity (DO), the urodynamic observation of phasic bladder contractions during the filling phase. On the face of it, this represents a more sound foundation for developing animal models. However, such contractions appear to be normal in many animal species. Indeed in humans, contractions that would be labelled as DO during conventional urodynamics are often observed when people with normal lower urinary tracts are studied with ambulatory urodynamics. Accordingly, the use of terms such as OAB and DO in animal contexts risks misinterpretation of the underlying processes. Nonetheless, animal models are an essential adjunct in endeavouring to address the considerable clinical challenges of OAB.

2 The Problem

OAB is a symptom-based diagnosis defined by the International Continence Society (ICS) as urgency with or without urgency incontinence usually with daytime frequency and nocturia (Abrams et al. 2002a). This definition presumes that infection and other causes for the storage symptoms have been excluded. The key symptom of OAB is urgency, defined as a sudden compelling desire to void, which is difficult to defer (Abrams et al. 2002b). Urgency with at least one of the other storage symptoms listed above is essential for the diagnosis of OAB to be made. Synonyms for the condition include urgency syndrome and urgency–frequency syndrome. OAB is a diagnosis of exclusion as there is no pathognomonic criterion available to confirm the diagnosis.

It is a common condition with an estimated overall prevalence of 11.8%, comprising 10.8% of men and 12.8% of women (Irwin et al. 2006a). Although the condition affects all ages, the prevalence of OAB increases with ageing (Irwin et al. 2006a). OAB symptoms have been shown in numerous studies to have a significant negative impact on the health-related quality of life, emotional well-being and work productivity of affected individuals (Abrams et al. 2000; Irwin et al. 2006b). OAB can be socially disabling as it foments low self-esteem and embarrassment, which can in turn lead to depression and withdrawal from social activities. Increased night-time voiding may cause significant sleep pattern disturbances, resulting in fatigue. Urgency incontinence is associated with an increase in the number of falls and fractures in the elderly population (Brown et al. 2000).

DO is a urodynamic observation characterised by involuntary contractions during the filling phase of cystometry and these can be spontaneous or provoked (Abrams et al. 2002b). Although the symptom of urgency described by OAB patients often correlates with DO seen on cystometry, it must be emphasised that the two terms are not synonymous with each other. A retrospective study published in 2006 found that only 64% of patients diagnosed with OAB using the new ICS definition had DO demonstrable on urodynamics (Hashim and Abrams 2006). The study also showed that more than 30% of patients with DO did not have OAB

(Hashim and Abrams 2006), and it is therefore understandable why the bladder has previously been described as an "unreliable witness" (Blaivas 1996).

3 Pathophysiology of OAB

The current understanding of the pathophysiology of OAB is limited, and much of what is known about the clinical aetiology of the condition has been derived from epidemiological data. The mechanisms involved are complex and comprise both peripheral and central nervous system factors. Research into the pathogenesis of OAB is hampered by the fact that it is a symptom-based diagnosis, and consequently, studies in this field have focussed on abnormalities of afferent signalling and the mechanisms underlying DO. Changes in afferent return and signal processing are presumed to be the basis of urgency, whereas cystometrically determined DO is likely to be a contributing factor for incontinence in many OAB sufferers.

Regulation of normal micturition is complex and involves both spinal and supraspinal control mechanisms. The pontine micturition centre is the prime determinant of lower urinary tract function as it sets the volume at which the lower urinary tract switches from storage to voiding mode, thereby effectively determining maximum bladder capacity. The main factor causing the switch between these two phases is the level of afferent activity arising from the bladder. The afferent nerves conveying sensory information from the bladder to the central nervous system comprise at least two major populations. Myelinated Aδ-fibres are mechanosensitive and are probably responsible for the sensation of bladder fullness. They are activated by both low (non-nociceptive) and high (nociceptive) intravesical pressures. Unmyelinated C-fibres are the second type of afferent and are activated by cold, heat or irritation of the bladder mucosa. C-fibres have primarily nociceptive functions and do not usually respond to bladder distention, but may do so under pathological conditions. It has been proposed that DO and OAB may arise when the levels of afferent activity are inappropriately high for any given degree of bladder distention (Abrams and Drake 2007).

Three main hypotheses have been proposed to explain the pathophysiological basis of DO: neurogenic (de Groat 1997), myogenic (Brading 1997; Brading and Turner 1994) and peripheral autonomy (integrative) (Drake et al. 2001). The neurogenic theory places the fundamental problem in the central nervous system, whereas the latter two hypotheses focus on the periphery (Drake et al. 2006). The neurogenic hypothesis proposes that DO is caused by neural plasticity within the central nervous system, which gives rise to generalised, nerve-mediated excitation of detrusor muscle (de Groat 1997). This may result from damage to descending inhibitory pathways arising from higher brain centres, inappropriate expression of primitive spinal bladder reflexes, or as a result of sensitisation of afferent terminals (de Groat 1997).

The myogenic hypothesis suggests that DO results from changes within the bladder smooth muscle that lead to increased excitability and likelihood of spontaneous contraction with enhanced electrical coupling between muscle cells (Brading 1997).

In keeping with this, studies have revealed characteristic ultrastructural changes associated with DO, which could potentially facilitate the propagation of electrical activity and contraction over a wider than normal proportion of the detrusor muscle (Haferkamp et al. 2003a, b).

The integrative hypothesis arises from the observation that the bladder shows complex autonomous activity when descending suppressive influences are removed (Coolsaet et al. 1993; Drake et al. 2003a, b). Resulting localised contractions and stretches (micromotions) have been demonstrated in several animal models (Drake et al. 2003a). These findings have led to the suggestion that the detrusor muscle is functionally modular in arrangement, conceptually determined by a myovesical plexus, analogous to the myenteric plexuses, which control gut motility. While localised modular activity during bladder filling may contribute to the normal generation of sensory information, exaggerated responses may give rise to urinary urgency. In keeping with this, an increased frequency in micromotion activity has been correlated with reported sensations of urgency in symptomatic women in the absence of intravesical pressure changes (the so-called "sensory urgency") (Drake et al. 2005; Van Os-Bossagh et al. 2001). The hypothesis goes on to suggest that while activity in individual modules would have little effect on intravesical pressure, the enhanced coordination of modular activity through the myovesical plexus would lead to contraction of a more substantial proportion of the bladder wall resulting in the emergence of DO (Drake et al. 2001). The integrative hypothesis proposes that other cell types in addition to muscle (interstitial cells, urothelium and peripheral nerves) contribute to normal generation of localised spontaneous activity, and thus low pressure sensing of filling state; in symptomatic people, the localised micromotions are exaggerated, giving rise to urgency, and the same cell types also lead to the wider propagation of spontaneous activity, seen as DO.

As a symptom-based diagnosis, more recent thinking has shifted emphasis towards sensory mechanisms. The concept of "afferent noise" signifies the existence of normal and pathological mechanisms capable of modulating the amount of neuronal traffic in bladder-related sensory nerves for any given filling volume (Andersson 2002; Gillespie 2005), such as urothelial interactions with subjacent interstitial cells and nerve endings (Birder 2005; Birder and de Groat 2007), or alterations in transmitter profiles in peripheral nerves. In addition, central nervous system circuitry, including the limbic system and other neural processes underpinning conscious attention, has a major impact on the effects perceived by the individual. Functional brain imaging techniques are beginning to yield intriguing insights into the relevant brain areas, the potential matrix of brain centres regulating the processing of lower urinary tract sensory perception and motor responses and the recognised interactions with other organ systems.

It is clear from the above that the mechanisms that underlie OAB and DO are still incompletely understood. The pathogenesis of OAB is multifactorial, differing for different individuals, and altering over time in any one person. Normal bladder function is dependent on the intricate interplay of neural, myogenic and other cell types, and disruption of any of these may lead to lower urinary tract

dysfunction, if not compensated for elsewhere in the control hierarchy. It is therefore not surprising that increasingly complex hypothetical insights into bladder function and control are emerging; consequently, animal models are a vital tool to study their validity.

4 Current Management of OAB

For the majority of OAB sufferers, treatment is aimed at symptom relief rather than cure; consequently, OAB is now recognised as a chronic medical condition. The principles of treatment are to reduce urinary urgency so as to decrease the number of episodes of incontinence and improve urinary frequency, nocturia and voided volumes (Hashim and Abrams 2007). Once alternative pathologies have been excluded, the treatment of individuals diagnosed with OAB involves lifestyle advice, behavioural training and pharmacological and surgical interventions. Lifestyle interventions include fluid restriction, avoidance of foods with a high water content, reduction of evening fluid and caffeine intake, smoking cessation and weight loss (Bulmer et al. 2001; Hannestad et al. 2003). Bladder training, supplemented by pelvic floor muscle exercises, aims to suppress involuntary detrusor contractions through feedback inhibition as intentional contraction of the pelvic floor muscles can inhibit detrusor contraction (Yamaguchi et al. 2009). Antimuscarinic medication is the mainstay of treatment for OAB symptoms, but a significant proportion of patients do not respond to therapy, or will discontinue drug treatment because of its side effects.

Patients whose OAB symptoms fail to respond to optimised first line measures should be managed in a specialist centre within the settings of a multidisciplinary team. Previously, standard alternatives for sufferers with refractory symptoms involved major reconstructive surgery, of which the main options are augmentation cystoplasty (Bramble 1982, 1990) or urinary diversion into a urostomy (Singh et al. 1997). These procedures have the potential for significant morbidity and risk, and long-term satisfactory outcome is uncertain. Surgery is viewed as the last resort, but it has a role to play in the management of sufferers with severe symptoms and who have failed to respond to all other treatments.

Several treatments have since been developed and adopted for use to bridge the gap between conservative and surgical interventions. These include intravesical injections of Botulinum toxin, tibial nerve stimulation and sacral neuromodulation. They are warranted in patients for whom quality of life is significantly impaired by severe symptoms, but who are unwilling to accept the risks associated with surgery or are medically insufficiently healthy to contemplate general anaesthesia. Despite the seemingly wide range of treatments available, a significant proportion of patients with OAB continue to be troubled by their symptoms, and therefore a wider range of more effective therapies are required.

5 Importance of Animal Models

Our understanding of lower urinary tract function remains incomplete as human studies and research using human material are inherently limited because of the legal, ethical and moral implications associated with such investigations. Much of our knowledge of bladder function has come from in vitro research such as that using muscle strips (Buckner et al. 2002; Oh et al. 1999; Yamanishi et al. 2000; Hashitani et al. 2001), but findings from these types of experiments are difficult to extrapolate to whole bladder function. Over the past century, experimental work looking at bladder function in humans has involved in vivo cystometric investigations on willing patients and volunteers as well as molecular and cellular research using biopsy material (Gillespie 2005). Advances in diagnostic and functional imaging have allowed brain mapping (Griffiths et al. 2007, 2009) in human subjects, and although this has been informative, the results generated by these studies are by their nature correlational.

Attempts to discover the origins of urgency, urgency incontinence and DO have been the major forces driving research into lower urinary tract function (Gillespie 2005). Improved understanding of these phenomena may lead to the discovery of more effective treatments for storage symptoms and OAB. Increasing use of animal models to study the integrative physiology of the lower urinary tract and its control may go some way to achieving this, but there are important limitations (see below). Hypotheses typically need animal models to test derived predictions. Animal modelling thus provides a crucial link to the clinical context, and its use in basic and clinical research is a necessary precursor to safe and ethically sound research in humans. It can be used to generate novel directions of research and corroborate findings obtained by other means/studies. Animal models are the way in which the results from simpler in vitro research can be tested in "intact" biological systems without direct human experimentation. This is important because findings obtained under artificial in vitro conditions may not apply in vivo, and the results generated using one cell type may not be applicable to other cell lines. It allows assessment of the relevance of any in vitro study findings to the living whole organism.

Animal models can be classified as exploratory, explanatory or predictive, depending on the type of information that they are designed to yield (Hau 2008). Exploratory models are used to study physiological processes and pathological mechanisms of action in order to generate novel ideas and theories about biological function. These hypotheses then require validation by replicating the findings in other models before returning to human studies to ensure that they are relevant to the disease or physiological process being studied. Explanatory models are developed and applied in an attempt to improve our understanding of the importance and relevance of findings generated by other research and the aim of studies using these models is to provide an explanation for a biological process. Predictive models are those used to discover and quantify the impact of novel treatments and therapeutic agents and to assess their toxicity to the living organism (Table 1).

Table 1 Criteria for animal models

Criteria	
Reliability	The model provides consistent results
Face validity	The model has a similar phenotype to the human condition it is imitating
Aetiologic validity	The model is derived from a cause known to trigger the condition in humans
Predictive validity	The model is able to predict clinical outcomes in humans such as the response to treatment

The majority of animal models used in research have been developed to study the aetiology, pathogenesis, natural history and potential treatments for human disorders. The findings they generate may drive medical advances and understanding, but the information gained must be interpreted within the limitations of the model. The validation required will depend on how closely the model reproduces a disease or condition (Table 2). A homologous model is one in which the animal replicates the symptoms, aetiology and natural history of the human condition (Hau 2008). Isomorphic models resemble the human phenotype, but the underlying mechanism in the animal is different from the clinical setting (Hau 2008). Most models in current use are neither homologous nor isomorphic, but are partial as they fail to mimic the human condition they are modelling but bear enough similarities to allow their use for studying aspects of a disease or its treatments (Mogensen and Holm 1989). Irrespective of the type of model used, careful validation of both the model itself and the results it generates ensure that any findings are correctly extrapolated from animal to human; too much weight should not be given to findings from any one animal model.

6 Difficulties Interpreting Results from Studies Using Animal Models

The fact that animal models often fail to predict the efficacy of novel therapeutic agents in patients highlights the difficulties in extrapolating data from animals to humans. Ideally, animal models should reproduce all the facets of the human condition, but it is inconceivable that any single animal model will replicate all the signs, symptoms, mechanisms, and consequences of a disease/condition including the physiological and pathological changes that occur. In the context of OAB, the range of facets of the clinical condition clearly exceeds the realistic characteristics of an animal model (Table 3). A model is usually only relevant for a limited number of aspects of the human condition. Rather than viewing modelling as an attempt to replicate the human condition exactly, a more productive approach involves developing specific models each tailored to answering a particular experimental hypothesis. When viewed as tools rather than replicas, the value of animal models that do not recapitulate the entire human disease becomes more obvious.

Table 2 Characteristics of OAB

Characteristics	
Subjective	Urgency; defined as a sudden, compelling desire to pass urine and differing in nature from strong desire to void
	Frequency; perception by the patient that they void too often by day
	Increased filling sensation seen during urodynamics in some patients
	Affective; impact on quality of life and state of mind
Objective	Urgency urinary incontinence in a proportion of OAB patients
	Frequent passage of varying voided volumes on a frequency volume chart
	Nocturia; the need to wake at night to pass urine
	DO seen during urodynamics in some patients
Additional factors	Varied association with voiding symptoms and post void residual
	Varied association with bowel and gynaecological symptoms/conditions
	Effects of ageing on OAB incidence and progression
	Correctable factors exacerbating symptom severity (e.g., diet)
	Environmental factors exacerbating symptom severity [e.g., cold (Imamura et al. 2008)]

Table 3 Classification of animal models of human disease (Hau 2008)

Model type	Description
Induced	The condition under investigation is experimentally induced in healthy animals
Spontaneous	Naturally occurring genetic variants (mutants) modelling similar conditions in humans
Genetically modified	Genetically modified animals expressing a condition/disease of interest
Negative	Species, strain or breed of animal, which demonstrates a lack of reactivity to a particular stimulus or one that does not develop a specific condition following a pathological challenge that induces the disease in other animals
Orphan	Functional disorder occurring in an animal species, which has not yet been described in humans

Given that the primary physiological functions of the mammalian bladder are urine storage and voiding at appropriate times, it is not unreasonable to presume that the general principles regulating its function should be similar among different species (Brading 2006). It is however important to be aware that there are a number of important differences in normal urinary tract structure and function between different animal species and humans. These must be considered and taken into account before extrapolating physiological and pathological findings back to humans. For example, most mammalian species, apart from humans and old world monkeys, possess a dual cholinergic and purinergic excitatory innervation to the detrusor muscle of the bladder, and this is thought to be due to the evolutionary requirement of certain species to mark their territory with urine (Craggs et al. 1986; Hashitani et al. 2000; Sibley 1984). Although nerve-mediated contractions in the human bladder are almost solely mediated by a cholinergic mechanism, normal human bladder smooth muscle cells express functional adenosine

triphosphate (ATP, purinergic) receptors (Inoue and Brading 1991), and non-cholinergic non-adrenergic (NANC) excitatory transmission appears to emerge in some pathological conditions (Sibley 1984; Sjogren et al. 1982).

Another important difference is the arrangement of the parasympathetic system in certain species. Rats are commonly used for animal studies, but it must be noted that the postganglionic parasympathetic cell bodies innervating the rat bladder are found entirely in the pelvic ganglia, whereas a substantial proportion of these cell bodies are located in the bladder wall in humans and other animal species (McMurray et al. 2006). Consequently, the pattern of denervation following partial bladder outlet obstruction (BOO) differs between species (McMurray et al. 2006; Gabella and Uvelius 1990).

Acetylcholine is the primary neurotransmitter effecting bladder emptying through its action on the muscarinic receptors on detrusor muscle. Muscarinic receptors are classified based on molecular and pharmacological criteria into five subtypes (M_1–M_5), and detrusor muscle, like other forms of smooth muscle, exhibits a heterogeneous distribution of these receptor subtypes. Muscarinic receptors are also present in other cell types in the bladder, including interstitial cells (Gillespie et al. 2003) and the urothelium (Hanna-Mitchell et al. 2007). Studies have shown that the combined density of M_2 and M_3 receptors is very close to the total density of muscarinic receptors in each species studied (rat, rabbit, guinea-pig and human), with a predominance of M_2 receptor expression (Wang et al. 1995). Immunoprecipitation data indicate that the M_3 receptor subtype density varied from 8% in rats to 32% in humans, and that the proportion of muscarinic M_2 and M_3 receptor subtypes is different for the various species. The M_2–M_3 receptor ratio has been reported as 3:1 in the bladders from humans, rabbits and guinea-pigs and 9:1 in the rat bladder (Wang et al. 1995). Although it seems probable that there is variable expression of muscarinic receptor subtypes in the lower urinary tract of different species, recent studies have shed doubt on the validity of this immunohistological data (Pradidarcheep et al. 2009; Jositsch et al. 2009). Studies have shown that the traditional criteria used to establish the specificity of the antiserum used for immunoprecipitation studies is unable to reliably predict specificity. This is evidenced by the false positive labelling of tissues taken from muscarinic receptor gene-deficient mice (Jositsch et al. 2009). Care must therefore be taken when interpreting results of immunohistochemical studies that predate the development of the appropriate knock-out strains. There are also significant differences in the composition of the urinary bladder wall between small and large animal bladders, with variability in urodynamic features.

Almost 4,000 organisms including a variety of mammals have now had their genomes sequenced (Venter 2010), but unfortunately, large animals are often of limited value in genomic studies. Non-human primates should be ideally suited for modelling the human condition, but generating genetically modified monkeys or apes is fraught with difficulties. Even though the genomes of several non-human primates have been completely mapped out (The Chimpanzee Sequencing and Analysis Consortium 2005; Gibbs et al. 2007; Varki and Altheide 2005), manipulating specific genes for the purpose of knock-out and knock-in studies has been hampered by the low efficiency and poor reliability of the available gene transfer methods used (Chan 2004). Mice, on the other hand, though more dissimilar from

humans, are ideally suited for transgenic and knock-out studies because they can be genetically manipulated with relative ease and have a shorter generation span.

The structural and functional differences discussed above are but a few examples of the interspecies variability, which are relevant to research into the lower urinary tract. It is important that these aspects are taken into account when using animal models, in order to distinguish between experimental findings that constitute general principles and are applicable to other species from those that are particular to the animal being examined. Failure to attend to species differences not only ignores the opportunity to better understand the mechanisms of diversity, but may lead researchers to make inadequately founded suppositions (Insel 2007).

Given the interspecies variability, the various animal models in current use have different strengths and weaknesses, which render them suitable for studying different aspects of disease. Animal studies can be viewed as hierarchical in nature as studies using rodents and small laboratory animals are relatively simpler to perform and maintain than studies done using larger animals and primates. Research done with small mammals is less expensive, more accessible and less time-consuming to carry out because of the shorter lifespan of these animals, but care must be taken when attempting to translate findings derived from "simple" species to more "complex" ones (Insel 2007).

Irrespective of the animal species and model used, it is vitally important to decide whether an experimental finding or change is contributory and relevant to the physiological or pathological process being studied. Whole organisms adapt to change and cope with pathological challenges through neural plasticity and cellular adaptations. Experimental findings could therefore be compensatory changes or collateral effects rather than aetiological or pathophysiological. It may also be that the changes seen are epiphenomena, being totally unrelated to the underlying pathological process and occurring by coincidence. Careful validation of findings is needed to ensure applicability of any study result to humans, and this is often an underestimated last step of published studies using animal models.

Despite the difficulties associated with animal modelling and the need for careful validation of any findings that these studies yield, there is no substitute for their use as tools to evaluate medical interventions in complex biological systems (Matute-Bello et al. 2008). To overcome the shortcomings associated with animal models, researchers must pay close attention when choosing an animal model to ensure that it is the one that is best suited for testing a hypothesis or answering a specific question. Any findings should then ideally be reproduced in more than one mammalian species before extrapolating data and contemplating research using human subjects.

6.1 The Difficulty in Developing Animal Models of OAB

Several animal models have been developed in a variety of species in an attempt to study the pathophysiology of OAB. However, OAB is a symptom-based

diagnosis in which the conscious perception of urgency is key to the diagnosis. There is no way of knowing for definite whether an animal is experiencing urgency, even if pseudoaffective changes in behaviour may suggest it. Animals cannot relate their symptoms to investigators, and consequently, it is not possible technically to create an animal model of OAB. The problem is essentially the difficulty of interpolating subjectivity.

The inability to quantify urgency in animals, or assert its presence in the first place, necessitates the use of surrogate markers. That is why most experimental models have focussed on DO because this can be objectively measured at cystometry. It must be remembered however that DO is not pathognomonic of OAB, as a significant proportion of patients diagnosed with the condition have no evidence of DO on urodynamic evaluation (Hashim and Abrams 2006). Furthermore, non-micturition contractions do not necessarily equate to DO; indeed, they may be normal in some species.

Other surrogate markers that have been used in lower urinary tract research as clinically meaningful endpoints are urinary frequency, voided volumes and changes in micturition habit. An example of the latter is the fact that most rodents tend to urinate in one particular part of their confinement area, and in some models of presumed overactivity, the rodents stop doing this and begin voiding in various places (Gevaert et al. 2007). This behavioural modification is taken to be a marker of a change in their lower urinary tract whereby the rodent is unable to defer micturition and therefore has to void where it is rather than being able to make it to its usual spot in time. This is a plausible concept that may be analogous to the behavioural changes seen in OAB sufferers who identify the location of their nearest toilet when away from the comfort and safety of their home.

Research into bladder overactivity is also hampered by the lack of a biological marker (biomarker), which is specific to the lower urinary tract and reflects the activity of the disease process. Some molecules/substances have been identified and measured in the urine, but none have proven to be diagnostic. Given the likely multifactorial aetiology of OAB and the chronic nature of the condition, it will be difficult to develop such a biomarker.

For some patients, such as those with neuropathic lower urinary tract dysfunction due to a cerebrovascular event or a spinal cord injury, the aetiology of storage symptoms is known. The inciting event can be recapitulated to induce equivalent changes in animal models. For idiopathic OAB, this is not currently possible, hindering research into mechanisms and therapy. Consequently, phenotypic (isomorphic) models are used in which an animal expressing features (signs and behavioural changes) resembling those seen in OAB sufferers are used to try and determine the underlying aetiology. Unfortunately, this approach to modelling is complicated by the fact that the bladder has a limited repertoire of responses to injury, and thus differing aetiological factors may produce a similar picture in affected individuals.

7 Animal Models of OAB

A wide range of animal species have been used for lower urinary tract research and to study urological disease. These include hamsters, mice, guinea-pigs, rats, rabbits, cats, dogs, pigs and non-human primates. The aim of this section is to provide a summary of the major types of animal models that have been used to study the mechanisms and treatments of OAB, and to highlight the areas of concern when interpreting results yielded by studies using these models.

The starting point for many of our animal models has come from what is known epidemiologically about the condition. It is well known that ageing is associated with a rise in prevalence of OAB for both genders and this has underpinned the study of ageing animals. There are recognised associations of storage symptoms and OAB with neurological conditions and BOO, and consequently, animal models have been developed with this in mind. All patients with neurological disorders have some form of consequence to their lower urinary tract function as no matter how mild their disease is, they inevitably develop a bladder-related problem. This has led to the development of large range of neurological models.

Most animal models used to study OAB are induced models, whereby a relevant pathological challenge is experimentally applied to a healthy animal. With advancing technology and the ability to genetically modify animals, there is an increasing trend towards the use of transgenic models.

7.1 Peripheral Versus Central Models

Animal models of OAB can be broadly divided into peripheral and central models based on the predominant site of the deficit. Peripheral models are those resulting from direct damage to the bladder, its peripheral innervation or blood supply, whereas central models develop following injuries to the spinal cord, brainstem or higher centres.

7.2 Induced Hypersensitivity/Inflammatory Models

These are commonly used models in which hypersensitivity and/or inflammation is induced in the bladder by either a surgical insult or chemically by instillation of a noxious substance intravesically for a short period of time. Numerous chemicals have been used for this purpose and include acetic acid, citric acid, hydrochloric acid, capsaicin, protamine sulphate, xylene and turpentine. The resulting effects are thought to be due to up-regulation and stimulation of nociceptive afferent C-fibres within the bladder wall (Fowler 2002). This leads to increased sensory activity, which is a mechanism that has been proposed as a potential cause of urgency.

Although these models have been used to try and elucidate the mechanisms leading to the development of storage symptoms, they are not a true model for OAB as symptoms arise by the entirely unrelated process of acute reactive inflammation. Indeed on histological examination, the bladder mucosa of OAB sufferers does not show evidence of the inflammatory response, which is triggered in these animals. The noxious stimuli used can affect other epithelial surfaces indicating that the effects on the urothelium are a non-specific response to injury. Thus, these models may better equate to different clinical conditions, such as infectious cystitis, radiation cystitis or ketamine cystitis. Modelling a chronic condition with an acute inflammatory challenge fails to recapitulate the mechanisms of neural plasticity and cellular adaptation, which are likely to arise in OAB clinically.

7.3 Bladder Outlet Obstruction Model

BOO is a common problem for the ageing male as a consequence of benign prostatic enlargement (BPE), in whom increasing prevalence of lower urinary tract symptoms is also apparent (Irwin et al. 2009). Storage symptoms often persist even after the obstruction is surgically corrected (Seaman et al. 1994), signifying that the association is not necessarily causative (Thomas and Abrams 2000). The mechanisms underlying the OAB seen in patients with BOO are thus not fully understood.

Effects similar to BOO in humans are relatively straightforward to replicate in animals. This has been achieved in a variety of animal species including the pig, rat, guinea-pig and rabbit by partial obstruction of the urethra using some form of ligature that either occludes/stenoses the urethra immediately or does so gradually as the animal grows (Drake et al. 2006; Jorgensen et al. 1983; Sibley 1985; Kato et al. 1988; Pampinella et al. 1997; Wolffenbuttel et al. 2001). These models show many of the structural and physiological bladder wall changes seen in human BOO, including muscle cell hypertrophy, altered responsiveness to stimuli, increased spontaneous myogenic activity with development of non-micturition contractions and enlarged sensory neurons and parasympathetic ganglia. There is also patchy denervation of detrusor muscle (Turner and Brading 1997), a key factor in the myogenic hypothesis (Brading and Turner 1994), though it is not seen in rats (Gabella and Uvelius 1990). Filling phase detrusor contractions can persist despite pharmacological blockade of peripheral neuronal activity, signifying a non-neuronal origin (Turner and Brading 1997; Igawa et al. 1992).

Many of the published studies based on research done in partial BOO animal models have used female rodents, which complicates their interpretation, given that they are derived by analogy to male BPE. Furthermore, the induced BOO is much more acute, and potentially more severe, than BPE – particularly if the urethra is crushed by a ligature tied firmly against a calibration rod. Notwithstanding, partial BOO appears to be a good model to study lower urinary tract symptoms as it can be reliably reproduced with good face and aetiological validity.

7.4 Spontaneous Hypertensive Rat

The spontaneous hypertensive rat (SHR) is a genetic model of multifactorial hypertension, which is considered to resemble human essential hypertension (McMurray et al. 2006). The SHR also exhibits abnormal bladder function and hyperactive behaviour (Jin et al. 2009). Compared to their genetic control, these animals have been shown to have a reduced bladder capacity and micturition volume, increased urinary frequency and a greater occurrence of non-voiding contractions analogous to DO (McMurray et al. 2006). This has led to the use of the SHR as a model to study DO and OAB.

The exact cause for the abnormal voiding function in the SHR is not known, but seems to involve both peripheral and spinal mechanisms. The major abnormality appears to lie in the central nervous system with changes in the noradrenergic control of the micturition reflex (Persson et al. 1998). Peripherally, there is both an increased detrusor muscle and decreased neuronal responsiveness to norepinephrine.

The majority of cystometric studies in animals measure intravesical pressure alone, so there is no way of knowing whether the bladder pressure changes recorded are actually due to transmitted rises in abdominal pressure. This uncertainty is relevant to the SHR, given the hyperactive behaviour of the rats. A study published in 2008 tackled this issue by performing simultaneous recordings of intra-abdominal and intravesical pressures in conscious male SHRs (Jin et al. 2009). The group found that the majority (76%) of the intravesical pressure rises recorded in the SHRs represented overactive detrusor waves with the remainder being caused by abdominal pressure changes (Jin et al. 2009). Although ideally simultaneous measurement of intra-abdominal and intravesical pressures should be done at cystometry, given that the vast majority of pressure changes recorded in these animals are due to non-voiding contractions of the detrusor, it seems reasonable to measure intravesical pressure alone.

7.5 The Hyperlipidaemic Rat

Epidemiological studies have shown correlation between lower urinary tract symptoms and erectile dysfunction (Ponholzer et al. 2004; Macfarlane et al. 1996; Boyle et al. 2003), suggesting that common mechanisms might be involved in the development of both these conditions. The role of hyperlipidaemia in erectile dysfunction has been extrapolated to derive a model of lower urinary tract symptoms and OAB. Rats fed with a high fat/cholesterol diet show an increase in urinary frequency and a greater number of non-voiding contractions during bladder filling on awake cystometry compared to control rats (Rahman et al. 2007; Son et al. 2007). The hyperlipidaemic rats show a significantly higher weight and cholesterol level compared to the controls, but with no statistically significant difference in glucose levels.

The underlying pathological mechanisms have not been clearly defined, but current evidence suggests that there is likely to be both a vascular and neurogenic

component (Rahman et al. 2007). Chronic bladder ischaemia secondary to atherosclerosis may be one aetiological factor as prolonged moderate bladder ischaemia has been shown to be associated with detrusor contractions during bladder filling in rabbits (Azadzoi et al. 1999). There is also an up-regulation of purinergic receptors in the urothelium and bladder nerve bundles compared to control rats (Rahman et al. 2007), perhaps corresponding to the increased NANC innervation seen in ageing humans and bladder conditions (Andersson and Pehrson 2003).

7.6 Neurological Models

Injuries or diseases of the central nervous system can disrupt the voluntary control of micturition, damage descending central inhibition and cause re-emergence of primitive voiding reflexes (Andersson and Pehrson 2003). The central control of micturition is highly complex, and the voiding disturbance that develops will depend on the location and extent of the neurological injury. The coordination between the detrusor smooth muscle and the sphincter mechanism of the bladder occurs in the pontine region of the brainstem. Consequently, patients with suprapontine or cortical lesions have co-ordinated voiding mechanisms without detrusor sphincter dyssynergia (DSD) but typically have neurogenic DO (i.e., DO associated with a relevant neurological condition) on urodynamic studies (Andersson and Pehrson 2003). On the other hand, patients with spinal cord injuries will present with a variety of lower urinary tract signs and symptoms depending on the level of injury and whether it is partial or complete. Features comprise neurogenic DO, DSD and various forms of urinary incontinence.

Neurological models are not directly relevant to idiopathic OAB, but they have improved our understanding of the complex pathways controlling micturition. Given that OAB may develop as a result of increased bladder sensation and altered processing of afferent information, increased knowledge of lower urinary tract dysfunction in neurological patients is likely to be relevant to OAB and other hypersensitive bladder syndromes such as interstitial cystitis.

7.6.1 Spinal Cord Transection/Injury

This is one of the commonest neurological models used. Immediately following spinal cord injury, a spinal shock phase of varied duration is followed by disinhibition hyperreflexia below the injury, including spasticity (skeletal muscle hyperreflexia), DO (bladder hyperreflexia) and DSD (sphincter hyperreflexia). In addition to disinhibition resulting from loss of inhibitory descending pathways from higher centres, neural plasticity is relevant. Thus, nociceptive C fibres, which do not usually respond to bladder distension, emerge as functionally active in the afferent limb, underpinning part of the development of neurogenic DO (Morrison et al. 2002). This may explain the response to intravesical vanilloid therapy (Kuo et al. 2006).

7.6.2 Suprapontine Animal Models

A number of central nervous system disorders cause voiding dysfunction in humans, including cerebrovascular events, dementia, Parkinson's disease (PD) and multiple sclerosis (MS). In stroke patients, the most common urodynamic abnormality is neurogenic DO. Animal models of cerebrovascular events have provided insights into the pathophysiology of stroke-associated OAB. Experimental cerebral infarction can be achieved by occlusion of the middle cerebral artery (MCA) in rats, producing ischaemia within the ipsilateral frontoparietal cortex and the caudate putamen (Belayev et al. 1996). This leads to the development of DO with an increase in micturition frequency and a reduction in bladder capacity (Yokoyama et al. 1997), and these changes persist for several months (Yokoyama et al. 1998a). These effects develop as soon as 30 min after MCA occlusion and it is therefore reasonable to conclude that bladder function is under tonic cortical control and that disinhibition leads to bladder overactivity (Yokoyama et al. 1998a). This is corroborated by studies of midcollicular decerebration in cats with an intact neuroaxis as this leads to a facilitatory effect on bladder function (Ruch and Tang 1956). However, decerebration of rats with MCA occlusion reduces the effects of cerebral infarction on bladder function, and this suggests that in addition to loss of tonic inhibition, the bladder overactivity that develops is mediated in part by upregulation of an excitatory pathway from the forebrain (Yokoyama et al. 2000). Intravenous administration of N-methyl-D-aspartate (NMDA) receptor antagonists reduce the effects of cerebral infarction in awake animals, indicating the involvement of glutamatergic pathways in the pathogenesis of stroke associated OAB (Yokoyama et al. 1998b). However, NMDA receptor antagonists cannot completely reverse these effects and therefore other neurotransmitter systems are likely to be involved. Pharmacological studies indicate that dopaminergic pathways are also involved in cerebral infarction-induced bladder hyperactivity, such that antagonism of D_2-like receptors in MCA-occluded animals increases bladder capacity (Yokoyama et al. 1999). This is unsurprising, given that in the normal rat, activation of D_1-like dopaminergic receptors inhibits micturition and activation of D_2-like receptors facilitates micturition (de Groat and Yoshimura 2001).

PD is a chronic and progressive degenerative disease of the brain characterised by selective destruction of striatal dopaminergic neurons that pass from the substantia nigra pars compacta to the putamen (Gerfen 2000). The majority of patients with the condition will eventually develop voiding dysfunction, characterised by neurogenic DO and impaired relaxation of the striated urethral sphincter. Parkinsonism can be induced in monkeys by administering the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which is selective for dopaminergic neurons in the substantia nigra (Burns et al. 1983). This animal model of PD has been used to study the pathogenesis of the disease and has increased our understanding of the dopaminergic pathways controlling micturition. In MPTP-treated monkeys, cystometry showed that injection of a dopamine D_1 receptor agonist significantly increases the bladder volume and pressure thresholds for inducing the micturition reflex, with no corresponding effect seen in normal monkeys (Yoshimura et al. 1993).

In contrast, administering a D_2 receptor agonist reduced the threshold volume of the bladder, which triggers the micturition reflex in both normal and MPTP-treated monkeys (Yoshimura et al. 1993). These results suggest that in PD, the degeneration of dopaminergic neurons in the substantia nigra leads to neurogenic DO, probably as a result of a failure of activation of D_1-like receptors, which then allows D_2 receptors to facilitate micturition.

7.6.3 Experimental Autoimmune Encephalomyelitis Model

Voiding dysfunction is a common problem for patients with MS and develops in over 90% of patients who have had MS for more than 10 years (Andersson and Pehrson 2003). The voiding abnormalities are due mainly to spinal demyelinating lesions, but cerebral defects may contribute. Neurogenic DO is the most common abnormality seen on urodynamic testing with 70% of patients affected, and this is associated with DSD in 50% of patients (Sirls et al. 1994).

Experimental autoimmune encephalomyelitis (EAE) is an inflammatory autoimmune condition, which targets nervous tissue and can be induced in various animal species by active immunisation with central nervous system immunogenic compounds or through passive transfer of encephalogenic T cell lines from affected animals (Petry et al. 2000). Animals with EAE can be used to study neuroinflammatory bladder dysfunction such as that which occurs with MS, though extrapolating data from these models has to take into account the uncertainties of transferring findings from an acute model to a chronic human condition. EAE is also a transient self-limiting condition that often recovers spontaneously, whereas MS is chronic and progressive. This must also be borne in mind when performing studies using this model.

7.7 Transgenic Animal Models

Advances in genetic engineering techniques have led to an increasing use of genetically modified organisms for research. Knock-out models and other transgenic animals are being used to study and understand the molecular mechanisms involved in both normal physiological processes and human disease. For example, knock-out mice have been used to determine the relative contribution of the different muscarinic receptor subtypes in normal bladder function and confirm that the M_3 subtype is the dominant receptor in vivo (Matsui et al. 2002).

The progress mapping the human genome, and various non-human organisms, such as *E. coli*, the fruit fly and the laboratory mouse, has yielded major insights in functional genomics and proteonomics (Hau 2008). The mouse is most commonly employed, given that it is widely available, easy to maintain and has a relatively short generation span. Several transgenic mouse models that bear particular relevance to lower urinary tract physiology and dysfunction have been developed. These knock-out models will be discussed briefly below but have clearly demonstrated that

molecular alterations of the urothelium, peripheral innervation and smooth muscle of the bladder leads to significant changes in its function (Hu et al. 2002).

7.7.1 Neuronal Nitric Oxide Synthase Knock-out Mouse

Compared with wild type mice, the neuronal nitric oxide synthase (nNOS) knock-out mouse demonstrates urinary frequency with no change in the total amount of urine produced (Burnett et al. 1997). This suggests changes in lower urinary tract function and is supported by the reduction in bladder capacity and induction of detrusor contractions seen in rats given intravesical nitric oxide scavengers or nitric oxide synthase inhibitors (Pandita et al. 2000; Masuda et al. 2007). Certain phosphodiesterases, through their effects on the nitric oxide-cyclic guanosine monophosphate pathway, are relevant to the lower urinary tract and may have clinical applicability (Andersson et al. 2007; Truss et al. 2000). It is not yet clear whether the changes seen in these animal models are due to bladder hypertrophy secondary to the impaired outlet relaxation or as a result of a lack of nitric oxide-induced modulation of the afferent pathways.

7.7.2 Uroplakin Knock-out Mouse

Uroplakins are integral components of the plaques that cover the apical surface of urothelial cells and are critical to the barrier function of the urothelium (Hu et al. 2002). Uroplakin IIIa and especially uroplakin II knock-out mice are associated with significant changes in bladder function including increased spontaneous activity and inter-micturition pressure and the development of non-voiding contractions on cystometry analogous to DO (Aboushwareb et al. 2009). The bladders of these knock-out mice show no significant change in their contractile response to carbachol, so an enhanced contractile response to endogenous neurotransmitters is unlikely to be a major contributing factor to the alterations in bladder function (Aboushwareb et al. 2009). On histological examination, uroplakin deficiency is associated with ultra-structural changes, which are implicated in the defective urothelial permeability seen in these models (Aboushwareb et al. 2009). These transgenic mice provide further evidence for the important role that the urothelium plays with regards to bladder physiology and dysfunction.

7.7.3 Prostaglandin Receptor Knock-out Mouse

Prostaglandins are produced by the constitutively expressed cyclooxygenase-(COX) 1 enzyme, and an inducible isozyme, COX-2. Expression of COX-2 is enhanced in models of bladder dysfunction, and prostaglandin E_2 (PGE_2) produced by this enzyme is increased in the urine of male and female patients with overactive bladders (Kim et al. 2005, 2006). PGE_2 mediates its effects by activating the EP

family (EP_1–EP_4 isoforms) of G-protein coupled receptors (Hao and Breyer 2008). When administered intravesically, PGE_2 induces non-voiding contractions and reduces bladder capacity in humans (Schussler 1990).

PGE_2 may have a physiological role to play as it is produced by detrusor muscle in response to stretch. Knock-out models of the EP_3 receptor have enlarged bladder capacity, which is unrelated to urine composition or volume (McCafferty et al. 2008). These knock-out animals do not display bladder overactivity when an EP_3 receptor agonist is infused intravesically. Thus, the EP_3 receptor may contribute to bladder dysfunction under conditions of enhanced PGE_2 release, as observed in OAB patients (McCafferty et al. 2008). The limited evidence currently available point towards an effect on peripheral afferent sensitivity.

7.7.4 Purinergic Receptor Knock-out Mouse

ATP is released from human and animal urothelium in response to mechanical stretch (Sun et al. 2001; Ferguson et al. 1997). ATP can act as a sensory neurotransmitter by binding to purinergic receptors (P_2X) on suburothelial afferent nerve endings. Indeed, intravesical instillation of ATP into normal awake, freely moving rats triggers bladder overactivity, with an increased frequency and amplitude of bladder contractions (Pandita and Andersson 2002). P_2X_3 receptor knock-out mice appear to have reduced bladder sensation, with reduced urinary frequency and larger voided volumes (Cockayne et al. 2000). This points towards a physiological role for ATP and purinergic receptors in normal bladder function. It may be that one of the contributory mechanisms in OAB could be an augmentation of the purinergic pathway with enhanced ATP levels and up-regulation of purinergic receptor expression.

7.7.5 Oestrogen Receptor Knock-out Mouse

Epidemiological studies have implicated oestrogen deficiency as a factor contributing to the increased prevalence of lower urinary tract symptoms seen with ageing in women (Iosif and Bekassy 1984). The influence of oestrogen on the bladder has consequently been the subject of several experimental and clinical studies. Oestrogen receptors (ERs) are members of the nuclear receptor superfamily, and two different subtypes (ERα and ERβ) have been identified (Kuiper et al. 1997; Kuiper et al. 1996). They are ligand-activated transcription factors that regulate target gene expression. Both ER subtypes are expressed in the lower urinary tract, but ERβ is thought to be the predominant isoform in the bladder (Makela et al. 2000).

Studies using knock-out mice lacking either the ERα, ERβ or both receptors showed no significant differences in voiding patterns or in any parameter measured on awake control cystometry compared to wild-type animals (Schroder et al. 2003). Use of in vitro muscle strips from sacrificed animals also showed no differences in contractile response to electrical field stimulation or carbachol for any of the

knock-out strains compared to control mice (Schroder et al. 2003). There is however variation in the response to capsaicin, as ERα knock-out mice did not develop the overactive cystometry patterns seen in the other ER knock-out strains and wild-type mice when capsaicin was instilled intravesically. This suggests that rather than an efferent or direct muscle effect, oestrogen may impact on lower urinary tract function through changes in afferent signalling (Schroder et al. 2003), and in keeping with this, ERs have been identified in several areas within the central nervous system known to be involved in micturition (VanderHorst et al. 1997, 1998, 2001).

Capsaicin binds the vanilloid receptor type 1 (VR-1) (Caterina et al. 1997), and this receptor is believed to play an important role in sensitisation and development of visceral pain (Wang et al. 2008). The absence of a response to capsaicin in the ERα knock-out mouse points towards an oestrogen-mediated alteration in VR-1 function and thus a possible role in pain mediation (Schroder et al. 2003). This may explain why painful bladder disorders such as interstitial cystitis are more common in women and why the symptoms of sufferers fluctuate in severity during the menstrual cycle.

The role of oestrogen in OAB is less clear. Although oestrogens have long been prescribed to treat postmenopausal lower urinary tract symptoms, the clinical efficacy of this approach has not been conclusively proven. The benefits patients experience with hormone replacement therapy may result from reversal of urogenital atrophy rather than being due to a direct action on the lower urinary tract. A double-blind placebo-controlled trial carried out in 40 postmenopausal women failed to demonstrate a statistically significant beneficial effect of an oestradiol implant over placebo in treating urinary urgency (Rufford et al. 2003). This adds further weight to the argument that oestrogen is unlikely to play a major role in OAB (Table 4).

8 Conclusions

OAB is a prevalent condition affecting the quality of life. Current management is limited to symptom relief and consequently is viewed as a chronic medical condition. If better treatments are to be developed with the eventual possibility of a cure, then an improved understanding of the integrative physiology of the lower urinary tract and its dysfunction are required.

Animal models are an important research tool for the study of whole organism physiology and pathology. Unfortunately, owing to the fact that OAB is a symptom-based diagnosis, development of an animal model of the condition is hindered by the lack of a corroborating biomarker-specific for OAB and one which predicts treatment outcome. The various models that have been used to date have different strengths and weaknesses but nevertheless have yielded findings that have advanced our understanding of lower urinary tract function.

Table 4 Animal models of OAB

Animal model	Model type	Advantages of model	Disadvantages of model
Hypersensitivity/ inflammatory models	Peripheral, induced model	• Easy to develop, surgery not required • Wide range of chemicals can be used to trigger injury • Exhibits many of the clinical features seen in OAB	• Poor aetiological validity, fails to recapitulate mechanisms that underlie OAB, symptoms due to non specific tissue response to injury • Acute model for chronic condition
BOO model	Peripheral, induced model	• Replicates many structural and physiological bladder changes seen in human BOO • Reliable model with good face and aetiological validity	• Surgical procedure required • Induced BOO more acute and potentially more severe than in the human condition • Female rodents often used
SHR	Genetic model	• Easily accessible • Expresses many of the clinical features seen in OAB	• Exact cause of abnormal voiding function not yet established • Hyperactive behaviour may exaggerate the animals' phenotype
Hyperlipidaemic rat	Peripheral and central model	• Easily reproducible • Aetiologically relevant to male patients with erectile dysfunction and lower urinary tract symptoms	• Underlying pathological mechanisms not clearly defined • Increased weight may be associated with a larger number of non-voiding contractions due to rises in intra-abdominal pressure
Spinal cord transection/ injury	Central, induced model	• Easy to develop • Aetiologically relevant to patients with spinal cord injury	• Surgical procedure required • Not directly relevant to idiopathic OAB
MCA occlusion	Central, induced model	• Improves understanding of the higher centres and pathways involved in micturition • Aetiologically relevant to patients with a total anterior circulation stroke	• Surgical procedure required • Ischaemic injury may be more severe than in the human condition • Less relevant to patients with strokes affecting other cerebral territories
MPTP toxin model	Central, induced model	• Easy to develop, as toxin is administered systemically and is lipophilic • Good face validity as the treated animals develop parkinsonian symptoms and associated lower urinary tract dysfunction	• Acute model for a chronic progressive condition • Limited aetiologic (construct) validity as PD is not commonly toxin induced • MPTP is highly toxic to humans (dangerous to administer)

EAE model	Central, induced model	• Good face validity, exhibits many of the clinical and histological features of MS • Useful for studying neuroinflammatory bladder dysfunction such as that which occurs in MS • Disease in EAE is easy to detect and can be relapsing	• Variable reliability as the clinical course of the animal model depends on the immunising antigen • Poor aetiological validity as animal immunisation does not induce MS, but acute disseminated encephalomyelitis • Acute model for chronic progressive conditions • Unlike MS, EAE is a self limiting condition • Limited predictive value for therapeutic agents
Transgenic animals (knock-out/knock-in)	Genetic model	• Allows the roles of specific gene products to be studied • Improves understanding of molecular mechanisms in normal bladder physiology and dysfunction	• Poor aetiologic validity, fails to recapitulate mechanisms that underlie OAB • Limited face validity • Sophisticated and costly procedure for development

It is unrealistic to expect that a single animal model will recapitulate all the signs, symptoms, mechanisms and consequences of a disease/condition. However, proper selection of the most appropriate model will allow most experimental questions to be addressed. Animal modelling is an iterative process in which information gained should be re-invested and used to modify and refine existing models. There is no question that animal modelling has been crucial in advancing medical knowledge. It is hoped that better treatments for OAB may be developed through ongoing development of robust animal models.

References

Aboushwareb T et al (2009) Alterations in bladder function associated with urothelial defects in uroplakin II and IIIa knockout mice. Neurourol Urodyn 28(8):1028–1033

Abrams P, Drake M (2007) Overactive bladder. In: Wein AJ et al (eds) Campbell-Walsh urology. Saunders, Philadephia, pp 2079–2090

Abrams P et al (2000) Overactive bladder significantly affects quality of life. Am J Manag Care 6(11 suppl):S580–S590

Abrams P et al (2002a) The standardisation of terminology of lower urinary tract function: report from the Standardisation Sub-committee of the International Continence Society. Neurourol Urodyn 21(2):167–178

Abrams P et al (2002b) The standardisation of terminology of lower urinary tract function: report from the Standardisation Sub-committee of the International Continence Society. Am J Obstet Gynecol 187(1):116–126

Andersson KE (2002) Bladder activation: afferent mechanisms. Urology 59(5 Suppl 1):43–50

Andersson KE, Pehrson R (2003) CNS involvement in overactive bladder: pathophysiology and opportunities for pharmacological intervention. Drugs 63(23):2595–2611

Andersson KE et al (2007) Phosphodiesterases (PDEs) and PDE inhibitors for treatment of LUTS. Neurourol Urodyn 26(6 Suppl):928–933

Azadzoi KM et al (1999) Overactivity and structural changes in the chronically ischemic bladder. J Urol 162(5):1768–1778

Belayev L et al (1996) Middle cerebral artery occlusion in the rat by intraluminal suture. Neurological and pathological evaluation of an improved model. Stroke 27(9):1616–1622; discussion 1623

Birder LA (2005) More than just a barrier: urothelium as a drug target for urinary bladder pain. Am J Physiol Renal Physiol 289(3):F489–F495

Birder LA, de Groat WC (2007) Mechanisms of disease: involvement of the urothelium in bladder dysfunction. Nat Clin Pract Urol 4(1):46–54

Blaivas JG (1996) The bladder is an unreliable witness. Neurourol Urodyn 15(5):443–445

Boyle P et al (2003) The association between lower urinary tract symptoms and erectile dysfunction in four centres: the UrEpik study. BJU Int 92(7):719–725

Brading AF (1997) A myogenic basis for the overactive bladder. Urology 50(6A Suppl):57–67; discussion 68–73

Brading AF (2006) Spontaneous activity of lower urinary tract smooth muscles: correlation between ion channels and tissue function. J Physiol 570(Pt 1):13–22

Brading AF, Turner WH (1994) The unstable bladder: towards a common mechanism. Br J Urol 73(1):3–8

Bramble FJ (1982) The treatment of adult enuresis and urge incontinence by enterocystoplasty. Br J Urol 54(6):693–696

Bramble FJ (1990) The clam cystoplasty. Br J Urol 66(4):337–341

Brown JS et al (2000) Urinary incontinence: does it increase risk for falls and fractures? Study of Osteoporotic Fractures Research Group. J Am Geriatr Soc 48(7):721–725
Buckner SA et al (2002) Spontaneous phasic activity of the pig urinary bladder smooth muscle: characteristics and sensitivity to potassium channel modulators. Br J Pharmacol 135(3):639–648
Bulmer P, Yang Q, Abrams P (2001) Does cigarette smoking cause detrusor instability in women? J Obstet Gynaecol 21(5):528–529
Burnett AL et al (1997) Urinary bladder-urethral sphincter dysfunction in mice with targeted disruption of neuronal nitric oxide synthase models idiopathic voiding disorders in humans. Nat Med 3(5):571–574
Burns RS et al (1983) A primate model of parkinsonism: selective destruction of dopaminergic neurons in the pars compacta of the substantia nigra by N-methyl-4-phenyl-1, 2, 3, 6-tetra-hydropyridine. Proc Natl Acad Sci USA 80(14):4546–4550
Caterina MJ et al (1997) The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 389(6653):816–824
Chan AW (2004) Transgenic nonhuman primates for neurodegenerative diseases. Reprod Biol Endocrinol 2:39
Cockayne DA et al (2000) Urinary bladder hyporeflexia and reduced pain-related behaviour in P2X3-deficient mice. Nature 407(6807):1011–1015
Coolsaet BL et al (1993) New concepts in relation to urge and detrusor activity. Neurourol Urodyn 12(5):463–471
Craggs MD, Rushton DN, Stephenson JD (1986) A putative non-cholinergic mechanism in urinary bladders of New but not Old World primates. J Urol 136(6):1348–1350
de Groat WC (1997) A neurologic basis for the overactive bladder. Urology 50(6A Suppl):36–52; discussion 53–56
de Groat WC, Yoshimura N (2001) Pharmacology of the lower urinary tract. Annu Rev Pharmacol Toxicol 41:691–721
Drake MJ, Mills IW, Gillespie JI (2001) Model of peripheral autonomous modules and a myovesical plexus in normal and overactive bladder function. Lancet 358(9279):401–403
Drake MJ, Harvey IJ, Gillespie JI (2003a) Autonomous activity in the isolated guinea pig bladder. Exp Physiol 88(1):19–30
Drake MJ et al (2003b) Partial outlet obstruction enhances modular autonomous activity in the isolated rat bladder. J Urol 170(1):276–279
Drake MJ et al (2005) Localized contractions in the normal human bladder and in urinary urgency. BJU Int 95(7):1002–1005
Drake M et al (2006) Muscarinic stimulation of the rat isolated whole bladder: pathophysiological models of detrusor overactivity. Auton Autacoid Pharmacol 26(3):261–266
Ferguson DR, Kennedy I, Burton TJ (1997) ATP is released from rabbit urinary bladder epithelial cells by hydrostatic pressure changes–a possible sensory mechanism? J Physiol 505(Pt 2): 503–511
Fowler CJ (2002) Bladder afferents and their role in the overactive bladder. Urology 59(5 suppl 1): 37–42
Gabella G, Uvelius B (1990) Urinary bladder of rat: fine structure of normal and hypertrophic musculature. Cell Tissue Res 262(1):67–79
Gerfen CR (2000) Molecular effects of dopamine on striatal-projection pathways. Trends Neurosci 23(10 Suppl):S64–S70
Gevaert T et al (2007) Deletion of the transient receptor potential cation channel TRPV4 impairs murine bladder voiding. J Clin Invest 117(11):3453–3462
Gibbs RA et al (2007) Evolutionary and biomedical insights from the rhesus macaque genome. Science 316(5822):222–234
Gillespie JI (2005) A developing view of the origins of urgency: the importance of animal models. BJU Int 96(suppl 1):22–28
Gillespie JI, Harvey IJ, Drake MJ (2003) Agonist- and nerve-induced phasic activity in the isolated whole bladder of the guinea pig: evidence for two types of bladder activity. Exp Physiol 88(3):343–357

Griffiths D et al (2007) Cerebral control of the bladder in normal and urge-incontinent women. Neuroimage 37(1):1–7
Griffiths DJ et al (2009) Cerebral control of the lower urinary tract: how age-related changes might predispose to urge incontinence. Neuroimage 47(3):981–986
Haferkamp A, Dorsam J, Elbadawi A (2003a) Ultrastructural diagnosis of neuropathic detrusor overactivity: validation of a common myogenic mechanism. Adv Exp Med Biol 539(Pt A): 281–291
Haferkamp A et al (2003b) Structural basis of neurogenic bladder dysfunction. III. Intrinsic detrusor innervation. J Urol 169(2):555–562
Hanna-Mitchell AT et al (2007) Non-neuronal acetylcholine and urinary bladder urothelium. Life Sci 80(24–25):2298–2302
Hannestad YS et al (2003) Are smoking and other lifestyle factors associated with female urinary incontinence? The Norwegian EPINCONT Study. BJOG 110(3):247–254
Hao CM, Breyer MD (2008) Physiological regulation of prostaglandins in the kidney. Annu Rev Physiol 70:357–377
Hashim H, Abrams P (2006) Is the bladder a reliable witness for predicting detrusor overactivity? J Urol 175(1):191–194; discussion 194–195
Hashim H, Abrams P (2007) Overactive bladder: an update. Curr Opin Urol 17(4):231–236
Hashitani H, Bramich NJ, Hirst GD (2000) Mechanisms of excitatory neuromuscular transmission in the guinea-pig urinary bladder. J Physiol 524(Pt 2):565–79
Hashitani H et al (2001) Origin and propagation of spontaneous excitation in smooth muscle of the guinea-pig urinary bladder. J Physiol 530(Pt 2):273–286
Hau J (2008) Animal models for human disease. In: Conn PM (ed) Sourcebook of models for biomedical research. Humana, New Jersey
Hu P et al (2002) Role of membrane proteins in permeability barrier function: uroplakin ablation elevates urothelial permeability. Am J Physiol Renal Physiol 283(6):F1200–F1207
Igawa Y, Mattiasson A, Andersson KE (1992) Is bladder hyperactivity due to outlet obstruction in the rat related to changes in reflexes or to myogenic changes in the detrusor? Acta Physiol Scand 146(3):409–411
Imamura T et al (2008) Cold environmental stress induces detrusor overactivity via resiniferatoxin-sensitive nerves in conscious rats. Neurourol Urodyn 27(4):348–352
Inoue R, Brading AF (1991) Human, pig and guinea-pig bladder smooth muscle cells generate similar inward currents in response to purinoceptor activation. Br J Pharmacol 103(4):1840–1841
Insel TR (2007) From animal models to model animals. Biol Psychiatry 62(12):1337–1339
Iosif CS, Bekassy Z (1984) Prevalence of genito-urinary symptoms in the late menopause. Acta Obstet Gynecol Scand 63(3):257–260
Irwin DE et al (2006) Population-based survey of urinary incontinence, overactive bladder, and other lower urinary tract symptoms in five countries: results of the EPIC study. Eur Urol 50 (6):1306–1314; discussion 1314–1315
Irwin DE et al (2006b) Impact of overactive bladder symptoms on employment, social interactions and emotional well-being in six European countries. BJU Int 97(1):96–100
Irwin DE et al (2009) Prevalence, severity, and symptom bother of lower urinary tract symptoms among men in the epic study: impact of overactive bladder. Eur Urol 56:14–20
Jin LH et al (2009) Substantial detrusor overactivity in conscious spontaneously hypertensive rats with hyperactive behaviour. Scand J Urol Nephrol 43(1):3–7
Jorgensen TM et al (1983) Experimental bladder hyperreflexia in pigs. Urol Res 11(5):239–240
Jositsch G et al (2009) Suitability of muscarinic acetylcholine receptor antibodies for immunohistochemistry evaluated on tissue sections of receptor gene-deficient mice. Naunyn Schmiedebergs Arch Pharmacol 379(4):389–395
Kato K et al (1988) The functional effect of mild outlet obstruction on the rabbit urinary bladder. J Urol 140(4):880–884
Kim JC et al (2005) Changes of urinary nerve growth factor and prostaglandins in male patients with overactive bladder symptom. Int J Urol 12(10):875–880

Kim JC et al (2006) Nerve growth factor and prostaglandins in the urine of female patients with overactive bladder. J Urol 175(5):1773–1776; discussion 1776
Kuiper GG et al (1996) Cloning of a novel receptor expressed in rat prostate and ovary. Proc Natl Acad Sci USA 93(12):5925–5930
Kuiper GG et al (1997) Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. Endocrinology 138(3):863–870
Kuo HC, Liu HT, Yang WC (2006) Therapeutic effect of multiple resiniferatoxin intravesical instillations in patients with refractory detrusor overactivity: a randomized, double-blind, placebo controlled study. J Urol 176(2):641–645
Macfarlane GJ et al (1996) The relationship between sexual life and urinary condition in the French community. J Clin Epidemiol 49(10):1171–1176
Makela S et al (2000) Differential expression of estrogen receptors alpha and beta in adult rat accessory sex glands and lower urinary tract. Mol Cell Endocrinol 170(1–2):219–229
Masuda H et al (2007) Effects of anaesthesia on the nitrergic pathway during the micturition reflex in rats. BJU Int 100(1):175–180
Matsui M et al (2002) Mice lacking M2 and M3 muscarinic acetylcholine receptors are devoid of cholinergic smooth muscle contractions but still viable. J Neurosci 22(24):10627–10632
Matute-Bello G, Frevert CW, Martin TR (2008) Animal models of acute lung injury. Am J Physiol Lung Cell Mol Physiol 295(3):L379–L399
McCafferty GP et al (2008) Enhanced bladder capacity and reduced prostaglandin E2-mediated bladder hyperactivity in EP3 receptor knockout mice. Am J Physiol Renal Physiol 295(2): F507–F514
McMurray G, Casey JH, Naylor AM (2006) Animal models in urological disease and sexual dysfunction. Br J Pharmacol 147(suppl 2):S62–S79
Mogensen J, Holm S (1989) Basic research and animal models in neuroscience – the necessity of co-evolution. Scand J Lab Anim Sci 16(Suppl 1):51
Morrison J, Steers WD, Brading A (2002) Neurophysiology and neuropharmacology. In: Abrams P et al (eds) Incontinence: 2nd international consultation on incontinence. Plymbridge, Plymouth, pp 85–161
Oh SJ et al (1999) Carbachol-induced sustained tonic contraction of rat detrusor muscle. BJU Int 84(3):343–349
Pampinella F et al (1997) Time-dependent remodeling of the bladder wall in growing rabbits after partial outlet obstruction. J Urol 157(2):677–682
Pandita RK, Andersson KE (2002) Intravesical adenosine triphosphate stimulates the micturition reflex in awake, freely moving rats. J Urol 168(3):1230–1234
Pandita RK, Mizusawa H, Andersson KE (2000) Intravesical oxyhemoglobin initiates bladder overactivity in conscious, normal rats. J Urol 164(2):545–550
Persson K et al (1998) Spinal and peripheral mechanisms contributing to hyperactive voiding in spontaneously hypertensive rats. Am J Physiol 275(4 Pt 2):R1366–R1373
Petry KG et al (2000) Experimental allergic encephalomyelitis animal models for analyzing features of multiple sclerosis. Pathol Biol 48(1):47–53
Ponholzer A et al (2004) Association between lower urinary tract symptoms and erectile dysfunction. Urology 64(4):772–776
Pradidarcheep W et al (2009) Lack of specificity of commercially available antisera against muscarinergic and adrenergic receptors. Naunyn Schmiedebergs Arch Pharmacol 379 (4):397–402
Rahman NU et al (2007) An animal model to study lower urinary tract symptoms and erectile dysfunction: the hyperlipidaemic rat. BJU Int 100(3):658–663
Ruch TC, Tang PC (1956) Localization of brain stem and diencephalic areas controlling the micturation reflex. J Comp Neurol 106(1):213–245
Rufford J et al (2003) A double-blind placebo-controlled trial on the effects of 25 mg estradiol implants on the urge syndrome in postmenopausal women. Int Urogynecol J Pelvic Floor Dysfunct 14(2):78–83

Schroder A et al (2003) Estrogen receptor subtypes and afferent signaling in the bladder. J Urol 170(3):1013–1016
Schussler B (1990) Comparison of the mode of action of prostaglandin E2 (PGE2) and sulprostone, a PGE2-derivative, on the lower urinary tract in healthy women. A urodynamic study. Urol Res 18(5):349–352
Seaman EK et al (1994) Persistence or recurrence of symptoms after transurethral resection of the prostate: a urodynamic assessment. J Urol 152(3):935–937
Sibley GN (1984) A comparison of spontaneous and nerve-mediated activity in bladder muscle from man, pig and rabbit. J Physiol 354:431–443
Sibley GN (1985) An experimental model of detrusor instability in the obstructed pig. Br J Urol 57(3):292–298
Singh G, Wilkinson JM, Thomas DG (1997) Supravesical diversion for incontinence: a long-term follow-up. Br J Urol 79(3):348–353
Sirls LT, Zimmern PE, Leach GE (1994) Role of limited evaluation and aggressive medical management in multiple sclerosis: a review of 113 patients. J Urol 151(4):946–950
Sjogren C et al (1982) Atropine resistance of transmurally stimulated isolated human bladder muscle. J Urol 128(6):1368–1371
Son H et al (2007) New unstable bladder model in hypercholesterolemia rats. Urology 69(1): 186–190
Sun Y et al (2001) Augmented stretch activated adenosine triphosphate release from bladder uroepithelial cells in patients with interstitial cystitis. J Urol 166(5):1951–1956
The Chimpanzee Sequencing and Analysis Consortium (2005) Initial sequence of the chimpanzee genome and comparison with the human genome. Nature 437(7055):69–87
Thomas AW, Abrams P (2000) Lower urinary tract symptoms, benign prostatic obstruction and the overactive bladder. BJU Int 85(suppl 3):57–68; discussion 70–71
Truss MC et al (2000) Initial clinical experience with the selective phosphodiesterase-I isoenzyme inhibitor vinpocetine in the treatment of urge incontinence and low compliance bladder. World J Urol 18(6):439–443
Turner WH, Brading AF (1997) Smooth muscle of the bladder in the normal and the diseased state: pathophysiology, diagnosis and treatment. Pharmacol Ther 75(2):77–110
Van Os-Bossagh P et al (2001) Micromotions of bladder wall in chronic pelvic pain (CPP): a pilot study. Int Urogynecol J Pelvic Floor Dysfunct 12(2):89–96
VanderHorst VG et al (1997) Estrogen receptor-immunoreactive neurons in the lumbosacral cord projecting to the periaqueductal gray in the ovariectomized female cat. Neurosci Lett 236(1): 25–28
VanderHorst VG et al (1998) Estrogen receptor-alpha-immunoreactive neurons in the periaqueductal gray of the adult ovariectomized female cat. Neurosci Lett 240(1):13–16
VanderHorst VG, Meijer E, Holstege G (2001) Estrogen receptor-alpha immunoreactivity in parasympathetic preganglionic neurons innervating the bladder in the adult ovariectomized cat. Neurosci Lett 298(3):147–150
Varki A, Altheide TK (2005) Comparing the human and chimpanzee genomes: searching for needles in a haystack. Genome Res 15(12):1746–1758
Venter JC (2010) Multiple personal genomes await. Nature 464(7289):676–677
Wang P, Luthin GR, Ruggieri MR (1995) Muscarinic acetylcholine receptor subtypes mediating urinary bladder contractility and coupling to GTP binding proteins. J Pharmacol Exp Ther 273(2):959–966
Wang ZY et al (2008) Lack of TRPV1 inhibits cystitis-induced increased mechanical sensitivity in mice. Pain 139(1):158–167
Wolffenbuttel KP et al (2001) Urodynamic follow-up of experimental urethral obstruction in individual guinea pigs. Neurourol Urodyn 20(6):699–713
Yamaguchi O et al (2009) Clinical guidelines for overactive bladder. Int J Urol 16(2):126–142
Yamanishi T et al (2000) The role of M(2)-muscarinic receptors in mediating contraction of the pig urinary bladder in vitro. Br J Pharmacol 131(7):1482–1488

Yokoyama O et al (1997) Influence of anesthesia on bladder hyperactivity induced by middle cerebral artery occlusion in the rat. Am J Physiol 273(6 Pt 2):R1900–R1907
Yokoyama O et al (1998a) Change in bladder contractility associated with bladder overactivity in rats with cerebral infarction. J Urol 159(2):577–580
Yokoyama O et al (1998b) Effects of MK-801 on bladder overactivity in rats with cerebral infarction. J Urol 159(2):571–576
Yokoyama O et al (1999) Glutamatergic and dopaminergic contributions to rat bladder hyperactivity after cerebral artery occlusion. Am J Physiol 276(4 Pt 2):R935–R942
Yokoyama O et al (2000) Role of the forebrain in bladder overactivity following cerebral infarction in the rat. Exp Neurol 163(2):469–476
Yoshimura N et al (1993) The dopamine D1 receptor agonist SKF 38393 suppresses detrusor hyperreflexia in the monkey with parkinsonism induced by 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP). Neuropharmacology 32(4):315–321

Animal Models of Stress Urinary Incontinence

Hai-Hong Jiang and Margot S. Damaser

Contents

Abstract Stress urinary incontinence (SUI) is a common health problem significantly affecting the quality of life of women worldwide. Animal models that simulate SUI enable the assessment of the mechanism of risk factors for SUI in a controlled fashion, including childbirth injuries, and enable preclinical testing of new treatments and therapies for SUI. Animal models that simulate childbirth are presently being utilized to determine the mechanisms of the maternal injuries of childbirth that lead to SUI with the goal of developing prophylactic treatments. Methods of assessing SUI in animals that mimic diagnostic methods used clinically have been developed to evaluate the animal models. Use of these animal models to

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test innovative treatment strategies has the potential to improve clinical management of SUI. This chapter provides a review of the available animal models of SUI, as well as a review of the methods of assessing SUI in animal models, and potential treatments that have been tested on these models.

Keywords Animal models · Bladder pressure · Leak point pressure · Mechanism of injury · Simulated childbirth · Stress urinary incontinence · Urethral pressure · Urinary incontinence · Urodynamics

1 Introduction

Stress urinary incontinence (SUI) is jointly defined by the International Continence Society and International Urogynecological Association as the complaint of involuntary loss of urine on effort or physical exertion (e.g., sporting activities), or on sneezing or coughing (Haylen et al. 2010). It results from a sudden increase of abdominal pressure in the absence of detrusor contraction and is associated with both intrinsic sphincter dysfunction and urethral hypermobility (McGuire 2004). SUI is a common health problem worldwide and significantly impacts both the society and individuals (Birnbaum et al. 2004; Hampel et al. 2004). It is correlated to various risk factors, including general disease, obesity, smoking, being female, advanced age, surgical trauma, and, most significantly, childbirth (DeLancey et al. 2008). However, its pathophysiology is not completely understood.

No animal model can completely simulate the human situation and multifactorial basis for SUI. Nonetheless, animal models can be utilized to further our understanding of the pathophysiology of SUI and enable preclinical testing of potential treatments. They also allow us to assess the mechanism of specific risk factors or contributing elements to SUI in a controlled fashion and to use this knowledge to improve or optimize the management strategy for SUI. Translational investigation of SUI using animal models could potentially lead to an understanding of the importance of each event during the progression of SUI and also to the development of preventive interventions to be delivered before the occurrence of significant events.

2 Methods of Determining SUI in Animal Models

SUI is a behavioral condition that involves unintentional urine leakage; however, animals cannot indicate intent. Therefore, the functional surrogate of urethral resistance to leakage has been used to assess SUI in animal models. In the clinical case, sophisticated urodynamics and other related tests may be performed to narrow diagnosis and treatment (McGuire 2004). The methods used to assess urethral resistance in animal models are based on clinical methods of diagnosing SUI; therefore, the principals of assessing SUI are relatively similar between the two situations.

Other differences between clinical and experimental animal methods arise because untrained animals do not easily follow instructions. Therefore, anesthesia is required for animal testing to immobilize the animal. In addition, invasive and nonsurvival studies examinations can be performed in animals, unlike in humans.

Urethral resistance contributing to urinary continence involves both passive and active mechanisms (Rovner and Wein 2004; Haab et al. 1996), including pressure transmission, urethral smooth muscle contraction, urethral striated muscle contraction, pelvic floor muscle contraction, as well as mucosal coaptation and elasticity of the urethra. Deficiency of these factors is associated with insufficient urethral resistance and may result in symptoms of SUI and decreased leak point pressure (LPP) during a urodynamics study (McGuire 2004). Several methods of determining and characterizing SUI in animal models have been established and developed in the last decade (Hijaz et al. 2008) and are reviewed below.

2.1 Sneeze Testing

The sneeze test was introduced by Lin et al. in 1998 for use in rats to test for decreased urethral resistance to leakage and presumptive SUI (Lin et al. 1998). To perform this test, chili powder or a clipped whisker is placed in the rat's nose, inducing sneezing (Lin et al. 1998). The sneezing response transiently increases abdominal pressure on the bladder and induces leakage in 30% of animals 4 weeks after simulated childbirth injury. No leakage was induced in uninjured rats (Lin et al. 1998). This method has been used extensively to study SUI in rodent models (Conway et al. 2005; Kaiho et al. 2007; Kamo et al. 2003, 2006) and has also been used in cats and other animals (Julia-Guilloteau et al. 2007; Bernabe et al. 2008; Wallois et al. 1995).

Sneeze testing has been utilized to investigate the nature of the continence reflex response to increased abdominal pressure due to sneezes (Kamo et al. 2003; Julia-Guilloteau et al. 2007). It is presumed that the bladder is squeezed by the abdominal wall during sneezing since bladder pressure was significantly decreased after opening the abdomen during sneeze testing (Kamo et al. 2003). Pressure in the middle urethra increased before bladder pressure increased from sneeze testing, suggesting that continence mechanisms are activated in preparation for sneezing (Kamo et al. 2003). These active closure pressure increases elicited by a reflex response to sneezing are believed to result from contraction of the external urethral sphincter and pelvic floor muscles occurring in the middle urethra in female rats (Kamo et al. 2003).

Urethral smooth muscle contraction also actively contributes to active closure mechanisms during a sneeze reflex since intrathecal phentolamine, prazosin, and intravenous nisoxetine decrease the increased pressure response to sneezing in the urethra of rats (Kaiho et al. 2007). Therefore, the continence response to sneezing includes the noradrenergic system, which can be enhanced with a norepinephrine

reuptake inhibitor to prevent SUI via α_1-adrenoceptors (Kaiho et al. 2007), as utilized by duloxetine in rats (Miyazato et al. 2008).

2.2 *Manual LPP Testing*

Manual LPP testing in rats, mimicking a Crede maneuver, has been developed to test decreased urethral resistance in animal models (Cannon et al. 2002). Because the procedure is relatively easy and highly repeatable, and LPP values can also be achieved from uninjured control animals (Cannon et al. 2002; Damaser et al. 2003), this method of SUI testing enables statistical comparisons of LPP between experimental groups and has therefore become popular (Hijaz et al. 2004; Woo et al. 2009; Wood et al. 2008; Kefer et al. 2008; Lin et al. 2010). Urethane anesthesia is usually used for manual LPP testing since it best maintains urological reflexes (Cannon et al. 2002). It is usually approved only for end-stage use, and animals must be euthanized prior to awakening. Therefore, other anesthetics, such as ketamine and xylazine, have been utilized, when repeat or survival experiments are necessary (Phull et al. 2007). However, ketamine affects activity of the sympathetic nervous system both indirectly, via inhibition of neuronal uptake of catecholamine, and directly by alpha-2 receptor agonism (Aroni et al. 2009), which may affect bladder and urethral function. As a result, use of ketamine and xylazine as an anesthetic has been shown to induce bladder overactivity in rats (Zhang et al. 2010; Cannon and Damaser 2001). However, the effects on urethral resistance have not been determined.

To minimize the effects on urethral resistance by placement of a urethral catheter, a suprapubic bladder catheter is usually placed several days before manual LPP testing (Cannon et al. 2002). However, transurethral catheters have been used for LPP testing in special circumstances, such as during simultaneous measurement of the external urethral sphincter (EUS) electromyogram (EMG) (Jiang et al. 2009a).

To perform manual LPP testing in rats, a passive vesical pressure increase is made by gradually and slowly pressing on the abdomen directly above the bladder until leakage is observed at the urethral meatus. At the moment of urine leakage, the external pressure is rapidly removed and bladder pressure quickly returns to baseline (Cannon et al. 2002). LPP testing is usually performed during filling cystometry with the bladder approximately half full, avoiding times just after voiding or during unstable contractions. If an active bladder pressure contraction is induced by LPP testing, the bladder should be refilled and LPP testing repeated. Bladder pressure increase during LPP testing can be easily differentiated from a bladder contraction or voiding since bladder pressure decreases more slowly after completion of voiding and more fluid is evacuated during voiding. Manual LPP testing has been validated in several SUI animal models (Kefer et al. 2008). Recent efforts to standardize the procedure led to the development of a device consisting of a soft-tipped force applicator with a force sensor, laser crosshairs, and a hand-held

remote control system (Shoffstall et al. 2008). Although the device decreased the variability of novices at the LPP test in rats, it had no impact on the variability of testing performed by experienced investigators (Shoffstall et al. 2008).

2.3 Vertical Tilt Table LPP Testing

In this method, a rat is mounted on a vertical tilt table mimicking the erect situation in humans. A saline reservoir is connected to a suprapubic catheter to increase bladder pressure when raised (Lee et al. 2003; Kamo et al. 2004; Kwon et al. 2006). Results using the tilt table technique have been compared to those using manual LPP testing and indicate that they are similar and repeatable (Conway et al. 2005). Prior to the procedure, the spinal cord is usually transected at T8–T9 to eliminate voiding reflexes. However, the test has also been utilized successfully without spinal cord transection (Takahashi et al. 2006).

Although the vertical position of the rats during this test better simulates the human erect body position, the bladder volume is significantly increased as the reservoir height is increased. This change in bladder volume increase may induce additional bladder reflexes and active responses, creating the need for the spinal cord transection. Although the spinal cord transection abolishes unwanted neurogenic activity of the bladder, it also eliminates supraspinal regulation of Onuf's nucleus and other spinal reflex centers important for continence (Sugaya et al. 2005). Thus, while this method of testing has advantages, such as the erect posture of the animals, it also has disadvantages, both of which must be considered when selecting a testing method for a specific investigation.

2.4 Electrical Stimulation LPP Testing

LPP testing by electrical stimulation of the abdominal muscles, inducing a sudden increase in abdominal pressure, was introduced recently in female rats (Kamo and Hashimoto 2007) to mimic a cough reflex (Widdicombe 1995). As with tilt table testing, to eliminate surpraspinal reflex voiding, the spinal cord is transected at the T8–T9 level. The abdominal skin near the right and left tips of the 11th–13th costae are cut to expose the abdominal oblique muscles, where stimulation needle electrodes are inserted. The only study using this method to date demonstrated that reflex urethral closure mechanisms via bladder–spinal cord–urethral sphincter and pelvic floor muscles greatly contribute to a continence reflex response in increased urethral resistance to prevent leakage (Kamo and Hashimoto 2007).

Electrical stimulation LPP testing is excellent for simulating a sudden increase in abdominal pressure. The LPP value is greater than that from other testing methods because of the contribution of pressure transmission from abdominal muscle contraction. A shortcoming of this method is that in some animals, there

is no leakage even at the maximum bearable stimulation level (Kamo and Hashimoto 2007). Moreover, as above, to inhibit bladder contraction, a spinal cord transection has to be performed, eliminating supraspinal regulation of continence reflexes.

2.5 Urethral Closure Pressure Testing

Direct testing of urethral closure pressure has also been performed via utilization of a special catheter, initially introduced transvesically through an incision in the bladder dome and then placed into the bladder neck or the urethra (Bae et al. 2001). This technique was further modified for retrograde urethral perfusion pressure (RUPP) testing in rats via introduction of a dual catheter system via the urethral meatus and placement of a watertight suture (Rodriguez et al. 2005). The pressure sensor is usually placed at the mid-urethra since the highest pressure and greatest continence reflex response in female rats corresponds with the location of urethral striated muscles (Kamo et al. 2003). In contrast, in cat (Julia-Guilloteau et al. 2007; Bernabe et al. 2008) and in dog (Thuroff et al. 1982), the greatest urethral closure pressure active response is located at the distal urethra.

In contrast to vesical pressure measurement, urethral pressure measurement can be inconsistent and difficult to reproduce. The clinical usefulness of urethral pressure profile remains unclear (Lose et al. 2002). To address this, microtransducer-tipped pressure-sensing catheters have been utilized for the measurement of urethral closure pressure (Kamo et al. 2003, 2004; Kamo and Hashimoto 2007) and other urethral pressure measurements (Phull et al. 2007). However, since the microtip is actually a point force sensor, different measurement sites, even with the same area, could lead to different results. Therefore, reproducibility of urethral closure pressure and RUPP measurements remains in question.

3 SUI Animal Models

Animal models for SUI in rodents were first introduced by performing vaginal distension (VD) to simulate the maternal injuries of childbirth in female rats (Lin et al. 1998; Sievert et al. 2001). Since then, more and more SUI animal models have been developed using various methods to injure different aspects of the urinary continence mechanism, such as nerve injury (Bernabe et al. 2008; Damaser et al. 2003, 2007; Hijaz et al. 2004; Peng et al. 2006; Ahn et al. 2005), urethral cauterization (Chermansky et al. 2004), urethrolysis (Rodriguez et al. 2005), and pubourethral ligament injury (Kefer et al. 2008, 2009). These animal models enable us to further understand how these factors contribute to development of SUI and to test novel potential therapies (Fig. 1).

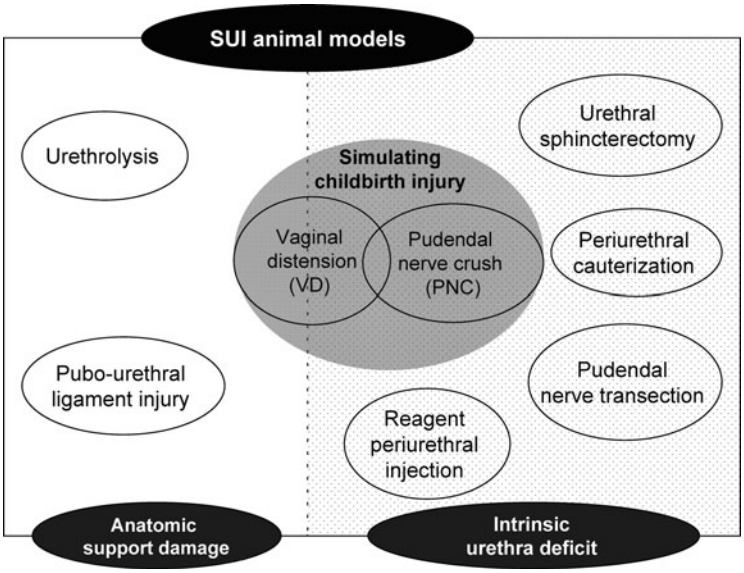

Fig. 1 Animal models for stress urinary incontinence (SUI) can be divided into two broad categories of those that create damage to anatomical support and those that create an intrinsic urethral deficit. Vaginal distension falls into both categories and, along with pudendal nerve crush, provides a simulation of the maternal injuries of childbirth

3.1 Models Simulating Childbirth Injury

3.1.1 Vaginal Distension

Most studies using VD to simulate the maternal injuries of childbirth use similar methods: a Foley catheter is inserted into the rat's vagina under anesthesia and the balloon is inflated to distend the vagina and injure organs and tissues nearby for an extended period of time (Hijaz et al. 2008). The VD model has became the most used model to study mechanisms of injury and tissue recovery in response to childbirth, since it was introduced over 10 years ago (Lin et al. 1998). Urethral resistance is significantly decreased after VD as confirmed by sneeze testing, LPP testing, and urethral closure pressure testing (Lin et al. 1998; Kamo et al. 2006; Pan et al. 2007). The decrease in urethral resistance recovers in a few weeks, depending primarily on the duration of time the balloon was left distended in the vagina (Pan et al. 2007).

VD has been widely adopted as a model of SUI in rats, however, with some variability in its execution. The selection of catheter size and balloon dilation volume should depend on the age, weight, and breeding status of the rat or on the damage level desired for a specific study. Lin et al. initially used a 12Fr Foley catheter inflated with 2.0 ml in virgin rats and 2.5 ml in retired breeder rats for 4 h (Lin et al. 1998). A larger 22Fr catheter was utilized immediately postpartum in rats

with a 5 ml balloon distension for 3 h (Sievert et al. 2004). In addition, the rat was place in a prone position with the symphysis at the edge of the table and the VD catheter attached to a 130 g weight to simulate stretch of tissues during vaginal delivery. Resplande et al. (2002) performed VD in rats immediately postpartum using an 18Fr catheter inflated to 5 ml for 4 h.

In our experience, VD is most repeatable when the vagina is first accommodated and predilated by inserting and removing increasing sizes of urethral dilators (24–32Fr.) (Cannon et al. 2002). We use a modified 10Fr. Foley catheter for VD, inflate it to 3 ml, secure it with a single suture in the skin, and leave it in place for up to 4 h (Damaser et al. 2003; Woo et al. 2009; Wood et al. 2008; Jiang et al. 2009a; Pan et al. 2007, 2009). We use virgin rats in our studies; whereas others use rats immediately postpartum (Sievert et al. 2001, 2004; Lin et al. 2008a). Even though no study has yet performed a head to head comparison of outcomes, the results are similar in the two studies with a predictable decrease in urethral resistance after VD (Hijaz et al. 2008; Damaser et al. 2003, 2005; Woo et al. 2009; Wood et al. 2008; Jiang et al. 2009a; Sievert et al. 2001, 2004; Pan et al. 2007, 2009; Lin et al. 2008a). These variations in VD methodology may correspond to differences in outcomes; however, it is generally accepted that, regardless of the specific methodology, VD damages urethral function in the short-term and reduces urethral resistance to leakage, resulting in SUI that recovers with time.

VD damages the muscular and neurological structures responsible for continence since these tissues are compressed between the dilated balloon and the pelvis, particularly the pubic symphysis (Sievert et al. 2004). Marked cellular swelling and edema in the levator muscles has been shown 1 day after VD (Lin et al. 1998). Smooth muscle between the vagina and the urethra in incontinent rats was also disrupted and thinned (Lin et al. 1998). Cannon et al. (2002) similarly documented extensive disruption of the skeletal muscle layer and marked thinning of the smooth muscle fibers in the urethra following 1 h VD.

VD also results in decreased blood flow to urethra, and hypoxia of the bladder, urethra, and vagina, supportive of hypoxic injury as a possible mechanism of injury leading to SUI (Damaser et al. 2005). Similarly, prolonged VD increases hypoxia-inducible factor 1α expression in the urethra (Wood et al. 2008). VD can also affect expression of other cytokines, including monocyte chemotactic protein-3 (MCP-3) and its receptors (Woo et al. 2007, 2009; Wood et al. 2008). Since MCP-3 attracts mesenchymal stem cells to the local region to facilitate repair (Schenk et al. 2007), this suggests participation of an innate stem cell-mediated repair mechanism after VD. The expression of MCP-3 is also correlated with duration of VD (Wood et al. 2008), confirming a duration-dependent injury and repair mechanism.

Kamo et al. (2006) reported that SUI after VD results from decreased active closure mechanism at the mid-urethra and does not alter abdominal pressure transmission below the bladder neck. Active middle urethra sneeze-induced reflex responses are present but decreased by approximately 50% after VD in rats (Kamo et al. 2006). Furthermore, Jiang et al. (2009a) demonstrated that EUS EMG activity and bladder-to-EUS response activity during LPP testing were significant reduced

4 days after VD. EUS EMG activity recovered by 3 weeks after VD, corresponding with recovery of LPP (Jiang et al. 2009a). These studies confirm that, similar to humans, active urethral closure participates in a sneeze or LPP continence reflex in rats and that the neuromuscular participants in this reflex are damaged by VD, particularly those in the urethra. Therefore, VD simulates mostly urethral mechanisms of SUI rather than hypermobility or pelvic floor mechanisms.

Since birth injury is one of the greatest risk factors in the etiology of SUI in women, VD simultating the maternal injuries of delivery currently provides the best model to study the mechanisms of urethral injury and recovery as well as its pathophysiology. This model further allows us to explore and improve potential treatments to accelerate functional recovery of SUI since insufficient recovery after vaginal delivery injury along with other injuries and factors are strongly correlated with a later development of the SUI (Snooks et al. 1990). Therefore, the best use of the VD model may be in combination with other pathologies, injuries, or genetic manipulations. Work to date in combinatorial models is reviewed later in this chapter.

3.1.2 Pudendal Nerve Injury

The pudendal nerve controls EUS activity, including tonic activity during continence, and activates to strengthen the guarding response to prevent urinary leakage (Park et al. 1997; Evans 1936). It can be trapped and injured during vaginal childbirth when coursing through Alcock's canal in the ischiorectal fossa, especially between the sacrospinous ligament and the sacrotuberous ligament (Snooks et al. 1986). Increased pudendal nerve terminal motor latency, indicative of nerve injury, correlates with vaginal delivery, advanced age, and SUI (Tetzschner et al. 1997; Olsen et al. 2003; Snooks et al. 1984). Several obstetric factors, such as multiparity, forceps delivery, increased duration of the second stage of labor, third degree perineal tear, and high birth mass, are specifically correlated with pudendal nerve damage (Snooks et al. 1986). While cesarean section can be sparing to the pudendal nerve, cesarean section during labor is not and can put a woman at risk of pudendal nerve damage (Sultan et al. 1994).

In addition to distal pudendal nerve injury at the EUS during vaginal delivery, clinical evidence also demonstrates damage to the pudendal nerve in Alcock's canal, particularly as the nerve reenters the pelvis at the sacral spine. A novel animal model, pudendal nerve crush (PNC), simulating this damage, has been developed (Kerns et al. 2000). In rats, the rising roots of the pudendal nerve are more proximal than in humans since the pudendal nerve arises from L6–S1 roots in rats and S2–S4 roots in humans (McKenna and Nadelhaft 1986; Pacheco et al. 1989). Nonetheless, innervation of the EUS from the pudendal nerve is similar between rats and humans. In female rats, the motor pudendal nerve bifurcates within Alcock's canal into separate fascicles that innervate the external anal sphincter and EUS (McKenna and Nadelhaft 1986; Kane et al. 2002).

For PNC, the pudendal nerve is accessed via the dorsal approach and is then crushed bilaterally in the ischiorectal fossa where it reenters the pelvis (Ahn et al. 2005; Kerns et al. 2000). In this model, all three branches of the pudendal nerve are crushed: sensory, EUS motor, and external anal sphincter motor, to simulate the injuries of childbirth. It is possible to injure each independently, however, potentially to test theories regarding reflexes or innervation.

PNC injury appears to result in acute SUI since LPP is significantly reduced until 2 weeks after the injury (Damaser et al. 2007). LPP then trends upward and returns to control levels with increasing time after injury, suggesting that nerve function begins to recover or compensatory changes in the urethra occur (Ahn et al. 2005; Damaser et al. 2007). Despite functional recovery 2 weeks after PNC, the distal nerve and EUS both show evidence of nerve degeneration and early signs of recovery at this time point (Damaser et al. 2007). Only 6 weeks after PNC is full neuroregeneration observed in the pudendal nerve and the EUS (Damaser et al. 2007), suggesting that 2 weeks represents an early time point of initial neuroregeneration and that there is redundancy in the innervation such that incomplete regeneration can lead to functional recovery. Estrogen has been shown to increase the neuroregenerative response of the injured pudendal nerve and promote and facilitate recovery of urethral function (Kane et al. 2004; Ahmed et al. 2006).

EUS EMG and pudendal nerve motor branch potential recordings after PNC support the redundancy theory since they demonstrate significant recovery 3 weeks afterward (Jiang et al. 2009a). However, the increase in amplitude and frequency of EUS EMG activity during LPP testing remains significantly different 3 weeks after PNC compared to normal rats, suggesting that nerve regeneration remains an ongoing process (Jiang et al. 2009a). Three months after PNC, approximately half the crushed pudendal neurons have regenerated to the EUS (Kerns et al. 2000), supporting the idea that incomplete regeneration is sufficient for functional recovery.

PNC injury in female rat, an SUI rodent model, results in a peripheral neurogenic and functional deficit followed by regeneration and recovery in the EUS. It allows evaluation of the pudendal nerve and neuromuscular recovery of the EUS and is useful for investigating mechanisms of neuromuscular recovery and for testing neuroregenerative agents or other potential treatments. It can also be utilized in conjunction with other pathologies, such as diabetes, aging, obesity, or genetic manipulation, to assess mechanism of injury and recovery in populations at increased risk for SUI.

3.1.3 Combination Models

During childbirth trauma, both muscles and nerves can be injured. Thus, the injuries occurring simultaneously during vaginal childbirth may represent a unique compound neuromuscular injury. These two injuries, along with injuries to the pelvic floor, are strongly correlated with later development of SUI (Snooks et al. 1990). Therefore, to further understand the mechanism of SUI after childbirth injury, both

VD and PNC have been implemented in combination with each other to investigate the interaction between the different risk factors for SUI.

A dual injury model utilizing both PNC and VD has been developed and may best mimic the injuries incurred due to vaginal delivery (Jiang et al. 2009a). Functional recovery including EUS EMG and pudendal nerve motor branch potentials recorded during both LPP testing and voiding occurs more slowly after both PNC and VD than after either PNC or VD alone (Jiang et al. 2009a, b). Similarly, histological analysis of the EUS and the pudendal nerve confirms this result with slowed anatomical recovery from a dual injury (Pan et al. 2009; Jiang et al. 2009b).

Neurotrophins, particularly brain-derived neurotrophic factor, are upregulated by the target muscle to regenerate peripheral nerves and reinnervate the target muscle after nerve injury; in contrast, they are downregulated after muscle injury (Yan et al. 1994; Friedman et al. 1995; Sakuma et al. 2001). One day after VD, brain-derived neurotrophic factor expression in the EUS was reduced, whereas it was dramatically upregulated in the EUS 1 day after PNC (Pan et al. 2009). After a dual injury of both PNC and VD, brain-derived neurotrophic factor expression was upregulated somewhat but not to the same extent as after PNC, suggesting that insufficient brain-derived neurotrophic factor upregulation may contribute to slowed recovery after dual injury (Pan et al. 2009). Therefore, this model could be utilized to test methods that produce a local increase in neurotrophic factors as potential methods of promoting neuroregeneration and reinnervation after childbirth.

VD has also been combined with other pathophysiologies, such as diabets mellitus or oophorectomy (OVX), to study their interactions. Streptozotocin-induced diabetes causes increased severity and delayed functional recovery after VD (Kim et al. 2007), suggesting that diabetic women likely recover more slowly from maternal birth injuries. When VD follows delivery of rat pups, a later OVX increases the incidence of SUI, although OVX does not increase SUI incidence without VD (Sievert et al. 2001). On the other hand, Kuo (2002)found that OVX affects the intrinsic urethral closure mechanism, but without rat pup delivery, OVX does not affect LPP after VD. Multiple VD procedures have been utilized to simulate multiple deliveries (Kuo 2002; Pauwels et al. 2009). Pauwels et al. (2009) applied repeat VD to postpone recovery of urethral resistance to leakage. LPP is significantly decreased when both OVX and multiple VD were applied (Kuo 2002).

VD has also been performed in mice to induce SUI with the expectation of combining VD with genetic manipulation to study the impact of genetics on SUI (Lin et al. 2008b, 2010). A distension volume of 0.1–0.3 ml results in significantly reduced LPP in C57BL/6 female mice in the short term (Lin et al. 2008b). The density of immunoreactive neurofilaments in the urethra are reduced after VD with a 0.3- or 0.2-ml balloon (Lin et al. 2010). VD in mice could be more useful in future when trangenic models are combined with VD to investigate the genetic dependence of SUI in women after delivery. For example, elastin metabolism can be altered in women with SUI (Chen et al. 2006), and mice with genetically altered elastin metabolism can develop pelvic organ prolapse and decreased LPP after pup delivery (Lee et al. 2008). Combining this genetic model with VD could potentially

elucidate the mechanism of elastin recovery after childbirth and identification of women at high risk for SUI.

3.2 Models Simulating Anatomic Support Damage

3.2.1 Urethrolysis

Urethrolysis, both retropubic and vaginal, has been performed for some patients with urethral obstruction or complications of urethral suspension procedures (Foster and McGuire 1993). It has also been utilized to create an animal model of durable urethral dysfunction or decreased urethral resistance, mimicking SUI. Transabdominal urethrolysis in female rats consists of circumferentially detaching the proximal urethra by incising the fascia (Rodriguez et al. 2005). The remaining urethra is then detached from the anterior vagina and pubis, which produces a significant decrease in both LPP and RUPP until 24 weeks after the procedure (Rodriguez et al. 2005). Denervation and an increase in the number of apoptotic cells have also been demonstrated 4–24 weeks after urethrolysis (Rodriguez et al. 2005). Pauwels et al. (2009) further refined this procedure as urethral transposition by surgically freeing the urethra and vagina from their fixation to the abdominal skin, resulting in a durable incontinence and a significant decrease in LPP 8 weeks after injury. Therefore, urethrolysis causes connective tissue damage and a significant diminishment of anatomic support and results in a long lasting or durable decrease in urethral resistance to leakage. It could be best suited for investigation of pathogenesis and treatment of the subset of SUI with obvious bladder neck or urethral hypermobility and could be utilized for preclinical evaluation of slings, injectables, and other potential treatments.

3.2.2 Pubourethral Ligament Injury

The pubourethral ligament (PUL) is believed to strongly attach the ventral surface of the urethra to the pubic bone, and its role in continence is described in the integral theory of continence and SUI (Petros 1998; Petros and Ulmsten 1990). Kefer et al. (2008, 2009) adopted the theory and created a PUL deficiency in female rats as a model of SUI. LPP is significantly decreased both in the short term (10 days) and in the long term (28 days) after suprapubic PUL transection (Kefer et al. 2008, 2009), validating PUL deficiency as an additional model of durable SUI. When transecting this ligament, it is almost impossible to eliminate potential injuries to the other structures, including other pelvic fascia and ligaments, as well as muscles, vessels, and nerves under the pubic symphysis. Both PUL transection and urethrolysis result in urethral mobility, but PUL transection appears to be a milder injury. Therefore, PUL transection has similar uses to urethrolysis as a model of SUI.

3.3 Models Simulating Intrinsic Urethra Deficit

3.3.1 Periurethral Cauterization

Periurethral cauterization was created as a model of SUI in rats by Chermansky et al. (2004). The procedure is performed via transabdominal access with a transurethral catheter for support. Electrocauterization of tissues lateral to the midurethra decreased LPP without affecting bladder function, reducing LPP 2 weeks after the procedure and maintaining it as decreased for up to 16 weeks (Chermansky et al. 2004). Histology suggests that damage to striated muscle and nerves contributes to the change in LPP in this model, potentially creating an intrinsic urethral deficit (Chermansky et al. 2004). It is also a durable model of SUI with more significant tissue damage and a long lasting decrease in urethral resistance. It is potentially most useful for investigation of potential interventions, especially those aimed at treating severe and/or complicated SUI.

3.3.2 Urethral Sphincterectomy

A canine SUI model of urethral sphincterectomy has recently been developed by Eberli et al. (2009) in which creation of irreversible damage to the sphincter was the goal. Approximately ¼ of the circumferential sphincter muscle, containing both smooth and striated muscle, was excised through a low midline abdominal incision (Eberli et al. 2009). Both histology and pudendal nerve stimulation indicated an absence of sphincter muscle and its response. Seven months after sphincterectomy, urethral resistance to leakage remained significantly decreased, as evidenced by decreased urethral pressure profile and stress urethral profile as well as decreased LPP. This SUI model represents an extreme and irreversible sphincter deficiency, which could be useful for preclinical evaluation of methods of entirely reproducing sphincter function, such as with an artificial or cell-based sphincter replacement procedure.

3.3.3 Pudendal Nerve Transection

Since PNC recovers relatively rapidly, bilateral pudendal nerve transection has been used to study permanent damage to the pudendal nerve (Conway et al. 2005; Kamo et al. 2003; Hijaz et al. 2004; Jiang et al. 2009a; Peng et al. 2006). The procedure is the same as PNC, except that the pudendal nerve is transected bilaterally instead of being crushed. A segment of nerve (~2 mm long) is removed from the transection site to prevent neuroregeneration. A significant decrease in LPP results, both in the short term and as long as 6 weeks after pudendal nerve transection (PNT) (Conway et al. 2005; Kamo et al. 2003; Jiang et al. 2009a; Peng et al. 2006). The decrease in LPP is only partial if a unilateral PNT is performed (Peng et al. 2006). Peng et al.

(2006) demonstrated that voiding efficiency also decreases after PNT, suggesting that EUS bursting activity during voiding facilitates emptying in female rats. These studies all support the idea that the pudendal nerve plays a critical role in the mechanism of urinary continence since as soon as it is injured, the urethral resistance to leakage decreases significantly.

Jiang et al. (2009b) confirmed that 6 weeks after PNT, the EUS showed neurogenic atrophy and contained thin small muscle bundles with no striations. No significant EUS EMG tonic activity and bursting during voiding was recorded 6 weeks after PNT (Jiang et al. 2009a, b). This is in contrast to a previous report by Peng et al., who demonstrated EUS EMG tonic activity and bursting during voiding 6 weeks after PNT despite a decrease in the diameter of striated muscle bundles in the EUS (Peng et al. 2006). This difference could be due to the use of different EMG electrodes in the two studies, suggesting a need to standardize electrodes used for measuring EUS EMG in models of SUI.

PNT has also been investigated in other female animals, such mice, cats, and dogs (Bernabe et al. 2008; Lin et al. 2010; li-El-Dein and Ghoneim 2001). Lin et al. (2010) confirmed permanent effects of PNT in female mice and that both LPP and the density of neurofilaments in the urethra were significantly reduced 4, 10, and 20 days after PNT. In female cats, after unilateral pudendal and pelvic nerve lesions, only distal urethral pressure decreased significantly. In contrast, bilateral pudendal and pelvic nerve lesions may cause urine leakage and significantly decrease the urethral pressure response (Bernabe et al. 2008). Bladder and urethral function after PNT has also been investigated in female dogs (li-El-Dein and Ghoneim 2001). While not specifically designed as an animal model of SUI, the results indicate a decrease in urethral resistance after PNT (li-El-Dein and Ghoneim 2001).

3.3.4 Reagent Periurethral Injection

Botulinum Toxin has been used as a reagent to induce chemical denervation of the urethral sphincter via periurethral injection in rats (Takahashi et al. 2006). A significant decrease in LPP occurred 2 weeks after injection and was supported by evidence of shrinkage of smooth and striated urethral muscle. Six weeks after injection, rats demonstrated functional recovery, making this a model of reversible SUI aimed specifically at injuring the function of smooth and striated muscle. It may be most useful for preclinical testing of treatments to accelerate recovery after chemical or radiation-induced SUI.

4 Treatment Testing on SUI Animal Models

Clinical treatments for SUI include conservative techniques, pharmacologic therapy, and surgical procedures. These therapies aim to strengthen urinary continence via specific or integrated deficiency correction. Although standard interventions

such as anti-incontinence surgery have been verifiably successful in restoration of anatomic support and improvement of intrinsic urethral deficiencies (Williams and Klutke 2008), it is still necessary to use animal models of SUI to test new surgical techniques and materials, pharmacologic targets, and therapeutic strategies, such as stem cell therapy. We review here the work to date using these animal models to test potential new treatments.

4.1 Pharmacological Therapy Testing

4.1.1 Serotonin and Norepinephrine Reuptake Inhibitors

Experiments in cats suggested that duloxetine, a serotonin and norepinephrine reuptake inhibitor, could enhance EUS activity through $5HT_2$ and α_1 adrenergic mechanisms (Thor and Katofiasc 1995), indicating promise for the treatment of SUI. After VD in rats, urethral resistance was significantly increased by duloxetine administered intravenously (Miyazato et al. 2008). Intrathecal administration of idazoxan, a selective α_2 adrenergic receptor antagonist, indicated that duloxetine can prevent SUI by facilitating noradrenergic and serotonergic systems in the spinal cord, enhancing the sneeze reflex (Miyazato et al. 2008). Other similar SNRIs, including venlafaxine (Bae et al. 2001) and nisoxetine (Kaiho et al. 2007), can also increase urethral pressure in rats and have also been suggested to be useful for treatment of SUI. This class of drug, however, has the potential for central nervous system complications, keeping it from being made available to the general public in the USA (2005).

4.1.2 Fibroblast Growth Factor Testing

Takahashi et al. (2006) evaluated the effects of sustained release of basic fibroblast growth factor (bFGF) in rat urethra denervated by botulinum-A toxin. They incorporated bFGF into 200 μl gelatin hydrogels and injected them into the urethral sphincter, enabling sustained release of bFGF. They determined that bFGF delivered in this fashion produced a significant improvement in urethral sphincter contractility and suggested bFGF as a promising therapy for SUI. Further testing in other animal models and experiments designed to elucidate the mechanism of effect of bFGF ought to be performed to assess the clinical utility of this potential treatment.

4.1.3 Angiotensin II Testing

Using the rat SUI models of PNT and urethrolysis, Phull et al. (2007) demonstrated that angiotensin I and II receptor inhibition significantly decreases urethral resistance to leakage as evidenced by decreased LPP and RUPP. Angiotensin II treatment

restored urethral tone in rats with intrinsic sphincter dysfunction, suggesting that angiotensin II may have a functional role in the maintenance of urethral tone and in continence function (Phull et al. 2007). This work could be continued using other models of SUI to determine the mechanism of the role of angiotensin II in continence and SUI in order to assess its clinical utility.

4.2 Sling Surgical Procedures

Since the last century, different urethral suspension techniques and multiple types of slings have been developed (Williams and Klutke 2008). However, there is no consensus on the ideal sling procedure since there is no consensus on the various theories and explanations for the urinary continence mechanism (DeLancey et al. 2008). Today, the tension-free vaginal tape procedure has become one of the most popular sling procedures (Rapp and Kobashi 2008). Nonetheless, animal models of a rat urethral sling procedure have been developed to further evaluate the mechanism of the sling procedure and its complications (Hijaz et al. 2004).

Using LPP testing on a rat PNT model, Hijaz et al. (2004, 2005a) determined that a vaginal sling procedure results in restoration of urethral resistance to leakage in both short- and long-term (5 weeks) testing. They further confirmed that the sling procedure may cause bladder outlet obstruction regardless of whether the mid-urethral segment of the sling was cut after the procedure (Hijaz et al. 2005b). They demonstrated histological changes as a result of both intact and cut slings, including inflammation, localized edema, and differential collagen remodeling, which could account for the preserved anti-incontinence mechanisms of cut or intact polypropylene slings observed clinically (Chen et al. 2009).

To test long-term continence recovery, Cannon et al. (2005) combined tissue engineering and a sling procedure in an SUI rat model in which the proximal sciatic nerve was transected bilaterally. They prepared tissue-engineered slings with muscle-derived cells obtained via the preplate technique and subsequently seeded for 2 weeks on a small intestinal submucosa scaffold. The sling procedure was performed via a transabdominal approach and resulted in significantly increased LPP with no urinary retention, suggesting that the cells may help prevent the outlet obstruction complications of slings (Cannon et al. 2005). Further work on the mechanism of this effect is needed to assess its clinical utility.

4.3 Cell-Based Therapy Testing

Cell-based therapies and tissue engineering hold potential as treatments and preventions for SUI, particularly treatment with autologous multipotent adult stem cells, including mesenchymal stem cells, muscle-derived stem cells (MDSC), and adipose-derived stem cells (ADSC) (Furuta et al. 2007; Nikolavsky and Chancellor 2010).

These adult stem cells can be derived from a given subject, expanded in culture, and given back to the subject. Stem cell strategies for SUI have been studied in animal models and are presently undergoing clinical trials (Carr et al. 2008). Although controversy and conflicting results have occurred in the field, preliminary results from a recent clinical trial appear to be promising (Nikolavsky and Chancellor 2010).

4.3.1 Muscle Precursor Cell Therapy

Implantation of muscle precursor cells (MPC) can be traced to three decades ago, when fusion between donor MPCs and host myofibers was first noted in skeletal muscle transfer (Partridge et al. 1978). Cannon et al. applied this theory and tested direct injection of allogenic MPC into rat urethral sphincters denervated by PNT (Cannon et al. 2003). Their results indicated that the cells significantly improved the fast-twitch muscle contraction amplitude of the denervated sphincter to 87% of normal animals. New skeletal muscle fiber formation at the injection site of the urethra was also indicated by immunohistochemistry (Cannon et al. 2003). MPC autografting in a murine model of urethral sphincter injury has also been reported (Yiou et al. 2002). The results demonstrated that this procedure may accelerate sphincter muscle repair by producing a significant increase in the diameter and number of myofibers (Yiou et al. 2002), suggesting that MPC autografting represents a potential new therapeutic approach to urethral sphincter insufficiency.

4.3.2 Stem Cell Homing Signals

Beside direct injection of cells, utilization of cytokines and stem cell homing signals as a treatment for SUI may represent an alternative treatment paradigm. Recently, using a VD rat model of SUI, Woo et al. (2007) demonstrated MCP-3 overexpression in the urethra immediately following VD, as well as a strong correlation between VD duration and the subsequent expression of MCP-3 and one of its associated receptors, CCR1, in the urethra (Wood et al. 2008). This result was consistent regardless of whether an inbred or outbred strain of rat was used (Woo et al. 2009). These studies could form the basis for further evaluation of MCP-3 and stem cell homing for treatment or prevention of SUI (Takacs 2007).

4.3.3 Adipose-Derived Stem Cell Therapy

ADSC can differentiate into adipogenic, myogenic, and osteogenic cells when specific induction factors are present (Zuk et al. 2002). ADSC treatment may also be a promising method for treatment of intrinsic urethral deficiency. Jack et al. (2005) found that human-processed lipoaspirate cells, an accessible source of pluripotent cells, remain viable up to 12 weeks after injection into the urethra

with incorporation and differentiation, suggesting that processed lipoaspirate cells may represent a feasible cell source for SUI treatment. They further reported LPP and urethral functional improvement in a rat urethrolysis model of SUI after ADSC injection with a biodegradable microbead carrier (Zeng et al. 2006). Further work is needed to determine the fate of these cells and the mechanism of this effect.

4.3.4 Muscle-Derived Stem Cell Therapy

MDSC display significant regenerative capacity and an improved transplantation capacity compared to MPC implantation (Furuta et al. 2007). In a rat PNT model of SUI, urethral resistance was restored in both the short- and long-term (12 weeks) with periurethral injection of MDSC (Lee et al. 2003, 2004). Although both MDSC and fibroblast injection increased LPP, only the MDSC treatment group had significantly improved urethral muscle contractility (Kwon et al. 2006). MDSC injection also significantly increased LPP 4 and 6 weeks after urethral injury by periurethral cauterization (Chermansky et al. 2004). Therefore, in addition to providing a bulking effect, direct injection of MDSC may physiologically improve urethral sphincter contraction and contribute to continence in animal models of SUI. Clinical trials have commenced, and some results remain controversial (Carr et al. 2008; Aboushwareb and Atala 2008). Therefore, further laboratory investigation is needed to elucidate the mechanism of this effect, and caution is advised in commencing with clinical trials.

5 Summary and Perspective on SUI Animal Models

Investigation into the mechanisms of SUI and development of animal models to test innovative treatments and preventions is relatively new and has blossomed over the course of the last decade. The models and methods of testing provide promise in utilization for preclinical testing of potential treatments and elucidation of the mechanism of SUI development and the mechanism of effect of potential treatments. Much of the testing to date has been observational, documenting the time course of events after an injury, a treatment, or both. Even as the field moves to clinical testing of innovative treatments, more laboratory research needs to be done using interventional studies aimed specifically at determining the mechanism of injury and the mechanism of potential treatments since many questions remain unanswered regarding the mechanism of observed effects.

References

(2005) Duloxetine: new drug. For stress urinary incontinence: too much risk, too little benefit. Prescrire Int 14(80):218–220

Aboushwareb T, Atala A (2008) Stem cells in urology. Nat Clin Pract Urol 5(11):621–631

Ahmed Y, Lin DL, Ferguson C, Esparza N, Damaser MS (2006) Effect of estrogen on urethral function and nerve regeneration following pudendal nerve crush in the female rat. J Urol 175(5):1948–1952

Ahn H, Lin DL, Esparza N, Damaser MS (2005) Short-term timecourse of bilateral pudendal nerve injury on leak-point pressure in female rats. J Rehabil Res Dev 42(1):109–114

Aroni F, Iacovidou N, Dontas I, Pourzitaki C, Xanthos T (2009) Pharmacological aspects and potential new clinical applications of ketamine: reevaluation of an old drug. J Clin Pharmacol 49(8):957–964

Bae JH, Moon DG, Lee JG (2001) The effects of a selective noradrenaline reuptake inhibitor on the urethra: an in vitro and in vivo study. BJU Int 88(7):771–775

Bernabe J, Julia-Guilloteau V, Denys P, Chartier-Kastler E, Alexandre L, Peeters M et al (2008) Peripheral neural lesion-induced stress urinary incontinence in anaesthetized female cats. BJU Int 102(9):1162–1167

Birnbaum HG, Leong SA, Oster EF, Kinchen K, Sun P (2004) Cost of stress urinary incontinence: a claims data analysis. Pharmacoeconomics 22(2):95–105

Cannon TW, Damaser MS (2001) Effects of anesthesia on cystometry and leak point pressure of the female rat. Life Sci 69:1193–1202

Cannon TW, Wojcik EM, Ferguson CL, Saraga S, Thomas C, Damaser MS (2002) Effects of vaginal distension on urethral anatomy and function. BJU Int 90(4):403–407

Cannon TW, Lee JY, Somogyi G, Pruchnic R, Smith CP, Huard J et al (2003) Improved sphincter contractility after allogenic muscle-derived progenitor cell injection into the denervated rat urethra. Urology 62(5):958–963

Cannon TW, Sweeney DD, Conway DA, Kamo I, Yoshimura N, Sacks M et al (2005) A tissue-engineered suburethral sling in an animal model of stress urinary incontinence. BJU Int 96(4):664–669

Carr LK, Steele D, Steele S, Wagner D, Pruchnic R, Jankowski R et al (2008) 1-year follow-up of autologous muscle-derived stem cell injection pilot study to treat stress urinary incontinence. Int Urogynecol J Pelvic Floor Dysfunct 19(6):881–883

Chen B, Wen Y, Zhang Z, Guo Y, Warrington JA, Polan ML (2006) Microarray analysis of differentially expressed genes in vaginal tissues from women with stress urinary incontinence compared with asymptomatic women. Hum Reprod 21(1):22–29

Chen CC, Hijaz A, Drazba JA, Damaser MS, Daneshgari F (2009) Collagen remodeling and suburethral inflammation might account for preserved anti-incontinence effects of cut polypropylene sling in rat model. Urology 73(2):415–420

Chermansky CJ, Cannon TW, Torimoto K, Fraser MO, Yoshimura N, de Groat WC et al (2004) A model of intrinsic sphincteric deficiency in the rat: electrocauterization. Neurourol Urodyn 23(2):166–171

Conway DA, Kamo I, Yoshimura N, Chancellor MB, Cannon TW (2005) Comparison of leak point pressure methods in an animal model of stress urinary incontinence. Int Urogynecol J Pelvic Floor Dysfunct 16(5):359–363

Damaser MS, Broxton-King C, Ferguson C, Kim FJ, Kerns JM (2003) Functional and neuroanatomical effects of vaginal distention and pudendal nerve crush in the female rat. J Urol 170(3):1027–1031

Damaser MS, Whitbeck C, Chichester P, Levin RM (2005) Effect of vaginal distension on blood flow and hypoxia of urogenital organs of the female rat. J Appl Physiol 98(5):1884–1890

Damaser MS, Samplaski MK, Parikh M, Lin DL, Rao S, Kerns JM (2007) Time course of neuroanatomical and functional recovery after bilateral pudendal nerve injury in female rats. Am J Physiol Renal Physiol 293(5):F1614–F1621

DeLancey JO, Trowbridge ER, Miller JM, Morgan DM, Guire K, Fenner DE et al (2008) Stress urinary incontinence: relative importance of urethral support and urethral closure pressure. J Urol 179(6):2286–2290

Eberli D, Andersson KE, Yoo JJ, Atala A (2009) A canine model of irreversible urethral sphincter insufficiency. BJU Int 103(2):248–253

Evans JP (1936) Observations on the nerves of supply to the bladder and urethra of the cat, with a study of their action potentials. J Physiol 86(4):396–414

Foster HE, McGuire EJ (1993) Management of urethral obstruction with transvaginal urethrolysis. J Urol 150(5 Pt 1):1448–1451

Friedman B, Kleinfeld D, Ip NY, Verge VM, Moulton R, Boland P et al (1995) BDNF and NT-4/5 exert neurotrophic influences on injured adult spinal motor neurons. J Neurosci 15(2): 1044–1056

Furuta A, Jankowski RJ, Pruchnic R, Yoshimura N, Chancellor MB (2007) The promise of stem cell therapy to restore urethral sphincter function. Curr Urol Rep 8(5):373–378

Haab F, Zimmern PE, Leach GE (1996) Female stress urinary incontinence due to intrinsic sphincteric deficiency: recognition and management. J Urol 156(1):3–17

Hampel C, Artibani W, Espuna PM, Haab F, Jackson S, Romero J et al (2004) Understanding the burden of stress urinary incontinence in Europe: a qualitative review of the literature. Eur Urol 46(1):15–27

Haylen BT, de Ridder D, Freeman RM, Swift SE, Berghmans B, Lee J et al (2010) An international Urogynecological Association (IUGA)/International Continence Society (ICS) joint report on the terminology for female pelvic floor dysfunction. Neurourol Urodyn 29(4):20

Hijaz A, Daneshgari F, Cannon T, Damaser M (2004) Efficacy of a vaginal sling procedure in a rat model of stress urinary incontinence. J Urol 172(5 Pt 1):2065–2068

Hijaz A, Bena J, Daneshgari F (2005a) Long-term efficacy of a vaginal sling procedure in a rat model of stress urinary incontinence. J Urol 173(5):1817–1819

Hijaz A, Daneshgari F, Huang X, Bena J, Liu G, Saffore L et al (2005b) Role of sling integrity in the restoration of leak point pressure in the rat vaginal sling model. J Urol 174(2):771–775

Hijaz A, Daneshgari F, Sievert KD, Damaser MS (2008) Animal models of female stress urinary incontinence. J Urol 179(6):2103–2110

Jack GS, Almeida FG, Zhang R, Alfonso ZC, Zuk PA, Rodriguez LV (2005) Processed lipoaspirate cells for tissue engineering of the lower urinary tract: implications for the treatment of stress urinary incontinence and bladder reconstruction. J Urol 174(5):2041–2045

Jiang HH, Pan HQ, Gustilo-Ashby MA, Gill B, Glaab J, Zaszczurynski P et al (2009a) Dual simulated childbirth injuries result in slowed recovery of pudendal nerve and urethral function. Neurourol Urodyn 28(3):229–235

Jiang HH, Gustilo-Ashby AM, Salcedo LB, Pan HQ, Sypert DF, Butler RS et al (2009b) Electrophysiological function during voiding after simulated childbirth injuries. Exp Neurol 215(2):342–348

Julia-Guilloteau V, Denys P, Bernabe J, Mevel K, Chartier-Kastler E, Alexandre L et al (2007) Urethral closure mechanisms during sneezing-induced stress in anesthetized female cats. Am J Physiol Regul Integr Comp Physiol 293(3):R1357–R1367

Kaiho Y, Kamo I, Chancellor MB, Arai Y, de Groat WC, Yoshimura N (2007) Role of noradrenergic pathways in sneeze-induced urethral continence reflex in rats. Am J Physiol Renal Physiol 292(2):F639–F646

Kamo I, Hashimoto T (2007) Involvement of reflex urethral closure mechanisms in urethral resistance under momentary stress condition induced by electrical stimulation of rat abdomen. Am J Physiol Renal Physiol 293(3):F920–F926

Kamo I, Torimoto K, Chancellor MB, de Groat WC, Yoshimura N (2003) Urethral closure mechanisms under sneeze-induced stress condition in rats: a new animal model for evaluation of stress urinary incontinence. Am J Physiol Regul Integr Comp Physiol 285(2):R356–R365

Kamo I, Cannon TW, Conway DA, Torimoto K, Chancellor MB, de Groat WC et al (2004) The role of bladder-to-urethral reflexes in urinary continence mechanisms in rats. Am J Physiol Renal Physiol 287(3):F434–F441

Kamo I, Kaiho Y, Canon TW, Chancellor MB, de Groat WC, Prantil RL et al (2006) Functional analysis of active urethral closure mechanisms under sneeze induced stress condition in a rat model of birth trauma. J Urol 176(6 Pt 1):2711–2715

Kane DD, Shott S, Hughes WF, Kerns JM (2002) Motor pudendal nerve characterization in the female rat. Anat Rec 266(1):21–29

Kane DD, Kerns JM, Lin DL, Damaser MS (2004) Early structural effects of oestrogen on pudendal nerve regeneration in the rat. BJU Int 93(6):870–878

Kefer JC, Liu G, Daneshgari F (2008) Pubo-urethral ligament transection causes stress urinary incontinence in the female rat: a novel animal model of stress urinary incontinence. J Urol 179(2):775–778

Kefer JC, Liu G, Daneshgari F (2009) Pubo-urethral ligament injury causes long-term stress urinary incontinence in female rats: an animal model of the integral theory. J Urol 181 (1):397–400

Kerns JM, Damaser MS, Kane JM, Sakamoto K, Benson JT, Shott S et al (2000) Effects of pudendal nerve injury in the female rat. Neurourol Urodyn 19(1):53–69

Kim JH, Huang X, Liu G, Moore C, Bena J, Damaser MS et al (2007) Diabetes slows the recovery from urinary incontinence due to simulated childbirth in female rats. Am J Physiol Regul Integr Comp Physiol 293(2):R950–R955

Kuo HC (2002) Effects of vaginal trauma and oophorectomy on the continence mechanism in rats. Urol Int 69(1):36–41

Kwon D, Kim Y, Pruchnic R, Jankowski R, Usiene I, de MF et al (2006) Periurethral cellular injection: comparison of muscle-derived progenitor cells and fibroblasts with regard to efficacy and tissue contractility in an animal model of stress urinary incontinence. Urology 68(2):449–454

Lee JY, Cannon TW, Pruchnic R, Fraser MO, Huard J, Chancellor MB (2003) The effects of periurethral muscle-derived stem cell injection on leak point pressure in a rat model of stress urinary incontinence. Int Urogynecol J Pelvic Floor Dysfunct 14(1):31–37

Lee JY, Paik SY, Yuk SH, Lee JH, Ghil SH, Lee SS (2004) Long term effects of muscle-derived stem cells on leak point pressure and closing pressure in rats with transected pudendal nerves. Mol Cells 18(3):309–313

Lee UJ, Gustilo-Ashby AM, Daneshgari F, Kuang M, Vurbic D, Lin DL et al (2008) Lower urogenital tract anatomical and functional phenotype in lysyl oxidase like-1 knockout mice resembles female pelvic floor dysfunction in humans. Am J Physiol Renal Physiol 295(2): F545–F555

li-El-Dein B, Ghoneim MA (2001) Effects of selective autonomic and pudendal denervation on the urethral function and development of retention in female dogs. J Urol 166(4):1549–1554

Lin AS, Carrier S, Morgan DM, Lue TF (1998) Effect of simulated birth trauma on the urinary continence mechanism in the rat. Urology 52(1):143–151

Lin G, Shindel AW, Banie L, Deng D, Wang G, Hayashi N et al (2008a) Molecular mechanisms related to parturition-induced stress urinary incontinence. Eur Urol 55:1213–1223

Lin YH, Liu G, Daneshgari F (2008b) A mouse model of simulated birth trauma induced stress urinary incontinence. Neurourol Urodyn 27(4):353–358

Lin YH, Liu G, Li M, Xiao N, Daneshgari F (2010) Recovery of continence function following simulated birth trauma involves repair of muscle and nerves in the urethra in the female mouse. Eur Urol 57(3):512–513

Lose G, Griffiths D, Hosker G, Kulseng-Hanssen S, Perucchini D, Schafer W et al, Standardization Sub-Committee, International Continence Society (2002) Standardization of urethral pressure measurement: report from the Standardization Sub-Committee of the International Continence Society. Neurourol Urodyn 21:258–260

McGuire EJ (2004) Pathophysiology of stress urinary incontinence. Rev Urol 6(suppl 5):S11–S17

McKenna KE, Nadelhaft I (1986) The organization of the pudendal nerve in the male and female rat. J Comp Neurol 248(4):532–549

Miyazato M, Kaiho Y, Kamo I, Chancellor MB, Sugaya K, de Groat WC et al (2008) Effect of duloxetine, a norepinephrine and serotonin reuptake inhibitor, on sneeze-induced urethral continence reflex in rats. Am J Physiol Renal Physiol 295(1):F264–F271

Nikolavsky D, Chancellor MB (2010) Stem cell therapy for stress urinary incontinence. Neurourol Urodyn 29(suppl 1):S36–S41

Olsen AL, Ross M, Stansfield RB, Kreiter C (2003) Pelvic floor nerve conduction studies: establishing clinically relevant normative data. Am J Obstet Gynecol 189(4):1114–1119
Pacheco P, Martinez-Gomez M, Whipple B, Beyer C, Komisaruk BR (1989) Somato-motor components of the pelvic and pudendal nerves of the female rat. Brain Res 490(1):85–94
Pan HQ, Kerns JM, Lin DL, Liu S, Esparza N, Damaser MS (2007) Increased duration of simulated childbirth injuries results in increased time to recovery. Am J Physiol Regul Integr Comp Physiol 292(4):R1738–R1744
Pan HQ, Kerns JM, Lin DL, Sypert D, Steward J, Hoover CR et al (2009) Dual simulated childbirth injury delays anatomic recovery. Am J Physiol Renal Physiol 296(2):F277–F283
Park JM, Bloom DA, McGuire EJ (1997) The guarding reflex revisited. Br J Urol 80(6):940–945
Partridge TA, Grounds M, Sloper JC (1978) Evidence of fusion between host and donor myoblasts in skeletal muscle grafts. Nature 273(5660):306–308
Pauwels E, De WS, Wyndaele JJ (2009) Evaluation of different techniques to create chronic urinary incontinence in the rat. BJU Int 103(6):782–785
Peng CW, Chen JJ, Chang HY, de Groat WC, Cheng CL (2006) External urethral sphincter activity in a rat model of pudendal nerve injury. Neurourol Urodyn 25(4):388–396
Petros PE (1998) The pubourethral ligaments–an anatomical and histological study in the live patient. Int Urogynecol J Pelvic Floor Dysfunct 9(3):154–157
Petros PE, Ulmsten UI (1990) An integral theory of female urinary incontinence. Experimental and clinical considerations. Acta Obstet Gynecol Scand Suppl 153:7–31
Phull H, Salkini M, Escobar C, Purves T, Comiter CV (2007) The role of angiotensin II in stress urinary incontinence: A rat model. Neurourol Urodyn 26(1):81–88
Rapp DE, Kobashi KC (2008) The evolution of midurethral slings. Nat Clin Pract Urol 5(4): 194–201
Resplande J, Gholami SS, Graziottin TM, Rogers R, Lin CS, Leng W et al (2002) Long-term effect of ovariectomy and simulated birth trauma on the lower urinary tract of female rats. J Urol 168(1):323
Rodriguez LV, Chen S, Jack GS, de AF, Lee KW, Zhang R (2005) New objective measures to quantify stress urinary incontinence in a novel durable animal model of intrinsic sphincter deficiency. Am J Physiol Regul Integr Comp Physiol 288(5):R1332–R1338
Rovner ES, Wein AJ (2004) Treatment options for stress urinary incontinence. Rev Urol 6(suppl 3):S29–S47
Sakuma K, Watanabe K, Sano M, Uramoto I, Nakano H, Li YJ et al (2001) A possible role for BDNF, NT-4 and TrkB in the spinal cord and muscle of rat subjected to mechanical overload, bupivacaine injection and axotomy. Brain Res 907(1–2):1–19
Schenk S, Mal N, Finan A, Zhang M, Kiedrowski M, Popovic Z et al (2007) Monocyte chemotactic protein-3 is a myocardial mesenchymal stem cell homing factor. Stem Cells 25(1):245–251
Shoffstall AJ, Zaszczurynski PJ, Butler RS, Damaser MS (2008) Development of a device to standardize leak point pressure experiments in rats. Neurourol Urodyn 27(6):553
Sievert KD, Emre BM, Tsai T, Dahms SE, Nunes L, Lue TF (2001) The effect of simulated birth trauma and/or ovariectomy on rodent continence mechanism. Part I: functional and structural change. J Urol 166(1):311–317
Sievert KD, Bakircioglu ME, Tsai T, Nunes L, Lue TF (2004) The effect of labor and/or ovariectomy on rodent continence mechanism–the neuronal changes. World J Urol 22 (4):244–250
Snooks SJ, Barnes PR, Swash M (1984) Damage to the innervation of the voluntary anal and periurethral sphincter musculature in incontinence: an electrophysiological study. J Neurol Neurosurg Psychiatry 47(12):1269–1273
Snooks SJ, Swash M, Henry MM, Setchell M (1986) Risk factors in childbirth causing damage to the pelvic floor innervation. Int J Colorectal Dis 1(1):20–24
Snooks SJ, Swash M, Mathers SE, Henry MM (1990) Effect of vaginal delivery on the pelvic floor: a 5-year follow-up. Br J Surg 77(12):1358–1360
Sugaya K, Nishijima S, Miyazato M, Ogawa Y (2005) Central nervous control of micturition and urine storage. J Smooth Muscle Res 41(3):117–132

Sultan AH, Kamm MA, Hudson CN (1994) Pudendal nerve damage during labour: prospective study before and after childbirth. Br J Obstet Gynaecol 101(1):22–28

Takacs EB (2007) The potential use of cytokines and stem cell homing signals in the treatment of stress urinary incontinence. J Urol 177(4):1227–1228

Takahashi S, Chen Q, Ogushi T, Fujimura T, Kumagai J, Matsumoto S et al (2006) Periurethral injection of sustained release basic fibroblast growth factor improves sphincteric contractility of the rat urethra denervated by botulinum-a toxin. J Urol 176(2):819–823

Tetzschner T, Sorensen M, Jonsson L, Lose G, Christiansen J (1997) Delivery and pudendal nerve function. Acta Obstet Gynecol Scand 76(4):324–331

Thor KB, Katofiasc MA (1995) Effects of duloxetine, a combined serotonin and norepinephrine reuptake inhibitor, on central neural control of lower urinary tract function in the chloralose-anesthetized female cat. J Pharmacol Exp Ther 274(2):1014–1024

Thuroff JW, Bazeed MA, Schmidt RA, Tanagho EA (1982) Mechanisms of urinary continence: an animal model to study urethral responses to stress conditions. J Urol 127(6):1202–1206

Wallois F, Gros F, Masmoudi K, Larnicol N (1995) C-Fos-like immunoreactivity in the cat brainstem evoked by sneeze-inducing air puff stimulation of the nasal mucosa. Brain Res 687(1–2):143–154

Widdicombe JG (1995) Neurophysiology of the cough reflex. Eur Respir J 8(7):1193–1202

Williams ER, Klutke CG (2008) Stress urinary incontinence: the evolution of the sling. Expert Rev Med Devices 5(4):507–523

Woo LL, Hijaz A, Kuang M, Penn MS, Damaser MS, Rackley RR (2007) Over expression of stem cell homing cytokines in urogenital organs following vaginal distention. J Urol 177 (4):1568–1572

Woo LL, Hijaz A, Pan HQ, Kuang M, Rackley RR, Damaser MS (2009) Simulated childbirth injuries in an inbred rat strain. Neurourol Urodyn 28(4):356–361

Wood HM, Kuang M, Woo L, Hijaz A, Butler RS, Penn M et al (2008) Cytokine expression after vaginal distention of different durations in virgin Sprague-Dawley rats. J Urol 180(2):753–759

Yan Q, Matheson C, Lopez OT, Miller JA (1994) The biological responses of axotomized adult motoneurons to brain-derived neurotrophic factor. J Neurosci 14(9):5281–5291

Yiou R, Dreyfus P, Chopin DK, Abbou CC, Lefaucheur JP (2002) Muscle precursor cell autografting in a murine model of urethral sphincter injury. BJU Int 89(3):298–302

Zeng X, Jack GS, Zhang R, Wu B, Rodriguez LV (2006) Treatment of SUI using adipose derived stem cells: Restoration of urethral function. J Urol 175(4):900

Zhang Y, Bicek AD, Wang G, Timm GW (2010) Effects of periurethral neuromuscular electrical stimulation on the voiding frequency in rats. Int Urogynecol J Pelvic Floor Dysfunct 21 (10):1279–1284

Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H et al (2002) Human adipose tissue is a source of multipotent stem cells. Mol Biol Cell 13(12):4279–4295

Animal Models for Benign Prostatic Hyperplasia

J. Paul Hieble

Contents

Abstract Although the etiology of benign prostatic hyperplasia (BPH) is unknown, various animal models have been used for several decades to identify potential therapeutic approaches. These models can be divided into those measuring smooth muscle tone and those measuring cellular proliferation. Animal models have played an important role in the development of the two drug classes currently approved for the treatment of BPH: the α-adrenoceptor antagonists and the steroid 5-α-reductase inhibitors. However, models measuring prostatic tone have not been particularly useful in the identification of new α-adrenoceptor antagonists having advantages over currently available drugs, and it is not certain that reduction of prostatic smooth muscle tone is responsible for the relief of BPH symptoms. A further limitation with BPH models is that prostatic hyperplasia similar to the human condition does not occur spontaneously or cannot be induced in any suitable animal species. The identification of a more useful BPH model is focused on cellular mechanisms of prostatic growth, looking similarities between humans and experimental animals.

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1 Introduction

The human prostate is normally a walnut-sized organ located at the base of the bladder surrounding the urethra. It is composed of glandular acini separated by fibromuscular stroma. In benign prostatic hyperplasia (BPH), stromal nodules appear and enlarge, eventually compressing the normal gland against the serous capsule to form a muscular pseudocapsule (Hieble 2004). This results in both obstructive voiding symptoms (e.g., reduced flow rate and hesitancy) resulting from urethral compression and irritative storage symptoms (e.g., increased frequency and urgency) resulting from changes in bladder function as a result of the sustained obstruction.

The initiating event in the development of BPH is not known; however, the nodules almost certainly result from a stromal–epithelial interaction (Cunha 2008). The development of BPH is androgen dependent, although once developed, the hyperplasia can only be partially reversed by androgen deprivation. Before 1980, treatment of BPH was almost entirely surgical, although chemical castration was occasionally used. The development of drugs for BPH was based on data from Marco Caine and co-workers showing that phenoxybenzamine can block the contractile action of norepinephrine in an isolated strip of rat prostate (Raz et al. 1973), and the observation that BPH did not develop in persons lacking the enzyme steroid 5-α-reductase, which is responsible for conversion of testosterone to dihydrotestosterone (Imperato-McGinley et al. 1974). These two observations led to the development of α-adrenoceptor antagonists and steroid 5-α-reductase inhibitors, still the only two drug classes approved for the treatment of BPH.

2 Measurement of Prostatic Smooth Muscle Tone

2.1 Development of α_1-Adrenoceptor Antagonists for BPH

The observation that phenoxybenzamine can block the constrictor activity of noradrenaline in the rat prostate was extended to human prostate tissue (Caine et al.1975) and led to clinical evaluation of well-known α-adrenoceptor antagonists such as phenoxybenzamine and phentolamine in BPH patients (Caine and Perlberg 1977; Caine et al. 1978). The limited clinical data obtained with these compounds suggested a reduction in the obstructive and irritative symptoms of BPH. This correlation of results from isolated tissue with clinical efficacy appeared to confirm the hypothesis of a "dynamic" component to the obstruction of bladder outflow by the enlarged prostate (Caine 1986, 1988). Further studies using isolated strips of human,

canine, and rabbit prostate showed that the α_1-adrenoceptor subtype mediated the contractile activity (Lepor and Shapiro 1984; Hieble et al. 1985, 1986; Honda et al. 1985). Terazosin and doxazosin, already approved for the treatment of hypertension, were shown to be effective for relief of BPH symptoms. Another potent and selective α_1-adrenoceptor antagonist, tamsulosin, was also developed for BPH. A natural consequence of these results was to assume that the α-adrenoceptor on prostatic smooth muscle represented a viable therapeutic target.

2.2 *Use of Animal Models to Identify New α_1-Adrenoceptor Antagonists*

Once it was established that prostatic smooth muscle contracts in response to α_1-adrenoceptor activation, the goal of smooth muscle studies was to determine whether prostatic tone could be decreased without relaxation of other smooth muscle tissues, in particular the resistance vessels controlling blood pressure. A common in vivo assay used an anesthetized dog to measure the potency of a compound against the pressure increase in the prostatic urethra induced by either an α-adrenoceptor agonist or sympathetic nerve stimulation. This was compared with either the potency against agonist or nerve-mediated increases in blood pressure or the dose required to reduce basal blood pressure to a specific degree (e.g., see Testa et al. 1997). The ratio between doses in the prostatic and vascular assays was used to calculate the "uroselectivity" of the α-adrenoceptor antagonist. The discovery of multiple α_1-adrenoceptor subtypes, and the development of radioligand binding assays for the determination of subtype selectivity, occurred while α_1-adrenoceptor antagonists were being developed for BPH. Of the α_1-antagonists known to be effective in BPH, doxazosin and terazosin did not differentiate between subtypes, while tamsulosin was equally potent at α_{1A}- and α_{1D}-adrenoceptor and at least an order of magnitude less potent at the α_{1B} subtype. An obvious path for the development of better drugs for BPH was to determine their subtype selectivity and uroselectivity. Many thousands of compounds, and drug candidates showing substantial uroselectivity, were tested in this manner. All of these compounds had some selectivity for the α_{1A}-adrenoceptor versus the α_{1D} and α_{1B} subtypes, although in vivo uroselectivity did not always require a high degree of α_{1A} selectivity (Testa et al. 1997). Several of these uroselective α-adrenoceptor antagonists were evaluated in rigorous double-blind clinical trials. Surprisingly, these new antagonists were less effective against BPH symptoms than the older compounds already in clinical use. The reason for this disparity is still not completely understood, but does not appear to result from differences in human and animal prostatic α-adrenoceptors, since data from isolated human and animal prostate correlate well. The only selective α_{1A}-adrenoceptor antagonist to successfully complete clinical trials is silodosin (KMD-3213). Although silodosin has about 50-fold selectivity for α_{1A}- versus α_{1D}-adrenoceptor (Tatemichi et al. 2006), the effective clinical doses would suggest that both α_{1A}- and α_{1D}-adrenoceptor are blocked. Another new

α-adrenoceptor antagonist showing efficacy in humans was naftopidil, which, like tamsulosin, has highest affinity for the α_{1A} and α_{1D} subtypes but with a small (threefold) preference for the α_{1D} (Takei et al. 1999). The only other new α_1-adrenoceptor antagonist to be developed was alfuzosin, which, like terazosin and doxazosin, had equal affinity at all three α_1-adrenoceptor subtypes (Kenny et al. 1994).

Hence, despite an extensive search by several pharmaceutical companies, a superior α_1-adrenoceptor antagonist for BPH was not found. However, it cannot be claimed that these results were helpful in the design of better drugs for BPH. In fact, this effort may have hindered the drug discovery process, since clinical resources were devoted to drugs that did not prove to be effective. This has led to the questioning of the correctness of the original hypothesis, i.e., is α-adrenoceptor-mediated prostatic tone contributory to BPH symptoms? It has been proposed (Lepor 2007) that, although the α_{1A}-adrenoceptor is responsible for contraction of prostatic smooth muscle inducing bladder outlet obstruction, another subtype is responsible for other BPH symptoms; hence an α_{1A}-selective compound is less effective than an α_{1A}/α_{1D}-antagonist (e.g., tamsulosin) or a non-subtype-selective α_1-antagonist (e.g., terazosin, doxazosin, or alfuzosin).

In view of the clinical failure of selective α_{1A}-antagonists, this class is no longer being pursued. There is still interest in α_{1A}/α_{1D}-antagonists (Chiu et al. 2007; Liu et al. 2009; Nanda et al. 2009), since some investigators feel that this profile offers a superior efficacy versus side-effect profile compared to the non-subtype-selective compounds. It has been proposed that bladder α_{1D}-adrenoceptors may be upregulated as a consequence of the chronic obstruction by the enlarged prostate and that blockade of this receptor subtype may reduce bladder hyperactivity (González Enguita et al. 2003; Schwinn and Roehrborn 2008). Other companies are no longer pursuing new drugs targeting prostatic smooth muscle in view of the apparent lack of correlation between animal and clinical results.

2.3 *Other Factors Relating to Measurement of Prostatic Muscle Contraction*

In contrast to other smooth muscle tissues, such as blood vessels, trachea, or intestine, the muscle fibers in prostate have no specific orientation. Therefore, the tone generated by a strip of prostatic muscle cannot be optimized by cutting the tissue in a specific manner. This results in lower maximal tension for a prostatic strip (typically 1–3 g for dog prostate) vis-à-vis a vascular tissue such as saphenous vein (typically >10 gm) where a ring or helical strip can be cut to measure tone of the circular smooth muscle layer.

Although the first in vitro study on prostatic muscle contractility used rat tissue, other species were more commonly used, since rat prostate has a low content of fibromuscular stroma (Mori et al. 2009). Most of the early studies measuring

contraction or relaxation of isolated prostate smooth muscle have been done in canine or rabbit tissue. Recently, rat prostate has been studied more often for in vitro studies, perhaps because it offers the advantage of testing the three α_1-adrenoceptor subtypes in tissues from a single species. Mouse prostate has been shown to contract in response to noradrenaline, with pharmacology similar to that observed in human prostate and prostate from other commonly used animal species (Gray and Ventura 2006). Mouse prostate has been used to test the effects of gene knockout on α-adrenoceptor-mediated increases in smooth muscle tone (Gray et al. 2008).

Abnormal ejaculation has been associated with some of the α_1-adrenoceptor antagonists used for the treatment of BPH. In vivo experiments in the dog compare the potency of these compounds against phenylephrine-induced increases in pressure within the prostatic urethra and vas deferens (Noguchi et al. 2008). All compounds tested were more potent in the vas deferens, providing a possible explanation for this side effect.

Contractile mechanisms can also be studied in isolated prostate smooth muscle cells (Lang et al. 2006; Boulbès et al. 2006; Nguyen et al. 2009). These techniques do not seem to offer any practical advantages for studying adrenoceptor-mediated contraction. They may be useful for investigation of other steps in the contractile process, although the ability to maintain functional α-adrenoceptors in cultured prostatic smooth muscle cells has not yet been established. The ultimate goal would be to identify a molecular target specific to prostate smooth muscle. It remains to be determined, however, whether more effective blockade of prostate muscle contraction would lead to more effective control of BPH symptoms in humans.

2.4 Hyperplastic Versus Normal Prostate

An ideal model for testing drug effects on prostatic tone would utilize an enlarged rather than a normal prostate. The dog develops age-related spontaneous prostatic hyperplasia. In contrast to human prostate, canine prostate is not divided into lobes, and the hyperplasia is observed throughout the gland, rather than as discrete nodules. Hyperplasia can also be induced in the dog by either testosterone or a combination of testosterone and estrogen. The histology of induced prostatic hyperplasia in the dog resembles spontaneous canine BPH, although addition of estrogen produces some increase in stromal proliferation. There is no evidence that the pharmacological properties of canine prostatic smooth muscle differ between normal and hyperplastic prostate. A comparison of the effects of an α_1-adrenoceptor antagonist (silodosin) on urethral pressure in young and old dogs with BPH showed no difference, even though the old dogs had larger prostates (Kobayashi et al. 2009). In the dog, prostatic enlargement does not often result in urethral obstruction. If the prostate is wrapped in a nylon mesh, which mimics the muscular capsule found in human BPH, testosterone-induced prostatic enlargement induces urethral obstruction (Broderick et al. 1994).

Commonly used strains of laboratory rat do not exhibit age-related prostatic hyperplasia, although 24-month-old Brown Norway rats show areas of focal epithelial hyperplasia in the dorsal and lateral prostatic lobes (Bethel et al. 2009). Androgenic stimulation induces prostatic hyperplasia in the rat. Some investigators attribute the increases in urethral resistance associated with this hyperplasia to the enlarged prostate; however the rat urethra does not pass through the prostate, and it seems more likely that increased urethral resistance results from hyperplasia of urethral smooth muscle.

Large nonhuman primates such as the baboon or chimpanzee have prostates showing anatomical and physiological similarity to that of the humans. Furthermore, these primates do develop spontaneous prostate hyperplasia (Mubiru et al. 2008; Steiner et al. 1999). An early experiment (Karr et al. 1984) showed that testosterone could induce BPH in the baboon and that the resulting hyperplastic prostate had characteristics resembling clinical BPH in humans. The similarity between biochemical markers such as PSA between baboon and human BPH suggests that non-invasive studies in this species may be useful (Mubiru et al. 2007). However, due to the length of time required for BPH to develop as well as ethical and logistical concerns, these species are unlikely to be useful for developing practical models for drug discovery. The effects of testosterone on urethral resistance in the primate have not been reported; however experiments in a few cynomolgus monkeys showed that 12-week testosterone treatment made passing a catheter through the urethra into the bladder difficult and that treatment with an α-adrenoceptor antagonist facilitated catheterization (Hieble, unpublished observation).

3 Measurement of Prostatic Growth

3.1 Induction of Cellular Proliferation

The ideal drug for BPH would prevent or possibly reverse the development of stromal nodules. It is difficult to evaluate this activity in animal models, since these nodules are not normally formed in prostates from the commonly used experimental animals. Androgenic stimulation is commonly used to induce prostatic hyperplasia. The addition of estrogen to androgen results in an enhanced stromal hyperplasia. The ratio of testosterone to estrogen appears to be important in achieving maximal effects on the stroma (Yokota et al. 2004). An estrogen/androgen ratio of 100 was found to be optimal for inducing stromal hyperplasia in the rat (Zhou et al. 2009).

While this type of model is commonly used for testing the effects of compounds on cellular proliferation in the prostate, it is relatively nonspecific. The active androgen for prostatic cells from both humans and experimental animals was identified as dihydrotesterone. Therefore, to test for steroid-5-α-reductase activity, the effect of a compound on dihydrotestosterone-induced hyperplasia is compared

with the effects against testosterone. A nonspecific androgen antagonist blocks the stimulatory activity of both androgens, while a steroid-5-α-reductase inhibitor blocks testosterone, but not dihydrotesterone. Both rat and dog models were used in the evaluation of the steroid 5-α-reductase inhibitors now approved for BPH. The lack of stromal proliferation in these models is not important, since these compounds act primarily on the glandular epithelium.

More specific models attempt to simulate the stromal–epithelial interaction thought to initiate human BPH. It has been known for many years that if fetal urogenital sinus is implanted into rat or mouse prostate, it will undergo hormonally dependent growth (Chung et al. 1984; Cunha 2008). A recent study (Mori et al. 2009) shows that the tissue derived from implanted embryonic rat urogenital sinus has a much higher stromal content (70%) compared with normal rat prostate (20%) or rat prostate in which hypertrophy has been induced by exogenous testosterone (15%). An antiandrogen was effective in preventing growth of the embryonic tissue, but with lower potency than observed against testosterone-induced epithelial hyperplasia. This model could potentially be useful for identifying agents capable of inhibiting stromal proliferation.

Another method for inducing hyperplasia in the mouse prostate is by gene knockout or growth factor overexpression. The liver X receptor (LXR) is a ligand-activated transcription factor, which is activated by oxysterols, including precursors to steroid hormones (Beltowski and Semczuk 2010). The LXR receptor knockout mouse develops prostate hyperplasia with several characteristics resembling human BPH (Kim et al. 2009). Likewise, overexpression of keratinocyte-derived chemokine, the murine analog of the chemokine IL-8, produces prostate hyperplasia with the stromal component similar to that found in humans (Schauer et al. 2009). Similar results were observed in mice overexpressing prolactin (Wennbo et al. 1997).

3.2 *Effects of α-Adrenoceptor Antagonists on Cellular Proliferation*

Despite the lack of a significant reduction in prostate size in clinical trials with α_1-adrenoceptor antagonists, there have been several reports showing a reduction in cellular proliferation in animal models. Thirty-day treatment of normal rats with a high dose of doxazosin produced a 20% reduction in prostatic weight and an increase in the density of stromal collagen fibers (Justulin et al. 2008). Using the model described above, where BPH was induced in the rat by implantation of fetal urogenital sinus, 21-day treatment with naftopidil, a preferential α_{1D}-adrenoceptor antagonist, inhibited cellular proliferation in both epithelia and stroma. The inhibition of stromal proliferation (60–75%) was especially impressive (Kojima et al. 2009). Although 120-day treatment of normal rats with a low dose of terazosin had no effect on prostate weight, expression of basic FGF on both epithelial and stromal

cells was reduced (Mitropoulos et al. 2009). Treatment of nude mice bearing a xenograft of the human prostate tumor LNCaP with an α_{1A}/α_{1D}-antagonist resulted in a dose-dependent reduction in LNCaP cell growth, and regular dosing with α-adrenoceptor antagonist suppressed tumor growth (Liu et al. 2009).

4 Other Animal Models

Recently, animal models have been developed to evaluate therapies for BPH, which may be considered as intermediate between traditional pharmacotherapy and surgery. Inducing a "prostatic infarct" via embolization of the arterial blood supply to the prostate may offer a less invasive alternative to the transurethral ablation techniques currently in common use. Furthermore, reducing prostatic volume in this manner preserves the urethra. This technique was evaluated in the dog; introduction of polyvinyl alcohol beads to the artery supplying the prostate produced a significant reduction in prostate volume, measured by magnetic resonance imaging, in dogs in which prostatic hyperplasia had been produced by treatment with dihydrotestosterone and estradiol (Jeon et al. 2009). A similar study in intact pigs showed a significant reduction in prostate volume (3.9 ± 1.3 vs. 7.3 ± 2.3 ml) following intra-arterial administration of 500–700 μM microspheres (Sun et al. 2008).

Another novel therapy for BPH is the intra-prostatic injection of botulinum toxin. Intra-prostatic injection of normal rats with purified botulinum toxin reduced prostatic weight, inducing atrophy and diffuse apoptosis (Nishiyama et al. 2009). This model may be useful for evaluating other neurotoxins or optimizing purification procedures.

5 Conclusions

Although the action of drugs on the prostate has been studied for over three decades, there is still a need for better animal models. The mechanism of action for the α1-adrenoceptor antagonists, first-line therapy for most BPH patients, is not yet understood. In contrast to long-held assumptions, their primary mechanism of action may not involve reduction of bladder outlet resistance (Barendrecht et al. 2008), and the commonly used models comparing effects on prostatic/urethral resistance and blood pressure probably do not correlate with clinical profile. If relaxation of bladder outlet resistance is not sufficient for clinical efficacy, how are new smooth muscle relaxant targets, such as PDE-5 inhibitors (Cornu and Rouprêt 2007), to be validated?

The ideal drug for BPH would combine effects on outlet resistance with inhibition of stromal cell proliferation. It is possible that some α_1-adrenoceptor antagonists may have such an action (Kojima et al. 2008), as well as other classes currently

under clinical evaluation, such as vitamin D receptor agonists (Adorini et al. 2007). A model allowing urodynamic evaluation in the presence of stromal hyperplasia could reflect the true clinical potential of such compounds.

References

Adorini L, Penna G, Amuchastegui S et al (2007) Inhibition of prostate growth and inflammation by the vitamin D receptor agonist BXL-628 (elocalcitol). J Steroid Biochem Mol Biol 103:689–693

Barendrecht MM, Abrams P, Schumacher H et al (2008) Do alpha1-adrenoceptor antagonists improve lower urinary tract symptoms by reducing bladder outlet resistance? Neurourol Urodyn 27:226–230

Beltowski J, Semczuk A (2010) Liver X receptor (LXR) and the reproductive system – a potential novel target for therapeutic intervention. Pharmacol Rep 62:15–27

Bethel CR, Chaudhary J, Anway MD, Brown TR (2009) Gene expression changes are age-dependent and lobe-specific in the brown Norway rat model of prostatic hyperplasia. Prostate 69:838–850

Boulbès D, Choquet A, Barrère-Lemaire S et al (2006) Differentiated rabbit prostatic stromal cells in primary culture display functional alpha1A-adrenoceptors. Neurourol Urodyn 25:168–178

Broderick GA, Longhurst PA, Juniewicz PE et al (1994) A novel canine model of partial outlet obstruction secondary to prostatic hypertrophy. World J Urol 12:245–248

Caine M (1986) Clinical experience with alpha-adrenoceptor antagonists in benign prostatic hypertrophy. Fed Proc 45:2604–2608

Caine M (1988) Alpha-adrenergic mechanisms in dynamics of benign prostatic hypertrophy. Urology 32(6 Suppl):16–20

Caine M, Perlberg S (1977) Dynamics of acute retention in prostatic patient and role of adrenergic receptors. Urology 9:399–403

Caine M, Raz S, Zeigler M (1975) Adrenergic and cholinergic receptors in the human prostate, prostatic capsule and bladder neck. Br J Urol 47:193–202

Caine M, Perlberg S, Meretyk S (1978) A placebo-controlled double-blind study of the effect of phenoxybenzamine in benign prostatic obstruction. Br J Urol 50:551–554

Chiu G, Li S, Connolly PJ, Pulito V et al (2007) (Arylpiperazinyl)cyclohexylsufonamides: discovery of alpha(1a/1d)-selective adrenergic receptor antagonists for the treatment of benign prostatic hyperplasia/lower urinary tract symptoms (BPH/LUTS). Bioorg Med Chem Lett 17:3292–3297

Chung LW, Matsuura J, Runner MN (1984) Tissue interactions and prostatic growth I. Induction of adult mouse prostatic hyperplasia by fetal urogenital sinus implants. Biol Reprod 31:155–163

Cornu JN, Rouprêt M (2007) Recent progress in the medical treatment of benign prostatic hyperplasia. Prog Urol 17(5 Suppl 1):1029–1032

Cunha GR (2008) Mesenchymal-epithelial interactions: past, present, and future. Differentiation 76:578–586

González Enguita C, Vela Navarrete R, Salcedo de Diego A et al (2003) Categorization of adrenergic alpha 1 receptors in detrusor of patients with obstructive BPH. Initial study on experimental animal model. Actas Urol Esp 27:684–691

Gray KT, Ventura S (2006) Alpha1L-adrenoceptors mediate contractions of the isolated mouse prostate. Eur J Pharmacol 540:155–161

Gray KT, Short JL, Ledent C, Ventura S (2008) Targeted disruption of the A2A adenosine receptor reduces in-vitro prostate contractility in mature mice. Eur J Pharmacol 592:151–157

Hieble JP (2004) Therapeutic strategies for benign prostatic hypertrophy. Drug Discov Today Ther Strateg 1:243–248

Hieble JP, Caine M, Zalaznik E (1985) In vitro characterization of the alpha-adrenoceptors in human prostate. Eur J Pharmacol 107:111–117
Hieble JP, Boyce AJ, Caine M (1986) Comparison of the alpha-adrenoceptor characteristics in human and canine prostate. Fed Proc 45:2609–2614
Honda K, Miyata-Osawa A, Takenaka T (1985) Alpha 1-adrenoceptor subtype mediating contraction of the smooth muscle in the lower urinary tract and prostate of rabbits. Naunyn Schmiedebergs Arch Pharmacol 330:16–21
Imperato-McGinley J, Guerrero L, Gautier T, Peterson RE (1974) Steroid 5 alpha-reductase deficiency in man: an inherited form of male pseudohermaphroditism. Science 186:1213–1215
Jeon GS, Won JH, Lee BM et al (2009) The effect of transarterial prostate embolization in hormone-induced benign prostatic hyperplasia in dogs: a pilot study. J Vasc Interv Radiol 20:384–390
Justulin LA Jr, Delella FK, Felisbino SL (2008) Doxazosin reduces cell proliferation and increases collagen fibers in rat prostatic lobes. Cell Tissue Res 332:171–183
Karr JP, Kim U, Resko JA et al (1984) Induction of benign prostatic hypertrophy in baboons. Urology 23:276–289
Kenny BA, Naylor AM, Carter AJ et al (1994) Effect of alpha 1 adrenoceptor antagonists on prostatic pressure and blood pressure in the anesthetized dog. Urology 44:52–57
Kim HJ, Andersson LC, Bouton D et al (2009) Stromal growth and epithelial cell proliferation in ventral prostates of liver X receptor knockout mice. Proc Natl Acad Sci USA 106:558–563
Kobayashi S, Tomiyama Y, Tatemichi S et al (2009) Effects of silodosin and tamsulosin on the urethra and cardiovascular system in young and old dogs with benign prostatic hyperplasia. Eur J Pharmacol 613:135–140
Kojima Y, Hayase M, Imura M, Okada S, Kubota Y, Sasaki S, Hayashi Y, Kohri K (2008) The role of alpha1-adrenoceptor subtypes in benign prostatic hyperplasia-from bed to bench. Hinyokika Kiyo 54:457–462
Kojima Y, Sasaki S, Oda N et al (2009) Prostate growth inhibition by subtype-selective alpha(1)-adrenoceptor antagonist naftopidil in benign prostatic hyperplasia. Prostate 2009 Jun 18. [Epub ahead of print]
Lang RJ, Nguyen DT, Matsuyama H et al (2006) Characterization of spontaneous depolarizations in smooth muscle cells of the Guinea pig prostate. J Urol 175:370–380
Lepor H (2007) Alpha blockers for the treatment of benign prostatic hyperplasia. Rev Urol 9:181–190
Lepor H, Shapiro E (1984) Characterization of alpha1 adrenergic receptors in human benign prostatic hyperplasia. J Urol 132:1226–1229
Liu CM, Lo YC, Tai MH et al (2009) Piperazine-designed alpha 1A/alpha 1D-adrenoceptor blocker KMUP-1 and doxazosin provide down-regulation of androgen receptor and PSA in prostatic LNCaP cells growth and specifically in xenografts. Prostate 69:610–623
Mitropoulos D, Kyroudi-Voulgari A, Christelli E et al (2009) Terazosin treatment suppresses basic fibroblast growth factor expression in the rat ventral prostate. Clin Invest Med 32:E1–7
Mori F, Oda N, Sakuragi M et al (2009) New histopathological experimental model for benign prostatic hyperplasia: stromal hyperplasia in rats. J Urol 181:890–898
Mubiru JN, Hubbard GB, Dick EJ Jr et al (2007) A preliminary study of the baboon prostate pathophysiology. Prostate 67:1421–1431
Mubiru JN, Hubbard GB, Dick EJ Jr et al (2008) Nonhuman primates as models for studies of prostate specific antigen and prostatic diseases. Prostate 68:1546–1554
Nanda K, Naruganahalli KS, Gupta S et al (2009) RBx 6198: a novel alpha1-adrenoceptor antagonist for the treatment of benign prostatic hyperplasia. Eur J Pharmacol 607:213–219
Nguyen DT, Lang RJ, Exintaris B (2009) Alpha(1)-adrenoceptor modulation of spontaneous electrical waveforms in the guinea-pig prostate. Eur J Pharmacol 608:62–70
Nishiyama Y, Yokoyama T, Tomizawa K et al (2009) Effects of purified newly developed Botulinum neurotoxin type A in rat prostate. Urology 74:436–439

Noguchi Y, Ohtake A, Suzuki M, Sasamata M (2008) In vivo study on the effects of alpha1-adrenoceptor antagonists on intraurethral pressure in the prostatic urethra and intraluminal pressure in the vas deferens in male dogs. Eur J Pharmacol 580:256–261

Raz S, Zeigler M, Caine M (1973) Pharmacological receptors in the prostate. Br J Urol 45:663–667

Schauer IG, Ressler SJ, Rowley DR (2009) Keratinocyte-derived chemokine induces prostate epithelial hyperplasia and reactive stroma in a novel transgenic mouse model. Prostate 69:373–384

Schwinn DA, Roehrborn CG (2008) a-Adrenoceptor subtypes and lower urinary tract symptoms. Int J Urol 15:193–199

Steiner MS, Couch RC, Raghow S, Stauffer D (1999) The chimpanzee as a model of human benign prostatic hyperplasia. J Urol 162:1454–1461

Sun F, Sánchez FM, Crisóstomo V et al (2008) Benign prostatic hyperplasia: transcatheter arterial embolization as potential treatment-preliminary study in pigs. Radiology 246:783–789

Takei R, Ikegaki I, Shibata K et al (1999) Naftopidil, a novel alpha1-adrenoceptor antagonist, displays selective inhibition of canine prostatic pressure and high affinity binding to cloned human alpha1-adrenoceptors. Jpn J Pharmacol 79:447–454

Tatemichi S, Kobayashi K, Maezawa A et al (2006) Alpha1-adrenoceptor subtype selectivity and organ specificity of silodosin (KMD-3213). Yakugaku Zasshi 126:209–216

Testa R, Guarneri L, Angelico P et al (1997) Pharmacological characterization of the uroselective alpha-1 antagonist Rec 15/2739 (SB 216469): role of the alpha-1L adrenoceptor in tissue selectivity, part II. J Pharmacol Exp Ther 281:1284–1293

Wennbo H, Kindblom J, Isaksson OG, Törnell J (1997) Transgenic mice overexpressing the prolactin gene develop dramatic enlargement of the prostate gland. Endocrinology 138:4410–4415

Yokota T, Honda K, Tsuruya Y et al (2004) Functional and anatomical effects of hormonally induced experimental prostate growth: a urodynamic model of benign prostatic hyperplasia (BPH) in the beagle. Prostate 58:156–163

Zhou Y, Xiao XQ, Chen LF et al (2009) Proliferation and phenotypic changes of stromal cells in response to varying estrogen/androgen levels in castrated rats. Asian J Androl 11:451–459

Use of Functional Imaging to Monitor Central Control of Voiding in Humans

Derek J. Griffiths

Contents

Abstract Bladder problems are frequently disorders of control, which is exercised from the brain. In such disorders, brain responses to bladder events are abnormal; therapy is accompanied by regional changes that may be measured by functional imaging and used to monitor the effect of treatment. The regional responses may be understood in terms of a tentative model of the bladder control system. The model helps also to interpret alterations in brain behavior (as imaged by functional scanning) that occur when afferent signals from bladder or urethra are changed experimentally or by an underlying disorder or treatment, for example, overactive bladder (urge/urgency incontinence). Successful treatment may either increase the

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ability to cope with the problem or may be curative. The direction of treatment-induced change of abnormal brain responses can distinguish these two possibilities and shed light on the therapeutic mechanism. In addition, brain activity in regions such as insula or dorsal anterior cingulate cortex may be regarded as a proxy for sensations such as desire to void or urgency, which are otherwise difficult to define or measure. Monitoring of brain responses in these regions offers an obvious way to test the effect of drugs.

Keywords Brain · Bladder control · Bladder filling sensation · Functional brain imaging · Urgency · Urge urinary incontinence

1 Introduction

Disorders of voluntary bladder control are prevalent and are of two main types: inability to empty the bladder (retention, urinary) and inability to store urine, manifested as the triad of symptoms making up the overactive bladder syndrome overactive bladder (OAB) (frequency, urgency, and urge or incontinence, urge(ncy)). In some cases, the symptoms are associated with neurological diseases such as multiple sclerosis or spinal cord injury, or with neuropathy (e.g., in longstanding diabetes), or with other peripheral changes (e.g., prostatic obstruction). Nevertheless in many cases the underlying pathology is unknown. Until recently the cause of such disorders has been sought in the peripheral organs or the afferent signals that they generate. For example, Fowler's syndrome (unexplained urinary retention in younger women) has been ascribed to an overactive urethral sphincter, while overactive bladder, as the name suggests, is usually supposed to be a manifestation of an underlying bladder abnormality, at least if no obvious pathological cause can be identified.

Correspondingly, and especially in OAB, most animal models and pharmacological approaches to therapy have focused on the bladder or on bladder afferents. Yet these symptoms are manifestations of loss of voluntary control of the bladder, and control is exerted by an extensive spinal and supraspinal neuronal network, of which the peripheral organs and their innervation form only a small part. On the face of it, pathology of the control system seems equally or more likely to be responsible for bladder control problems than an intrinsic bladder defect. This possibility was for a long time ignored, however, because of the lack of technical means to study it. Undoubtedly, this neglect has limited the range of potential therapeutic targets.

Recent advances in functional brain imaging, and especially the widespread accessibility of these techniques during the past decade, have provided a basic understanding of the supraspinal bladder control system, which makes it possible to recognize normal and abnormal states of the brain and monitor response to therapy (Kavia et al. 2005; Griffiths and Tadic 2008). Functional imaging has great advantages: the ability to monitor the control system itself rather than just the behavior of the peripheral organs and their afferent signals; and the ability to do this in human beings, with or without disease, instead of experimental animals. Indeed, good animal models of OAB symptoms such as urge incontinence are difficult to devise because lack of communication makes it difficult to draw the critical distinction

between involuntary loss of bladder control and voluntary voiding, or even to know whether such a distinction is meaningful in an animal. Furthermore, functional imaging may enable us to recognize neural correlates of clinically important but difficult-to-define concepts, thus offering easily measured proxy markers that might be used to monitor therapeutic effect. As we shall see moreover the direction of the changes in brain activity brought about by therapy provides helpful information about the therapeutic mechanism.

Functional brain imaging may be carried out by a number of techniques, including SPECT scanning (single photon emission tomography), PET scanning (positron emission tomography), and fMRI (functional magnetic resonance imaging). All provide an indirect measure of regional cerebral blood flow, believed to represent local neuronal activity. One potential problem that should be borne in mind when interpreting functional imaging studies is that neuronal activity may be either excitatory or inhibitory: without further information it is not possible to distinguish these two possibilities. Both SPECT and PET require injection of a radioactive tracer. SPECT has relatively poor temporal and spatial resolution. PET is good for examining long-lived states and slow changes in brain activity and has been used to investigate both storage and voiding. fMRI has better temporal and spatial resolution than PET. It is relatively inexpensive and noninvasive, but produces a weak and noisy signal. It is good for examining relatively short events, but frequent repetitions are required in order to average out random errors due to noise. This requirement has so far precluded fMRI measurements during actual voiding [imagined voiding has been examined (Kuhtz-Buschbeck et al. 2005)], but repetitive infusion and withdrawal of a small amount of water in and out of the bladder has proved a fruitful way of mimicking bladder filling so as to investigate the storage phase (Griffiths et al. 2005). (Technically, the fMRI BOLD [blood oxygen level dependent] signal during withdrawal of fluid is subtracted from the signal during infusion, and the result is averaged over numerous repetitions.) Limitation to the storage phase has not proved a problem because disorders of bladder control such as OAB are disorders of storage.

The following Sect. 2 continues with a description of a tentative model of the normal bladder control system. Its purpose is to set in an overall framework the many regions of the brain that respond to bladder events. There is then a summary of the potential effects of pharmacological therapy and the existing evidence concerning alterations in brain behavior (as imaged by functional scanning) when afferent signals from bladder or urethra are changed experimentally, by disease, or by treatment. There follows a more detailed description of brain responses to bladder filling in subjects with OAB and how they are altered by therapy (although unfortunately not pharmacological therapy).

2 The Bladder Control Network

The nodes of the supraspinal network that controls the bladder (the “centers” that are involved) have been established during more than a decade of functional imaging and to a large extent confirm the conclusions drawn from older animal

experiments and clinical observations (Andrew and Nathan 1964; Torrens and Feneley 1982). Merely to list the centers and ascribe a function to each has rightly been labeled "phrenology." Although the interpretation of functional scanning results has frequently not gone far beyond this point, there have been attempts to identify aspects of the connectivity of the various centers and to place them in a model network with some indication of their function and interactions (Blok 2002; Fowler et al. 2008; Tadic et al. 2008). During storage the ultimate result of the network is tonic inhibition of detrusor contraction, preventing voiding and maintaining continence. Only for voiding is this inhibition lifted.

Based on these findings and some imaginative extensions, a tentative model of the bladder control network has several neural circuits working in parallel (Fig. 1). They determine whether voiding is (1) mechanically appropriate (i.e., the bladder is adequately filled), (2) emotionally safe, and (3) socially appropriate. If they concur then voiding may take place. Neural circuit 1 of the network is part of the well-known spinobulbospinal (long-loop) voiding reflex. It is driven by spinal afferent signals that are believed to originate from mechanical transducers in the bladder wall or urothelium and to increase with bladder filling (Fowler et al. 2008). Thus circuit 1 is a mechanical reflex which by itself would ensure automatic and involuntary voiding whenever the bladder volume reached a critical level. It includes regions in the brainstem (pons) and midbrain.

Circuit 2 is concerned with the safety of the organism during voiding, a time of increased vulnerability. Lack of safety engenders an emotion, fear, and the derived concept anxiety. Correspondingly this circuit includes emotional (limbic) regions of the brain. It receives input from circuit 1 and spinal afferents, evaluates safety, and produces an output signal that prevents voiding unless the situation is deemed safe. It still makes no provision for voluntary control of voiding.

Circuit 3 takes input from the previous circuits and from spinal afferents to generate sensations of bladder filling (desire to void) and to evaluate the social propriety of voiding. It modifies the primary emotion, fear, derived from circuit 2, to generate secondary social emotions (Damasio 2003) such as fear of leakage (associated with urgency) and embarrassment or shame about incontinence. If voiding is judged acceptable then circuit 3 facilitates the lifting of voiding inhibition. Thus this circuit is involved in conscious sensation and voluntary control in the social setting. It includes cortical and cingulate parts of the brain. Comparison with recent publications in the cardiovascular field (Wager et al. 2009) suggests that circuit 3 should be divided into two parts, as in Fig. 1, which are the origins of pathways that become, respectively, the parasympathetic and sympathetic output controlling bladder and urethral behavior. If confirmed, this hypothesis would provide the missing link between brain activity and motor innervation at the peripheral level.

A considerable number of regions in these various circuits have been identified and some are shown in Fig. 1. Their interconnections, especially in circuit 3, are known to be numerous but are still largely unstudied in the context of bladder control. Some of the most important regions and their probable functions are as follows.

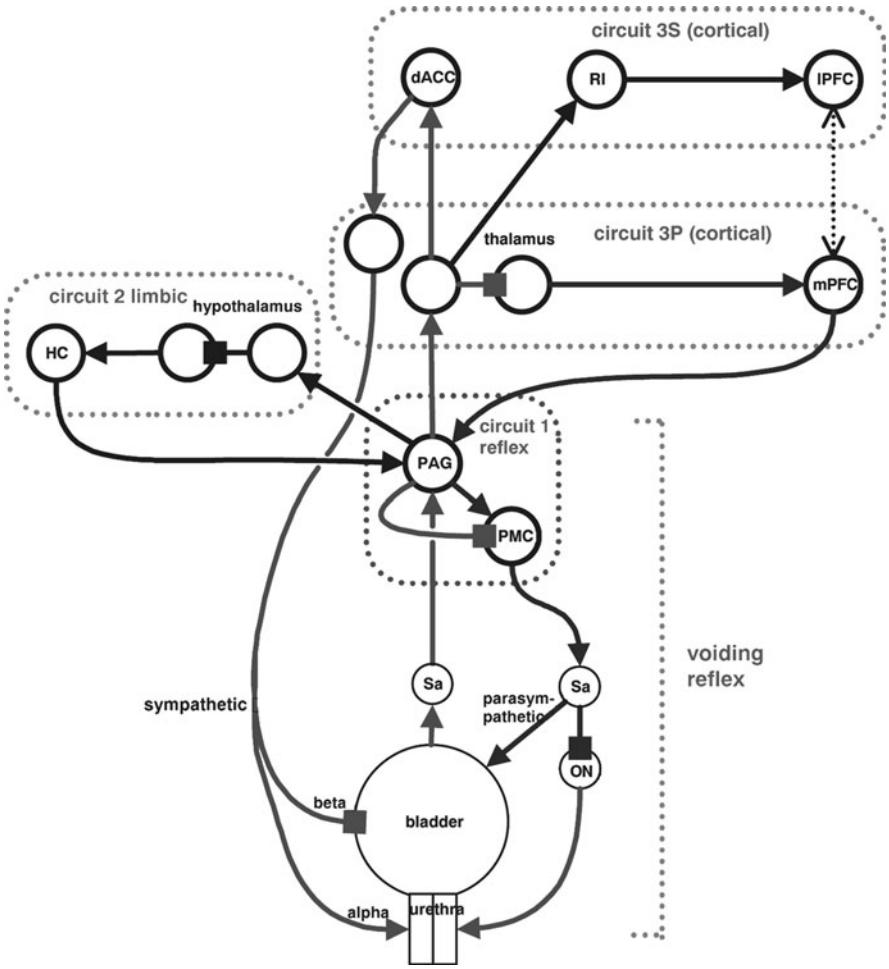

Fig. 1 Diagram suggesting how cerebral control may be exercised via neural circuits fulfilling different functions, showing postulated circuits 1 (mechanical, reflex), 2 (emotional, limbic), and 3 (voluntary, social, cortical). In each circuit, specific regions known to be concerned with bladder control are shown, together with a few of the many possible interconnections. Circuit 3 is divided into 2 branches, 3P and 3S. In circuit 3P deactivation of vmPFC (see Sect. 2) inhibits the voiding reflex at the PAG, thus suppressing voiding via an ultimately parasympathetic pathway. Activation of dACC in circuit 3S suppresses voiding by a different pathway, bypassing circuit 1, that acts via the sympathetic innervation to inhibit the detrusor and contract the urethral smooth muscle. *Arrow paths* excitatory neural link; *box paths* inhibitory neural link; *HC* hippocampus and parahippocampal complex; *mPFC* medial prefrontal cortex; *lPFC* lateral prefrontal cortex; *RI* (right) insula; *dACC* dorsal anterior cingulate cortex; *PMC* pontine micturition center; *PAG* periaqueductal gray; *Sa* parasympathetic regions of sacral cord

In circuit 1
- Midbrain periaqueductal gray (PAG), receives bladder afferents (Blok and Holstege 1998)
- Pontine micturition center (PMC; also known as Barrington's nucleus or M-region), on excitation sends a descending signal that promotes synergic voiding by relaxing the urethral sphincter and contracting the detrusor (Blok and Holstege 1998)

In circuit 2
- Hypothalamus (preoptic region), provides safe/unsafe signal controlling voiding (Blok and Holstege 1998)
- Parahippocampal complex, provides emotional basis of bladder sensations; the posterior parts of the cortex seem to function similarly

In circuit 3
- Insula, records bladder filling sensation (Craig 2002)
- Dorsal anterior cingulate cortex, dorsal (dACC), encodes motivation, e.g., urgency to void and concurrent motor output (Critchley et al. 2003) (see also Fig. 1)
- Medial prefrontal cortex, medial (mPFC), concerned with automatic reaction to bladder filling and decision to void in social context; produces output that inhibits voiding except when it is voluntarily desired
- Lateral prefrontal cortex, lateral (lPFC), concerned with voluntary regulation of emotion and bladder sensation

In addition, the thalamus seems to serve as a relay/interaction station between many regions.

When the bladder is filled, these regions become excited in a predictable way (Fig. 2). The PAG is activated, suggesting reception of increased bladder afferents. The thalamus lights up, consistent with its role in relaying these signals elsewhere in the brain. The insula is activated, especially on the right, consistent with increased bladder filling sensation (desire to void). The dACC is activated, relatively weakly in normal subjects (Fig. 2) but much more strongly in those with urge incontinence (see Sect. 5.2), consistent with a role as the neural correlate of urgency (a sensation not experienced by normal subjects). The somatosensory area (Brodmann Area 40) is activated bilaterally. The mPFC and pregenual anterior cingulate cortex, pregenual ACC are deactivated, weakly in normals but more strongly in urge incontinence, suggesting a unique role in controlling the bladder (see Fig. 1) and/or in the genesis of urge incontinence (Griffiths et al. 2009).

3 Potential Effects of Drugs on Central Control

Up to the present there has been little actual measurement of drug effects on central control of the bladder and none using the combination of fMRI, urodynamics, and repetitive infusion/withdrawal which has proved fruitful in

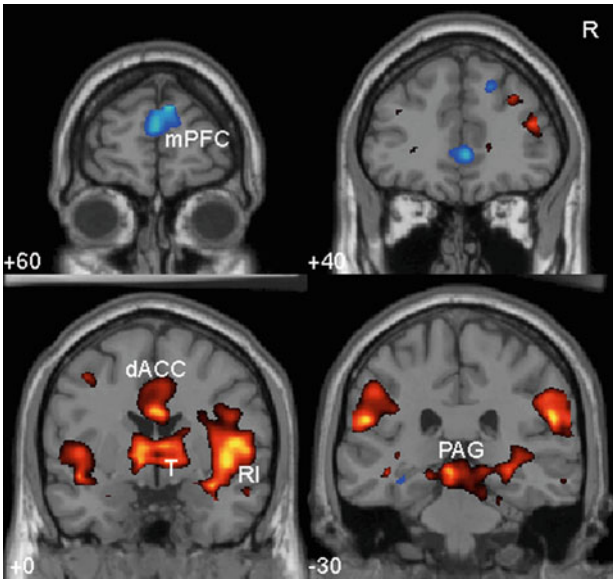

Fig. 2 For a group of ten normal females, regions responding to bladder filling when the bladder is near full and sensation is strong. *Red/yellow*: activation (probability threshold $P < 0.01$, uncorrected for multiple comparisons); *blue*: deactivation ($P < 0.05$, uncorrected). *PAG* periaqueductal gray; *T* thalamus; *RI* right insula; *dACC* dorsal anterior cingulate cortex; *mPFC* medial prefrontal cortex; *R* right. *Numbers* indicate Montreal Neurological Institute (MNI) *y*-coordinates

understanding how the control system operates (Griffiths et al. 2005; Mehnert et al. 2008; Kavia et al. 2010). Nevertheless there have been a few studies of the effects of nonpharmacological treatments on regional brain responses to bladder events (Blok et al. 2006; Kavia et al. 2010) (see also Sect. 5.5). In addition, there have been studies in situations that can be viewed as natural experiments mimicking alterations – especially alterations of bladder afferents – that might also occur with drugs used to treat overactive bladder symptoms, such as antimuscarinics or botulinum toxin. These studies offer guidance about what changes to expect in brain responses and how to interpret them. One factor that should be borne in mind when designing new studies of the effect of treatment is that successive brain scanning sessions will yield progressively different results whether or not therapy is performed, purely because of a changing memory trace in the brain related to repetition of sessions. Thus either an untreated control group should be included (note that, from a brain control point of view, placebo is not equivalent to no treatment), or else the changes in brain response that are significantly correlated with clinical success of therapy should be examined, since they show where therapy has an effect regardless of changing memory.

4 Effects of Changes in Afferent Signals

4.1 Aging

In old age, detrusor overactivity and urge incontinence become very common. Among those who maintain normal bladder function, however, sensation becomes weaker with increasing age (Pfisterer et al. 2006). Cortical – especially insula – activation in response to bladder filling also becomes weaker (Griffiths et al. 2007) as one would expect if insular activation represented the neural correlate of bladder filling sensation (desire to void). Possible reasons for diminished sensation in old age include diminished bladder afferents or impaired cerebral processing. Measurements made at smaller bladder volumes show a similar reduction in insular responses, which in this case is clearly related to weaker afferent signals. Thus peripheral changes could be a contributing factor to the age-associated decrease in response. Of course, supraspinal (or spinal) changes may also contribute.

At first sight it appears that pharmacotherapy with drugs that reduce bladder afferent signals – e.g., antimuscarinics or botulinum toxin – might have a similar effect. If so, one would expect to be able to monitor this effect by measuring the decreasing insular response to bladder filling during the course of treatment. However, the above observations were made in normal subjects. The situation may be different in patients with overactive bladder problems, as discussed in Sect. 5.

4.2 Fowler's Syndrome

Young women with Fowler's syndrome present with unexplained urinary retention, inability to void, and reduced bladder sensation. There is an underlying overactivity of the urethral sphincter, as shown by an abnormal sphincter EMG and elevated urethral pressure (DasGupta and Fowler 2004). Based on the bladder findings, one might expect reduced bladder afferents and reduced or absent brain responses to bladder filling. In fact, however, there are widespread negative responses, especially with near-empty bladder and without treatment (Fig. 3a) (Kavia et al. 2010). The interpretation of such negative responses is still widely debated (Shih et al. 2009), but we shall refer to them as deactivations. Deactivation during bladder filling occurs even in the PAG (see Fig. 1), suggesting that the normal bladder afferents are not reaching the brain at all. It occurs also in the insula, implying that sensation is suppressed by bladder filling instead of being increased. One possible interpretation is that in Fowler's syndrome, the overactive urethral sphincter responds to bladder filling with strong urethral afferents that inhibit bladder signals via a spinal pro-continence reflex, thus causing deactivation of many brain regions.

It would be interesting to compare these results with those obtained after injection of botulinum toxin in the bladder wall (a successful treatment for intractable

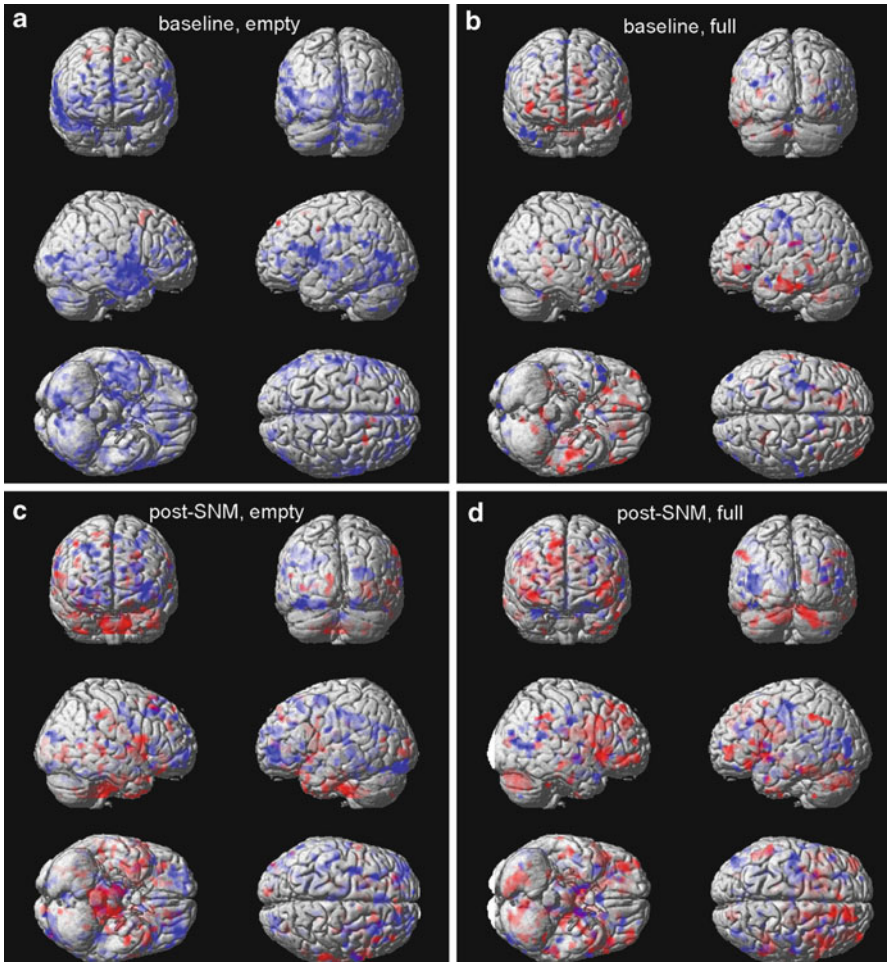

Fig. 3 Results for a group of six women with Fowler's syndrome with and without treatment by SNM. *Top panels*, **A** and **B**: Responses to bladder filling without treatment (with near-empty and with full bladder respectively). *Bottom panels*, **C** and **D**: responses after SNM. *Red* activation; *blue* deactivation; projected on surface of standard brain. Adapted from (Kavia et al. 2010) with permission

incontinence, urge), which presumably affects bladder afferents, and therefore should reduce responses to bladder filling without evoking abnormal deactivations.

4.3 Effect of Sacral Neuromodulation in Fowler's Syndrome

In women with Fowler's syndrome, deactivations are less pronounced if bladder afferents are strengthened, for example, by prefilling the bladder (Fig. 3b). Therapy with sacral neuromodulation (SNM), which presumably strengthens afferent signals

in a similar way, reenables voiding and improves sensation (DasGupta and Fowler 2004). Correspondingly, it reduces sacral neuromodulation and increases activation of cortical regions, as well as PAG (Kavia et al. 2010) (Fig. 3c, d). It appears therefore that treatment with SNM, although it does not cure the underlying urethral sphincter abnormality (DasGupta et al. 2005), is partially curative in the sense that it makes the brain responses more nearly normal. The effect of treatment could be monitored by determining the relative extent of deactivation vs. activation.

4.4 Ice Water (Bladder Cooling) Test

In addition to the Aδ fibers that convey afferent information about bladder filling to the CNS, there are C-fibers that transmit information about bladder pain and temperature. Instillation of cold fluid ("ice water") into the bladder has long been used to provoke and to test for neurogenic detrusor overactivity (Geirsson et al. 1993). Brain responses to this bladder cooling (presumably mediated by C-fibers) have been measured with PET (Matsuura et al. 2002). They are different from those evoked by bladder filling using room- or body-temperature liquid, mediated by Aδ fibers. In particular, there are cortical activations in regions different from those shown in Fig. 2 and no significant changes in brainstem or PAG. These results suggest that different afferent signals indeed activate different brain regions, and possibly that thermal and pain signals may bypass the PAG and pass directly to the thalamus (Mayer et al. 2006).

It would be interesting to study responses to bladder filling in patients with painful bladder syndrome; presumably regions similar to those activated by ice water would respond.

5 Overactive Bladder Syndrome: Urge Incontinence, Urgency, and Detrusor Overactivity

5.1 Overactive Bladder: Symptoms and Urodynamics

In patients with OAB symptoms, urodynamic testing frequently reveals involuntary bladder contractions (detrusor overactivity (DO)) during bladder filling. The contractions may be associated with the sensation of urgency (see Sect. 5.2) or with urine leakage (detrusor overactivity incontinence). Moreover cystometric capacity may be limited by the onset of DO, because further filling is hindered by strong bladder sensation or urine leakage. Together, these observations mimic the symptoms of the overactive bladder syndrome and therefore DO is often regarded as the cause of OAB. Although DO itself is usually viewed clinically as idiopathic, the

terms used to refer to it ("detrusor overactivity" and "overactive bladder") seem to imply an underlying bladder abnormality. This view has limited the direction of research and the choice of treatment targets for the past 50 years.

In practice, detrusor overactivity is frequently accompanied by involuntary urethral sphincter relaxation, or involuntary urethral relaxation may occur with urgency but without DO (McLennan et al. 2001). Thus the behavior of the urethra is probably just as important clinically as that of the bladder, a complication that should be borne in mind but is not emphasized in this chapter.

5.2 Urgency

As the bladder of a normal subject is filled, a sensation of desire to void is generated which fluctuates but gradually becomes stronger and more difficult to ignore (Abrams et al. 2002). It is associated with increased cortical response to bladder filling in the insula, especially on the right (see Figs. 1 and 2). The insula is believed to map afferents from the bladder and other visceral systems and thus to represent the degree of bladder filling and the strength of the desire to void (Craig 2002; Griffiths et al. 2007).

Patients with OAB symptoms, however, complain of an apparently different sensation called urgency. One of the goals of treatment is to reduce this bothersome sensation. Moreover urgency is a defining characteristic of incontinence, urge(ncy), "leakage of urine accompanied or preceded by urgency" (Abrams et al. 2002). Thus this sensation is a key symptom of the overactive bladder syndrome. In practice, however, it has been difficult to define, as is reflected in a change of definition: originally "a strong desire to void with fear of leakage [or pain]" (Abrams et al. 1988), but currently "a sudden compelling desire to void that is difficult to defer and may be accompanied or preceded by detrusor overactivity" (Abrams et al. 2002). Thus urgency seems to have several different aspects – a compelling nature, a sudden onset, and an emotional component (fear of leakage). Indeed it has been suggested that there are two types of urgency, one that is an intensification of the normal strong desire to void and another, not experienced by all patients, which is of a different nature (Blaivas et al. 2009). Clearly, it is difficult to use urgency, however important it may be, as an outcome measure for treatment of OAB if it is not unambiguously defined (Lowenstein et al. 2008). The above argument suggests that reliable clinical assessment of urgency from patient reports may remain elusive until the neural basis of the sensation – a particular state or states of brain activity – has been established. It is therefore worthwhile to find objective neural correlates of the various aspects of urgency and other sensations, which may help to identify and ultimately to more precisely define them. Such neural correlates may ultimately be viewed not so much as proxies for sensations such as urgency but their actual definitions, of which patient reports are an ambiguous reflection.

The probable neural correlate of the normal desire to void has been identified above: it is the increased activation of the insula provoked by bladder filling (Figs. 1 and 2). In female patients who suffer from urge incontinence and therefore experience urgency, however, brain responses to bladder filling are abnormal. In addition to insular activation there is a greatly enhanced response in dACC, especially if the bladder is well filled and sensation is strong (Griffiths et al. 2007) (Fig. 4). This abnormally strong reaction occurs in the absence of concurrent DO: i.e., prior to any actual loss of bladder control. dACC activation is believed to indicate an emotional response (e.g., fear of leakage) and to encode the motivation driven by this emotion (Critchley et al. 2003), as well as provide concomitant motor output that inhibits contraction of the bladder via its sympathetic innervation (see Fig. 1). Therefore increased dACC response appears to be the neural correlate of at least one aspect of urgency. At the same time, there is widespread activation of other parts of the cortex that probably indicates recruitment of other motor output (e.g., to the striated urethral sphincter) in an attempt to cope with imminent loss of control (Griffiths et al. 2005). Therefore, dACC activation can be used as a proxy for urgency or indeed as a defining characteristic of a type of urgency that includes the emotional aspect (fear) and its compelling nature but not sudden onset. Thus insular activation (by itself) seems to represent normal sensation, which has a relatively mild

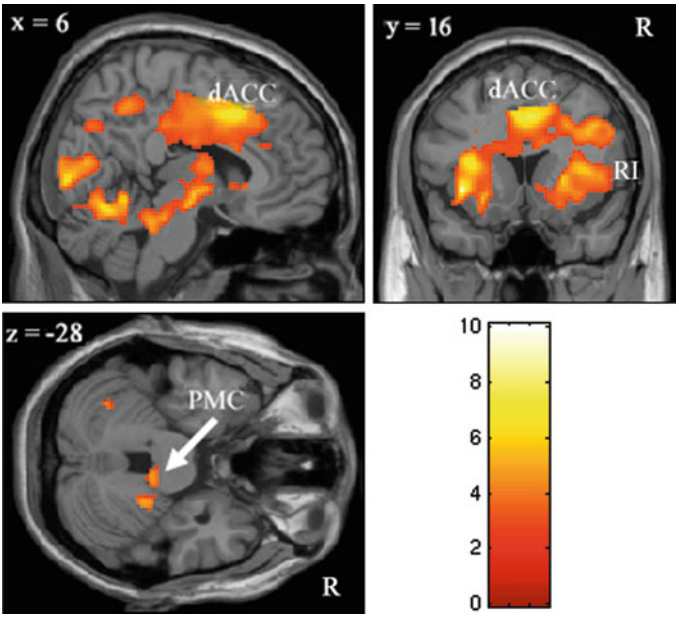

Fig. 4 Brain responses to bladder filling in ten urge-incontinent women (with strong sensation but in the absence of DO) ($P < 0.01$ uncorrected). Activation of dorsal anterior cingulate cortex (dACC) is markedly more pronounced than in normal subjects (Griffiths et al. 2007). *RI* right insula; *PMC* pontine micturition center; *R* right. *x*, *y*, *z* represent MNI coordinates of each section. Adapted from (Griffiths et al. 2007) with permission

emotional content (Fig. 1), while addition of dACC activation appears to correspond to an intensification of the normal desire to void with greater emotional and motivational content, perhaps corresponding to Blaivas's first type of urgency (Blaivas et al. 2009). The other aspect of urgency – sudden onset – may correspond to a different situation, the onset of an involuntary detrusor contraction (DO) or involuntary urethral relaxation, which signifies that loss of bladder control is not just threatened but is actually occurring. Interestingly, the pattern of brain activation when DO develops is apparently quite different from Fig. 4 (see Sect. 5.3).

Even at our present level of understanding, it is clear that measurement of dACC response to bladder filling offers a way to monitor therapeutically induced changes in one type or aspect of urgency. This can be done with fMRI, but in the future it may be possible to monitor dACC activation more simply with near-infrared spectroscopy, electroencephalography, or magnetoencephalography. Other parts of the brain (e.g., prefrontal cortex) may be even more accessible to such technologies and may offer opportunities for noninvasive monitoring of the effect of treatment.

Clearly, a therapy that is curative should reduce urgency and thus weaken dACC activation. On the other hand, if dACC activation is a coping reaction to threatened loss of bladder control, an alternative therapeutic mechanism would be to strengthen this reaction by reinforcing dACC activation. Thus measurement of dACC responses, and whether they are reduced or increased by successful therapy, may be used not only to monitor the progress of therapy, but also to shed light on the therapeutic mechanism.

5.3 Detrusor Overactivity

Detrusor overactivity (DO) (involuntary bladder contraction) is a urodynamic observation that by definition is identified by monitoring bladder (detrusor) pressures. Functional imaging in female subjects suggests that brain activation when DO develops is quite different from the abnormally strong dACC responses seen prior to development of DO and identified as urgency. There are several ways to perform brain-imaging of DO. One is to study brain responses to infusion and withdrawal during periods when DO (of whatever amplitude) is present; this is simple but unphysiological. Another is to study the association between bladder pressure (as it waxes and wanes with DO) and brain activity, regardless of infusion or withdrawal; this requires florid and prolonged DO which may not be easy to provoke in the scanner (see below).

Preliminary observations suggest that DO has a distinct neural signature involving reduced responses to bladder infusion in the prefrontal cortex (Griffiths et al. 2007), possibly suggesting abandonment of the attempt to control the bladder by neural circuit 3. A critical question is whether the PMC is activated during DO. If so, it would suggest that overactivity of the long-loop voiding reflex was responsible for idiopathic DO; if not, DO might be due to overactive spinal segmental

reflexes of the type that is usually present in infants but suppressed in continent adults (Fowler et al. 2008). So far the observations suggest that the PMC is not activated during DO, although more measurements are required to be certain of this rather surprising result.

DO is difficult to evoke in the scanner, presumably because circuit 2 assesses the scanner environment as "unsafe" and inhibits detrusor contraction. Thus no neural correlate of DO is likely to be useful clinically for monitoring the effects of therapy. The main importance of observations made during DO would be to show that there is a difference between the neural correlate of urgency viewed as the harbinger of imminent loss of control (i.e., just prior to onset of DO) and the neural activation pattern corresponding to actual DO. The present definition of urgency confuses the two and will probably need to be revised.

5.4 Effect of Sacral Neuromodulation in Urge Incontinence

Blok has made PET observations of the acute and chronic effects of SNM on patients with intractable urge incontinence (Blok et al. 2006). The acute effects, as one might expect if SNM stimulates afferent signals, are increased brain activity in a number of locations. However, after 6 or more months of SNM, there are notable reductions in cerebral activity evoked by bladder filling, especially in dACC at a location quite near to that shown in Fig. 2. Since dACC activation is believed to represent urgency and to be part of a coping reaction to threatened loss of control, this observation is consistent with a curative effect of SNM, leading to a gradual reduction of urgency as the brain learns that loss of control is less likely to occur.

An important observation is that acute and chronic effects of SNM are different, presumably because of the learning process mentioned above. The situation may be similar for drug therapy, which has an immediate effect on symptoms followed by a slower long-term improvement. These changes could be monitored by studying dACC response to bladder filling over the first few weeks and months of treatment.

5.5 Effect of Biofeedback Therapy on Urge Incontinence

Biofeedback therapy (BFB) is a recommended first-line conservative treatment for urge incontinence (Abrams et al. 2009). In older patients (women), it evokes relatively small changes in the properties of bladder and urethra, but seems to improve urge incontinence mainly by teaching the patients how better to control their bladder (unpublished observation). Thus it might be expected that fMRI should register the improvement in control. If BFB is curative, for example, we might expect abnormally pronounced dACC activation, representing urgency, to diminish. In fact recent preliminary results suggest that after 12 weeks of BFB,

there may be significant increases in response to bladder filling among those who are successfully treated, in dACC as well as right insula and other parts of the cortex. If this result is confirmed in larger numbers of subjects, dACC activation representing urgency may prove to be a coping reaction to impending loss of control, which is reinforced by biofeedback (BFB) training. Thus therapy may not necessarily cure the condition, but may enable the individual to better cope with it, presumably reflecting the feeling of improved control evoked by successful BFB.

Pharmacotherapy for urge incontinence presumably works differently. Indeed, recent unpublished results suggest that there may be phenotypes of urge UI that respond differently to drug or behavioral treatment. In a phenotype that responds well to antimuscarinics, the drug may be curative and so may reduce abnormal dACC activation (urgency). In contrast, in a phenotype that responds well to BFB, successful treatment may improve coping by an increase in dACC activation, as described above. In either case, it is clear that changes brought about by therapy, whether drug or behavioral, can be monitored by functional brain imaging, especially of the dACC.

6 Conclusion

Symptoms such as unexplained urinary retention or the overactive bladder syndrome, including urge(ncy) incontinence, reflect defects in the voluntary control of bladder and urethra. The control system is largely cerebral, and functional brain imaging shows that the brain does indeed respond differently to bladder filling in patients with such symptoms, as opposed to normal subjects. In principle, the abnormal responses may be causal or they may represent a coping reaction to threatened loss of bladder control. Observations of the effect of therapy (but unfortunately not pharmacological therapy) indicate that some types of successful treatment may strengthen the abnormal responses, suggesting that they increase the ability to cope with the problem; while other types may normalize the responses, suggesting that they are curative, reducing a causal abnormality. Thus monitoring of regional brain activity enables one not only to follow the effect of treatment, but also sheds light on the therapeutic mechanism.

In addition, enough is now known about brain control of the bladder to identify regions that are particularly important and their functions. Moreover, activation of these same regions is significantly correlated with the severity of urge incontinence as measured clinically (by bladder diary or pad test) (Tadic et al 2010). Consequently, brain activity in certain regions (e.g., insula or dACC) can be regarded as a proxy for clinically important concepts, such as desire to void or the abnormal sensation of urgency, which are expected to change when treatment is successful but are otherwise difficult to define or measure. Monitoring of brain responses to bladder filling in these regions offers an obvious way to test the effects of drugs.

We therefore stand on the threshold of a new era in testing of pharmacological and nonpharmacological therapies which will produce an avalanche of new data.

Inevitably, it will lead to new methods of treatment aimed not directly at the end organs but at their control, the seat of so many of our patients' symptoms.

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References

Abrams P, Blaivas JG, Stanton S, Andersen JT (1988) The standardisation of terminology of lower urinary tract function. Neurourol Urodyn 7:403–426

Abrams P, Cardozo L, Fall M, Griffiths D, Rosier P, Ulmsten U, van Kerrebroeck P, Victor A, Wein A (2002) The standardisation of terminology of lower urinary tract function: report from the Standardisation Sub-committee of the International Continence Society. Neurourol Urodyn 21:167–178

Abrams P, Cardozo L, Khoury S, Wein A (2009) Incontinence: 4th international consultation on incontinence, Editions 21. Health, Paris, France

Andrew J, Nathan PW (1964) Lesions of the anterior frontal lobes and disturbances of micturition and defaecation. Brain 87:233–262

Blaivas JG, Panagopoulos G, Weiss JP, Somaroo C (2009) Two types of urgency. Neurourol Urodyn 28:188–190

Blok BF (2002) Central pathways controlling micturition and urinary continence. Urology 59:13–17

Blok BF, Holstege G (1998) The central nervous system control of micturition in cats and humans. Behav Brain Res 92:119–125

Blok BFM, Groen J, Bosch JLHR, Veltman DJ, Lammersma AA (2006) Different brain effects during chronic and acute sacral modulation in urge incontinent patients with implanted neurostimulators. BJU Int 08:1238–1243

Craig AD (2002) How do you feel? Interoception: the sense of the physiological condition of the body. Nat Rev Neurosci 3:655–666

Critchley HD, Mathias CJ, Josephs O, O'Doherty J, Zanini S, Dewar BK, Cipolotti L, Shallice T, Dolan RJ (2003) Human cingulate cortex and autonomic control: converging neuroimaging and clinical evidence. Brain 126:2139–2152

Damasio AR (2003) Looking for Spinoza: joy, sorrow, and the feeling brain. Harcourt, Orlando, FL

DasGupta R, Fowler CJ (2004) Urodynamic study of women in urinary retention treated with sacral neuromodulation. J Urol 171:1161–1164

DasGupta R, Critchley HD, Dolan RJ, Fowler CJ (2005) Changes in brain activity following sacral neuromodulation for urinary retention. J Urol 174:2268–2272

Fowler CJ, Griffiths D, De Groat WC (2008) The neural control of micturition. Nat Rev Neurosci 9:453–466

Geirsson G, Fall M, Lindstrom S (1993) The ice-water test–a simple and valuable supplement to routine cystometry. BJU 71:681–685

Griffiths D, Tadic SD (2008) Bladder control, urgency, and urge incontinence: evidence from functional brain imaging. Neurourol Urodyn 27:466–474

Griffiths D, Derbyshire S, Stenger A, Resnick N (2005) Brain control of normal and overactive bladder. J Urol 174:1862–1867

Griffiths D, Tadic SD, Schaefer W, Resnick NM (2007) Cerebral control of the bladder in normal and urge-incontinent women. Neuroimage 37:1–7

Griffiths DJ, Tadic SD, Schaefer W, Resnick NM (2009) Cerebral control of the lower urinary tract: how age-related changes might predispose to urge incontinence. Neuroimage 47:981–986

Kavia RB, Dasgupta R, Fowler CJ (2005) Functional imaging and the central control of the bladder. J Comp Neurol 493:27–32

Kavia RB, DasGupta R, Critchley HD, Fowler CJ, Griffiths D (2010) An fMRI study of the effect of sacral neuromodulation on brain responses in women with Fowler's Syndrome. BJU Int 105:366–372

Kuhtz-Buschbeck JP, van der Horst C, Pott C, Wolff S, Nabavi A, Jansen O, Junemann KP (2005) Cortical representation of the urge to void: a functional magnetic resonance imaging study. J Urol 174:1477–1481

Lowenstein L, FitzGerald MP, Kenton K, Hatchett L, Durazo-Arvisu R, Mueller ER, Goldman K, Brubaker L (2008) Evaluation of urgency in women, with a validated urgency, severity and impact questionnaire (USIQ). Int Urogynecol J 20:301–307

Matsuura S, Kakizaki H, Mitsui T, Shiga T, Tamaki N, Koyanagi T (2002) Human brain region response to distention or cold stimulation of the bladder: a positron emission tomography study. J Urol 168:2035–2039

Mayer EA, Naliboff BD, Craig AD (2006) Neuroimaging of the brain-gut axis: from basic understanding to treatment of functional GI disorders. Gastroenterology 131:1925–1942

McLennan MT, Melick C, Bent AE (2001) Urethral instability: clinical and urodynamic characteristics. Neurourol Urodyn 20:653–660

Mehnert U, Boy S, Svensson J, Michels L, Reitz A, Candia V, Kleiser R, Kollias S, Schurch B (2008) Brain activation in response to bladder filling and simultaneous stimulation of the dorsal clitoral nerve – an fMRI study in healthy women. Neuroimage 41:682–689

Pfisterer M, Griffiths D, Schaefer W, Resnick N (2006) The effect of age on lower urinary tract function: a study in women. J Am Geriatr Soc 54:405–412

Shih YI, Chen CV, Shyu B, Lin Z, Chiang Y, Jaw F, Chen Y, Chang C (2009) A new scenario for negative functional magnetic resonance signals: endogenous neurotransmission. J Neurosci 29:3036–3044

Tadic SD, Griffiths D, Schaefer W, Resnick NM (2008) Abnormal connections in the supraspinal bladder control network in women with urge urinary incontinence. Neuroimage 39:1647–1653

Tadic SD, Griffiths D, Schaefer W, Cheng CI, Resnick NM (2010) Brain activity as measured by functional magnetic resonance imaging (fMRI) is related to patient reported severity of urge urinary incontinence. J Urol 183:221–228

Torrens M, Feneley RCL (1982) Rehabilitation and management of the neuropathic bladder. In: Illis LS, Sedgwick EM, Glanville HJ (eds) Rehabilitation of the neurological patient. Blackwell Scientific, Oxford

Wager TD, van Ast VA, Hughes BL, Davidson ML, Lindquist MA, Ochsner KN (2009) Brain mediators of cardiovascular responses to social threat, part II: Prefrontal-subcortical pathways and relationship with anxiety. Neuroimage 47:836–851

Neuroanatomy of the Lower Urinary Tract

Jonathan M. Beckel and Gert Holstege

Contents

Abstract The lower urinary tract (LUT), which consists of the urinary bladder and its outlet, the urethra, is responsible for the storage and periodic elimination of bodily waste in the form of urine. The LUT is controlled by a complex set of peripheral autonomic and somatic nerves, which in turn are controlled through neural pathways in the spinal cord and brain. This influence of the central nervous system allows for the conscious control of the bladder, allowing the individual to choose an appropriate place to urinate. Defects in the CNS pathways that control the LUT can lead to incontinence, an embarrassing condition that affects over 200 million people worldwide. As a first step in understanding the neural control of the bladder, we will discuss the neuroanatomy of the LUT, focusing first on the peripheral neural pathways, including the sensory pathways that transmit information on bladder filling and the motoneurons that control LUT muscle contractility. We will also discuss the organization of the central pathways in the spinal cord and

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brainstem that are responsible for coordinating bladder activity, promoting continuous storage of urine except for a few short minutes per day when micturition takes place. To conclude, we will discuss current studies underway that aim to elucidate the higher areas of the brain that control the voluntary nature of micturition in higher organisms.

Keywords Autonomic nervous system · Lower urinary tract · Periaqueductal gray · Pontine micturition center · Somatic nervous system

1 Introduction

The lower urinary tract (LUT), which is commonly referred to as simply the bladder although it also includes the urethra, is responsible for the storage and periodic elimination of waste products in the form of urine. Similar to other visceral organs, the LUT is controlled by the autonomic nervous system. However, proper function of the LUT also relies on neural pathways located in the brain, brainstem, and spinal cord, which sets it apart from other visceral organs that generally maintain some function independent of extrinsic neural input. This control of the LUT by the central nervous system allows the individual to choose the appropriate time and place to urinate.

In the present chapter, the central and peripheral neural pathways controlling micturition will be discussed, paying particular attention to the role of the brain in the voluntary control of the LUT. We will first outline the anatomy of the bladder as well as the neuroanatomy of the peripheral nerve fibers and central pathways controlling the LUT, and then describe how these pathways are organized to form the neural circuits responsible for both storage and voiding. We will also discuss the centers in the brain that control the "switch" between storage and voiding, giving a person voluntary control of micturition, allowing them to urinate only when it is appropriate.

2 The Anatomy and Innervation of the Lower Urinary Tract

2.1 The Gross Anatomy of the Lower Urinary Tract

The LUT consists of two functional structures (1) a reservoir responsible for holding urine, called the bladder and (2) an outlet to release urine, consisting of the bladder neck, urethra, and external urethral sphincter (Brooks 2007). The bladder is a sac-like organ with a wall consisting of three layers of smooth muscle, called the detrusor. Lining the lumen of the bladder is a layer of transitional epithelium, called the urothelium, which forms an almost impermeable barrier, allowing the bladder to hold urine. Between the detrusor and the urothelium lies the

submucosa and the lamina propria; layers of connective tissue that also house capillaries, afferent nerve terminals, lymph vessels, and immune cells.

Urine is released from the bladder through an outlet, a tube of smooth muscle called the urethra (Patel and Chapple 2008). The urethra is surrounded by the striated muscles of the pelvic floor. That part of the pelvic floor which lies immediately around the urethra is known as the external urethral sphincter. The urethra joins the bladder at its neck with a circular ring of smooth muscle called the internal urethral sphincter. During urine storage, both the internal and external urethral sphincter muscles are contracted, closing the bladder outlet thereby holding urine in the bladder. For a more detailed description of the structure and function of the bladder and urethra, see Chapter 7.

2.2 Peripheral Innervation of the Lower Urinary Tract

The LUT is innervated by three sets of nerves: (1) parasympathetic sacral pathways that travel the pelvic nerve, (2) sympathetic thoracolumbar pathways that travel the hypogastric nerve, and (3) sacral somatic motoneurons that travel the pudendal nerve (Fowler et al. 2008). These pathways work in concert in a reciprocal fashion to control the two functions of the LUT; storage being mediated through the sympathetic and somatic motoneurons, while voiding is induced via the parasympathetic pathways. Another important role is played by sensory afferent nerves, which carry information on bladder filling to the spinal cord.

2.2.1 Sympathetic Pathways

Sympathetic preganglionic motoneurons that innervate the bladder are located in the thoracolumbar levels of the spinal cord (T_{11}–L_2) of humans (Fowler et al. 2008; Vera and Nadelhaft 1992). These neurons exit the spinal cord and pass along the paravertebral sympathetic chain to the inferior splanchnic nerve and the inferior mesenteric ganglion (Fig. 1). Although some fibers synapse on postganglionic neurons in the inferior mesenteric ganglion, most preganglionic fibers continue along the hypogastric nerve to terminate on postganglionic neurons in the pelvic ganglion of the major pelvic plexus. From there postsynaptic fibers innervate the bladder and urethra. Additionally, both the pelvic and pudendal nerves, at least in the rat and cat, contain postganglionic sympathetic fibers from the paravertebral chain ganglia (Kuo et al. 1984). The sympathetic innervation of the bladder is divided into two distinct groups, those that innervate the main portion of the bladder and those that innervate the bladder neck and the urethra (Vera and Nadelhaft 1992). Of these two areas, the bladder neck and sphincter receive the strongest innervation. The postganglionic sympathetic fibers release norepinephrine to activate α-adrenergic receptors which constrict the smooth

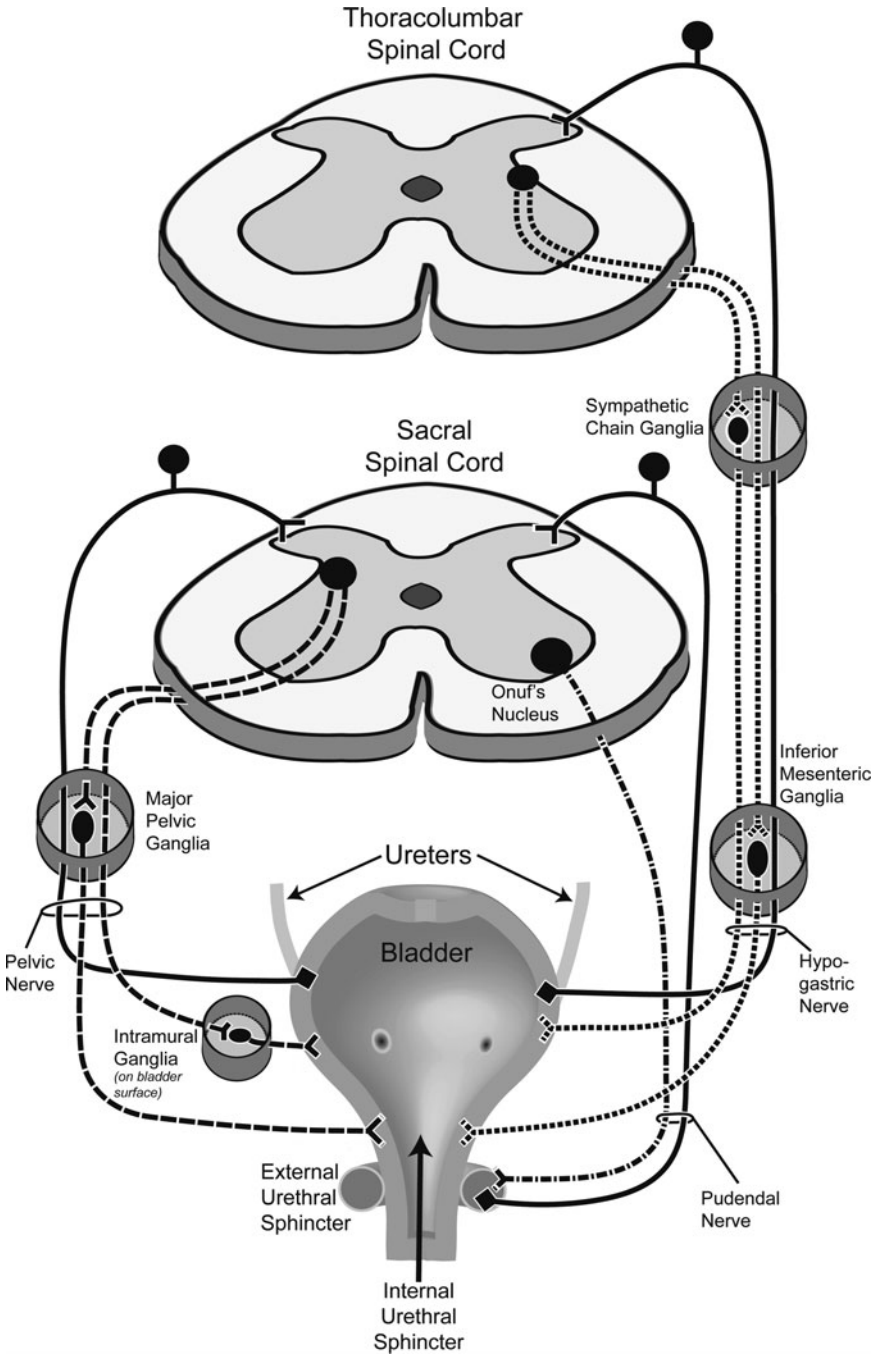

Fig. 1 Peripheral innervation of the LUT. Diagram depicting the peripheral innervation of the lower urinary tract. *Solid lines* afferent nerve fibers; *dashed lines* parasympathetic pathways; *Dotted lines* sympathetic pathways; *dashed with dots* somatic motoneurons

muscle of the bladder neck and internal urethral sphincter. β-adrenergic receptors on the detrusor also receive sympathetic innervation, and stimulation of these receptors causes relaxation of the detrusor smooth muscle (Rohner and Hannigan 1980; Todd and Mack 1969).

2.2.2 Parasympathetic Pathways

Preganglionic parasympathetic motoneurons originate in the sacral spinal cord (S_2–S_4 in humans, S_2–S_3 in the cat), although in some species, such as the rodent, they may also originate in the caudal lumbar spinal cord (L_6–S_2) (Andersson and Arner 2004; Petras and Cummings 1978). From the spinal cord, preganglionic motoneurons send fibers along the pelvic nerve to release acetylcholine through synapses on postganglionic neurons in the pelvic plexus or continue on to intramural ganglia located in the wall of the bladder (Fig. 1) (de Groat and Saum 1976). Postganglionic parasympathetic fibers release acetylcholine and ATP on smooth muscle fibers in the bladder by activating muscarinic (M_3) and purinergic (P2X) receptors, respectively. Parasympathetic fibers of the pelvic nerve also innervate the smooth muscle of the internal urethral sphincter where they release nitric oxide (NO), which has an inhibitory effect (Bennett et al. 1995).

2.2.3 Somatic Pathways

The innervation of the striated muscle of the external urethral sphincter originates in a specific region of the lateral ventral horn of the sacral spinal cord, generally centered in the human at the S_2 segment, but also in the caudal end of the S_1 segment and the middle of S_3. In the cat, these neurons are present from the caudal half of S_1 and the rostral half of S_2 (Vanderhorst and Holstege 1997). This region, which is concentrated in lamina IX, has been termed Onuf's Nucleus (ON), in honor of Bronislaw Onufrowicz, a Polish scientist who discovered the nucleus in 1899 (Mannen 2000; Pullen et al. 1997). Motoneurons in ON send axons through the pudendal nerve to the pelvic floor muscles, including the external anal and external urethral sphincter (Fowler et al. 2008; Nyo 1969; Patel and Chapple 2008; Vanderhorst and Holstege 1997). These neurons are cholinergic, releasing acetylcholine to activate postjunctional nicotinic receptors on the sphincter striated muscle fibers.

There has been some debate as to the nature of the motoneurons of ON (Mannen 2000; Mannen et al. 1982). They are generally thought of as somatic motoneurons, as they innervate striated muscle that can be voluntarily controlled and share similar morphological traits to motoneurons, such as the presence of large Nissl granules and the absence of a dense core of vesicles typically seen in preganglionic motoneurons. However, ON motoneurons also have autonomic properties. For example, they receive afferents from the paraventricular nucleus of the hypothalamus, as all autonomic, but not somatic, motoneurons do (Holstege 1987). Furthermore, there

is no evidence that ON motoneurons receive propriospinal afferents from interneurons in the adjacent intermediate zone, as all other somatic motoneurons do (Holstege and Tan 1987). Additionally, in patients that suffer from Shy–Drager syndrome, a disease that destroys autonomic motoneurons, ON motoneurons are also obliterated, leading to urinary incontinence. In contrast, ON motoneurons have been found to be undamaged in patients that suffer from amyotrophic lateral sclerosis (ALS), a condition that destroys somatic motoneurons that control striated muscle fibers. ALS patients rarely suffer from urinary incontinence (Mannen et al. 1982). While these results may bring into question the nature of the neurons populating ON, they stress the importance of ON in the control of the external urethral sphincter; when these neurons degenerate, urinary incontinence occurs.

2.2.4 Afferent Sensory Pathways

Sensory afferent nerves innervating the bladder and urethra travel along all three of the previously discussed nerves (pelvic, pudendal, and hypogastric). Of these three nerves, pelvic afferents play the most significant role in LUT function (Bahns et al. 1986; Janig and McLachlan 1986). Bladder afferent nerves are comprised of two types, myelinated A–δ fibers and unmyelinated C-fibers. Generally, these two subtypes of afferent nerves can be differentiated by the stimulus that activates them. A–δ fibers respond to stretch of the bladder wall as the bladder fills with urine and to bladder contraction when voiding occurs (Janig and Morrison 1986). The threshold pressure to activate these fibers is relatively low; approximately 5–15 mm Hg (Rong et al. 2002), which corresponds to the pressure in the bladder when most humans first report sensations of bladder filling. C-fibers, on the other hand, are generally labeled as silent, as they have very high thresholds for firing and are not activated by physiologically relevant bladder pressures. C-fibers respond to nociceptive stimulation by chemicals, such as capsaicin or menthol, or in response to inflammation (Habler et al. 1990). Additionally, chemical stimulation can sensitize C-fibers, allowing them to become mechano-sensitive (Cheng et al. 1993; Chuang et al. 2001). These findings suggest that the two types of afferent nerves perform separate roles in transmitting sensory information from the bladder; A–δ fibers transmit information on bladder filling to the central nervous system, while C-fibers, in case of pathological conditions in the bladder, signal nociceptive information, leading to feelings of pain in the bladder or bladder sphincter.

A number of studies have been conducted on dorsal root ganglion cells (DRGs) that pair axonal tracing with patch clamp techniques to classify the populations of afferent nerves innervating the LUT. These studies estimate that approximately 70% of the afferents innervating the LUT are of the C-fiber type, based on their response to capsaicin (Yoshimura et al. 2003). A number of transmitters and chemical mediators have been indentified that are capable of either modulating the threshold of firing of afferent nerves or producing an action potential themselves. These substances include ATP (Dang et al. 2005), acetylcholine (Genzen

et al. 2001), prostaglandins (England et al. 1996), tachykinins (Hong-Sheng and Zhi-Qi 1998), neurotrophic factors (Carroll et al. 1998), and inflammatory mediators (Dang et al. 2008; Wang et al. 2007). These substances can be released from a number of sources in the bladder wall, such as urothelial cells, inflammatory cells, smooth muscle cells, and endothelial cells in blood vessels (Apodaca et al. 2007). These findings have led to the hypothesis that the sensory limb of the LUT is not limited to only afferent nerves, but instead includes a number of cells types, such as urothelial cells and myofibroblasts contained in the suburothelium, that act as a sensory web, releasing chemical mediators to influence afferent excitability. For more information on how afferent nerves are influenced by other cell types in the LUT, see Chapters 9–11.

2.3 Central Circuits Controlling the Lower Urinary Tract

Neuronal tracing studies with retrograde tracers such as wheat germ agglutinin–horseradish peroxidase (WGA–HRP), cholera toxin b, or pseudorabies virus (PRV) have done much to elucidate the organization of micturition-related neurons in the central nervous system. These agents, when injected into the bladder or urethra, travel in a retrograde fashion through the peripheral nerves that relay information from the bladder into the spinal cord (Oztas 2003). In some cases, these tracers can also travel across synapses, allowing visualization of subsequent neurons in the micturition pathway. Given enough time, it is possible for these tracers to travel into the brain, labeling the centers in the brain that may be important in micturition. A problem, however, is that PRV not only crosses synapses but also reach the neurophil and hence reach nearby axons of cells not involved in micturition control. An example is retrograde labeling of rubrospinal neurons in the mesencephalon which terminate on premotor interneurons near the sympathetic or parasympathetic motoneurons of the bladder, but do not terminate on them (Holstege and Kuypers 1982). Nevertheless, these tracers have given researchers a look into the organization of the neural pathways controlling bladder function in the central nervous system. These experiments, however, only give an idea as to the layout of the neurons in the central nervous system that may play a role in micturition, they do not tell us what that role might be (i.e., excitatory or inhibitory). Therefore, further experiments are needed to elucidate those roles. Generally, data on the role of particular areas of the spinal cord and brain are obtained through electrophysiological experiments in animals, where neurons are electrically (or chemically) stimulated in order to determine their effect on micturition. It is also possible to glean information on the role of a particular group of neurons in humans through functional imaging, such as functional magnetic resonance imaging (fMRI) and PET scanning or through examination of patients that develop abnormal bladder function following discernable injuries to the central nervous system (i.e., a lesion to a particular area of the brain).

2.3.1 Organization of the Spinal Pathways Controlling the LUT

Using retrograde tracing techniques, it is possible to elucidate the location in the spinal cord of the motoneurons innervating the bladder. For example, it has been shown that the parasympathetic preganglionic neurons that send axons along the pelvic nerve are present in the intermediolateral nucleus of the lumbosacral spinal cord (Morgan et al. 1993) (Fig. 2). These preganglionic neurons send dendrites into a cell group ventrolateral to the dorsal horn, as well as medially into the

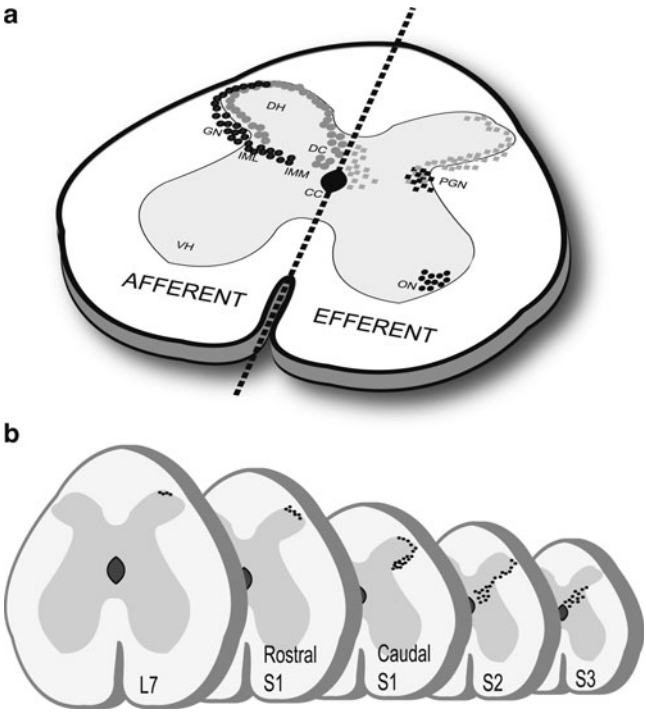

Fig. 2 Organization of neurons in the spinal cord. (**a**) Artist's rendition of the organization of the afferent, efferent, and interneurons in the sacral spinal cord are important for micturition. Spinal innervation is bilateral; however, we have lateralized the depiction of afferent and efferent pathways in order to better visualize their distribution. *On the left*, the locations of afferent nerve terminals are depicted. *Black circles* denote the A–δ fiber terminals, while *grey circles* denote C fiber terminals. *On the right side*, cell bodies of interneurons and efferent motoneurons are depicted. *Black squares*: preganglionic parasympathetic motoneurons; *grey squares*: spinal interneurons; *Black triangles*: somatic motoneurons that control the urethra. Abbreviations: *DH* dorsal horn; *VH* ventral horn; *GN* Gert's Nucleus; *IML* Intramediolateral cell group; *IMM* Intramediomedial cell group; *CC* central canal; *DC* Dorsal commissure; *PGN* preganglionic neurons; *ON* Onuf's Nucleus. (**b**) Diagram depicting the location of afferent nerve terminals in Gert's Nucleus of the cat. Note the change in distribution from the apex of the dorsal horn through the lateral dorsal horn, the intermediolateral, and finally the intermediomedial cells groups as the spinal section progress caudally

intermediomedial cell group and the dorsal commissure (Morgan et al. 1991). Sympathetic preganglionic motoneurons are found in a similar arrangement, originating in the intermediolateral nucleus of the caudal thoracic and rostral lumbar levels of the spinal cord (T11–L2) (Vizzard et al. 2000). Retrograde tracing studies have also confirmed the presence of somatic motoneurons that innervate the external urethral sphincter in the ventrolateral portion of ON (Nadelhaft et al. 1992; Sasaki 1994).

Terminals in the spinal cord of afferent nerve fibers from the LUT enter the spinal cord in the dorsal horn, terminating in Rexed's lamina I and along the lateral edge of the dorsal horn and in the intermediate zone (laminae V–VII) (Morgan et al. 1981; Thor et al. 1989). Additionally, LUT afferent nerves terminate in the central portion of the spinal cord, in the intermediomedial cell group, and in the dorsal commissure including lamina X, dorsal to the central canal. The two types of afferent nerves of the LUT have distinct termination patterns in the spinal cord (Birder and de Groat 1993; Vizzard 2000; Yamada et al. 2007). As shown in Fig. 2, A–δ fibers terminate in a cell group in the lateral funiculus, just lateral to the dorsal horn, extending caudally into the intermediate zone. The cells of this cell group, in turn, send long fibers to the central part of the midbrain periaqueductal gray (PAG) bilaterally, but mainly contralaterally (Klop et al. 2005; Vanderhorst et al. 1996). The cell group also receives dendrites from parasympathetic preganglionic motoneurons located in the intermediolateral nucleus (Holstege and Griffiths 1990) as well as descending projections from the pontine micturition center (PMC) (Holstege et al. 1979). Due to the number of pathways that converge here, this area is thought to play an important role in the control of micturition and has been termed "Gert's nucleus" after Gert Holstege, whose lab was the first to identify this cell group anatomically (Vanderhorst et al. 1996). The nociceptive C-fiber afferents, on the other hand, terminate in the superficial dorsal horn, in an area starting in the dorsal commissure and extending laterally through lamina I and superficial part of lamina II into lamina V. From here nociceptive information is carried through ascending interneurons to the thalamus, where nociceptive stimuli are processed (Nishii et al. 2008).

Given these neuroanatomical studies, it is clear that afferent nerves innervating the LUT terminate in close proximity to the preganglionic motoneurons that control LUT activity. Additional tracing studies have also demonstrated the presence of interneurons in the dorsal horn, dorsal commissure, and intermediolateral nucleus of the spinal cord that bridge the gap between afferent nerves and the preganglionic motoneurons (Nadelhaft et al. 1992; Vizzard et al. 1995). For example, sympathetic preganglionic motoneurons receive a strong innervation from enkephalin-positive interneurons (Llewellyn-Smith et al. 2005). The origin of these interneurons is not known; however, spinal cord transection does not alter the prevalence of enkephalin terminals in the sympathetic nucleus, suggesting that the source is spinal and not supraspinal. An electrophysiological study in the cat (Araki and De Groat 1996) has demonstrated groups of interneurons that synapse on sacral parasympathetic neurons, such as a group located in the intermediomedial nucleus. Another group of interneurons, which also have excitatory effects on parasympathetic preganglionic neurons, is located just

dorsal to the intermediolateral nucleus. These interneurons may play a role during development in the cat, when micturition occurs in response to stimulation of perineal afferents (i.e., when the mother cat licks the perineum of the neonate).

Interneurons present in the intermediomedial cell group of the lumbosacral spinal cord are thought to coordinate the urinary bladder with other visceral functions such as defecation, as it has been shown that afferents from both the distal colon and the urinary bladder converge on spinal interneurons in the intermediomedial cell group of the sacral spinal cord (McMahon and Morrison 1982; Qin and Foreman 2004; Vizzard et al. 2000). There is also some convergence between the pathways controlling bladder function and those controlling the sexual organs, such as the penis (de Groat and Booth 1993).

Very little is currently known about the spinal interneurons that project to the motoneurons present in ON. It is known that these motoneurons receive afferent projections from segmental afferents, not directly but through interneurons, which most likely consist of a polysynaptic pathway that includes at least two interneurons based on the latency of evoked action potentials recorded from the urethral motoneurons following electrical stimulation of the contralateral pudendal nerve (McMahon et al. 1982). These evoked action potentials can be greatly reduced by increasing intravesical pressure, suggesting that they are tonically active during storage and are only turned off during micturition to allow proper voiding. There may also be inhibitory interneurons controlling the activity of ON motoneurons (Blok et al. 1997a, b). The most direct evidence for these inhibitory neurons comes from research demonstrating a group of GABAergic neurons present in the intermediomedial cell group of the sacral spinal cord (Blok et al. 1997a). Electrical or chemical stimulation of these neurons in the cat resulted in significant decreases of urethral pressure. Additionally, there exist glycinergic neurons that inhibit ON motoneurons, as the glycine receptor antagonist strychnine prevents sphincter relaxation during micturition (Shefchyk et al. 1998). These glycinergic neurons are also located in the intermediomedial cell group and may be identical to the GABAergic neurons described previously, as studies have indicated a 50% colocalization of GABAergic and glycinergic staining in the intermediolateral cell group (Sie et al. 2001).

2.3.2 Supraspinal Control of Voiding

The first description of an area of the brain that played a role in micturition was in 1925 by Barrington, who characterized a region of the pons in the cat that influenced urinary bladder function (Barrington 1925). Electrical or chemical stimulation of this area, located in the dorsolateral pontine tegmentum, ventral to the mesencephalic trigeminal tract and locus coeruleus, results in a decrease in urethral pressure, followed immediately by a contraction of the detrusor, mimicking micturition (Holstege et al. 1986; Sugaya et al. 1987). Bilateral ablation of this area through electrically induced lesions results in the loss of bladder control and leads

to complete urinary retention. Given its ability to cause micturition, it has been termed the pontine micturition center (PMC) although it is also referred to as "Barrington's nucleus" in honor of its original investigator or the "M-region" for its medial location in the pons (Griffiths et al. 1990; Holstege et al. 1986). While PMC stimulation studies have been performed mostly in the cat, functional studies in the human using fMRI or PET have also demonstrated an area in the dorsal pontine tegmentum that is activated during voiding, suggesting that the PMC is also located in this area of the brainstem in humans as well (Blok et al. 1997b, 1998b).

In the cat, there also exists an area of the pons more lateral to the PMC that has an excitatory effect on the LUT. This area is known as the "L-region" because of its lateral position in the pons. The L-region is also called the "pontine storage center" or "continence center," as stimulation of this region promotes urine storage. The L-region is directly connected to the motoneurons of ON by a monosynaptic, descending pathway (Holstege and Kuypers 1982; Holstege et al. 1979, 1986); hence its effects are mediated through the control of the urethral sphincter and other parts of the pelvic floor. While the L-region has an effect on urethral pressure in the cat, its importance in the human is questionable. Transection of the spinal cord, which damages the descending pathways from the L-region, does not affect the bladder's ability to store urine.

It has been shown using tracing experiments that the PMC is connected to the parasympathetic preganglionic motoneurons controlling bladder contraction through long, descending, monosynaptic pathways (Blok and Holstege 1997). This descending pathway is likely to use glutamate as the major excitatory transmitter (Matsumoto et al. 1995). In addition to this excitatory pathway, descending neurons from the PMC also synapse on inhibitory, GABAergic, and glycinergic interneurons located in the intermediomedial cell group of the sacral spinal cord (Blok et al. 1997a). These interneurons synapse on and inhibit ON motoneurons, causing a relaxation of the urethral sphincter. Because it can activate voiding, the PMC was originally thought to be the main "switch" in the brain controlling micturition, receiving afferent signals from the brain and activating voiding when the bladder was full. However, it has been shown that the PMC only receives afferent input from the LUT in the rat (Blok and Holstege 2000); in higher organisms such as the cat or human, the PMC receives no direct input from bladder afferent nerves (Blok et al. 1995). Therefore, while the PMC plays a role as the ultimate efferent nucleus of the micturition pathway, another portion of the brain must receive sensory input from the bladder and determine when to commence voiding through activation of the PMC. In simple terms, the PMC is the switch that turns on voiding, but another part of the brain decides when to throw that switch.

Holstege's group was the first to demonstrate that the sensory control center in the micturition pathway is a region of the brain known as the periaqueductal gray (PAG) (Blok et al. 1995). The PAG consists of the gray matter encircling the cerebral aqueduct in the mesencephalon, an area of the brain found to be important in controlling a number of somatic and visceral responses to stress or fear in the cat, such as freezing, jumping, tachycardia, and increased blood pressure

(Bandler et al. 1991; Behbehani 1995). It has also been shown to be important in the nociception, and chemical or electrical stimulation of the PAG can lead to analgesia. Furthermore, the PAG has been shown to control respiration and sound production in animals and humans (Holstege 1989; Subramanian et al. 2008). Therefore, the PAG is a brain center that simultaneously receives sensory information as well as modulates premotor interneurons that control a variety of organs, making it an ideal candidate to play a role in micturition.

Structurally, the PAG appears to be divided into two subsections. For example, it has been shown in the cat that ascending fibers from GN terminate in the central area of the PAG, while most PAG neurons that project to the PMC are located more laterally (Blok and Holstege 1994, 2000; Blok et al. 1995). This suggests that the PAG possesses the ability to process the sensory information coming from the bladder and "decides" when to activate the PMC and commence voiding. It has been hypothesized that this "control center" is the basis for the voluntary control of the LUT, as it receives projections from many higher brain centers (Holstege 2005). These brain centers have been implicated in such aspects as introspective awareness and appropriate social behavior. Thus the PAG may represent the major control center which combines bladder filling information with higher levels of conscious thought to determine if voiding is appropriate.

2.4 Higher Brain Centers Involved in Voluntary Voiding

Electrophysiological and anatomical studies in the cat have implicated a number of areas of the brain, such as the medial frontal cortex and the preoptic area, paraventricular nucleus, and periventricular nucleus of the hypothalamus (Gjone 1966; Holstege 1987; Holstege et al. 1986) (Fig. 3). Functional imaging of the human brain during storage or voiding has added a few more areas to those found in animal studies, such as the insula, thalamus, and anterior cingulate gyrus (Kavia et al. 2005). In some cases, these connections have been verified through injection of an anterograde tracer into the brain to identify their terminals in the spinal cord. For example, it has been shown that the paraventricular hypothalamic nucleus sends fibers to the sacral parasympathetic nucleus, as well as the motoneurons of ON (Holstege 1987; Holstege and Mouton 2003).

It is interesting to note that the majority of the higher brain centers labeled following injections of retrograde tracers into the bladder are connected through projections to the PAG, but not to the PMC (Kuipers et al. 2006). This further supports the proposed role of the PAG as the major control center for micturition, acting as the "switch" between storage and voiding depending on signals from the brain. One exception to this would be the hypothalamic preoptic area, which sends its projections directly to the PMC (Ding et al. 1999; Holstege 1987). The medial preoptic area has been shown to be important for sexual function, specifically in males where stimulation of this area in animals elicits penile erection (Giuliano et al. 1996). Thus, the medial preoptic nucleus may be responsible for inhibiting

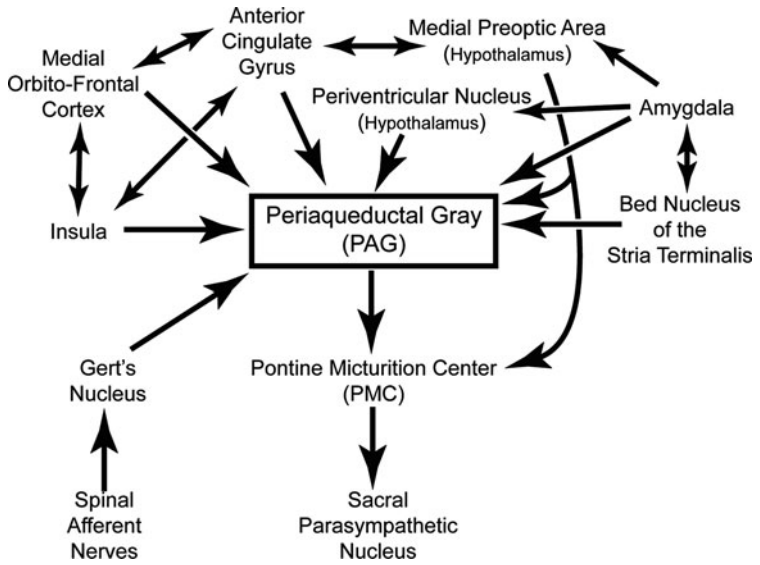

Fig. 3 Brain centers involved in micturition. Diagram depicting the connections in the brain that may play a role in controlling micturition. Note that areas of the brain all send projections to the periaqueductal gray (PAG); with only the medial preoptic area of the hypothalamus sending projections directly to the pontine micturition center (PMC). This convergence of projections on the PAG makes it the ideal center for coordinating sensory information from the bladder with environmental and emotional conditions of the individual in order to determine if the timing is right to activate the PMC and initiate micturition

bladder activity during sexual activity. The ventrolateral preoptic area is known to be important for sleep control (Sallanon et al. 1989) and therefore may play a role in preventing micturition during sleep. This area may also represent an area that coordinates micturition and sexual arousal, as stimulation of cholinergic nerves in the lateral preoptic nucleus with carbachol can elicit erections in male rats (Schmidt et al. 2000).

The areas of the brain that are connected to the PAG have functions that fit well with playing a role in micturition. For example, the hypothalamus, the anterior cingulate gyrus, and the prefrontal cortex are parts of, or related to, the so-called "limbic system," which is thought to be involved in emotion, behavior, and long-term memory (Holstege 1998). Proper toilet etiquette is a behavior learned early in a child's development, and instances where improper control of the bladder results in an accident can be highly embarrassing. The anterior cingulate gyrus and the hippocampus have profound influences on autonomic functions such as blood pressure and respiration (Pool and Ransohoff 1949). Finally, areas such as the thalamus and the insula are involved in the perception of sensations, including nociception, from the viscera (Derbyshire 2003). Therefore, these areas would play a significant role in introspection, or the phenomena where one is not aware of the state of one's bladder unless the situation calls for it (i.e., the bladder is full).

Retrograde tracing studies have also indicated connections between the PAG and the raphe nuclei, the locus coeruleus, and the A5 noradrenergic cell group. These areas of the brain are responsible for integration and "level-setting" of autonomic and somatomotor responses in the body (Aston-Jones and Cohen 2005; Holstege 1991, 1992; Lovick 1997; Maiorov et al. 2000). This means that while these areas of the brain are not directly involved in micturition, they play a role as a dynamic gain control, modulating the level of excitability required from sensory pathways to trigger awareness of the bladder's fullness. A common example of these "level-setting" actions is the "fight or flight" response, a response to acute emergency situations such as an attack by a predator. In these cases, the survival of the animal depends on the ability of certain physiological systems, such as the cardiovascular system or the somatomotor pathways controlling the skeletal muscle of the limbs, to respond quickly. In these cases, such systems as digestion, nociception, or micturition take a low priority as to not distract or divert vital resources away from the systems that require them. Under a "fight or flight" situation, these areas of the brain would be responsible for increasing the "level" of sensory input required to cause conscious awareness of the bladder's fullness. Therefore, even though the bladder may be very full, the animal does not feel the desire to void until the threat has passed.

3 Conclusions

The LUT is innervated by both autonomic and somatic motoneurons, which control its function. These motoneurons are controlled by a complex set of neurons in the spinal cord, brainstem, and brain, which coordinate the different parts of the LUT in a reciprocal manner, maintaining proper urinary function. While some of these neural systems have been well characterized, such as the peripheral nerves innervating the bladder, others are not yet completely understood, such as the brain centers that control voluntary voiding. Advanced imaging techniques, such as fMRI and PET scanning, however, are rapidly expanding our knowledge of the brain's control of micturition. As we increase our understanding of the brain's control of the LUT, the more likely we are to understand the causes of disorders where voluntary control of the LUT is lost and find ways to treat these diseases of which so many humans deeply suffer from.

References

Andersson KE, Arner A (2004) Urinary bladder contraction and relaxation: physiology and pathophysiology. Physiol Rev 84:935–986

Apodaca G, Balestreire E, Birder LA (2007) The uroepithelial-associated sensory web. Kidney Int 72:1057–1064

Araki I, De Groat WC (1996) Unitary excitatory synaptic currents in preganglionic neurons mediated by two distinct groups of interneurons in neonatal rat sacral parasympathetic nucleus. J Neurophysiol 76:215–226

Aston-Jones G, Cohen JD (2005) Adaptive gain and the role of the locus coeruleus-norepinephrine system in optimal performance. J Comp Neurol 493:99–110

Bahns E, Ernsberger U, Janig W et al (1986) Functional characteristics of lumbar visceral afferent fibres from the urinary bladder and the urethra in the cat. Pflugers Arch 407:510–518

Bandler R, Carrive P, Zhang SP (1991) Integration of somatic and autonomic reactions within the midbrain periaqueductal grey: viscerotopic, somatotopic and functional organization. Prog Brain Res 87:269–305

Barrington F (1925) The effect of lesions of the hind- and mid-brain on micturition in the cat. Q J Exp Physiol 15:81–102

Behbehani MM (1995) Functional characteristics of the midbrain periaqueductal gray. Prog Neurobiol 46:575–605

Bennett BC, Kruse MN, Roppolo JR et al (1995) Neural control of urethral outlet activity in vivo: role of nitric oxide. Br J Urol 153:2004–2009

Birder LA, de Groat WC (1993) Induction of c-fos expression in spinal neurons by nociceptive and nonnociceptive stimulation of LUT. Am J Physiol 265:R326–R333

Blok BF, Holstege G (1994) Direct projections from the periaqueductal gray to the pontine micturition center (M-region). An anterograde and retrograde tracing study in the cat. Neurosci Lett 166:93–96

Blok BF, Holstege G (1997) Ultrastructural evidence for a direct pathway from the pontine micturition center to the parasympathetic preganglionic motoneurons of the bladder of the cat. Neurosci Lett 222:195–198

Blok BF, Holstege G (2000) The pontine micturition center in rat receives direct lumbosacral input. An ultrastructural study. Neurosci Lett 282:29–32

Blok BF, De Weerd H, Holstege G (1995) Ultrastructural evidence for a paucity of projections from the lumbosacral cord to the pontine micturition center or M-region in the cat: a new concept for the organization of the micturition reflex with the periaqueductal gray as central relay. J Comp Neurol 359:300–309

Blok BF, de Weerd H, Holstege G (1997a) The pontine micturition center projects to sacral cord GABA immunoreactive neurons in the cat. Neurosci Lett 233:109–112

Blok BF, Willemsen AT, Holstege G (1997b) A PET study on brain control of micturition in humans. Brain 120(Pt 1):111–121

Blok BF, van Maarseveen JT, Holstege G (1998a) Electrical stimulation of the sacral dorsal gray commissure evokes relaxation of the external urethral sphincter in the cat. Neurosci Lett 249:68–70

Blok BF, Sturms LM, Holstege G (1998b) Brain activation during micturition in women. Brain 121(Pt 11):2033–2042

Brooks JD (2007) Anatomy of the lower urinary tract and male genitalia. In: Wein AJ, Kavoussi LR, Novick AC et al (eds) Cambell-Walsh urology. Philadelphia, Saunders Elsevier

Carroll P, Lewin GR, Koltzenburg M et al (1998) A role for BDNF in mechanosensation. Nat Neurosci 1:42–46

Cheng CL, Ma CP, de Groat WC (1993) Effects of capsaicin on micturition and associated reflexes in rats. Am J Physiol 265:R132–R138

Chuang YC, Fraser MO, Yu Y et al (2001) The role of bladder afferent pathways in bladder hyperactivity induced by the intravesical administration of nerve growth factor. J Urol 165:975–979

Dang K, Bielefeldt K, Gebhart GF (2005) Differential responses of bladder lumbosacral and thoracolumbar dorsal root ganglion neurons to purinergic agonists, protons, and capsaicin. J Neurosci 25:3973–3984

Dang K, Lamb K, Cohen M et al (2008) Cyclophosphamide-induced bladder inflammation sensitizes and enhances P2X receptor function in rat bladder sensory neurons. J Neurophysiol 99:49–59

de Groat WC, Booth AM (1993) Neural control of penile erection. In: Maggi CA (ed) Nervous control of the urogenital system. London, Harwood

de Groat WC, Saum WR (1976) Synaptic transmission in parasympathetic ganglia in the urinary bladder of the cat. J Physiol 256:137–158

Derbyshire SW (2003) A systematic review of neuroimaging data during visceral stimulation. Am J Gastroenterol 98:12–20

Ding YQ, Wang D, Xu JQ et al (1999) Direct projections from the medial preoptic area to spinally-projecting neurons in Barrington's nucleus: an electron microscope study in the rat. Neurosci Lett 271:175–178

England S, Bevan S, Docherty RJ (1996) PGE2 modulates the tetrodotoxin-resistant sodium current in neonatal rat dorsal root ganglion neurones via the cyclic AMP-protein kinase A cascade. J Physiol 495(Pt 2):429–440

Fowler CJ, Griffiths D, de Groat WC (2008) The neural control of micturition. Nat Rev Neurosci 9:453–466

Genzen JR, Van Cleve W, McGehee DS (2001) Dorsal root ganglion neurons express multiple nicotinic acetylcholine receptor subtypes. J Neurophysiol 86:1773–1782

Giuliano F, Rampin O, Brown K et al (1996) Stimulation of the medial preoptic area of the hypothalamus in the rat elicits increases in intracavernous pressure. Neurosci Lett 209:1–4

Gjone R (1966) Excitatory and inhibitory bladder responses to stimulation of 'limbic', diencephalic and mesencephalic structures in the cat. Acta Physiol Scand 66:91–102

Griffiths D, Holstege G, Dalm E et al (1990) Control and coordination of bladder and urethral function in the brainstem of the cat. Neurourol Urodyn 9:63–82

Habler HJ, Janig W, Koltzenburg M (1990) Activation of unmyelinated afferent fibres by mechanical stimuli and inflammation of the urinary bladder in the cat. J Physiol 425:545–562

Holstege G (1987) Some anatomical observations on the projections from the hypothalamus to brainstem and spinal cord: an HRP and autoradiographic tracing study in the cat. J Comp Neurol 260:98–126

Holstege G (1989) Anatomical study of the final common pathway for vocalization in the cat. J Comp Neurol 284:242–252

Holstege G (1991) Descending motor pathways and the spinal motor system: limbic and nonlimbic components. Prog Brain Res 87:307–421

Holstege G (1992) The emotional motor system. Eur J Morphol 30:67–79

Holstege G (1998) The emotional motor system in relation to the supraspinal control of micturition and mating behavior. Behav Brain Res 92:103–109

Holstege G (2005) Micturition and the soul. J Comp Neurol 493:15–20

Holstege G, Griffiths D (1990) Neuronal organization of micturition. In: Paxinos G (ed) The human nervous system. Academic, New York

Holstege G, Kuypers HG (1982) The anatomy of brain stem pathways to the spinal cord in cat. A labeled amino acid tracing study. Prog Brain Res 57:145–175

Holstege G, Mouton LJ (2003) Central nervous system control of micturition. Int Rev Neurobiol 56:123–145

Holstege G, Tan J (1987) Supraspinal control of motoneurons innervating the striated muscles of the pelvic floor including urethral and anal sphincters in the cat. Brain 110(Pt 5):1323–1344

Holstege G, Kuypers HG, Boer RC (1979) Anatomical evidence for direct brain stem projections to the somatic motoneuronal cell groups and autonomic preganglionic cell groups in cat spinal cord. Brain Res 171:329–333

Holstege G, Griffiths D, de Wall H et al (1986) Anatomical and physiological observations on supraspinal control of bladder and urethral sphincter muscles in the cat. J Comp Neurol 250:449–461

Hong-Sheng L, Zhi-Qi Z (1998) Small sensory neurons in the rat dorsal root ganglia express functional NK-1 tachykinin receptor. Eur J Neurosci 10:1292–1299
Janig W, McLachlan EM (1986) The sympathetic and sensory components of the caudal lumbar sympathetic trunk in the cat. J Comp Neurol 245:62–73
Janig W, Morrison JF (1986) Functional properties of spinal visceral afferents supplying abdominal and pelvic organs, with special emphasis on visceral nociception. Prog Brain Res 67:87–114
Kavia RB, Dasgupta R, Fowler CJ (2005) Functional imaging and the central control of the bladder. J Comp Neurol 493:27–32
Klop EM, Mouton LJ, Kuipers R et al (2005) Neurons in the lateral sacral cord of the cat project to periaqueductal grey, but not to thalamus. Eur J Neurosci 21:2159–2166
Kuipers R, Mouton LJ, Holstege G (2006) Afferent projections to the pontine micturition center in the cat. J Comp Neurol 494:36–53
Kuo DC, Hisamitsu T, de Groat WC (1984) A sympathetic projection from sacral paravertebral ganglia to the pelvic nerve and to postganglionic nerves on the surface of the urinary bladder and large intestine of the cat. J Comp Neurol 226:76–86
Llewellyn-Smith IJ, Dicarlo SE, Collins HL et al (2005) Enkephalin-immunoreactive interneurons extensively innervate sympathetic preganglionic neurons regulating the pelvic viscera. J Comp Neurol 488:278–289
Lovick TA (1997) The medullary raphe nuclei: a system for integration and gain control in autonomic and somatomotor responsiveness? Exp Physiol 82:31–41
Maiorov DN, Malpas SC, Head GA (2000) Influence of pontine A5 region on renal sympathetic nerve activity in conscious rabbits. Am J Physiol Regul Integr Comp Physiol 278:R311–R319
Mannen T (2000) Neuropathological findings of Onuf's nucleus and its significance. Neuropathology 20(Suppl):S30–S33
Mannen T, Iwata M, Toyokura Y et al (1982) The Onuf's nucleus and the external anal sphincter muscles in amyotrophic lateral sclerosis and Shy-Drager syndrome. Acta Neuropathol 58:255–260
Matsumoto G, Hisamitsu T, de Groat WC (1995) Role of glutamate and NMDA receptors in the descending limb of the spinobulbospinal micturition reflex pathway of the rat. Neurosci Lett 183:58–61
McMahon SB, Morrison JF (1982) Two group of spinal interneurones that respond to stimulation of the abdominal viscera of the cat. J Physiol 322:21–34
McMahon SB, Morrison JF, Spillane K (1982) An electrophysiological study of somatic and visceral convergence in the reflex control of the external sphincters. J Physiol 328:379–387
Morgan C, Nadelhaft I, de Groat WC (1981) The distribution of visceral primary afferents from the pelvic nerve to Lissauer's tract and the spinal gray matter and its relationship to the sacral parasympathetic nucleus. J Comp Neurol 201:415–440
Morgan CW, de Groat WC, Felkins LA et al (1991) Axon collaterals indicate broad intraspinal role for sacral preganglionic neurons. Proc Natl Acad Sci USA 88:6888–6892
Morgan CW, De Groat WC, Felkins LA et al (1993) Intracellular injection of neurobiotin or horseradish peroxidase reveals separate types of preganglionic neurons in the sacral parasympathetic nucleus of the cat. J Comp Neurol 331:161–182
Nadelhaft I, Vera PL, Card JP et al (1992) Central nervous system neurons labelled following the injection of pseudorabies virus into the rat urinary bladder. Neurosci Lett 143:271–274
Nishii H, Nomura M, Fujimoto N et al (2008) Thalamic neural activation in the cyclophosphamide-induced visceral pain model in mice. Neurosci Res 60:219–227
Nyo M (1969) Innervation of the bladder and urethra. J Anat 105:210
Oztas E (2003) Neuronal tracing. Neuroanatomy 2:2–5
Patel AK, Chapple CR (2008) Anatomy of the lower urinary tract. Surgery 26:127–132
Petras JM, Cummings JF (1978) Sympathetic and parasympathetic innervation of the urinary bladder and urethra. Brain Res 153:363–369

Pool JL, Ransohoff J (1949) Autonomic effects on stimulating rostral portion of cingulate gyri in man. J Neurophysiol 12:385–392
Pullen AH, Tucker D, Martin JE (1997) Morphological and morphometric characterisation of Onuf's nucleus in the spinal cord in man. J Anat 191:201–213
Qin C, Foreman RD (2004) Viscerovisceral convergence of urinary bladder and colorectal inputs to lumbosacral spinal neurons in rats. Neuroreport 15:467–471
Rohner TJ, Hannigan J (1980) Effect of norepinephrine and isoproterenol on in vitro detrusor muscle contractility and cyclic AMP content. Invest Urol 17:324–327
Rong W, Spyer KM, Burnstock G (2002) Activation and sensitisation of low and high threshold afferent fibres mediated by P2X receptors in the mouse urinary bladder. J Physiol 541:591–600
Sallanon M, Denoyer M, Kitahama K et al (1989) Long-lasting insomnia induced by preoptic neuron lesions and its transient reversal by muscimol injection into the posterior hypothalamus in the cat. Neuroscience 32:669–683
Sasaki M (1994) Morphological analysis of external urethral and external anal sphincter motoneurones of cat. J Comp Neurol 349:269–287
Schmidt MH, Valatx JL, Sakai K et al (2000) Role of the lateral preoptic area in sleep-related erectile mechanisms and sleep generation in the rat. J Neurosci 20:6640–6647
Shefchyk SJ, Espey MJ, Carr P et al (1998) Evidence for a strychnine-sensitive mechanism and glycine receptors involved in the control of urethral sphincter activity during micturition in the cat. Exp Brain Res 119:297–306
Sie JA, Blok BF, de Weerd H et al (2001) Ultrastructural evidence for direct projections from the pontine micturition center to glycine-immunoreactive neurons in the sacral dorsal gray commissure in the cat. J Comp Neurol 429:631–637
Subramanian HH, Balnave RJ, Holstege G (2008) The midbrain periaqueductal gray control of respiration. J Neurosci 28:12274–12283
Sugaya K, Matsuyama K, Takakusaki K et al (1987) Electrical and chemical stimulations of the pontine micturition center. Neurosci Lett 80:197–201
Thor KB, Morgan C, Nadelhaft I et al (1989) Organization of afferent and efferent pathways in the pudendal nerve of the female cat. J Comp Neurol 288:263–279
Todd JK, Mack AJ (1969) A study of human bladder detrusor muscle. Br J Urol 41:448–454
Vanderhorst VG, Holstege G (1997) Organization of lumbosacral motoneuronal cell groups innervating hindlimb, pelvic floor, and axial muscles in the cat. J Comp Neurol 382:46–76
Vanderhorst VG, Mouton LJ, Blok BF et al (1996) Distinct cell groups in the lumbosacral cord of the cat project to different areas in the periaqueductal gray. J Comp Neurol 376:361–385
Vera PL, Nadelhaft I (1992) Afferent and sympathetic innervation of the dome and the base of the urinary bladder of the female rat. Brain Res Bull 29:651–658
Vizzard MA (2000) Alterations in spinal cord Fos protein expression induced by bladder stimulation following cystitis. Am J Physiol Regul Integr Comp Physiol 278:R1027–R1039
Vizzard MA, Erickson VL, Card JP et al (1995) Transneuronal labeling of neurons in the adult rat brainstem and spinal cord after injection of pseudorabies virus into the urethra. J Comp Neurol 355:629–640
Vizzard MA, Brisson M, de Groat WC (2000) Transneuronal labeling of neurons in the adult rat central nervous system following inoculation of pseudorabies virus into the colon. Cell Tissue Res 299:9–26
Wang JG, Strong JA, Xie W et al (2007) Local inflammation in rat dorsal root ganglion alters excitability and ion currents in small-diameter sensory neurons. Anesthesiology 107:322–332
Yamada Y, Daneshgari F, Liu G, et al (2007) Spinal c-fos expression induced by neuroselective electrostimulation of bladder afferent pathways (abstract). Society for Neuroscience Annual Meeting, Program # 821.2 (www.sfn.org)
Yoshimura N, Seki S, Erickson KA et al (2003) Histological and electrical properties of rat dorsal root ganglion neurons innervating the lower urinary tract. J Neurosci 23:4355–4361

Anatomy and Histology of the Lower Urinary Tract

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Contents

Abstract The function of the lower urinary tract is basically storage of urine in the bladder and the at-will periodic evacuation of the stored urine. Urinary incontinence is one of the most common lower urinary tract disorders in adults, but especially in the elderly female. The urethra, its sphincters, and the pelvic floor are key structures

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in the achievement of continence, but their basic anatomy is little known and, to some extent, still incompletely understood. Because questions with respect to continence arise from human morbidity, but are often investigated in rodent animal models, we present findings in human and rodent anatomy and histology. Differences between males and females in the role that the pelvic floor plays in the maintenance of continence are described. Furthermore, we briefly describe the embryologic origin of ureters, bladder, and urethra, because the developmental origin of structures such as the vesicoureteral junction, the bladder trigone, and the penile urethra are often invoked to explain (clinical) observations. As the human pelvic floor has acquired features in evolution that are typical for a species with bipedal movement, we also compare the pelvic floor of humans with that of rodents to better understand the rodent (or any other quadruped, for that matter) as an experimental model species. The general conclusion is that the "Bauplan" is well conserved, even though its common features are sometimes difficult to discern.

Keywords Bladder · Lower urinary tract · Man · Mouse · Pelvic floor · Prostate · Ureter · Urethra · Urethral sphincter · Vestibular glands

1 Introduction

The urinary tract produces, stores, and evacuates urine. In all vertebrates, urine is produced by the kidneys, transported to the bladder for temporary storage via the ureters, and evacuated from the bladder via the urethra (Fig. 1). The lower urinary tract (LUT) is basically organized for urinary storage in the bladder and the at-will periodic evacuation of stored urine. It consists of two ureters, the bladder, and the urethra.

1.1 Sex Differences

Major differences between males and females mostly concern the urethra. The best-known differences concern the prostate gland, which surrounds the urethra in its proximal portion in males and the distal urethra, which is much longer in males than in females due to its distal extension into the urethral plate of the penis. The almost epidemic levels of urinary incontinence in peri- and postmenopausal multiparous women (see below) have also implicated the pelvic floor as an important determinant of continence. The anatomy of the pelvic floor and the pronounced and well-defined differences in makeup between females and males are, therefore, also discussed in detail.

1.2 Species Differences

A major species difference is the encapsulation of the prostate within a well-formed prostatic fascia in human, which is not found in, e.g., rodent males. In addition, the

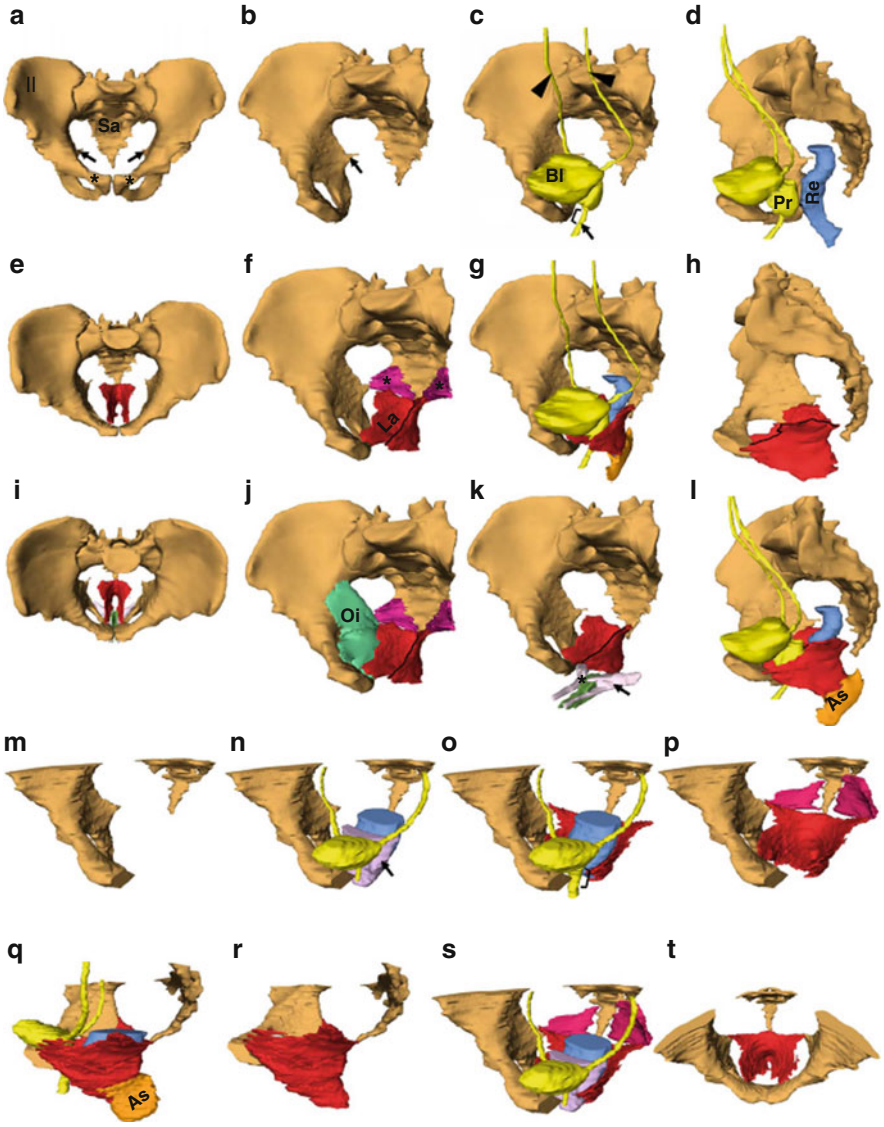

Fig. 1 Anatomy of the male (panels **a**–**l**) and female (panels **m**–**t**) adult human pelvis, pelvic organs, and pelvic floor. Colors are identical for anatomical structures in the male and female. The *bracket* in **c** and **o** illustrates the approximate level of the external urethral sphincter muscle. Small structures are illustrated by *asterisks*, *arrows, or arrowheads*: pubic bone (*asterisks* in **a**), ischial spine (*arrows* in **a** and **b**), ureters (*arrowheads* in **c**), urethra (*arrow* in **c**), coccygeal muscle (*asterisks* in **e**), bulbospongious muscle (*asterisk* in **k**), ischiocavernous muscle (*arrow* in **k**), vagina (*arrow* in **n**). *As* external anal sphincter muscle, *Bl* urinary bladder, *Il* ilium, *La* levator ani muscle, *Oi* internal obturator muscle, *Pr* prostate, *Ps* pubic symphysis, *Re* rectum, *Sa* sacrum, *U* urethra

architecture of the bony pelvis and pelvic floor corresponds directly to a bi- or quadruped way of locomotion. These structural differences obviously affect the functional outcome of experimental modification in animal models of prostate size or pelvic floor atrophy. Since the widespread availability of genetically modified strains makes mice an increasingly more important experimental animal model, the differences in LUT and pelvic floor anatomy between mice and humans are discussed in detail.

1.3 Embryology

Tissue interactions and responses can often be predicted from their developmental history. Advances in techniques to genetically modify gene expression in mice have yielded much insight into similarities and differences in the signaling cascades that govern development and remain important in the adult period. The embryology of the mouse LUT, therefore, is also summarized.

2 Anatomy of the Human LUT and Pelvic Floor

The ureters are 25–35 cm long tubular structures that connect the renal pelvis to the base of the bladder. The wall of the ureter is composed of an inner, longitudinal and an outer, circular smooth muscle layer. Urine is stored in the bladder, a hollow, smooth muscular, and elastic organ. Anteriorly, the bladder base lies against the pubic symphysis. Posteriorly, the bladder neighbors the genital tract in the female (uterus and vagina) and the rectum in the male. The detrusor muscle is the smooth muscular layer of the bladder wall. During the filling phase, the detrusor is stretched slowly and at a certain level of filling, the urge to void increases. The parasympathetic nervous system is then responsible for initiating the opening of the bladder outlet and the contraction of the detrusor. This leads to opening of the bladder outlet and to expulsion of urine through the urethra (micturition).

2.1 The Urethra and Its Accessory Glands

In both sexes, the urethra connects the urinary bladder to the outside world to pass urine. Furthermore, it is a conduit for the passage of semen in the male. The urethra is sexually highly dimorphic with regard to the accessory glands that drain on it and in the extension of the urethra into the penis in the male (penile urethra).

2.1.1 Male

The ~20 cm-long-male urethra is subdivided into the pelvic urethra, with prostatic and membranous portions, and the penile urethra, which includes the bulbar portion. The prostatic portion of the urethra connects to the bladder neck and runs through the core of the prostatic mass and is lined with transitional cell epithelium ("urothelium"; for a definition, see section "Urothelium"). This segment contains the openings of the ejaculatory ducts and the numerous openings of the prostatic ducts. The relatively narrow membranous portion of the urethra is surrounded by the external urethral sphincter proximally and the perineal membrane (for a definition, see section "External urethral sphincter muscles") distally. The penile urethra courses through the spongious body within the body of the penis to end at the external meatus. The ducts of the bulbourethral glands enter the urethra just distal to the perineal membrane. The membranous urethra and the proximal parts of the penile urethra up to the fossa navicularis are lined with pseudostratified columnar epithelium. The fossa itself and the external meatus are lined by stratified squamous epithelium. The ducts of the numerous small (peri-) urethral glands (glands of Littré) drain into the penile urethra.

Accessory Glands

The male accessory glands consist of the vesicular glands (formerly called the seminal vesicles), the prostate, the bulbourethral glands, and the (peri-) urethral glands. The secretions from the vesicular glands and the prostate together constitute the seminal fluid, with only small contributions from the bulbourethral and peri-urethral glands.

Vesicular Glands

The paired vesicular glands are saccular structures that are apposed to the postero-inferior surface of the urinary bladder. Each gland consists internally of mucosal folds that are lined with simple columnar epithelium. Each gland has a short duct that drains into the ampulla of the deferent duct. Between the ampulla and its outflow into the urethra, the deferent duct is called the ejaculatory duct.

Prostate Gland

The prostate gland is situated immediately inferior to the bladder and surrounds the most proximal portion of the urethra (Fig. 1). The prostate gland produces a slightly alkaline fluid, which is the major component of ejaculated semen. The adult prostate is a firm, dense structure that rests on the anterior portion of the pelvic floor (Myers et al. 2010). Only its posterior portion can be palpated during a rectal examination. The glands of the prostate drain via numerous short ducts into the prostatic portion of the urethra. The normal development of the prostate is dependent

on a sufficient level of testosterone secretion by the fetal testis. From birth to puberty, the prostate weighs 1–2 g, but increases to ~20 g or the size of a chestnut or small plum during puberty (Haddad et al. 2001).

As in other mammals, the human prostate develops from separate lobes (see below), but this common origin is not reflected in the four anatomically distinct zones that are now used to describe the adult prostate anatomically (Fig. 2). The peripheral zone comprises 70–75% of the gland, the central zone 20–25%, and the transitional zone 5–10%, while the anterior surface consists of fibromuscular stroma (McNeal 1968; Myers et al. 2010). The peripheral zone occupies the posterior aspect of the prostate, while the central zone occupies and surrounds the superior, juxtavesical part around the ejaculatory ducts. The transitional zone surrounds the urethra laterally and ventrally. The anterior fibromuscular zone is usually devoid of glandular tissue and largely composed of smooth muscle and fibrous tissue.

The prostate doubles in size during the 50 years following puberty, but the increase differs between the different zones: the central zone triples in size (Well et al. 2007), whereas the peripheral zone remains similar in size (McNeal 1968). Approximately 70% of prostatic cancers are found in the peripheral zone, while ~25% develop in the central zone (McNeal et al. 1988). The transitional zone grows throughout life, but is rarely associated with carcinoma. Instead, its growth underlies benign prostatic hyperplasia (BPH) as a part of the aging process and causes the symptoms associated with this process (Sampson et al. 2007).

The prostate is richly innervated. Gross dissection and immunostaining have shown that the peripheral zone is more densely innervated than the transition zone, and the posterior portion of the prostatic capsule is more densely innervated than the anterior portion. Furthermore, innervation declines with aging (Xue et al. 2000; Powell et al. 2005).

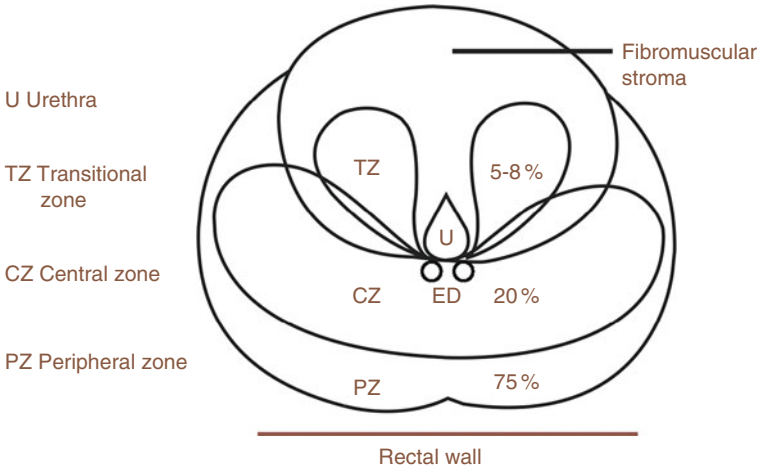

Fig. 2 Schematic diagram of the zones of the human prostate. Anterior is at the *upper side* of the drawing and posterior at the lower, rectal side. *ED* ejaculatory duct. Adapted from http://network.nature.com/people/basanta/blog/2008/01/14/facts-about-prostate-cancer

Microscopically, the glandular tissue in the entire prostate is tubuloalveolar in architecture. The pseudostratified epithelium contains stem, transit-amplifying, basal, secretory, and neuroendocrine cells (McNeal 1988; Xue et al. 2000; Tokar et al. 2005). The smooth muscle cells surrounding the prostatic acini are thinner than those surrounding the seminal vesicle. The stromal fibroblasts express androgen (AR) and estrogen receptor β (ERβ), as well as 5α-reductase and aromatase, which catalyze the local synthesis of 5α-dihydrotestosterone and of estrogens, respectively. (Sampson et al. 2007). In aging men with BPH, the stromal compartment in the transition zone of the prostate becomes enlarged and altered in its cellular composition. These changes are followed by focal proliferation of smooth muscle cells and basal epithelial cell hyperplasia (Sampson et al. 2007; Well et al. 2007).

The secretory epithelial cells also express AR and ERβ. In the elderly, the epithelial cells in the peripheral zone are reported to contain approximately twofold higher AR levels than the epithelial cells in the central and transitional zones (Sanchez-Visconti et al. 1995; Sampson et al. 2007). The epithelial acinar cells contribute to the seminal fluid. One of the products is prostate-specific antigen (PSA), a glycoprotein with a trypsin-like protease function that helps to keep the semen liquefied. PSA is a marker of prostate cellular activity and serves as a nonspecific plasma biomarker for prostate cancer (LeBeau et al. 2009), but is also increased in (chronically) inflamed prostates. In the absence of cancer and prostatitis, PSA is a useful marker for (changes in) prostate size (for review, see Roehrborn 2008). Within the lumina of prostatic acini, dense calcified proteinaceous concretions or *corpora amylacea* accumulate with aging.

Bulbourethral Gland

The pea-sized bulbourethral (Cowper's) glands are located postero-superiorly to the penal bulb. A short duct from each gland enters the urethra at the bulb. The lining of these paired, compound, tubuloalveloar glands is simple columnar epithelium. A capsule of dense connective tissue with some smooth and skeletal muscle of the bulbocavernous and urethral muscles is present around them.

Urethral Glands

The male urethral (Littré's) glands are mucous glands in the wall of the penile urethra.

2.1.2 Female

The ~4 cm-long-female urethra ends between the clitoris and the vagina. The mucosal lining of the urethra is composed of pseudostratified columnar epithelium proximally and of stratified squamous epithelium distally. The female urethra is surrounded anteriorly by the external urethral sphincter.

Accessory Glands

The female accessory glands consist of the lesser and the greater vestibular glands. The ducts of the lesser vestibular glands end distally near the mouth of the urethra, whereas the ducts of the greater vestibular glands drain on the vaginal vestibule at either side of the vaginal orifice. Strictly speaking, the greater vestibular glands are, therefore, not accessory glands of the urethra.

The Lesser Vestibular Glands

The lesser vestibular (Skene's) glands are located on the anterior wall of the vagina, around the distal end of the urethra. They drain into the urethra or near the urethral opening. Skene's gland is also known as the female prostate (see below). The microscopic anatomy and the composition of the secretions of the female lesser vestibular glands and the male prostate are similar (Zaviacic and Ablin 2000; Zaviacic et al. 2000). The secretions of Skene's gland also include a low concentration of PSA and the plasma PSA increases in women with, e.g., Skene's gland carcinoma. It should be kept in mind, though, that PSA in the female is also produced by, e.g., diseased breast tissue (Zaviacic and Ablin 2000).

The Greater Vestibular Glands

The greater vestibular (Bartholin's) glands are homologous to the bulbourethral glands in males. These paired glands are nonpalpable structures that are located in the labia minora.

2.2 The Human Pelvic Floor

2.2.1 The Pelvic Floor and Continence

Lower urinary tract symptoms (LUTS) are highly prevalent. The cost of illness due to LUTS is a substantial human and economic burden and will increase further with the aging of the general population in future, stressing the need for effective forms of management (Milsom 2009). Loss of urine (urinary incontinence) is one of the most common LUTS in adults, especially in the female (Coyne et al. 2009). Drug treatment for urinary incontinence, particularly stress urinary incontinence (SUI), until now has met only limited success (Alhasso et al. 2005). In most cases, pelvic floor dysfunction is considered to be a major factor in the development of incontinence. For this reason, the anatomical properties of the pelvic floor are highlighted here.

The pelvic floor plays not only an important role in pelvic organ support, but also in urinary and fecal continence (Bharucha 2003; Madoff et al. 2004; Norton and Brubaker 2006; Wald 2007; Jelovsek et al. 2007; Ashton-Miller and DeLancey

2007). Pelvic floor dysfunction is a serious public health problem. Depending on the definition used, at least 30% of (post) menopausal women suffer from urinary incontinence (Hunskaar et al. 2003) and as many as 10% of all women may suffer from pelvic floor dysfunction that requires surgery (Olsen et al. 1997; DeLancey 2005). In the United States alone, almost 300,000 operations for pelvic organ prolapse and SUI are performed each year (Boyles et al. 2003a, b).

2.2.2 The Anatomy of the Pelvic Floor

Anatomically, the pelvic floor is an intricate complicated part of the human body. In the last few years, several functional and anatomical studies were performed that have provided new insights into its function and dysfunction. The urethra and prostate are embedded in the pelvic floor, while the bladder lies on top of it (Fig. 1). The following components of the pelvic floor are discussed: (1) levator ani muscle (LAM) and coccygeal muscle, (2) urethral sphincter muscles, (3) bulbospongious and ischiocavernous muscles, and (4) connective tissue and smooth muscle components of the levator hiatus.

The Levator Ani Muscle

The LAM is the main muscle of the pelvic floor and is believed to play an important role in both urinary (Bharucha 2003; Norton and Brubaker 2006) and fecal continence (Bharucha 2003; Wald 2007). The superior part of the LAM is attached to the pubic bone, the ischial spine, the sacrum/coccyx, and the tendinous arch of the levator ani between the pubic bone and ischial spine (Fig. 1). Because its primary function is support rather than movement, fibrous tissue often replaces the muscular component later in life. The puborectal part of the LAM, which is situated inferior to the LAM's posterior attachment to the coccyx (Wallner et al. 2009a), is attached to the pubic bone anteriorly and forms a sling around the dorsal aspect of the rectum. If the puborectal muscle contracts, the rectum is pulled anteriorly and closed to retain feces (Padda et al. 2007).

Different populations of patients with SUI were shown to have a thinner (Kirschner-Hermanns et al. 1993; Hoyte et al. 2001, 2004; Stoker et al. 2003; Aukee et al. 2004) or damaged LAM (Tunn et al. 1998), or have damage to the nerve supply to the pelvic floor (Snooks et al. 1984, 1985). Women with a levator ani trauma are not only prone to develop SUI (Kirschner-Hermanns et al. 1993; DeLancey et al. 2003; Stoker et al. 2003), but also often suffer from pelvic organ prolapse (Dietz and Lanzarone 2005; DeLancey et al. 2007). The LAM is particularly prone to damage during vaginal delivery. Imaging studies of postdelivery females showed that the LAM can be damaged especially at its pubic attachment (DeLancey et al. 2003; Dietz and Lanzarone 2005; Margulies et al. 2007; Dietz and Simpson 2008). Different stretch models of the LAM – a skeletal muscle – during passage of the fetal head through the pelvic outlet have been suggested and predict a

maximum muscle stretch of 160% (Martins et al. 2007; Parente et al. 2007), 326% (Lien et al. 2004), and 350% (Hoyte et al. 2008). The last numbers equal or exceed the 150% limit that is physiologically permissible before overstretching the actin–myosin complexes injures the muscle fibers structurally (Brooks et al. 1995). If the outcome of the two studies that predict maximum stretches of >300% would apply to real-life deliveries, every delivery should then result in a major trauma to the LAM. Two anatomical aspects seem crucial for the predictions of these models: (1) the attachments of the different parts of the LAM; and (2) the muscle fiber direction within this muscle.

The superior part of the LAM is fixed by its attachments to the pubic bone, the ischial spine, and the coccyx. During delivery, this part of the LAM will not be stretched much. The portion of the LAM that is subject to stretch is the puborectal muscle.

In one model (Lien et al. 2004), the muscle with the highest stretch (326%) is a part of the pubococcygeal muscle that inserts into the perineal body between the vagina and the rectum. This relatively short muscular sling would have to stretch enormously when the fetal head passes through the vagina. However, we were not able to confirm the presence of this specific part of the pubococcygeal muscle as a striated muscle (Wallner et al. 2009a). The model, which predicts a maximum stretch of 350%, was developed from a 3D reconstruction of an MRI of a nulliparous woman (Hoyte et al. 2008). The LAM was modeled as a single muscle that was equally stretchable in all directions, even though muscles can only stretch physiologically in the direction of the muscle fibers. Stretch in another direction will not result in overstretched muscle fibers, but in the loss of the integrity of the endo-, peri-, and epimysial connective tissue.

This observation reveals that a universally accepted, detailed topographic description of the anatomy of the LAM is not yet available. The only model in the literature that did implement muscle fiber direction was the one that showed a maximum stretch of 160% (Janda et al. 2003; d'Aulignac et al. 2005; Martins et al. 2007; Parente et al. 2007). This figure comes close to the maximally allowable stretch without injury to skeletal muscle. This model assumes that the muscle fibers in the iliococcygeal and puborectal muscles have a parallel orientation (Janda et al. 2003; d'Aulignac et al. 2005; Martins et al. 2007; Parente et al. 2007). By contrast, in the model predicting a 326% stretch, the muscle fibers in the iliococcygeal muscle have a latero-medial orientation as opposed to the antero-posterior orientation of the fibers in the puborectal muscle (Lien et al. 2004). Even if the boundary conditions of the "160%" model are confirmed, this finding demonstrates that giving birth touches the physiological limits of the human "Bauplan." The divergent findings in these cited studies emphasize that it is important not only to determine the proper topographic anatomy of the pelvic muscles, but also to take into account the regional differences in fiber orientation.

The interpretation of the measurements of stretch during vaginal deliveries may have been biased by the fact that >300%-stretch models of the pelvic floor are based on MRI images. The normal pelvic floor of a person in an upright position is subject to intra-abdominal pressure. In the supine position, the intra-abdominal pressure is

lower, which may relax the LAM and induce the typical funnel configuration of the LAM seen on MRI studies. The 160%-stretch model, on the other hand, was based on cadaver measurements (Janda et al. 2003; d'Aulignac et al. 2005; Martins et al. 2007; Parente et al. 2007). It is self-evident that the anatomical basis of this discrepancy between MRI findings and cadaveric models needs to be resolved.

Interestingly, the part that is stretched the most in the most detailed model (Lien et al. 2004) is positioned at the level where smooth muscle anchors the pelvic organs to the pelvic floor (see section "Connective tissue and smooth muscle components of the levator hiatus"). Such an extreme stretch is detrimental for striated muscle, but can be readily accommodated by smooth muscle (An and Fredberg 2007; Bosse et al. 2008).

The Coccygeal Muscle

The coccygeal muscle arises from the tip of the ischial spine, posterior to the LAM. The muscle fibers fan out and attach to the lateral side of the coccyx and the inferior part of the sacrum. The sacrospinous ligament is situated inferior to this muscle and is tightly fused with it (Stoker and Wallner 2008).

The External Urethral Sphincter Muscles

Women with SUI often have a weakened or asymmetric contractility of the pelvic floor (Kirschner-Hermanns et al. 1993; Stoker et al. 2003), which implies a role for the LAM in urinary continence. The mechanism by which the LAM aids in urinary continence is less well delineated than in fecal continence. The established model states that the "intrinsic continence mechanism" causes urethral closure by contraction of the external urethral sphincter muscle (striated muscle) and the internal urethral sphincter muscle (smooth muscle). The "extrinsic continence mechanism," which is formed by the pelvic floor muscles, organs, and support structures surrounding the urethra and urethral sphincter muscles (DeLancey 1988), is thought to support the intrinsic continence mechanism. In our opinion, however, such a functional dichotomy does not exist.

In females, the EUS is a horseshoe-shaped muscle rather than a "true" circular sphincter (for a comparison of the basic male and female morphology, see Fig. 3). The EUS has no direct muscular attachment to the bony pelvis (Wallner et al. 2009a). The EUS consists of a superior part (adjacent to the bladder) and an inferior part (distal from the bladder). In females, the superior part covers only the urethra, while the inferior part covers both the urethra and an antero-lateral portion of the vagina. This latter part of the EUS is firmly attached to the puborectal part of the LAM by a tendinous connection (Wallner et al. 2009a). In males, the EUS also originates as a horseshoe-shaped structure with the opening on the dorsal side, but the involution of the Müllerian tubes in the male fetus allows the inferior part of the muscle to fold back on the posterior surface of the urethra. As a result, a fibrous

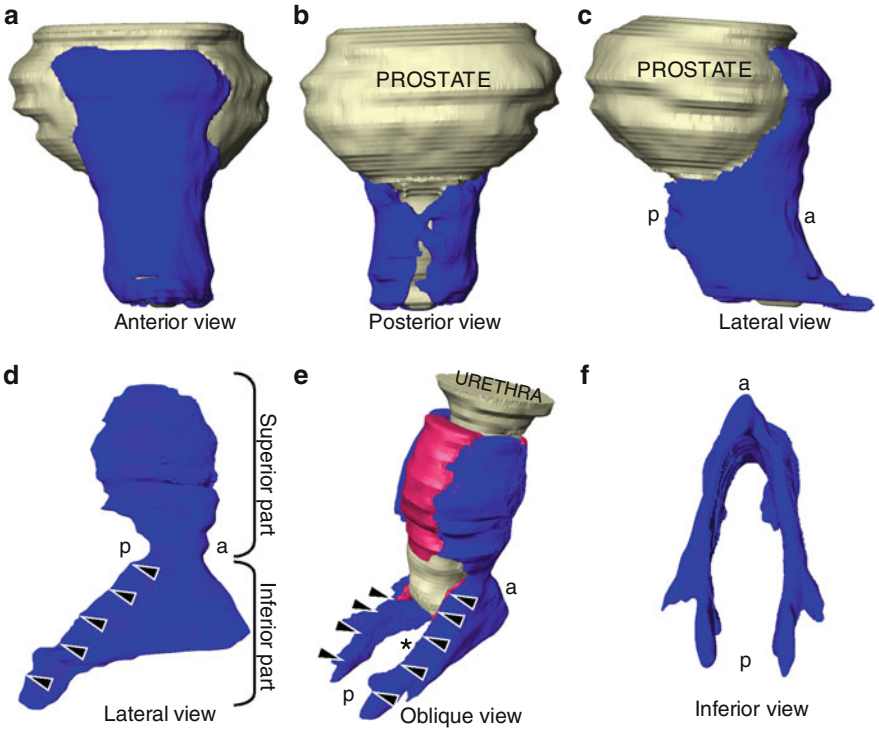

Fig. 3 The external urethral sphincter (EUS) in a male foetus (**a**–**c**, 22 weeks of gestation) and a female foetus. Panels **a**–**c**: 3D reconstruction of the male EUS (*blue*) in anterior view (**a**), posterior view (**b**), and right-lateral view (**c**). The EUS is shown in *blue* and the urethra and prostate are shown in *light grey*. Note that the EUS partly covers the ventral part of the prostate. Panels **d**–**f**: 3D reconstruction of the EUS (*blue*) and the smooth internal urethral sphincter muscle (*pink*) in right-lateral view (**d**), oblique view (**e**, with urethra in *light grey*), and inferior view (**f**). Note the superior and inferior part of the EUS (shown in **e**) and the absence of striated muscle on posterior aspect of the urethra. The inferior part of the EUS exists of large muscular "wings" that laterally also surround the vagina (position of the vagina illustrated by asterisk in **e**). The *arrowheads* in **d** and **e** illustrate the level at which the inferior part of the EUS is firmly attached to the puborectal part of the LAM by a tendinous connection. Anterior and posterior directions are represented by "a" and "p." This figure has been adapted from Wallner et al. (2009a 600/id)

connection with the LAM does not develop (Wallner et al. 2009a). Only the most ventral and inferior aspect of the prostate is enclosed by the EUS (Fig. 3). In all probability, the male EUS functions as a "true sphincter," as it is not attached to the LAM, but, instead, almost completely surrounds the urethra.

In females, contraction of the puborectal muscle pulls the rectum and vagina anteriorly (Padda et al. 2007), so that increased pressure develops on the posterior urethral wall, compressing the urethral lumen (DeLancey 1988; Wallner et al. 2009a). In addition, the contraction of the puborectal sling moves the recto-vaginal complex anteriorly and superiorly toward the urethra in a plane that lies parallel, but superior to that of the EUS (Wallner et al. 2009a). Simultaneous contraction of the

LAM and EUS, therefore, causes an anteriorly convex bend in the mid-urethra, i.e., at the position where the greatest increase in intraurethral pressure upon voluntary pelvic floor contraction develops (Hutch and Rambo 1967; DeLancey 1986). Interestingly, this is exactly the level where the tension-free vaginal tape is advised to be placed (Rahn et al. 2006).

This description shows that EUS function in females is dependent on an intact LAM. If this muscle is damaged, stretched, or has its attachments loosened during delivery, the EUS loses its fixation point on the LAM and can no longer function properly. In our view, this dependence of the EUS on the integrity of the LAM explains why urinary continence is so often affected by LAM lesions.

The anatomy and presence of the "transverse perineal muscle" has long been a controversial issue. Anatomically, it can be concluded that the "deep transverse perineal muscle" is, in fact, the inferior part of the EUS, and not a separate muscle of the pelvic floor (Kato et al. 2008; Stein and DeLancey 2008; Wallner et al. 2009a). The "urogenital diaphragm" [also known as the "perineal membrane" (Stein and DeLancey 2008)], which is described as formed by the deep transverse perineal muscle and surrounding connective tissue fascia, is therefore not a separate anatomical structure in the pelvic floor. As explained above, the inferior part of the EUS in males bends dorsally around the urethra, so that a separate deep transverse perineal muscle or perineal membrane does not exist in males. These conclusions explain why previous descriptions of the urogenital diaphragm were contradictory in the sense that some studies clearly identified a urogenital diaphragm, whereas others denied its existence (Kaye et al. 1997; Dorschner et al. 1999; Haderer et al. 2002; Mirilas and Skandalakis 2004; Nakajima et al. 2007; Stein and DeLancey 2008; Kato et al. 2008; Wallner et al. 2009a).

Bulbospongious, Ischiocavernous, and "Transverse Perineal" Muscles

The weak point in the pelvic organ support system is the levator hiatus, through which the organs pass the LAM. The bulbospongious and ischiocavernous muscles are traditionally thought to have a sexual function (Ballard 1997). However, since these muscles partly fill the space inferior to the levator hiatus, they can also support the pelvic floor during episodes of increased intra-abdominal pressure, such as coughing, laughing, or during weight lifting. This putative role of the bulbospongious and ischiocavernous muscles is underscored by the marked hypertrophy of these muscles in the male individual of the Visible Human Project, who was an active bodybuilder (Spitzer et al. 1996). In females, these muscles are less developed (Francis 1952) and may therefore represent an additional weak spot in the pelvic organ support system.

Connective Tissue and Smooth Muscle Components of the Levator Hiatus

While the striated muscles of the pelvic floor give active support, the connective tissue fascia of the pelvic floor is considered to provide continuous passive support

to counterbalance the effects of constant and intermittent elevations of intra-abdominal pressure (Ashton-Miller and DeLancey 2007). Connective tissue fascias cover the superior and inferior surface of the pelvic floor. Additionally, the different muscles of the pelvic floor are connected to each other by connective tissue and to the bony pelvis.

From several studies (Von Hayek 1969; Albers et al. 1973; Wilson et al. 1983; Petros 1998; Jones et al. 2003; Suzme et al. 2007; Nakajima et al. 2007; Reisenauer et al. 2008; Wallner 2008; Wallner et al. 2009b), it is established that the connective tissue of the adult pelvis contains smooth muscle cells. Smooth muscle has also been demonstrated in the adult pubourethral, pubovesical, puboprostatic (Albers et al. 1973; Wilson et al. 1983; Petros 1998), and uterosacral "ligaments" (Jones et al. 2003; Suzme et al. 2007; Reisenauer et al. 2008), as well as in the tendinous arch of the pelvic fascia (Von Hayek 1969; Pit et al. 2003). The presence of ligaments between the urethra, bladder, prostate, and the pubic bone has, however, been questioned (Fritsch et al. 2006). Also structures in the pelvis that are called "ligaments," such as the cardinal ligament, uterosacral ligament, and the lateral ligament of the rectum, are actually not genuine ligaments, but nerves and vessels surrounded or covered by strands of connective tissue (De Caro et al. 1998).

We have recently demonstrated that, in the fetus, smooth muscle cells in the levator hiatus connect the medial surface of the LAM to the pelvic organs (Wallner 2008; Wallner et al. 2009b). Around the rectum, this smooth "levator hiatus muscle" (LHM) merges with and is part of the longitudinal smooth muscle layer of the rectum. The LHM remains present throughout life, expanding coincident with the increasing size of the pelvis and pelvic organs. Additional myofibroblasts in the adult pelvic floor may be recruited from fibroblasts (Wallner 2008; Wallner et al. 2009b).

The LHM anchors the urethra, prostate, vagina, and rectum to the LAM at the level of the levator hiatus. This architecture prevents these organs from descending through the hiatus. Although women with prolapse have a larger levator hiatus than women without prolapse (DeLancey and Hurd 1998), prolapses are believed not to originate at the level of the hiatus itself, but more superiorly and descend through the vagina (Jelovsek et al. 2007). The pelvic organs are further supported by an endopelvic fascia. As stated previously, the pelvic floor includes this complex system of ligamentous supports known as the endopelvic fascia, which envelopes the pelvic organs and attaches them to the pelvic sidewall. Many parts of this endopelvic fascia have been separately named as the uterosacral and cardinal ligaments, and the pubocervical and rectovaginal fascias. The endopelvic fascia differs from fascias in other parts of the body in that it is not a uniform sheet of densely woven collagen fibers, but rather a heterogeneous mesh of collagen, elastin, perivascular smooth muscle, fibroblasts, vessels, and nerves (Handa et al. 1996). The contribution of these components needs to be clarified.

Smooth muscle tissue is usually present in the wall of organs that are hollow. To accommodate volume changes, smooth muscle tissue can maintain stretch over at least threefold of its resting length (An and Fredberg 2007; Bosse et al. 2008). Smooth muscle can stretch to such a length, because it is continuously remodeling:

length adaptation starts with a partial disassembly of the contractile and cytoskeletal units followed by reassembly of the units at the new cell length (Bosse et al. 2008).

Nerve Supply of the Pelvic Floor

The pelvic floor is innervated by the levator ani and pudendal branches of the sacral plexus. The levator ani nerve courses superior to the pelvic floor and is the main nerve to the LAM (Wallner et al. 2006a, 2006b, 2008a, 2008b). The pudendal nerve courses inferior to the pelvic floor and innervates the striated portions of the urethral and anal sphincters, as well as the bulbospongious and ischiocavernous muscles. The pudendal nerve contributes to the innervation of the LAM only in ~50% of cases (Wallner et al. 2008b). The levator ani nerve is especially prone to damage due to stretch during vaginal delivery. It can also be damaged during pelvic surgery (Wallner et al. 2006b, 2008a, b).

3 Anatomy and Development of the Rodent LUT and "Pelvic Floor"

3.1 The Components of the Rodent LUT

It is well established that the "Bauplan," that is, the blueprint of the body architecture, is similar in all mammals, including primates and rodents, but substantial quantitative differences, particularly with respect to the accessory sex glands, exist. Even larger differences exist between the pelvic floor of bipeds and quadrupeds. A general difference between rodents and humans is the intraperitoneal location of the abdominal organs, including the kidneys and the bladder, in rodents, whereas these organs are retroperitoneal in humans. Several useful dissection guides of the adult rat and mouse exist (Walker 1995; The Staff of the Jackson Laboratory 1996) (http://www.informatics.jax.org/greenbook/). In addition, the "GenitoUrinary Development Molecular Anatomy Project" (McMahon et al. 2008) (http://www.gudmap.org/) provides useful background information, while a dissection guide for the embryonic rodent urinary tract is also available (Staack et al. 2003). Here, we briefly review the gross anatomy of the rodent urinary tract and expand in more detail on a couple of aspects of rodent anatomy and development that may be of relevance if rodents are used as an animal model.

3.1.1 Gross Anatomy of the Kidney, Ureter, and Bladder

Rodent kidneys differ from their human counterparts in having a single renal papilla that is surrounded by a funnel-shaped calyx, whereas human kidneys are

multipapillate with major and minor calyces. A further difference exists in the more cranial location of the right kidney as compared with the left one in rodents, whereas the reverse is true of humans (Yokota et al. 2005). Interestingly, the right renal artery does have a more cranial origin from the aorta than the left one in all mammals (Yokota et al. 2005), indicating a similar level of origin from the aorta, but a different definitive location in humans.

The ureter begins at the renal pelvis and courses dorsal to the uterine horn or deferent duct from the kidney to the base of the bladder. The bladder occupies the lower (pelvic) portion of the abdomen, but can rise, when filling, into the main portion of the abdomen. Parts of the bladder that are often distinguished are the dome, the trigone at the base of the bladder between the entrance of both ureters craniolaterally and the deferent ducts caudomedially, and the bladder neck with the smooth internal urethral sphincter at the transition to the urethra.

3.1.2 Ureter, Vesicoureteral Junction, and Bladder Trigone

The upper urinary tract (UUT) derives from the ureteric bud, an epithelial tube that emerges from the caudal end of the Wolffian (or mesonephric) duct between embryonic day (ED) 10.5 and 11.5 in the mouse embryo. Its distal end grows into the adjacent kidney mesenchyme and forms the renal collecting duct, while its more proximal parts form the renal pelvis and the ureter [for review, see, e.g., Costantini (2006)]. Initially, the ureter and the Wolffian duct drain into the bladder via the "common nephric duct." According to the widely accepted, but never tested "ureteral bud theory" of Mackie and Stephens (1975), the wall of the common nephric duct would expand and unfold to become the bladder trigone (Fig. 4a, b) in such a way that the ureteric orifice becomes located craniolaterally in the wall of the base of the bladder and the orifice of the Wolffian (deferent) duct caudomedially in the dorsal wall of the intraprostatic part of the urethra at the level of the colliculus ("hill") seminalis. If the ureteral bud develops too cranially from the Wolffian duct, the common nephric duct does not become integrated in its entirety into the trigone and the ureter drains into the urethra. If, on the other hand, the ureteral bud develops too caudally from the Wolffian duct, the ureteric orifice does become located more caudally and medially in the trigone. The first condition often interferes with normal urine drainage, whereas the second condition is accompanied by urinary reflux due to too straight a course of the distal end of the ureter through the bladder wall (the valve mechanism of the vesicoureteral junction is largely dependent on the oblique course of the ureter through the bladder wall and the passive closure of the junction due to the intraluminal bladder pressure (Fig. 4a). If two ureteric buds develop on the same side, the upper usually drains into the urethra and the lower is inserted at its proper position.

The hypothesis of Mackie and Stephens confers a mesodermal origin to the trigonal wall, whereas the remaining bladder wall is of endodermal origin. Recently, very elegant work with Hoxb7-Gfp and Hoxb7-Cre transgenic mice, which specifically label the epithelial cells of the Wolffian duct and ureter, has

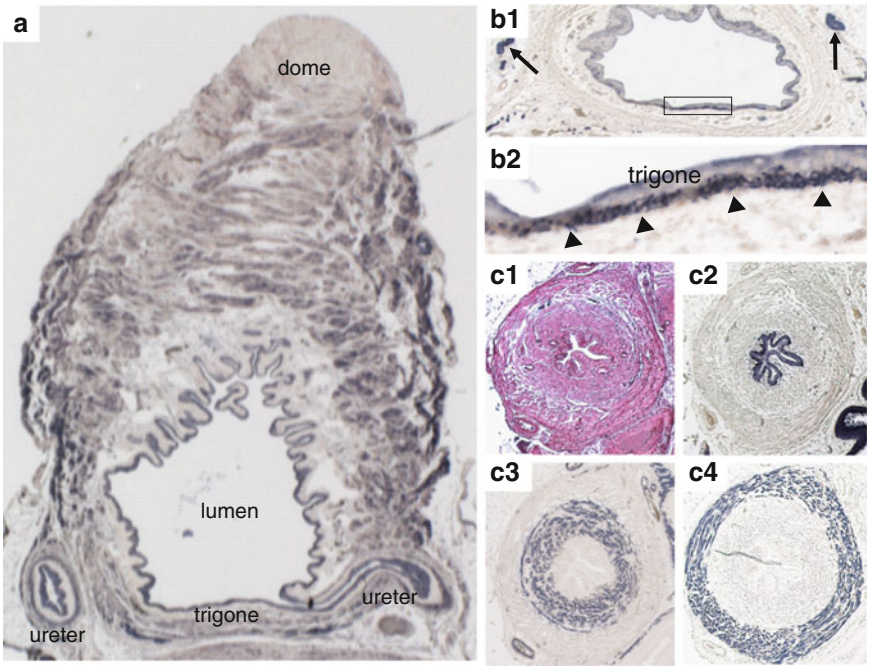

Fig. 4 Functional anatomy of the ureters, bladder, and urethral sphincter. Panel **a**: Frontal section through main axis of rat bladder, with the trigone and both ureters at the bottom and the bladder dome at the *top*. The urothelium and smooth muscle components are stained. Note the long intramural course of the ureter with the flap valve. The longitudinal muscle layer of the intramural portion of the ureter facilitates fluid discharge into the bladder lumen (Roshani et al. 1996). Panels **b**: Rich innervation of basal trigonal urothelium with neurofilament-positive fibers. Panel **b**2 is a magnification of the boxed area in panel **b**1. Arrows in **b**1 indicate nerves; arrowheads in **b**2 indicate neurofilament-positive plexus in epithelio-mesenchymal junction. Panels **c**: Serial sections of the urethral sphincter, stained with haematoxylin and azophloxin (**c**1), or with antisera identifying the urothelium (**c**2), the smooth muscular internal urethral sphincter (**c**3), and the striated external urethral sphincter (**c**4)

confirmed the insertion of the common nephric duct into the bladder wall, but has also shown that this tissue then regresses by programmed cell death (Viana et al. 2007; Mendelsohn 2009). The wall of the bladder trigone, therefore, does not differ in embryonic origin from the remaining part of the bladder wall, but remains unique because of its transient interaction with the cells of the common nephric duct.

These findings indicate that the site of sprouting of the ureteric bud is critical for its subsequent developmental fate and must be tightly regulated. The position where the ureteric bud emerges from the Wolffian duct is determined by the Gdnf/Ret signaling pathway (see (Murawski and Gupta 2006; 2008; Reidy and Rosenblum 2009) for review). Glial-derived neurotrophic factor (Gdnf) that is expressed in the mesenchyme along the Wolffian duct becomes restricted to the site of budding by a rather complex signaling cascade [reviewed by Bouchard (2004), Airik et al. (2006), Reidy and Rosenblum (2009)]. Gdnf promotes budding by binding to the

receptor tyrosine kinase Ret and the GPI-linked cell surface co-receptor Gfrα1 that are expressed in the epithelium of the mesonephric duct (reviewed in Costantini and Shakya 2006; Murawski and Gupta 2006). The resulting position of the ureteric bud is a quantitative trait (Murawski and Gupta 2008), in the sense that its location follows a continuous distribution and, consequently, that the intravesicular course of the ureter varies on both sides and between individuals, as does proneness to reflux. Mouse reflux models have abnormally located ureteric buds (Murawski and Gupta 2008), but reflux also develops if the ureteric orifice is enlarged due to, e.g., a uroplakin deficiency (Wu et al. 2009).

3.1.3 Urothelium

The smooth muscle layers that surround the ureter, bladder, and pelvic urethra need a signal (probably sonic hedgehog) from the overlying epithelium to differentiate from the mesenchyme. Remarkably, this signal remains expressed throughout life and is not specific for the urothelium (Cao et al. 2008). In contrast, the urothelium itself is highly specific for the urinary tract and covers the surface of the renal pelvis, ureter, bladder, and pelvic urethra proximal to the outlet of the deferent ducts in rodents. Urothelium, also known as "transitional epithelium," consists of a basal cell layer, one (in rodents) or more (in larger animals such as cattle and humans) intermediate cell layers, and a superficial cell layer. The "umbrella" cells that form the highly characteristic superficial layer of the urothelium do not extend to the basement membrane. Pseudostratified epithelium covers, instead, the collecting ducts and papilla of the kidney (Romih et al. 2005), that is, the most distal part of the ureteric bud (humans, however, differ from rodents in that the epithelium covering the papilla is covered with urothelium rather than pseudostratified epithelium). Although the urothelium turns over very slowly (turnover rate of ~200 days (Wu et al. 2009), progenitor cells are found throughout the urothelium. However, their density differs regionally and is approximately twofold higher in the bladder base than in the bladder dome (Nguyen et al. 2007).

Two physiological characteristics of the urothelium are its ability to accommodate stretch and its very high transepithelial electric resistance (Wu et al. 2009). The so-called urothelial "plaques" account for both properties. Urothelial plaques are concave-shaped two-dimensional crystalline structures that are 0.3–1 mm in diameter and occupy ~90% of the urothelial apical surface. The plaques are aggregates of hexameric 16-nm particles that, in turn, consist of four different uroplakin subunits. The plaques of asymmetric, outwardly concave unit membranes are joined by narrow "hinge" regions (Wu et al. 2009). Urothelia differ quantitatively at different anatomical sites (for review, see Romih et al. 2005). The ureteral urothelium has, for instance, a lower content of cytoplasmic fusiform vesicles and uroplakin proteins than the bladder urothelium (Wu et al. 2009). However, whether or not the urothelium differs qualitatively in different regions remains to be settled. The plaques also have clinical relevance, because they allow adherence of uropathogenic *Escherichia coli* to their surface (Wu et al. 2009).

The late fetal and early postnatal period of rodents is associated with intense remodeling of the lining of the bladder. In this period, programmed cell death, desquamation, and proliferation of the urothelium are much more active than at any other moment thereafter (Erman et al. 2001, 2006). Uroplakins and cytokeratin7 are permanently expressed in the urothelium, but the transition of cytokeratin18 to cytokeratin20 expression (Erman et al. 2006) and the appearance of polyploidy (Walker 1958) in the urothelial cells coincide with the onset of the remodeling period on the last prenatal day. Uroplakin-positive cells always co-express cytokeratin20 (Romih et al. 2005).

The urothelium expresses many types of receptors, including cholinergic receptors. These receptors have a sensory function and regulate the activity of the suburothelial nerve plexus and, thus, local changes in vascular perfusion and/or reflex bladder contractions (reviewed in Fowler et al. 2008]). The suburothelial nerve plexus is especially well developed in the trigone area (Fig. 4b).

3.1.4 Urethra

The urethra connects the bladder neck with the external world. Its anatomy and that of the accessory sex glands that drain into it are similar in different rodents (Pinheiro et al. 2003a). The rodent male urethra is usually divided into two portions, namely, the pelvic and the penile urethra. Both portions are connected at the penile bulb, a ventrocaudal diverticle of the urethra just distal to its passage through the pelvic floor. The pelvic urethra corresponds with the prostatic and membranous urethra in humans. The penile bulb in rodents is well developed and larger (relative to body size) than the human equivalent. The pelvic urethra of rodents is surrounded by the prostate, a layer of smooth muscle (which extends to and includes the bulb), and a well-developed striated urethral sphincter (Fig. 4c). The penile urethra and bulb are surrounded by the corpus spongiosum urethrae (Ciner et al. 1996) and supported by both corpora cavernosa dorsolaterally. While both corpora cavernosa appose in the pendular part of the penis, they separate near the penile bulb to curve laterally toward the ischial bone. As in humans, the urethra of rodents curves 35–40° ventrally during its passage through the prostate and again almost 90° after passing through the pelvic floor, just before entering the penile bulb (Pinheiro et al. 2003a). Since the latter location is also the narrowest (the so-called urethral isthmus), it forms the major obstacle to catheterizing or instrumenting the bladder in male animals. In rodents, a penile bone is present in the distal part of the septum separating both corpora cavernosa.

3.1.5 Urethral Epithelium

The urethra is covered with urothelium in its juxtavesicular portion, with stratified squamous epithelium at its external orifice on the penile glans, and with pseudostratified columnar epithelium in between. The female urethra corresponds to the

pelvic portion of the male urethra, with its external orifice located at the base of the clitoris. The pseudostratified columnar epithelium contains basal cells with staining properties of neurosecretory cells (Pinheiro et al. 2003b). Using Cre-Lox-mediated cell marking, it has now been definitively settled that the penile urethra develops in its entirety from the endodermal urethral plate, without material contribution of the ectoderm of the genital tubercle (Kurzrock et al. 1999; Seifert et al. 2008). The urethral plate is transformed into the urethral tube by the bilateral formation of "urethral folds," which fuse ventrally in a proximal-to-distal direction starting at the penile bulb (which has no homologue in the female). Defects of fusion explain the occurrence of different forms of hypospadias in males.

3.1.6 Urethral Glands

The pelvic urethra, including the urethral bulb, is surrounded by urethral glands, most of which are small and located immediately below the urethral epithelium inside the smooth muscle sheath (Hall 1936). The larger glands are the prostate and bulbourethral (Cowper's) glands [Skene's paraurethral (Shehata 1974; dos Santos et al. 2003) and Bartholin's (bulbourethral) glands, respectively, in the female]. The accessory glands of the urethra develop extensively in the perinatal period, with a strong quantitative influence of androgens (Cooke et al. 1987; Timms 2008). The ducts of the prostate and bulbourethral glands perforate the smooth muscle layer, but the paraurethral glands do not, because the lack of androgens allows the development of a continuous intervening smooth muscle layer in perinatal females (Thomson et al. 2002). The bulbourethral glands are located external to the pelvic floor and are covered by the striated bulbospongious muscle medially and the striated ischiocavernous muscle laterally. The ducts of the bulbourethral glands enter the urethra just proximal to the urethral bulb in the male and on the papilla of the urethral orifice in the female. The position of the orifice of these ducts is, therefore, a convenient landmark to delineate the exclusively male portion of the urethra.

The preputial (male) or clitoral (female) glands are sebaceous glands that lie in the subcutis and drain on the base of the preputial fossa. Their secretions confer the typical odor to especially male mouse urine and are said to function as territorial marker. It is somewhat confusing that both the peri-urethral glands and the sebaceous glands of the genital tubercle are associated with the name of Littré.

3.1.7 Prostate Gland

The rodent prostate (Fig. 5) lacks a well-developed capsule, so that it is difficult to discern it macroscopically at the neck of the bladder. The epithelium of the prostate glands is of the columnar type. Although the prostate glands drain via many ducts into the urethra, these ducts can be grouped as belonging to four lobes, i.e., a ventral, two lateral, and a dorsal lobe (Price 1963; Jesik et al. 1982; Timms 2008).

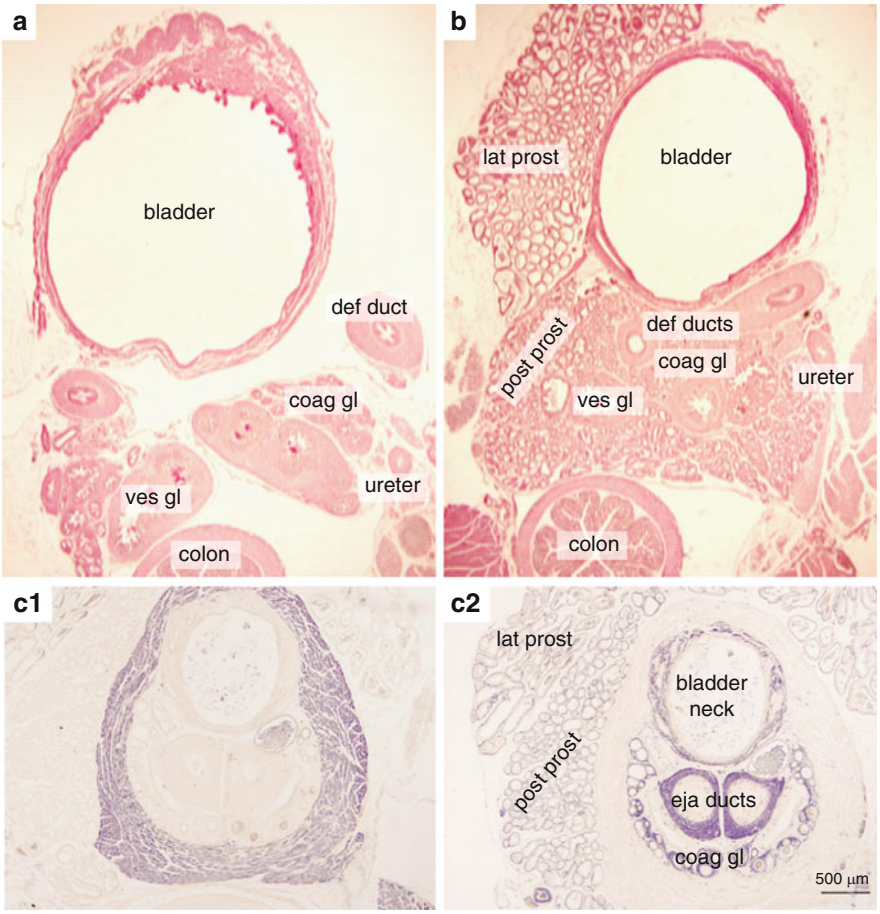

Fig. 5 Transverse sections through the bladder and bladder neck to demonstrate topography of accessory male glands in the rat. The *top* is ventral and the *bottom* (colon) dorsal. Between the bladder and the colon (panel **a**), the deferent ducts (def duct), coagulating glands (coag gl), vesicular glands (ves gl), and ureters are seen. In panel **b**, the lateral and posterior lobes of the prostate are also present. Panel **c** is a section through the bladder neck just superior to the entrance of the ejaculatory ducts (eja ducts) into the urethra. Panel **c**1 is stained for the presence of the striated external urethral sphincter, while panel **c**2 is stained for the presence of smooth muscle

Among these lobes, the dorsal and lateral lobes resemble each other more closely than the ventral lobe (Jesik et al. 1982). This difference may correspond to the differences in hormonal sensitivity and secretory properties of the mesenchyme surrounding the ventral lobe ("ventral mesenchymal pad") from that surrounding the dorsolateral parts of the developing prostate (Timms 2008). The initial development of the prostate gland occurs largely in the perinatal period (Thomson and Marker 2006; Timms 2008). Although large increases in size of the gland still occur thereafter, the number and position of the ductal openings into the prostate do not

change anymore (Price 1963). In agreement, the stem cells of the prostate have been localized in the proximal regions of the glandular ducts (Tsujimura et al. 2002; Leong et al. 2008).

Even though the developmental anatomy of the four lobes of the prostate is similar in rodents and humans, the nomenclature used in human clinical practice differs markedly. In human anatomy, a "central zone" of glands adjacent to the bladder neck and surrounding the ejaculatory ducts (the intraprostatic portions of the deferent ducts) is distinguished from a "peripheral zone" that surrounds the central zone dorsocaudally (McNeal 1968). The central zone therefore corresponds to the cranial portion of the dorsal lobe, while the peripheral lobe corresponds to the caudal dorsal and lateral lobes.

3.1.8 Vesicular and Coagulating Glands

The deferent duct (inside the prostate named the ejaculatory duct) drains into the urethra at what is called the colliculus seminalis. The large, whitish, semilunar-shaped vesicular gland (previously known as seminal vesicle) develops from the ampullary portion of the deferent duct (Pinheiro et al. 2003a) and extends dorso-laterally from the bladder. The vesicular gland drains into the urethra via the ejaculatory duct or, more rarely, independently just lateral to the ejaculatory ducts. The more translucent coagulating gland is found in the medial, concave border of the vesicular gland. This gland drains independently into the urethra between the colliculus seminalis and the bladder neck (Pinheiro et al. 2003a). The epithelium of the vesicular and coagulating glands (Fig. 5) is of the columnar type.

3.2 The Muscles of the Rodent LUT

3.2.1 Smooth Muscle

Smooth muscle surrounds the parts of the LUT that are covered with urothelium. In the renal pelvis, bundles of smooth muscle cells adjacent to the urothelium increase in numbers and thickness with increasing distance from the papillar base. Along the ureter, the smooth muscle layer forms an inner longitudinal and an outer circular layer. In larger animals, including humans, the third layer, which is typical for the bladder, becomes identifiable in the juxtavesical portion of the ureter. Smooth muscle also covers the pelvic portion of the urethra. The smooth muscle layers are not as regularly oriented as in the gut and differ in preferential orientation: the inner layer is mostly longitudinal in orientation and the outer circular.

In addition to the "typical" spindle-shaped smooth muscle cells, a further outer layer of atypical smooth muscle cells is found between the base of the renal papilla and the uretero-pelvic junction. These "interstitial cells" function as the pacemaker cells for pyeloureteric peristalsis (Lang et al. 2006). Similar interstitial cells form,

together with peripheral neurons, the myovesical plexus of the bladder wall, which appear to represent the functional contractile units of the bladder (Drake 2007). This suburothelial nerve plexus, which has some terminal fibers projecting into the urothelium, is particularly prominent at the bladder neck, but relatively sparse at the dome of the bladder (Fowler et al. 2008). The urothelium, the suburothelial regular and interstitial smooth muscle cells, and the suburothelial nerve plexus are hypothesized to function as a stretch-receptor organ [reviewed in Fowler et al. (2008)]

3.2.2 Urethral Sphincter

The general topography of the male and female urethral sphincter (of the rat) is similar in that the urethral epithelium is surrounded by a submucous layer containing a well-developed venous plexus and smooth internal urethral sphincter muscle, while the striated external urethral sphincter muscle forms the outer layer (Figs. 4c and 5c). The prostate glands are not covered by striated muscle (Fig. 5a, b) (Bierinx and Sebille 2006). This was also observed in humans (Wallner et al. 2009b). Striated muscle covers the remaining part of the pelvic urethra in the male (Bierinx and Sebille 2006) and the lower 60–70% of the urethra in the female (Kim et al. 2007). The striated urethral sphincter is circular in its upper part with only a fibrous raphe connecting both sides on its dorsal aspect. A ventral fibrous tissue strip connecting the left- and a right-sided portion of the urethral sphincter (Bierinx and Sebille 2006) was never observed by us in sections of either rat or mouse. In the female rat, the lower part differs from the upper part in that it has dorsolateral wings in its lower part that cover the vagina laterally (Praud et al. 2003; Kim et al. 2007). Our findings on the anatomy of the male and female urethral sphincter in fetal and neonatal mice confirm the description in the rat. In humans, the lower part of the external urethral sphincter forms the so-called urethrovaginal" sphincter (Wallner et al. 2009a). The additional third fascicle of the urethral sphincter described by Biérinx c.s. (Bierinx and Sebille 2006) is, in our opinion, the ischiourethral muscle (see section "Anatomy of the perineal muscles").

3.3 The Pelvic Floor Muscles in the Mouse

3.3.1 The Bauplan

The main pelvic floor muscles in humans (LAM and coccygeal muscle) are homologous with the tail-moving muscles in quadruped vertebrates (Schimpf and Tulikangas 2005). In humans, upright walking and the tail remnant lashed between the legs no longer allows isotonic contractions of these muscles (with the exception of the puborectal muscle). This new configuration apparently changed the function of these muscles adaptively toward support of the pelvic organs instead and to assist the connective tissue in this role. Such a support function could, however, also be

performed in an more energy-efficient way by connective tissue fascia (McNeill Alexander 2002), which may explain why there is a relatively strong development of connective tissue support in the pelvic floor as compared with the weaker development of pelvic floor musculature in humans (Stewart 1984; Abitbol 1988; Schimpf and Tulikangas 2005).

The human LAM is composed of the pubococcygeal, the iliococcygeal, and the puborectal muscle. The coccygeal muscle is also part of the human pelvic floor. The muscles in rodents that are homologous to the human pubococcygeal, iliococcygeal, and coccygeal muscles are the pubocaudal, iliocaudal, and coccygeal muscles (Fig. 6) (Bremer et al. 2003). The pubocaudal, iliocaudal, and coccygeal muscles are functionally tail muscles and attach to the medial surface of the bony pelvis at one end and via long tendons to the tail vertebrae on the other hand. The pubocaudal and iliocaudal muscles develop as separate muscle units but fuse during later development. The muscles can then only be distinguished by their separate attachments to the superior portion of the pubic bone (pubocaudal muscle) and the ilium (iliocaudal muscle; Fig 6b). The muscles are termed "-caudal" because of the attachment to the caudal (tail) vertebrae. The coccygeal muscle is termed "coccygeal" in homology to the muscle in humans. It is, however, still attached to the tail vertebrae. A puborectal portion of the pubocaudal muscle, which forms a sling around the rectum (homologous to the human puborectal muscle), cannot, however, be identified in the rodents.

3.3.2 Anatomy of the Perineal Muscles

The ischiocavernous and bulbospongious muscles are topographically separated from the pubo- and iliocaudal muscles (Fig. 6). The development of the ischiocavernous and bulbospongious muscles is androgen-dependent in rodents (Hayes 1965; Cihak et al. 1967, 1970; Sachs 1982; Holmes and Sachs 1994; Joubert et al. 1994; Nnodim 1999; Yiou et al. 2001), that is, the muscles develop in both sexes, but regress postnatally in the female. In the male, the bulbospongious muscle consists of two pairs of muscle bellies and encircles both the urethra and the rectum (Fig. 6d–f). The ventral muscle bellies enclose the bulb of the penis, while the dorsal muscle belly (dorsal bulbospongious muscle) forms a sling around the dorsal aspect of the rectum. Erroneously (Poortmans and Wyndaele 1998), the dorsal portion of the bulbospongious muscle in rodents has previously been called the "levator ani muscle" (Hayes 1965; Cihak et al. 1967, 1970; Sachs 1982; Holmes and Sachs 1994; Joubert et al. 1994; Nnodim 1999; Yiou et al. 2001). The muscle is, however, not homologous to the human LAM but to the dorsal component of the bulbospongious muscle, which, like the ventral part of the bulbospongious muscle, is exquisitely androgen-dependent and regresses in the female due to the very low concentration of circulating androgens (Joubert et al. 1994).

The prenatal development of ventral and dorsal portions of the bulbospongious muscle is similar in the male and female. However, already in 7-day-old female mice (equivalent to a human neonate) the muscles have become strongly reduced in

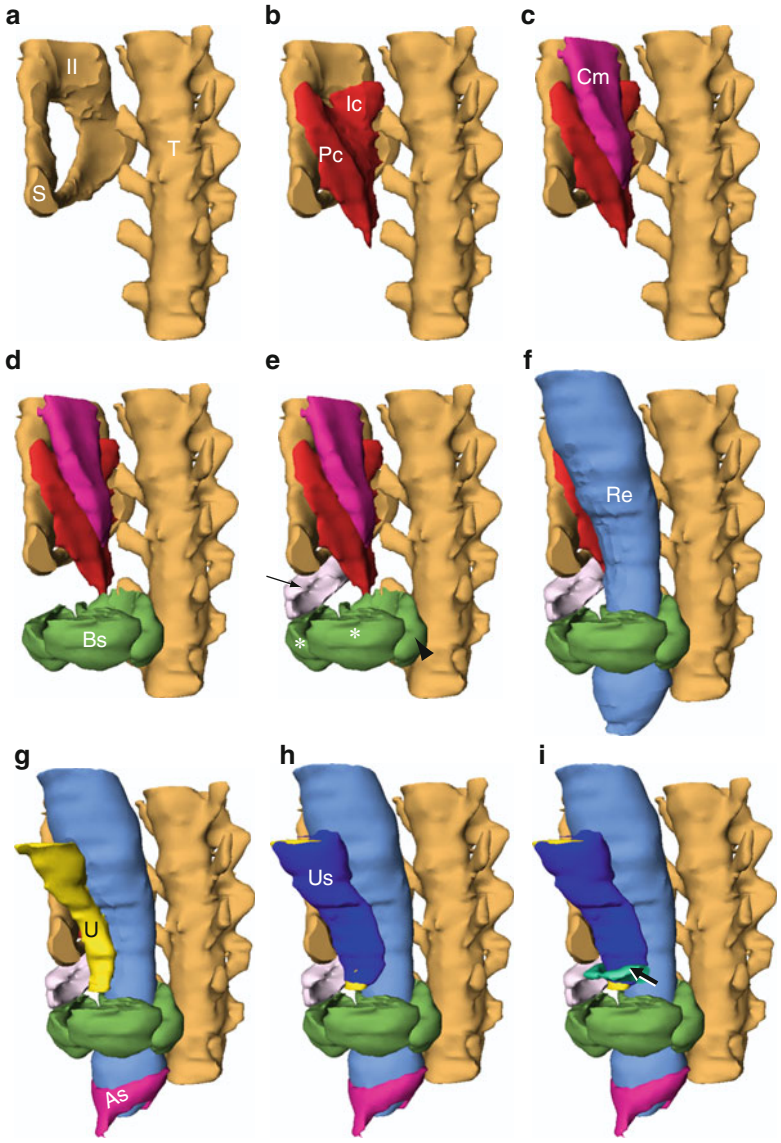

Fig. 6 3D reconstruction of 7-day-old male mouse pelvis in an oblique view from the front/left side. Note that the pubo- and iliocaudal muscle are fused (**b**). The bulbospongious muscle has two ventral bellies (*asterisks*) and one dorsal belly (*arrowhead*; **e**). The ischiocavernous and ischiourethral muscles are illustrated by *arrows* in **e** and **i**, respectively. *As* external anal sphincter muscle, *Bs* bulbospongious muscle, *Cm* right side coccygeal muscle, *Ic* right side iliocaudal muscle, *Il* right side ilium, *Pc* right side pubocaudal muscle, *Re* rectum, *U* urethra, *Us* external urethral sphincter muscle

size: the volume of the female bulbospongious muscle at that age is only 6% of the male bulbospongious muscle. For comparison, the volume of the non-androgen-dependent iliocaudal in the female is 95% of that in a male of the same age. Similarly, the ischiocavernous muscle regresses in the female (Yiou et al. 2001).

The ischiourethral muscle (Fig. 6i) lies ventral to the urethra and spanning itself between the left and right ischiocavernosus muscles. It forms a sling around the ventral aspect of the deep dorsal vein of the clitoris/penis, ventrally. The muscle presumably has a sexual role, by contributing to intense erection by compressing the deep dorsal vein (Dail and Sachs 1991). This muscle does not exist in humans.

The external anal sphincter is a completely circular muscle surrounding the anorectum. The muscular tissue of the external anal sphincter has no muscular connections to the muscles of the pelvic floor, i.e., the bulbospongious muscle (including the dorsal bulbospongious muscle) or the ischiocavernous muscle (Fig. 6g).

The pubocaudal, iliocaudal, and coccygeal muscles are all innervated directly by nerve branches from the sacral plexus on their medial surface (Bremer et al. 2003). These nerve branches are, therefore, homologous to the levator ani nerve in the human. The somatic nerve supply to the bulbospongious and ischiocavernous muscles, and to the striated urethral and anal sphincter muscles, occurs through the pudendal nerve that courses inferior to the pubocaudal, iliocaudal, and coccygeal muscles. In humans, the pudendal nerve contributes to innervation of the LAM in about 50% of cases.

References

Abitbol MM (1988) Evolution of the ischial spine and of the pelvic floor in the hominoidea. Am J Phys Anthropol 75:53–67

Airik R, Bussen M, Singh MK, Petry M, Kispert A (2006) Tbx18 regulates the development of the ureteral mesenchyme. J Clin Invest 116:663–674

Albers DD, Faulkner KK, Cheatham WN, Elledge EF, Coalson RE (1973) Surgical anatomy of the pubovesical (puboprostatic) ligaments. J Urol 109:388–392

Alhasso A, Glazener CM, Pickard R, N'dow J (2005) Adrenergic drugs for urinary incontinence in adults. Cochrane Database Syst Rev; CD001842

An SS, Fredberg JJ (2007) Biophysical basis for airway hyperresponsiveness. Can J Physiol Pharmacol 85:700–714

Ashton-Miller JA, DeLancey JO (2007) Functional anatomy of the female pelvic floor. Ann NY Acad Sci 1101:266–296

Aukee P, Usenius JP, Kirkinen P (2004) An evaluation of pelvic floor anatomy and function by MRI. Eur J Obstet Gynecol Reprod Biol 112:84–88

Ballard DJ (1997) Treatment of erectile dysfunction: can pelvic muscle exercises improve sexual function? J Wound Ostomy Continence Nurs 24:255–264

Bharucha AE (2003) Fecal incontinence. Gastroenterology 124:1672–1685

Bierinx AS, Sebille A (2006) The urethral striated sphincter in adult male rat. Anat Embryol (Berl) 211:435–441

Bosse Y, Sobieszek A, Pare PD, Seow CY (2008) Length adaptation of airway smooth muscle. Proc Am Thorac Soc 5:62–67

Bouchard M (2004) Transcriptional control of kidney development. Differentiation 72:295–306
Boyles SH, Weber AM, Meyn L (2003a) Procedures for pelvic organ prolapse in the United States, 1979–1997. Am J Obstet Gynecol 188:108–115
Boyles SH, Weber AM, Meyn L (2003b) Procedures for urinary incontinence in the United States, 1979–1997. Am J Obstet Gynecol 189:70–75
Bremer RE, Barber MD, Coates KW, Dolber PC, Thor KB (2003) Innervation of the levator ani and coccygeus muscles of the female rat. Anat Rec A Discov Mol Cell Evol Biol 275A:1031–1041
Brooks SV, Zerba E, Faulkner JA (1995) Injury to muscle fibres after single stretches of passive and maximally stimulated muscles in mice. J Physiol 488(Pt 2):459–469
Cao M, Liu B, Cunha G, Baskin L (2008) Urothelium patterns bladder smooth muscle location. Pediatr Res 64:352–357
Cihak R, Gutmann E, Hanzlikova V (1967) Morphological, physiological characteristics, development and homology of the m. levator ani of the rat. Anat Anz 120:492–506
Cihak R, Gutmann E, Hanzlikova V (1970) Involution and hormone-induced persistence of the *M. sphincter* (levator) ani in female rats. J Anat 106:93–110
Ciner M, van Vorstenbosch CJ, Dijkstra G, van den Hurk R (1996) Penile bulb and its relationship with the pelvic urethra and the penile urethra in the rat: light and scanning electron microscopical observations. Anat Rec 244:452–469
Cooke PS, Young PF, Cunha GR (1987) Androgen dependence of growth and epithelial morphogenesis in neonatal mouse bulbourethral glands. Endocrinology 121:2153–2160
Costantini F (2006) Renal branching morphogenesis: concepts, questions, and recent advances. Differentiation 74:402–421
Costantini F, Shakya R (2006) GDNF/Ret signaling and the development of the kidney. Bioessays 28:117–127
Coyne KS, Sexton CC, Thompson CL, Milsom I, Irwin D, Kopp ZS, Chapple CR, Kaplan S, Tubaro A, Aiyer LP, Wein AJ (2009) The prevalence of lower urinary tract symptoms (LUTS) in the USA, the UK and Sweden: results from the Epidemiology of LUTS (EpiLUTS) study. BJU Int 104:352–360
Dail WG, Sachs BD (1991) The ischiourethralis muscle of the rat - anatomy innervation, and function. Anat Rec 229:203–208
D'Aulignac D, Martins JAC, Pires EB, Mascarenhas T, Natal Jorge RM (2005) A shell finite element model of the pelvic floor muscles. Comput Meth Biomech Biomed Eng 8:339–347
De Caro R, Aragona F, Herms A, Guidolin D, Brizzi E, Pagano F (1998) Morphometric analysis of the fibroadipose tissue of the female pelvis. J Urol 160:707–713
DeLancey JOL (1986) Correlative study of paraurethral anatomy. Obstet Gynecol 68:91–97
DeLancey JOL (1988) Structural aspects of the extrinsic continence mechanism. Obstet Gynecol 72:296–301
DeLancey JOL (2005) The hidden epidemic of pelvic floor dysfunction: achievable goals for improved prevention and treatment. Am J Obstet Gynecol 192:1488–1495
DeLancey JOL, Hurd WW (1998) Size of the urogenital hiatus in the levator ani muscles in normal women and women with pelvic organ prolapse. Obstet Gynecol 91:364–368
DeLancey JOL, Kearney R, Chou Q, Speights S, Binno S (2003) The appearance of levator ani muscle abnormalities in magnetic resonance images after vaginal delivery. Obstet Gynecol 101:46–53
DeLancey JOL, Morgan DM, Fenner DE, Kearney R, Guire K, Miller JM, Hussain H, Umek W, Hsu Y, Ashton-Miller JA (2007) Comparison of levator ani muscle defects and function in women with and without pelvic organ prolapse. Obstet Gynecol 109:295–302
Dietz HP, Lanzarone F (2005) Levator trauma after vaginal delivery. Obstet Gynecol 106:707–712
Dietz H, Simpson J (2008) Levator trauma is associated with pelvic organ prolapse. BJOG 115:979–984
Dorschner W, Biesold M, Schmidt F, Stolzenburg JU (1999) The dispute-about the external sphincter and the urogenital diaphragm. J Urol 162:1942–1945

dos Santos FC, Carvalho HF, Goes RM, Taboga SR (2003) Structure, histochemistry, and ultrastructure of the epithelium and stroma in the gerbil (*Meriones unguiculatus*) female prostate. Tissue Cell 35:447–457

Drake MJ (2007) The integrative physiology of the bladder. Ann R Coll Surg Engl 89:580–585

Erman A, Jezernik K, Stiblar-Martincic D, Romih R, Veranic P (2001) Postnatal restoration of the mouse urinary bladder urothelium. Histochem Cell Biol 115:309–316

Erman A, Veranic P, Psenicnik M, Jezernik K (2006) Superficial cell differentiation during embryonic and postnatal development of mouse urothelium. Tissue Cell 38:293–301

Fowler CJ, Griffiths D, de Groat WC (2008) The neural control of micturition. Nat Rev Neurosci 9:453–466

Francis CC (1952) The human pelvis. Mosby, St. Louis, MO

Fritsch H, Pinggera GM, Lienemann A, Mitterberger M, Bartsch G, Strasser H (2006) What are the supportive structures of the female urethra? Neurourol Urodyn 25:128–134

Haddad S, Restieri C, Krishnan K (2001) Characterization of age-related changes in body weight and organ weights from birth to adolescence in humans. J Toxicol Environ Health A 64:453–464

Haderer JM, Pannu HK, Genadry R, Hutchins GM (2002) Controversies in female urethral anatomy and their significance for understanding urinary continence: observations and literature review. Int Urogynecol J 13:236–252

Hall K (1936) The structure and development of the urethral sinus in the male white mouse, with notes on its occurrence in other rodents. J Anat 70(413–428):411

Handa VL, Harris TA, Ostergard DR (1996) Protecting the pelvic floor: obstetric management to prevent incontinence and pelvic organ prolapse. Obstet Gynecol 88:470–478

Hayes KJ (1965) The so-called 'levator ani' of the rat. Acta Endocrinol 48:337–347

Holmes GM, Sachs BD (1994) Physiology and mechanics of rat levator ani muscle - evidence for a sexual function. Physiol Behav 55:255–266

Hoyte L, Damaser MS, Warfield SK, Chukkapalli G, Majumdar A, Choi DJ, Trivedi A, Krysl P (2008) Quantity and distribution of levator ani stretch during simulated vaginal childbirth. Am J Obstet Gynecol 199(2):198.e1–e5

Hoyte L, Jakab M, Warfield SK, Shott S, Flesh G, Fielding JR (2004) Levator ani thickness variations in symptomatic and asymptomatic women using magnetic resonance-based 3-dimensional color mapping. Am J Obstet Gynecol 191:856–861

Hoyte L, Schierlitz L, Zou K, Flesh G, Fielding JR (2001) Two- and 3-dimensional MRI comparison of levator ani structure, volume, and integrity in women with stress incontinence and prolapse. Am J Obstet Gynecol 185:11–19

Hunskaar S, Burgio K, Diokno A, Herzog AR, Hjalmas K, Lapitan MC (2003) Epidemiology and natural history of urinary incontinence in women. Urology 62:16–23

Hutch JA, Rambo ON (1967) A new theory of the anatomy of the internal urinary sphincter and the physiology of micturition. 3. Anatomy of the urethra. J Urol 97:696–704

Janda S, van der Helm FCT, de Blok SB (2003) Measuring morphological parameters of the pelvic floor for finite element modelling purposes. J Biomech 36:749–757

Jelovsek JE, Maher C, Barber MD (2007) Pelvic organ prolapse. Lancet 369:1027–1038

Jesik CJ, Holland JM, Lee C (1982) An anatomic and histologic study of the rat prostate. Prostate 3:81–97

Jones NHJR, Healy JC, King LJ, Saini S, Shousha S, Allen-Mersh TG (2003) Pelvic connective tissue resilience decreases with vaginal delivery, menopause and uterine prolapse. Br J Surg 90:466–472

Joubert Y, Tobin C, Lebart MC (1994) Testosterone-induced masculinization of the rat levator ani muscle during puberty. Dev Biol 162:104–110

Kato M, Matsubara A, Murakami G, Abe SI, Ide Y, Sato I, Usui T (2008) Female perineal membrane: a study using pelvic floor semiserial sections from elderly nulliparous and multiparous women. Int Urogynecol J Pelvic Floor Dysfunct 19(12):1663–1670

Kaye KW, Milne N, Creed K, van der Werf B (1997) The 'urogenital diaphragm', external urethral sphincter and radical prostatectomy. Aust N Z J Surg 67:40–44
Kim RJ, Kerns JM, Liu S, Nagel T, Zaszczurynski P, Lin DL, Damaser MS (2007) Striated muscle and nerve fascicle distribution in the female rat urethral sphincter. Anat Rec (Hoboken) 290:145–154
Kirschner-Hermanns R, Wein B, Niehaus S, Schaefer W, Jakse G (1993) The contribution of magnetic resonance imaging of the pelvic floor to the understanding of urinary incontinence. Br J Urol 72:715–718
Kurzrock EA, Baskin LS, Cunha GR (1999) Ontogeny of the male urethra: theory of endodermal differentiation. Differentiation 64:115–122
Lang RJ, Tonta MA, Zoltkowski BZ, Meeker WF, Wendt I, Parkington HC (2006) Pyeloureteric peristalsis: role of atypical smooth muscle cells and interstitial cells of Cajal-like cells as pacemakers. J Physiol 576:695–705
LeBeau AM, Singh P, Isaacs JT, Denmeade SR (2009) Prostate-specific antigen is a "chymotrypsin-like" serine protease with unique P1 substrate specificity. Biochemistry 48:3490–3496
Leong KG, Wang BE, Johnson L, Gao WQ (2008) Generation of a prostate from a single adult stem cell. Nature 456:804–808
Lien KC, Mooney B, DeLancey JOL, Ashton-Miller JA (2004) Levator ani muscle stretch induced by simulated vaginal birth. Obstet Gynecol 103:31–40
Mackie GG, Stephens FD (1975) Duplex kidneys: a correlation of renal dysplasia with position of the ureteral orifice. J Urol 114:274–280
Madoff RD, Parker SC, Varma MG, Lowry AC (2004) Faecal incontinence in adults. Lancet 364:621–632
Margulies RU, Huebner M, DeLancey JOL (2007) Origin and insertion points involved in levator ani muscle defects. Am J Obstet Gynecol 196:251
Martins JA, Pato MP, Pires EB, Jorge RM, Parente M, Mascarenhas T (2007) Finite element studies of the deformation of the pelvic floor. Ann NY Acad Sci 1101:316–334
McMahon AP, Aronow BJ, Davidson DR, Davies JA, Gaido KW, Grimmond S, Lessard JL, Little MH, Potter SS, Wilder EL, Zhang P (2008) GUDMAP: the genitourinary developmental molecular anatomy project. J Am Soc Nephrol 19:667–671
McNeal JE (1968) Regional morphology and pathology of the prostate. Am J Clin Pathol 49:347–357
McNeal JE (1988) Normal histology of the prostate. Am J Surg Pathol 12:619–633
McNeal JE, Redwine EA, Freiha FS, Stamey TA (1988) Zonal distribution of prostatic adenocarcinoma. Correlation with histologic pattern and direction of spread. Am J Surg Pathol 12:897–906
McNeill Alexander R (2002) Tendon elasticity and muscle function. Comp Biochem Physiol A Mol Integr Physiol 133:1001–1011
Mendelsohn C (2009) Using mouse models to understand normal and abnormal urogenital tract development. Organogenesis 5:306–314
Milsom I (2009) Lower urinary tract symptoms in women. Curr Opin Urol 19:337–341
Mirilas P, Skandalakis JE (2004) Urogenital diaphragm: an erroneous concept casting its shadow over the sphincter urethrae and deep perineal space. J Am Coll Surg 198:279–290
Murawski IJ, Gupta IR (2006) Vesicoureteric reflux and renal malformations: a developmental problem. Clin Genet 69:105–117
Murawski IJ, Gupta IR (2008) Gene discovery and vesicoureteric reflux. Pediatr Nephrol 23:1021–1027
Myers RP, Cheville JC, Pawlina W (2010) Making anatomic terminology of the prostate and contiguous structures clinically useful: historical review and suggestions for revision in the 21st century. Clin Anat 23:18–29
Nakajima F, Takenaka A, Uchiyama E, Hata F, Suzuki D, Murakami G (2007) Macroscopic and histotopographic study of the deep transverse perineal muscle (musculus transversus perinei profundus) in elderly Japanese. Ann Anat 189:65–74

Nguyen MM, Lieu DK, deGraffenried LA, Isseroff RR, Kurzrock EA (2007) Urothelial progenitor cells: regional differences in the rat bladder. Cell Prolif 40:157–165
Nnodim JO (1999) Quantitative study of the effects of denervation and castration on the levator ani muscle of the rat. Anat Rec 255:324–333
Norton P, Brubaker L (2006) Urinary incontinence in women. Lancet 367:57–67
Olsen AL, Smith VJ, Bergstrom JO, Colling JC, Clark AL (1997) Epidemiology of surgically managed pelvic organ prolapse and urinary incontinence. Obstet Gynecol 89:501–506
Padda BS, Jung SA, Pretorius D, Nager CW, Den-Boer D, Mittal RK (2007) Effects of pelvic floor muscle contraction on anal canal pressure. Am J Physiol Gastrointest Liver Physiol 292: G565–G571
Parente MP, Jorge RM, Mascarenhas T, Fernandes AA, Martins JA (2007) Deformation of the pelvic floor muscles during a vaginal delivery. Int Urogynecol J Pelvic Floor Dysfunct. doi:10.1007/s00192-007-0388-7
Petros P (1998) The pubourethral ligaments–an anatomical and histological study in the live patient. Int Urogynecol J 9:147–154
Pinheiro PF, Almeida CC, Segatelli TM, Martinez M, Padovani CR, Martinez FE (2003a) Structure of the pelvic and penile urethra–relationship with the ducts of the sex accessory glands of the Mongolian gerbil (*Meriones unguiculatus*). J Anat 202:431–444
Pinheiro PF, Almeida CC, Segatelli TM, Martinez M, Padovani CR, Martinez FE (2003b) Ultrastructure of the urethra of the Mongolian gerbil. World J Urol 20:378–384
Pit MJ, De Ruiter MC, Nijeholt AABL, Marani E, Zwartendijk J (2003) Anatomy of the arcus tendineus fasciae pelvis in females. Clin Anat 16:131–137
Poortmans A, Wyndaele JJ (1998) M. Levator ani in the rat: does it really lift the anus? Anat Rec 251:20–27
Powell MS, Li R, Dai H, Sayeeduddin M, Wheeler TM, Ayala GE (2005) Neuroanatomy of the normal prostate. Prostate 65:52–57
Praud C, Sebe P, Mondet F, Sebille A (2003) The striated urethral sphincter in female rats. Anat Embryol (Berl) 207:169–175
Price D (1963) Comparative aspects of development and structure in the prostate. Natl Cancer Inst Monogr 12:1–27
Rahn DD, Marinis SI, Schaffer JI, Corton MM (2006) Anatomical path of the tension-free vaginal tape: reassessing current teachings. Am J Obstet Gynecol 195:1809–1813
Reidy KJ, Rosenblum ND (2009) Cell and molecular biology of kidney development. Semin Nephrol 29:321–337
Reisenauer C, Shiozawa T, Oppitz M, Busch C, Kirschniak A, Fehm T, Drews U (2008) The role of smooth muscle in the pathogenesis of pelvic organ prolapse–an immunohistochemical and morphometric analysis of the cervical third of the uterosacral ligament. Int Urogynecol J Pelvic Floor Dysfunct 19:383–389
Roehrborn CG (2008) The utility of serum prostatic-specific antigen in the management of men with benign prostatic hyperplasia. Int J Impot Res 20:S19–S26
Romih R, Korosec P, de Mello W Jr, Jezernik K (2005) Differentiation of epithelial cells in the urinary tract. Cell Tissue Res 320:259–268
Roshani H, Dabhoiwala NF, Verbeek FJ, Lamers WH (1996) Functional anatomy of the human ureterovesical junction. Anat Rec 245:645–651
Sachs BD (1982) Role of striated penile muscles in penile reflexes, copulation, and induction of pregnancy in the rat. J Reprod Fertil 66:433–443
Sampson N, Untergasser G, Plas E, Berger P (2007) The ageing male reproductive tract. J Pathol 211:206–218
Sanchez-Visconti G, Herrero L, Rabadan M, Pereira I, Ruiz-Torres A (1995) Ageing and prostate: age-related changes in androgen receptors of epithelial cells from benign hypertrophic glands compared with cancer. Mech Ageing Dev 82:19–29
Schimpf M, Tulikangas P (2005) Evolution of the female pelvis and relationships to pelvic organ prolapse. Int Urogynecol J 16:315–320

Seifert AW, Harfe BD, Cohn MJ (2008) Cell lineage analysis demonstrates an endodermal origin of the distal urethra and perineum. Dev Biol 318:143–152
Shehata R (1974) Urethral glands in the wall of the female urethra of rats, mice and closely related rodents. Acta Anat (Basel) 90:381–387
Snooks SJ, Badenoch DF, Tiptaft RC, Swash M (1985) Perineal nerve damage in genuine stress urinary incontinence. An electrophysiological study. Br J Urol 57:422–426
Snooks SJ, Barnes PRH, Swash M (1984) Damage to the innervation of the voluntary anal and periurethral sphincter musculature in incontinence: an electrophysiological study. J Neurol Neurosurg Psychiatry 47:1269–1273
Spitzer V, Ackerman MJ, Scherzinger AL, Whitlock D (1996) The visible human male: a technical report. J Am Med Inform Assoc 3:118–130
Staack A, Donjacour AA, Brody J, Cunha GR, Carroll P (2003) Mouse urogenital development: a practical approach. Differentiation 71:402–413
Stein TA, DeLancey JOL (2008) Structure of the perineal membrane in females: gross and microscopic anatomy. Obstet Gynecol 111:686–693
Stewart DB (1984) The pelvis as a passageway. I. Evolution and adaptations. Br J Obstet Gynaecol 91:611–617
Stoker J, Rociu E, Bosch JLHR, Messelink EJ, van der Hulst VPM, Groenendijk AG, Eijkemans MJC, Lameris JS (2003) High-resolution endovaginal MR imaging in stress urinary incontinence. Eur Radiol 13:2031–2037
Stoker J, Wallner C (2008) The anatomy of the pelvic floor and sphincters. Imaging pelvic floor disorders. In: Stoker J, Taylor SA, DeLancey JOL (eds) Springer, Berlin, Heidelberg, New York, pp 1–29
Suzme R, Yalcin O, Gurdol F, Gungor F, Bilir A (2007) Connective tissue alterations in women with pelvic organ prolapse and urinary incontinence. Acta Obstet Gynecol Scand 86:882–888
The Staff of the Jackson Laboratory (1996) Biology of the laboratory mouse. Dover, New York
Thomson AA, Timms BG, Barton L, Cunha GR, Grace OC (2002) The role of smooth muscle in regulating prostatic induction. Development 129:1905–1912
Thomson AA, Marker PC (2006) Branching morphogenesis in the prostate gland and seminal vesicles. Differentiation 74:382–392
Timms BG (2008) Prostate development: a historical perspective. Differentiation 76:565–577
Tokar EJ, Ancrile BB, Cunha GR, Webber MM (2005) Stem/progenitor and intermediate cell types and the origin of human prostate cancer. Differentiation 73:463–473
Tsujimura A, Koikawa Y, Salm S, Takao T, Coetzee S, Moscatelli D, Shapiro E, Lepor H, Sun TT, Wilson EL (2002) Proximal location of mouse prostate epithelial stem cells: a model of prostatic homeostasis. J Cell Biol 157:1257–1265
Tunn R, Paris S, Fischer W, Hamm B, Kuchinke J (1998) Static magnetic resonance imaging of the pelvic floor muscle morphology in women with stress urinary incontinence and pelvic prolapse. Neurourol Urodyn 17:579–589
Viana R, Batourina E, Huang H, Dressler GR, Kobayashi A, Behringer RR, Shapiro E, Hensle T, Lambert S, Mendelsohn C (2007) The development of the bladder trigone, the center of the anti-reflux mechanism. Development 134:3763–3769
Von Hayek H (1969) Das Bindegewebe und die glatte Muskulatur des Beckenbodens. In: Alken CE, Dix VW, Goodwin WE, Wildbolz E (eds) Handbuch der Urologie - 1. Anatomie und Embryologie. Springer, Berlin, Heidelberg, New York, pp 279–313
Wald A (2007) Fecal incontinence in adults. N Engl J Med 356:1648–1655
Walker BE (1958) Polyploidy and differentiation in the transitional epithelium of mouse urinary bladder. Chromosoma 9:105–118
Walker W (1995) Anatomy and dissection. WH Freeman, New York
Wallner C (2008) Development of the pelvic floor - implications for clinical anatomy. PhD thesis, University of Amsterdam, The Netherlands
Wallner C, Maas CP, Dabhoiwala NF, Lamers WH, DeRuiter MC (2006a) Evidence for the innervation of the puborectalis muscle by the levator ani nerve. Neurogastroenterol Motil 18:1121–1122

Wallner C, Maas CP, Dabhoiwala NF, Lamers WH, DeRuiter MC (2006b) The innervation of the pelvic floor muscles: a reappraisal for the levator ani nerve. Obstet Gynecol 108:529–534

Wallner C, Lange MM, Bonsing BA, Maas CP, Wallace CN, Dabhoiwala NF, Rutten HJ, Lamers WH, DeRuiter MC, van de Velde CJH (2008a) Causes of fecal and urinary incontinence after total mesorectal excision for rectal cancer based on cadaveric surgery: a study from the cooperative clinical investigators of the Dutch Total Mesorectal Excision Trial. J Clin Oncol 26:4466–4472

Wallner C, van Wissen J, Maas CP, Dabhoiwala NF, DeRuiter MC, Lamers WH (2008b) The contribution of the levator ani nerve and the pudendal nerve to the innervation of the levator ani muscles; a study in human fetuses. Eur Urol 54:1136–1142

Wallner C, Dabhoiwala NF, DeRuiter MC, Lamers WH (2009a) The anatomical components of urinary continence. Eur Urol 55:932–944

Wallner C, Dabhoiwala NF, DeRuiter MC, Lamers WH (2009b) Smooth-muscle anchors the pelvic organs in the pelvic floor

Well D, Yang H, Houseni M, Iruvuri S, Alzeair S, Sansovini M, Wintering N, Alavi A, Torigian DA (2007) Age-related structural and metabolic changes in the pelvic reproductive end organs. Semin Nucl Med 37:173–184

Wilson PD, Dixon JS, Brown AD, Gosling JA (1983) Posterior pubo-urethral ligaments in normal and genuine stress incontinent women. J Urol 130:802–805

Wu XR, Kong XP, Pellicer A, Kreibich G, Sun TT (2009) Uroplakins in urothelial biology, function, and disease. Kidney Int 75:1153–1165

Xue Y, van der Laak J, Smedts F, Schoots C, Verhofstad A, de la Rosette J, Schalken J (2000) Neuroendocrine cells during human prostate development: does neuroendocrine cell density remain constant during fetal as well as postnatal life? Prostate 42:116–123

Yiou R, Delmas V, Carmeliet P, Gherardi RK, Barlovatz-Meimon G, Chopin DK, Abbou CC, Lefaucheur JP (2001) The pathophysiology of pelvic floor disorders: evidence from a histomorphologic study of the perineum and a mouse model of rectal prolapse. J Anat 199:599–607

Yokota E, Kawashima T, Ohkubo F, Sasaki H (2005) Comparative anatomical study of the kidney position in amniotes using the origin of the renal artery as a landmark. Okajimas Folia Anat Jpn 81:135–142

Zaviacic M, Ablin RJ (2000) The female prostate and prostate-specific antigen. Immunohistochemical localization, implications of this prostate marker in women and reasons for using the term “prostate” in the human female. Histol Histopathol 15:131–142

Zaviacic M, Jakubovska V, Belosovic M, Breza J (2000) Ultrastructure of the normal adult human female prostate gland (Skene’s gland). Anat Embryol (Berl) 201:51–61

Neurophysiology of the Lower Urinary Tract

Jonathan M. Beckel and Gert Holstege

Contents

Abstract The lower urinary tract (LUT) has two functions: (1) the storage of waste products in the form of urine and (2) the elimination of those wastes through micturition. The LUT operates in a simple "on–off" fashion, either storing urine or releasing it during voiding. While this activity may seem simple, micturition is controlled by a complex set of peripheral neurons that are, in turn, coordinated by cell groups in the spinal cord, brainstem, and brain. When this careful coordination is interrupted, the control of the bladder is lost, resulting in incontinence or retention of urine. The purpose of this chapter is to review how the neural systems coordinating the activity of the lower urinary tract form neural circuits that are

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responsible for either maintaining continence (the storage reflex) or inducing micturition (the voiding reflex). We will also discuss the brain centers that enable higher organisms to voluntarily choose the time and place for voiding. Finally, we will discuss how defects in the pathways controlling micturition can lead to urinary incontinence and which treatments may normalize LUT function.

Keywords Autonomic nervous system · Bladder physiology · Lower urinary tract · Periaqueductal gray · Spinal cord injury · Urinary incontinence

1 Introduction

The lower urinary tract (LUT) has two functions: (1) the storage of waste products in the form of urine and (2) the elimination of those waste products from the body through micturition. These functions are controlled by autonomic and somatic motoneurons that innervate the bladder and urethra. These motoneurons control the components of the LUT in a reciprocal manner to maintain proper function. For example, during storage bladder smooth muscle is relaxed while the muscles of the internal and external urethral sphincters are contracted, preventing urine from leaving the bladder. During micturition, the opposite occurs, with contraction of the bladder and relaxation of the urethral sphincters. In this chapter, we will discuss the pathways that are responsible for storage and those responsible for voiding, including the higher centers of the brain that allow an individual to voluntarily control urination. Finally, we will put forward some ideas about how defects in these systems can lead to pathophysiological conditions of the LUT.

2 Neural Circuits Controlling the Lower Urinary Tract

The LUT is innervated by both autonomic and somatic motoneurons (de Groat 2006). The autonomic motoneurons consist of both sympathetic preganglionic motoneurons that send their fibers through the hypogastric nerve and parasympathetic motoneurons that send their fibers through the pelvic nerve, while somatic motoneurons send their fibers through the pudendal nerve. As discussed in Chap. 6, each type of motoneuron has a specific role in the physiology of the LUT. For example, sympathetic motoneurons are responsible for relaxation of the bladder detrusor muscle and for the contraction of the internal urethral sphincter, while parasympathetic activation contracts the bladder and relaxes the internal urethral sphincter (Fowler et al. 2008). Somatic motoneurons control the striated muscle of the external urethral sphincter. In order for the LUT to operate properly, these peripheral nerves must act in concert to either promote storage or eliminate urine. This coordination is achieved by neurons in the spinal cord, brainstem, and brain (Holstege and Mouton 2003).

2.1 The Storage Reflex

During filling, the bladder acts as a reservoir for urine, maintaining relatively low intravesical pressures. This is due to a lack of parasympathetic nerve activity and the viscoelastic properties of the bladder wall that allow the bladder to stretch to accommodate a continuously growing volume of urine (Yoshimura and de Groat 1997). During filling, mechano-sensitive receptors present in the bladder wall activate bladder afferent nerves, resulting in increased neural activity in the hypogastric and pelvic nerves (de Groat and Lalley 1972; Floyd et al. 1976; Sengupta and Gebhart 1994) (Fig. 1). Activation of bladder afferent nerves in animal studies, either through electrical stimulation of the pelvic of hypogastric nerve or through stretch of the bladder wall, results in increased activity in lumbar sympathetic preganglionic and postganglionic fibers that innervate the bladder and internal urethral sphincter (Andersson et al. 1990; de Groat and Theobald 1976; Floyd et al. 1982; Schondorf et al. 1983). Activation of sympathetic postganglionic fibers releases norepinephrine, which acts on β-adrenergic receptors present on bladder smooth muscle fibers to cause relaxation (Andersson and Arner 2004). Sympathetic postganglionic neurons also release norepinephrine to activate α-adrenergic receptors on the internal urethral sphincter smooth muscle fibers, contracting the bladder neck. There is also some evidence that sympathetic preganglionic neurons have access to parasympathetic postganglionic neurons, where they inhibit or excite them depending on which receptor is activated (α_2- or α_1-adrenergic receptors, respectively) (de Groat and Saum 1976; Keast et al. 1990; Nakamura et al. 1984).

Physiological experiments in animals indicate a link between pelvic afferents and lumbar sympathetic preganglionic neurons (de Groat and Lalley 1972; de Groat and Theobald 1976; Schondorf et al. 1983). For example, it has been demonstrated that increased afferent activity in the pelvic nerve induces increased activity in sympathetic efferent fibers in the hypogastric nerve (de Groat and Theobald 1976; Floyd et al. 1982). It is thought that these pathways are connected through a putative interneuronal connection between the sacral spinal cord, where pelvic afferent nerves terminate, and the intermediolateral nucleus of the rostral lumbar spinal cord, where sympathetic preganglionic neurons are located (de Groat 2006). Indeed, evoked sympathetic activity following pelvic nerve stimulation is not affected by spinal cord transection above the lumbar level (de Groat and Lalley 1972; de Groat and Theobald 1976). Moreover, there is electrophysiological evidence of interneurons in the sacral spinal cord that receive input from the pelvic, hypogastric, and pudendal nerves that send ascending projections to the lumbar spinal cord (McMahon and Morrison 1982). On the other hand, there is no anatomical evidence that supports the existence of ascending fibers between sacral cord interneurons and sympathetic preganglionic motoneurons. Injection of retrograde viral tracers into the bladder or major pelvic ganglion of the cat or rat labels sympathetic preganglionic motoneurons in the lumbar spinal cord, parasympathetic preganglionic motoneurons in the sacral spinal cord, as well as a significant number of interneurons in the dorsal commissure and intermediolateral nucleus of the sacral spinal cord (de Groat 1998; Marson 1997;

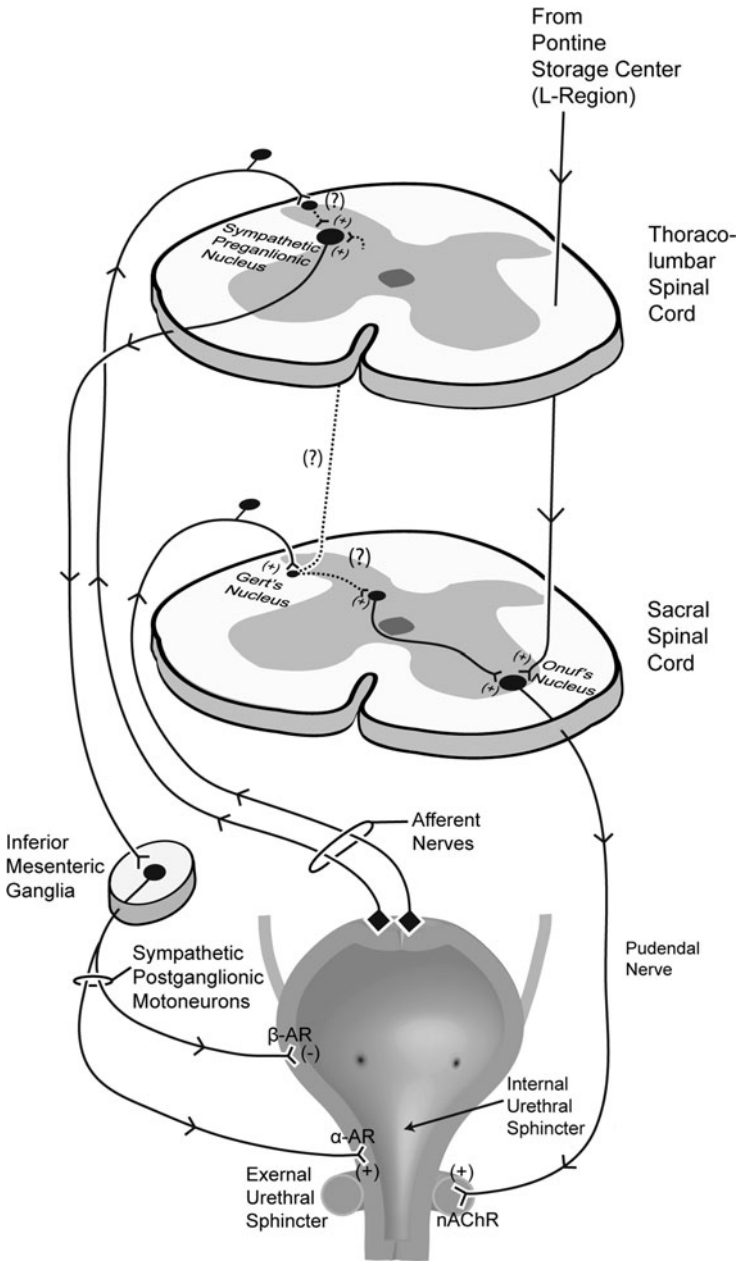

Fig. 1 *Organization of the storage reflexes in the spinal cord*. Diagram depicting the proposed organization of neural pathways responsible for continence. Continence is maintained through sympathetic and somatic motoneurons that innervate the bladder. Sympathetic preganglionic neurons originating in the lumbar spinal cord synapse on postganglionic neurons in the inferior mesenteric ganglia. These postganglionic neurons innervate the bladder detrusor and the internal

Nadelhaft et al. 1992). Bladder specific interneurons in the spinal cord were also visualized using *c-fos* expression following stimulation of bladder afferents (Birder and de Groat 1993). However, in these same studies only very sparse labeling of terminals in the lumbar spinal cord near sympathetic preganglionic motoneurons was found. Sympathetic preganglionic motoneurons are innervated by many glutamatergic terminals, the cell bodies of which orginate in the thoracic and lumbar segments of the spinal cord, but not in the sacral segments (Llewellyn-Smith et al. 2007). There also exists a strong enkephalinergic innervation of bladder sympathetic preganglionic motoneurons (Llewellyn-Smith et al. 2005). These enkephalinergic interneurons appear to be located in the spinal cord, as transection of the spinal cord at the T_{10} level did not eliminate enkephalin containing terminals innervating the sympathetic preganglionic bladder motoneurons. However, the exact origin of these enkephalinergic interneurons was not elucidated. Therefore, while physiological experiments support a connection between interneurons receiving pelvic nerve afferent terminals and sympathetic preganglionic motoneurons, the existence of such a connection is still questionable.

One possible explanation may lie in the convergence of peripheral nerves outside of the spinal cord. For example, the pelvic nerve in rat and cat contains a large number of sympathetic fibers that originate in the lumbar paravertebral sympathetic chain (Giuliano et al. 1997; Kuo et al. 1984). Additionally, sympathetic fibers have been shown that travel to the hypogastric nerve through the sympathetic chain and pelvic nerve (Nadelhaft and Vera 1991). Therefore, the evoked responses observed in sympathetic fibers of the hypogastric nerve following pelvic nerve stimulation may not be due to connections within the spinal cord, but to convergence of the peripheral nerves outside the spinal cord. Electrical stimulation of hypogastric afferent nerves in the cat inhibits the bladder, causing a relaxation of bladder smooth muscle and a contraction of the internal urethral sphincter (Vaughan 1992). Hypogastric afferents are mechanosensitive and respond to stretch of the bladder wall (Floyd et al. 1976, 1982). Given this mechanosensitivity and their ability to drive sympathetic motoneuron activity, hypogastric afferents may play a major role in maintaining continence.

Little is also known about the excitatory interneurons that control the somatic motoneurons of Onuf's Nucleus (ON). During storage, pudendal motoneurons are tonically active, releasing acetylcholine to activate α_1 nicotinic receptors present on

Fig. 1 (continued) urethral sphincter, where they release norepinephrine. Norepinephrine has inhibitory actions on bladder smooth muscle fibers through β-adrenergic receptor (β-AR) activation and excitatory actions on the internal urethral sphincter through α-adrenergic receptor (α-AR) activation. During storage, somatic motoneurons in Onufs Nucleus are also activated, releasing acetylcholine to contract the striated muscle of the external urethral sphincter through nicotinic receptors (nAChRs). These actions are driven by low level activity in bladder afferent nerves; however, the spinal interneurons that connect afferent and efferent pathways are not yet fully understood. Therefore, *question marks* and *dashed lines* indicate putative neural pathways that have not yet been confirmed. (*Plus*) Indicates an excitatory postsynaptic action while (*Minus*) indicates an inhibition

the striated muscle fibers of the external urethral sphincter, causing continuous contraction and closing of the urethra (Fraser and Chancellor 2003; Nyo 1969). Given the fact that activity of motoneurons in ON increases as the bladder fills, it is thought that tonic activity of these motoneurons is also driven by bladder afferent activity. This idea was strengthened when a group of interneurons in the sacral cord was discovered that was excited by perineal afferents and inhibited by pelvic nerve stimulation (McMahon et al. 1982). This suggests that external sphincter motoneurons are tonically activated by spinal interneurons which are inhibited during voiding. Additional research by Fedirchuk and colleagues isolated an area in the intermediomedial nucleus of the sacral spinal cord that contains excitatory interneurons that innervate ON (Fedirchuk et al. 1992). However, whether these interneurons also receive bladder afferents and project to ON is not known.

It is often stated that the neural pathways that control continence are contained in the spinal cord, with little influence from supraspinal areas. This is due to the observations that only voiding, not storage, is affected when the spinal cord is transected rostral to the T_{10} spinal segment (de Groat 1995). It should be noted, however, that ON motoneurons do receive afferents from the brainstem, specifically from the so-called "pontine storage center," also known as the L-region because of its lateral location in the pons (Griffiths 2002). The L-region sends direct projections to ON in the sacral spinal cord, and stimulation of this pontine region in cats results in contraction and of the pelvic floor musculature including the external urethral sphincter. Lesions in the L-region of the cat resulted in complete incontinence, further supporting its role for maintaining continence. A homologous area in the lateral pons of humans is activated in patients with a "full" bladder who are emotionally withholding micturition (Griffiths and Tadic 2008). Therefore, this region of the pons may play a role in the continuous closure of the bladder sphincter. There is also evidence that the nucleus retroambiguus controls pelvic floor muscles in response to abdominal pressure. The nucleus retroambiguus projects directly to Onuf's Nucleus (Vanderhorst and Holstege 1997) and, in cases of high abdominal pressure, further increases the contraction of the muscles innervated by Onuf' Nucleus (i.e., the external urethral sphincter, as well as the anal sphincter and other muscles of the pelvic floor) (Holstege and Tan 1987).

2.2 Voiding Reflexes

Voiding is initiated in a cell group located dorsally in the pontine medial tegmentum known as the pontine micturition center (PMC) (Blok and Holstege 1997; Griffiths 2002). It has also been called the "M-region" for its medial location in the pontine tegmentum as compared to the more laterally located L-region. The PMC is also called "Barrington's nucleus" after F.J.F Barrington (1884–1956), who first characterized the region in 1925 (Barrington 1925). The PMC is the ultimate efferent nucleus in the micturition pathway, as electrical or chemical stimulation of the PMC causes contraction of the detrusor and relaxation of the urethra, mimicking

micturition (Sugaya et al. 1987). To induce micturition, the PMC sends long descending fibers to the intermediolateral nucleus of the sacral spinal cord, where they terminate on parasympathetic preganglionic motoneurons (Fig. 2) (Blok and Holstege 1997). This activation results in the excitation of two groups of parasympathetic postganglionic neurons that innervate the LUT: (1) those that release acetylcholine and ATP to contract the bladder smooth muscle and (2) those that release nitric oxide, which relaxes the smooth muscle of the internal urethral sphincter (de Groat and Yoshimura 2001). Activation of the PMC also causes relaxation of the external urethral sphincter (Blok 2002). This relaxation is not mediated through direct interactions between descending fibers from the PMC and the urethral motoneurons of Onuf's nucleus, but on PMC terminations on inhibitory GABAergic and glycinergic interneurons located in the intermediomedial cell group of the sacral spinal cord. These interneurons, in turn, inhibit the somatic motoneurons of Onuf's nucleus (Blok et al. 1997b; Sie et al. 2001). The combined effect of activation of the PMC, thus, is a relaxation of the smooth and striated muscle of the internal and external urethral sphincters and a contraction of the bladder itself, resulting in the expulsion of urine.

There is some debate as to the role of the sympathetic nervous system during voiding. Studies indicate that activity in sympathetic hypogastric motoneurons decreases during micturition, suggesting that the sympathetic innervation of the bladder maintaining storage is inhibited during voiding (de Groat and Lalley 1972). This hypothesis proposes that the PMC acts as a neurological "switch," turning off the sympathetic and somatic storage circuit and turning on the parasympathetic voiding pathway. However, in tracing studies on the projections of the PMC, which show strong connections with parasympathetic preganglionic motoneurons, no projections to sympathetic preganglionic motoneurons in the thoracolumbar spinal cord could be found. Another possibility may be that micturition does not require inhibition of sympathetic motoneurons. In this case, the PMC's activation of parasympathetic motoneurons and inhibition of ON motoneurons would overwhelm the inhibitory action of sympathetic motoneurons during the short time of micturition.

While the PMC is the ultimate efferent nucleus of the neural pathway controlling voiding, its neurons do not receive direct afferent input from the LUT (Blok et al. 1995). Sensory information on bladder filling is carried by A-δ fibers from the bladder to a distinct cell group, called Gert's Nucleus (GN) located ventrolateral to the dorsal horn of the S_1–S_2 spinal cord (Holstege 2005; Klop et al. 2005). GN, in turn, sends ascending projections to a region of the mesencephalon known as the periaqueductal gray (PAG) (Klop et al. 2005; Vanderhorst et al. 1996). The PAG has been shown to play a critical role in the control of micturition, as it is strongly connected to the PMC (Blok and Holstege 1994). Additionally, lesions in the PAG in both cats and humans have resulted in urinary retention (Griffiths et al. 1990; Komiyama et al. 1998). Therefore, the PAG serves as an important regulator of the LUT, receiving sensory information from the bladder, processing it, and choosing whether to activate the PMC to initiate voiding (Holstege and Mouton 2003). Put into layman's terms, if the PMC is the "switch" that initiates the voiding circuitry, the PAG is the operator who decides when to throw that switch.

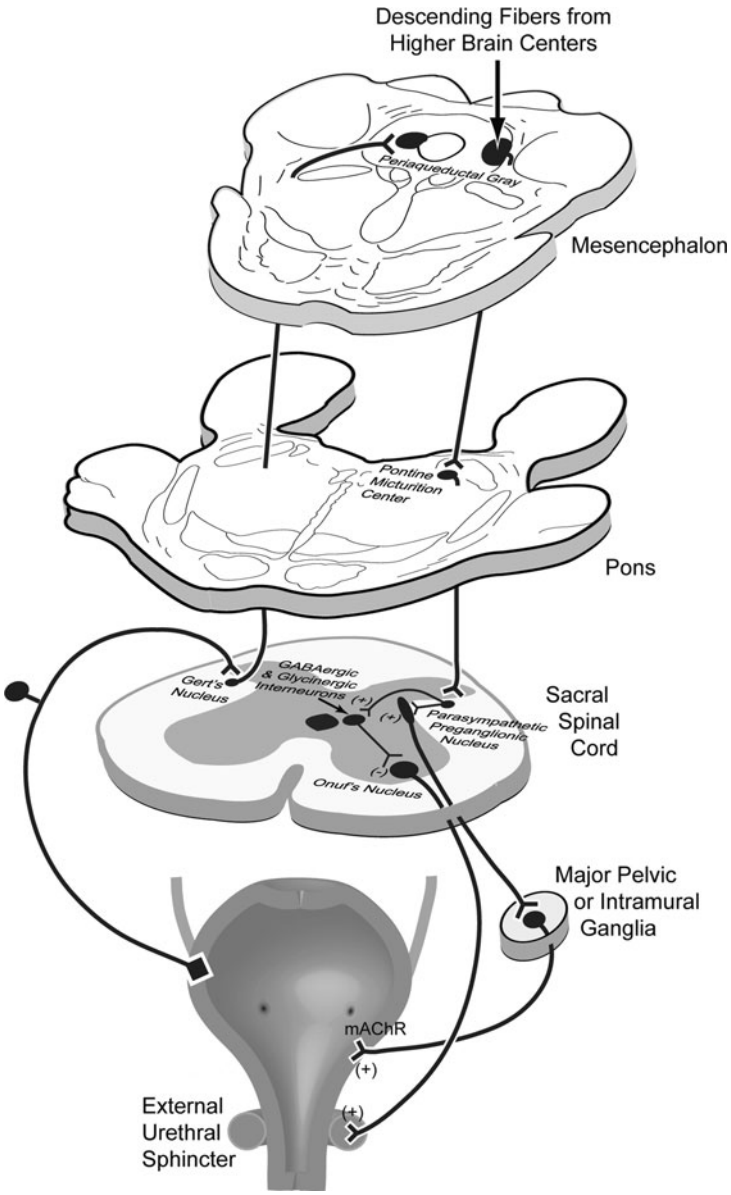

Fig. 2 *Neural pathways controlling voiding reflexes*. Diagram depicting the proposed neuronal pathways responsible for the voiding reflex. Sensory information from the bladder is carried to the sacral spinal cord by afferent fibers of the pelvic nerve where they synapse on interneurons in Gert's Nucleus. From here, ascending interneurons carry sensory information to the central part of the lateral periaqueductal gray in the mesencephalon. When the bladder reaches a threshold pressure, descending neurons from the lateral periaqueductal gray to the pontine micturition center are activated. The pontine micturition center activates parasympathetic preganglionic motoneurons in the sacral spinal cord which, in turn, activates postganglionic neurons that innervate the

While voiding is controlled through supraspinal mechanisms, there is some evidence of spinal circuits that may aid in micturition without supraspinal input. For example, electrical stimulation of pudendal afferents resulted in bladder contractions in healthy (Shefchyk and Buss 1998; Yoo et al. 2008) as well as in spinal cord transected cats (Tai et al. 2007). It has also been demonstrated that electrical stimulation of the urethra can excite the detrusor in humans with spinal cord injury (Gustafson et al. 2004). It is hypothesized that this reflex supports micturition by strengthening the excitatory signal to the detrusor when urine is passing through the urethra, promoting a complete emptying of the bladder. There is also a spinal reflex connecting somatosensory neurons innervating the skin of the perigenital area to the LUT (Tai et al. 2008). Mechanical stimulation of the perigenital area in postnatal kittens excites the bladder and relaxes the urethra, leading to micturition. The mother cat commonly activates this reflex by licking the perigenital region of the kitten to induce voiding. This reflex is lost during development of the kitten, when the supraspinal regions controlling voluntary voiding take over. In the adult, this reflex excites the external urethral sphincter, promoting storage.

3 The Role of the Brain in Voluntary Voiding

The neural circuits described above are responsible for controlling reflex bladder function; that is, the involuntary voiding exhibited by rodents or by infants. In humans over the age of 2 or 3, however, the timing of voiding can be voluntarily decided (de Groat et al. 1998). That is, an individual can choose to delay voiding even when the fullness of the bladder is past that where sensations become uncomfortable. There must then exist centers of the brain that can prevent the reflex activation of the PMC, allowing the individual to consciously determine the appropriate time to void. As discussed in Chap. 6, very few areas of the brain connect directly to the PMC; however, a number of brain centers, including the lateral, caudal, and anterior parts of the hypothalamus, the central nucleus of the amygdala, the bed nucleus of the stria terminalis, the medial and lateral preoptic region, and large portions of the prefrontal cortex, project strongly to the PAG (Blok and Holstege 1998; de Groat 1998; Nadelhaft et al. 1992). These areas all take part in the limbic system of the brain, which plays a role in emotion, memory, and social cognition and is responsible for regulating the autonomic nervous system in response to emotion (such as the "fight or flight" response) (Holstege 1998, 2005). Therefore, it is thought that the PAG is responsible for determining when to activate voiding, not based solely on the afferent information from the bladder,

Fig. 2 (continued) bladder smooth muscle, releasing acetylcholine to evoke a bladder contraction through muscarinic receptors (mAChR). Descending interneurons from the PMC also activate GABAergic and glycinergic inhibitory interneurons in the sacral spinal cord that inhibit the motoneurons of Onuf's Nucleus. This results in the relaxation of the external urethral sphincter

but in conjunction with information from higher brain centers. This could include information on environment ("Am I in the proper place to begin voiding?"), emotion (the fear of voiding in public), memory ("where is the closest bathroom?"), and proper social behavior ("Is this an acceptable place to void?"). If this information tells the PAG that it is inappropriate to void at that particular time, then the PAG does not activate the PMC and voiding does not occur, no matter how full the bladder is.

It has only been recently possible to examine the role of higher brain centers in micturition in humans, as functional imaging techniques such as PET and fMRI have become available that allow a noninvasive look into which brain centers are activated during storage and voiding (Kavia et al. 2005). A number of imaging studies have been completed, with the majority giving evidence of two main areas of the forebrain as being important during storage and/or micturition in the human: the anterior cingulate gyrus and the prefrontal cortex (For more information, refer to Chap. 5).

3.1 The Anterior Cingulate Gyrus

The anterior cingulate gyrus (ACG) is connected to a number of brain centers such as the amygdala, hippocampus, hypothalamus, insula, the dorsal medial nucleus of the thalamus, frontal motor areas, the caudate nucleus and putamen, and the PAG (Musil and Olson 1988). These connections have prompted the hypothesis that the ACG is a major control center integrating all information regarding the environment of the individual with emotional and motor stimuli and is of crucial importance for initiating goal oriented behavior (Bush et al. 2000). The ACG, therefore, plays a role in cognitive functions, such as attention and introspection (i.e., being aware that the bladder is full and it is time to void), and executive control (i.e., only voiding in an "appropriate" place).

All of the current functional imaging studies have shown an upregulation of activity in the anterior cingulate gyrus during filling or voiding, indicating a role for this area in micturition (Athwal et al. 2001; Blok et al. 1997a, 1998; Matsuura et al. 2002). Some discrepancies exist between studies on what part of the ACG is important to bladder function, with earlier studies mapping activation of the posterior ACG during bladder filling than later studies (Athwal et al. 2001; Matsuura et al. 2002). This is thought to highlight a functional difference in the two regions of the ACG; activity was increased in the posterior/dorsal portion of the ACG when patients were asked to rate the level of their desire to void, a cognitive task. The dorsal portion of the ACG also seems to be strongly activated in subjects that had poor bladder control, possibly indicating that these patients exhibit a greater awareness of the volume of their bladder as they dread an embarrassing accident. A more anterior/ventral area of the ACG is activated when the bladder is merely full, without the patient being required to think about it. The ACG is also activated during micturition. Because the ACG projects heavily to the PAG, this

activation during micturition may indicate that the ACG may also play a role in the activation of the PMC by the PAG (Blok et al. 1997a, 1998).

Experiments in animals support the role of the ACG in micturition. For example, electrical stimulation of the ACG in the anesthetized cat causes a contraction of the detrusor muscle (Gjone 1966; Gjone and Setekleiv 1963). In the rat, a chemical lesion of the ACG can cause an inhibition in the timing of bladder contractions, lengthening the interval between reflex bladder contractions without altering the strength of the contraction (Matsumoto et al. 2006). Clinically, patients with lesions in the ACG report having symptoms of urge incontinence (Andrew and Nathan 1964; Duffau and Capelle 2005). There is also a report of urinary retention following a cerebral infarct in the right caudal ACG (Funakoshi et al. 2005). Finally, SPECT brain scans of patients that had symptoms of urge incontinence exhibited decreased blood flow in the ACG as compared to normal patients (Griffiths 1998).

3.2 Prefrontal Cortex

The prefrontal cortex, an area of the frontal lobe anterior to the motor and premotor cortices of the brain, is also thought to play a role in controlling voluntary micturition. The prefrontal cortex has been implicated in the planning of complex cognitive behaviors that involve the expression of appropriate social behavior (Miller and Cohen 2001). This could also relate to the executive control of the urinary bladder, i.e., the ability to prevent urination until an acceptable place can be found to void, based on social expectations and consequences of actions.

The prefrontal cortex is connected with the ACG, the PAG, the hypothalamus, the thalamus, and the insula, i.e., all brain regions that have been implicated in the control of micturition (Carmichael and Price 1995). During brain imaging, a number of studies have demonstrated an activation of the prefrontal cortex, specifically the right inferior frontal gyrus, during both the voiding and filling phases (Athwal et al. 2001; Blok et al. 1997c, 1998; Matsuura et al. 2002; Nour et al. 2000). It is not known what significance of this lateralization of the prefrontal cortex may play. Clinically, lesions in the prefrontal cortex cause an inability to withhold urine, resulting in symptoms of urge incontinence, including frequent urination and nocturesis (Griffiths et al. 2005).

4 Central Nervous System Pharmacology Controlling Micturition

Research has indicated that a number of transmitters in the central nervous system play a role in micturition control. For example, glutamic acid, a major excitatory transmitter in the central nervous system, is crucial for proper micturition. Excitatory

glutamatergic signaling is mediated both by *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) receptors in the spinal cord, as both NMDA and AMPA receptor antagonists have an inhibitory effect on bladder contractions (Maggi et al. 1990; Sugaya and de Groat 1994; Yoshiyama et al. 1993a, b, 1994, 1995a, b). Glutamatergic signaling in the spinal cord plays a significant role in the descending pathways from the PMC that activate micturition. Intrathecal administration of NMDA or AMPA antagonists can block reflex voiding in the anesthetized rat (Yoshiyama et al. 1993a, b, 1995a, b). These antagonists can also block evoked micturition reflexes following electrical stimulation of the PMC (Matsumoto et al. 1995). Glutamatergic signaling may also play a role in the activation of spinal interneurons by bladder afferent nerves, as *c-fos* production in spinal interneurons in response to bladder afferent activation is diminished by NMDA or AMPA antagonists (Birder and de Groat 1992).

Noradrenergic signaling in the spinal cord can also influence bladder activity (de Groat et al. 1999). The locus coeruleus sends numerous noradrenergic projections to the sacral spinal cord, where they may participate in modulating the excitability of spinal neurons controlling micturition. For example, activation of spinal α_2 receptors promotes detrusor contraction and inhibits external urethral sphincter activity in the rat and cat (Galeano et al. 1986; Ishizuka et al. 1996a). Additionally, α_1 adrenergic receptors may play a role in storage, as intrathecal administration of prazosin (an α_1 antagonist) in cats suppresses activity in both sympathetic motoneurons and the motoneurons of ON (Danuser and Thor 1995). However, this role is not yet certain, as α_1 antagonists also have an inhibitory effect on bladder contractions in the anesthetized rat (Ishizuka et al. 1996b). Bladder contractions evoked by stimulation of the PMC in the cat are also blocked by intrathecal prazosin, further supporting an excitatory role for these receptors in the spinal cord (Yoshimura et al. 1990a, b, 1988).

Endogenous opioids have various inhibitory effects on the spinal pathways controlling micturition. This has been demonstrated in animals where intrathecal administration of opioid antagonists had an excitatory effect on the bladder, indicating the presence of a tonic inhibition of the bladder through spinal opioid mechanisms (de Groat et al. 1983). This tonic inhibition of the bladder is mediated through δ receptors in the spinal cord. Additionally, intrathecal administration of κ opioid receptor agonists suppresses external urethral sphincter activity (Thor et al. 1989).

There is evidence for inhibitory neurotransmission in the spinal cord, mediated through GABAergic signaling. GABAergic interneurons in the sacral intermediomedial cell group send fibers to the sacral parasympathetic nucleus, where $GABA_A$ receptor activation inhibits parasympathetic motoneuron activity (Araki 1994). Clinically, intrathecal administration of baclofen, a $GABA_A$ receptor agonist, increases the pressure threshold needed to activate micturition (Steers et al. 1992). Glycine is another inhibitory amino acid important for micturition. Its role in inhibiting parasympathetic neurons is debated, as activation of glycinergic pathways can inhibit activity in parasympathetic preganglionic neurons of the rat (Araki 1994), yet no such pathway has been discovered in the cat (Kruse et al. 1990). In the

cat, glycinergic interneurons can inhibit external sphincter motoneurons (Shefchyk et al. 1998; Sie et al. 2001) and are thought to be responsible for relaxing the sphincter in response to activation of descending pathways from the PMC during micturition.

Various studies have elucidated a number of other transmitters in the brain that are important to micturition. Stimulation of the PMC with acetylcholine, carbachol, dopamine, glutamate, and norepinephrine can evoke micturition (Sugaya et al. 1987). Additionally, injection of naloxone (a μ opioid receptor antagonist) (Noto et al. 1991) or bicuculline (a $GABA_A$ receptor antagonist) (Mallory et al. 1991) into the PMC can excite the micturition reflex, indicating that these receptors have a tonic inhibitory role in the PMC. Injection of fentanyl or leucine-enkephalin into the PMC has an inhibitory effect (Noto et al. 1991; Sugaya et al. 1987), further supporting the inhibitory role for opioid receptors in the PMC. While research has elucidated a number of transmitters important for signaling in the PMC, no significant research has yet been performed to examine the transmitters important in the other areas of the brain hypothesized to play a role in micturition.

5 Central Causes of Pathophysiology of the Lower Urinary Tract

Voluntary control of voiding requires supraspinal neural pathways, if these pathways are interrupted, the individual suffers from incontinence. Urinary incontinence is a generic term which refers to "any involuntary leakage of urine" and includes a number of different disorders. In the following section, we will discuss a few of the pathological conditions that can occur when central nervous system pathways controlling micturition are interrupted.

5.1 Spinal Cord Transection

An interruption of the brainstem-spinal pathways controlling micturition can occur when the spinal cord is injured. Loss of voluntary bladder control represents a major quality of life issue for patients with spinal cord transection (SCT). When SCT patients were asked in a recent survey which functional recovery would most greatly improve their lives, bladder and bowel function was ranked higher than regaining the use of their legs to walk (Anderson 2004). Therefore, bladder research has become a major focus of SCT researchers.

When SCT occurs rostral to the lumbosacral spinal cord, the immediate result is complete urinary retention (de Groat 1995). During this stage, the bladder cannot empty and the individual must be continuously catheterized to drain urine. If urine is not drained by catheterization, high bladder pressure will cause vesicoureteral

reflux and eventually renal failure and death. However, after a period of a few weeks in animals to a few months in humans, the bladder recovers its function much like that of an infant, where the bladder empties reflexively after reaching a threshold pressure (de Groat et al. 1998). For this to occur, pathways in the spinal cord that connect bladder afferent nerves to the parasympathetic neurons controlling bladder contractions must be activated (Fig. 3). It is thought that these spinal pathways play a role during infancy and are subsequently overruled during the development of the descending pathways of the supraspinal regions that control bladder function in the adult. Following SCT, these dormant pathways might be activated once again to control reflex voiding.

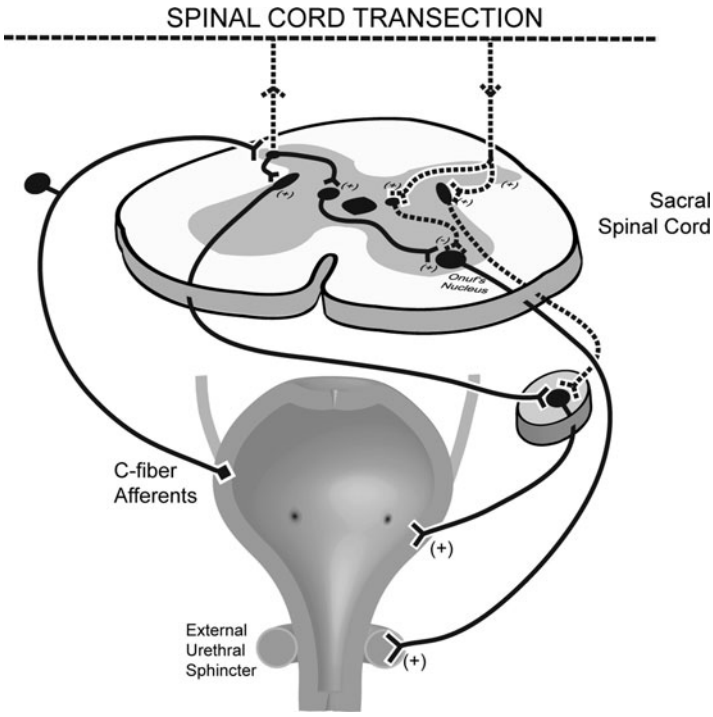

Fig. 3 *Reflex control of the lower urinary tract following spinal cord transection*. Diagram depicting the proposed changes in the organization of the neural pathways controlling micturition following spinal cord transection. When the spinal cord is transected, both the ascending afferent pathways to the brain and the descending efferent pathways from the pontine micturition center are cut off. As a result, neural pathways that would normally excite parasympathetic motoneurons and inhibit motoneurons in Onuf's Nucleus are not activated. However, a few weeks after spinal cord transection a spinal reflex forms that is driven by C-fiber afferent nerves. C-fiber activation results in excitation of spinal interneurons that directly activate parasympathetic motoneurons, contracting the bladder. However, these interneurons also activate Onuf's nucleus, contracting the external urethral sphincter, causing detrusor–sphincter dyssynergia. *Dotted lines* indicate neural pathways silenced following spinal cord transection

This reflex voiding, however, is inefficient for a number of reasons. First, the bladder smooth muscle is highly active, exhibiting small, nonvoiding contractions during the filling phase, called detrusor hyperreflexia (de Groat 1995). This increased bladder smooth muscle activity can be so strong that it results in urine leakage. Detrusor hyperreflexia is caused by a shift in the electrophysiological and chemical properties of bladder afferent nerves following SCT (Yoshimura 1999). In the normal bladder, capsaicin-sensitive, unmyelinated C-fiber afferent nerves are mechano-insensitive, reacting only to noxious stimuli. However, following SCT, C-fiber afferents become sensitized to mechanical stimuli and respond to stretch of the bladder wall as the bladder fills (de Groat et al. 1990). The increased sensitivity of bladder C-fiber afferents is thought to be the driving force behind detrusor hyperreflexia, as desensitization of C-fibers through the application of capsaicin can completely block bladder contractions in the spinalized cat (de Groat et al. 1990). These experiments also suggest that the change in afferent nerve phenotype following SCT is complete, with A-δ fibers no longer playing any role in micturition.

Voiding following SCT is also inefficient due to detrusor sphincter dyssynergia (DSD). In the normal bladder, descending pathways from the PMC excite the parasympathetic neurons responsible for contracting the bladder and indirectly inhibit the motoneurons responsible for keeping the urethra contracted (Griffiths et al. 1990). This coordination of bladder contraction and urethral relaxation allows for efficient voiding. However, when the supraspinal control is eliminated, this coordination disappears. Activation of bladder afferent nerves not only activates a spinal reflex that contracts the detrusor, but it also activates spinal reflexes that contract the external urethral sphincter (de Groat 1995; de Groat et al. 1998; Yoshimura and de Groat 1997). This simultaneous contraction of the detrusor and the sphincter prevents complete emptying of the bladder. For the patient this means intermittent catheterization to drain the residual urine in the bladder, which greatly increases the chances of acquiring a bladder infection. Additionally, the bladder must exert a greater force in order to expel urine through the closed urethra, which leads to a substantial thickening of the bladder smooth muscle (Kruse et al. 1993). This hypertrophy of the bladder wall reduces its compliance and decreases the bladder's capacity. It is also thought that this hypertrophy of the bladder wall is responsible for sensitization of C-fiber afferents in the bladder following SCT (Yoshimura 1999). Hypertrophied bladder smooth muscle releases increased levels of nerve growth factor, which has been shown to alter the expression of ion channels in bladder C-fiber afferents, increasing their excitability (Yoshimura et al. 2006).

5.2 Urge Urinary Incontinence

Urge urinary incontinence is defined as "the complaint of involuntary leakage of urine accompanied by or immediately preceded by urgency" (Abrams et al. 2002). Because the urgency experienced by the patient often occurs with little warning and

at volumes that are lower than the normal threshold for causing urgent bladder sensations, researchers have focused on peripheral sensory nerves for an etiology to urge urinary incontinence (Andersson 2002; Cruz 2004; Fowler 2002). However, research has indicated that defects in the central nervous system may be responsible for the pathophysiology of the disease.

For example, lesions in the centers of the brain thought to play a role in voluntary control of micturition, such as the prefrontal cortex or the anterior cingulate gyrus can lead to urge urinary incontinence (Andrew and Nathan 1964; Duffau and Capelle 2005; Griffiths et al. 2005). Additionally, it is known that some individuals develop small cerebral white matter lesions as they age, a condition known as leukoaraiosis (Brant-Zawadzki et al. 1985). Leukoaraiosis often occurs during aging, mainly due to vascular disease, such as atherosclerosis and subsequent hypertension and heart disease (Pantoni and Garcia 1997). It is also thought to play a role in a number of neurological conditions such as Alzheimer's and Parkinson's disease. Patients suffering from these neurological conditions often suffer from urinary incontinence (Kuchel et al. 2009); therefore, it is thought that this urinary dysfunction may also be due to leukoaraiosis and may represent a first stage of the other neurological disorders, as it appears that the urological symptoms often occur before leukoaraiosis is pronounced (Sakakibara et al. 1999). Therefore, it is becoming clear that urge incontinence is caused by an interruption of the inhibitory signals from the prefrontal cortex, the anterior cingulate gyrus and/or other areas of the brain to the PAG, preventing activation of the PMC by the PAG when the bladder is full. The result is an inability of the patient to maintain continence when they feel their bladder is full because they lack the time to find an acceptable place to void. Therefore, urge urinary incontinence is not a "bladder disease" as much as it is a "brain disease." Successful treatments for urge urinary incontinence might therefore be found in attempts to restore tonic inhibition to the PAG, either through pharmacological intervention or through electrical deep brain stimulation, similar to that currently being studied to treat Parkinson's patients (Weaver et al. 2009).

6 Conclusions

The motoneurons controlling the function of the LUT are carefully controlled by neurons in the spinal cord, brainstem, and brain. If these CNS pathways should become perturbed, the resulting loss of voluntary bladder control can drastically reduce one's quality of life. Given the multitude of transmitters in the central nervous system that play a role in controlling micturition, a number of potential drug targets for the treatment of bladder disorders exist. Only through better understanding of the central pathways controlling micturition can we develop novel techniques for the treatment for bladder pathology, such as that seen following spinal cord injury or leukoaraiosis.

References

Abrams P, Cardozo L, Fall M et al (2002) The standardisation of terminology of lower urinary tract function: report from the Standardisation Sub-committee of the International Continence Society. Neurourol Urodyn 21:167–178

Anderson KD (2004) Targeting recovery: priorities of the spinal cord-injured population. J Neurotrauma 21:1371–1383

Andersson KE (2002) Bladder activation: afferent mechanisms. Urology 59:43–50

Andersson KE, Arner A (2004) Urinary bladder contraction and relaxation: physiology and pathophysiology. Physiol Rev 84:935–986

Andersson PO, Sjogren C, Uvnas B et al (1990) Urinary bladder and urethral responses to pelvic and hypogastric nerve stimulation and their relation to vasoactive intestinal polypeptide in the anaesthetized dog. Acta Physiol Scand 138:409–416

Andrew J, Nathan PW (1964) Lesions on the anterior frontal lobes and disturbances of micturition and defaecation. Brain 87:233–262

Araki I (1994) Inhibitory postsynaptic currents and the effects of GABA on visually identified sacral parasympathetic preganglionic neurons in neonatal rats. J Neurophysiol 72:2903–2910

Athwal BS, Berkley KJ, Hussain I et al (2001) Brain responses to changes in bladder volume and urge to void in healthy men. Brain 124:369–377

Barrington F (1925) The effect of lesions of the hind- and mid-brain on micturition in the cat. Q J Exp Physiol 15:81–102

Birder LA, de Groat WC (1992) The effect of glutamate antagonists on c-fos expression induced in spinal neurons by irritation of the lower urinary tract. Brain Res 580:115–120

Birder LA, de Groat WC (1993) Induction of c-fos expression in spinal neurons by nociceptive and nonnociceptive stimulation of LUT. Am J Physiol 265:R326–R333

Blok BF (2002) Central pathways controlling micturition and urinary continence. Urology 59:13–17

Blok BF, Holstege G (1994) Direct projections from the periaqueductal gray to the pontine micturition center (M-region). An anterograde and retrograde tracing study in the cat. Neurosci Lett 166:93–96

Blok BF, Holstege G (1997) Ultrastructural evidence for a direct pathway from the pontine micturition center to the parasympathetic preganglionic motoneurons of the bladder of the cat. Neurosci Lett 222:195–198

Blok BF, Holstege G (1998) The central nervous system control of micturition in cats and humans. Behav Brain Res 92:119–125

Blok BF, De Weerd H, Holstege G (1995) Ultrastructural evidence for a paucity of projections from the lumbosacral cord to the pontine micturition center or M-region in the cat: a new concept for the organization of the micturition reflex with the periaqueductal gray as central relay. J Comp Neurol 359:300–309

Blok BF, Sturms LM, Holstege G (1997a) A PET study on cortical and subcortical control of pelvic floor musculature in women. J Comp Neurol 389:535–544

Blok BF, de Weerd H, Holstege G (1997b) The pontine micturition center projects to sacral cord GABA immunoreactive neurons in the cat. Neurosci Lett 233:109–112

Blok BF, Willemsen AT, Holstege G (1997c) A PET study on brain control of micturition in humans. Brain 120:111–121

Blok BF, Sturms LM, Holstege G (1998) Brain activation during micturition in women. Brain 121:2033–2042

Brant-Zawadzki M, Fein G, Van Dyke C et al (1985) MR imaging of the aging brain: patchy white-matter lesions and dementia. AJNR Am J Neuroradiol 6:675–682

Bush G, Luu P, Posner MI (2000) Cognitive and emotional influences in anterior cingulate cortex. Trends Cogn Sci 4:215–222

Carmichael ST, Price JL (1995) Limbic connections of the orbital and medial prefrontal cortex in macaque monkeys. J Comp Neurol 363:615–641

Cruz F (2004) Mechanisms involved in new therapies for overactive bladder. Urology 63:65–73
Danuser H, Thor KB (1995) Inhibition of central sympathetic and somatic outflow to the lower urinary tract of the cat by the alpha 1 adrenergic receptor antagonist prazosin. J Urol 153:1308–1312
de Groat WC (1995) Mechanisms underlying the recovery of lower urinary tract function following spinal cord injury. Paraplegia 33:493–505
de Groat WC (1998) Anatomy of the central neural pathways controlling the lower urinary tract. Eur Urol 34(Suppl 1):2–5
de Groat WC (2006) Integrative control of the lower urinary tract: preclinical perspective. Br J Pharmacol 147(Suppl 2):S25–40
de Groat WC, Lalley PM (1972) Reflex firing in the lumbar sympathetic outflow to activation of vesical afferent fibres. J Physiol 226:289–309
de Groat WC, Saum WR (1976) Synaptic transmission in parasympathetic ganglia in the urinary bladder of the cat. J Physiol 256:137–158
de Groat WC, Theobald RJ (1976) Reflex activation of sympathetic pathways to vesical smooth muscle and parasympathetic ganglia by electrical stimulation of vesical afferents. J Physiol 259:223–237
de Groat WC, Yoshimura N (2001) Pharmacology of the lower urinary tract. Annu Rev Pharmacol Toxicol 41:691–721
de Groat WC, Kawatani M, Hisamitsu T et al (1983) The role of neuropeptides in the sacral autonomic reflex pathways of the cat. J Auton Nerv Syst 7:339–350
de Groat WC, Kawatani M, Hisamitsu T et al (1990) Mechanisms underlying the recovery of urinary bladder function following spinal cord injury. J Auton Nerv Syst 30(Suppl):S71–7
de Groat WC, Araki I, Vizzard MA et al (1998) Developmental and injury induced plasticity in the micturition reflex pathway. Behav Brain Res 92:127–140
de Groat WC, Yoshiyama M, Ramage AG et al (1999) Modulation of voiding and storage reflexes by activation of alpha1-adrenoceptors. Eur Urol 36(Suppl 1):68–73
Duffau H, Capelle L (2005) Incontinence after brain glioma surgery: new insights into the cortical control of micturition and continence. Case report. J Neurosurg 102:148–151
Fedirchuk B, Song L, Downie JW et al (1992) Spinal distribution of extracellular field potentials generated by electrical stimulation of pudendal and perineal afferents in the cat. Exp Brain Res 89:517–520
Floyd K, Hick VE, Morrison JF (1976) Mechanosensitive afferent units in the hypogastric nerve of the cat. J Physiol 259:457–471
Floyd K, Hick VE, Morrison JF (1982) The influence of visceral mechanoreceptors on sympathetic efferent discharge in the cat. J Physiol 323:65–75
Fowler CJ (2002) Bladder afferents and their role in the overactive bladder. Urology 59:37–42
Fowler CJ, Griffiths D, de Groat WC (2008) The neural control of micturition. Nat Rev Neurosci 9:453–466
Fraser MO, Chancellor MB (2003) Neural control of the urethra and development of pharmacotherapy for stress urinary incontinence. BJU Int 91:743–748
Funakoshi K, Fukutake T, Nishino H et al (2005) Urinary retention caused by a small cortical infarction. J Neurol Neurosurg Psychiatry 76:457–458
Galeano C, Jubelin B, Germain L et al (1986) Micturitional reflexes in chronic spinalized cats: the underactive detrusor and detrusor-sphincter dyssynergia. Neurourol Urodyn 5:45–63
Giuliano F, Facchinetti P, Bernabe J et al (1997) Evidence of sympathetic fibers in the male rat pelvic nerve by gross anatomy, retrograde labeling and high resolution autoradiographic study. Int J Impot Res 9:179–185
Gjone R (1966) Excitatory and inhibitory bladder responses to stimulation of 'limbic', diencephalic and mesencephalic structures in the cat. Acta Physiol Scand 66:91–102
Gjone R, Setekleiv J (1963) Excitatory and inhibitory bladder responses to stimulation of the cerebral cortex in the cat. Acta Physiol Scand 59:337–348

Griffiths D (1998) Clinical studies of cerebral and urinary tract function in elderly people with urinary incontinence. Behav Brain Res 92:151–155
Griffiths DJ (2002) The pontine micturition centres. Scand J Urol Nephrol Suppl 210:21–26
Griffiths D, Tadic SD (2008) Bladder control, urgency, and urge incontinence: evidence from functional brain imaging. Neurourol Urodyn 27:466–474
Griffiths D, Holstege G, Dalm E et al (1990) Control and coordination of bladder and urethral function in the brainstem of the cat. Neurourol Urodyn 9:63–82
Griffiths D, Derbyshire S, Stenger A et al (2005) Brain control of normal and overactive bladder. J Urol 174:1862–1867
Gustafson KJ, Creasey GH, Grill WM (2004) A urethral afferent mediated excitatory bladder reflex exists in humans. Neurosci Lett 360:9–12
Holstege G (1998) The emotional motor system in relation to the supraspinal control of micturition and mating behavior. Behav Brain Res 92:103–109
Holstege G (2005) Micturition and the soul. J Comp Neurol 493:15–20
Holstege G, Mouton LJ (2003) Central nervous system control of micturition. Int Rev Neurobiol 56:123–145
Holstege G, Tan J (1987) Supraspinal control of motoneurons innervating the striated muscles of the pelvic floor including urethral and anal sphincters in the cat. Brain 110(Pt 5):1323–1344
Ishizuka O, Mattiasson A, Andersson KE (1996a) Role of spinal and peripheral alpha 2 adrenoceptors in micturition in normal conscious rats. J Urol 156:1853–1857
Ishizuka O, Persson K, Mattiasson A et al (1996b) Micturition in conscious rats with and without bladder outlet obstruction: role of spinal alpha 1-adrenoceptors. Br J Pharmacol 117:962–966
Kavia RB, Dasgupta R, Fowler CJ (2005) Functional imaging and the central control of the bladder. J Comp Neurol 493:27–32
Keast JR, Kawatani M, De Groat WC (1990) Sympathetic modulation of cholinergic transmission in cat vesical ganglia is mediated by alpha 1- and alpha 2-adrenoceptors. Am J Physiol 258: R44–50
Klop EM, Mouton LJ, Kuipers R et al (2005) Neurons in the lateral sacral cord of the cat project to periaqueductal grey, but not to thalamus. Eur J Neurosci 21:2159–2166
Komiyama A, Kubota A, Hidai H (1998) Urinary retention associated with a unilateral lesion in the dorsolateral tegmentum of the rostral pons. J Neurol Neurosurg Psychiatry 65:953–954
Kruse MN, Noto H, Roppolo JR et al (1990) Pontine control of the urinary bladder and external urethral sphincter in the rat. Brain Res 532:182–190
Kruse MN, Belton AL, de Groat WC (1993) Changes in bladder and external urethral sphincter function after spinal cord injury in the rat. Am J Physiol 264:R1157–63
Kuchel GA, Moscufo N, Guttmann CR et al (2009) Localization of brain white matter hyperintensities and urinary incontinence in community-dwelling older adults. J Gerontol A Biol Sci Med Sci 64:902–909
Kuo DC, Hisamitsu T, de Groat WC (1984) A sympathetic projection from sacral paravertebral ganglia to the pelvic nerve and to postganglionic nerves on the surface of the urinary bladder and large intestine of the cat. J Comp Neurol 226:76–86
Llewellyn-Smith IJ, Dicarlo SE, Collins HL et al (2005) Enkephalin-immunoreactive interneurons extensively innervate sympathetic preganglionic neurons regulating the pelvic viscera. J Comp Neurol 488:278–289
Llewellyn-Smith IJ, Martin CL, Fenwick NM et al (2007) VGLUT1 and VGLUT2 innervation in autonomic regions of intact and transected rat spinal cord. J Comp Neurol 503:741–767
Maggi CA, Giuliani S, Giachetti A et al (1990) The effect of MK-801 on the micturition reflex in anesthetized rats. Eur J Pharmacol 181:105–109
Mallory BS, Roppolo JR, de Groat WC (1991) Pharmacological modulation of the pontine micturition center. Brain Res 546:310–320
Marson L (1997) Identification of central nervous system neurons that innervate the bladder body, bladder base, or external urethral sphincter of female rats: a transneuronal tracing study using pseudorabies virus. J Comp Neurol 389:584–602

Matsumoto G, Hisamitsu T, de Groat WC (1995) Role of glutamate and NMDA receptors in the descending limb of the spinobulbospinal micturition reflex pathway of the rat. Neurosci Lett 183:58–61

Matsumoto S, Hanai T, Yoshioka N et al (2006) Medial prefrontal cortex lesions inhibit reflex micturition in anethetized rats. Neurosci Res 54:66–70

Matsuura S, Kakizaki H, Mitsui T et al (2002) Human brain region response to distention or cold stimulation of the bladder: a positron emission tomography study. J Urol 168:2035–2039

McMahon SB, Morrison JF (1982) Two group of spinal interneurones that respond to stimulation of the abdominal viscera of the cat. J Physiol 322:21–34

McMahon SB, Morrison JF, Spillane K (1982) An electrophysiological study of somatic and visceral convergence in the reflex control of the external sphincters. J Physiol 328:379–387

Miller EK, Cohen JD (2001) An integrative theory of prefrontal cortex function. Annu Rev Neurosci 24:167–202

Musil SY, Olson CR (1988) Organization of cortical and subcortical projections to anterior cingulate cortex in the cat. J Comp Neurol 272:203–218

Nadelhaft I, Vera PL (1991) Neurons labelled after the application of tracer to the distal stump of the transected hypogastric nerve in the rat. J Auton Nerv Syst 36:87–96

Nadelhaft I, Vera PL, Card JP et al (1992) Central nervous system neurons labelled following the injection of pseudorabies virus into the rat urinary bladder. Neurosci Lett 143:271–274

Nakamura T, Yoshimura M, Shinnick-Gallagher P et al (1984) alpha 2 and alpha 1-Adrenoceptors mediate opposing actions on parasympathetic neurons. Brain Res 323:349–353

Noto H, Roppolo JR, de Groat WC et al (1991) Opioid modulation of the micturition reflex at the level of the pontine micturition center. Urol Int 47(Suppl 1):19–22

Nour S, Svarer C, Kristensen JK et al (2000) Cerebral activation during micturition in normal men. Brain 123:781–789

Nyo MM (1969) Innervation of the bladder and urethra. J Anat 105:210

Pantoni L, Garcia JH (1997) Pathogenesis of leukoaraiosis: a review. Stroke 28:652–659

Sakakibara R, Hattori T, Uchiyama T et al (1999) Urinary function in elderly people with and without leukoaraiosis: relation to cognitive and gait function. J Neurol Neurosurg Psychiatry 67:658–660

Schondorf R, Laskey W, Polosa C (1983) Upper thoracic sympathetic neuron responses to input from urinary bladder afferents. Am J Physiol 245:R311–R320

Sengupta JN, Gebhart GF (1994) Mechanosensitive properties of pelvic nerve afferent fibers innervating the urinary bladder of the rat. J Neurophysiol 72:2420–2430

Shefchyk SJ, Buss RR (1998) Urethral pudendal afferent-evoked bladder and sphincter reflexes in decerebrate and acute spinal cats. Neurosci Lett 244:137–140

Shefchyk SJ, Espey MJ, Carr P et al (1998) Evidence for a strychnine-sensitive mechanism and glycine receptors involved in the control of urethral sphincter activity during micturition in the cat. Exp Brain Res 119:297–306

Sie JA, Blok BF, de Weerd H et al (2001) Ultrastructural evidence for direct projections from the pontine micturition center to glycine-immunoreactive neurons in the sacral dorsal gray commissure in the cat. J Comp Neurol 429:631–637

Steers WD, Meythaler JM, Haworth C et al (1992) Effects of acute bolus and chronic continuous intrathecal baclofen on genitourinary dysfunction due to spinal cord pathology. J Urol 148:1849–1855

Sugaya K, de Groat WC (1994) Effects of MK-801 and CNQX, glutamate receptor antagonists, on bladder activity in neonatal rats. Brain Res 640:1–10

Sugaya K, Matsuyama K, Takakusaki K et al (1987) Electrical and chemical stimulations of the pontine micturition center. Neurosci Lett 80:197–201

Tai C, Wang J, Wang X et al (2007) Bladder inhibition or voiding induced by pudendal nerve stimulation in chronic spinal cord injured cats. Neurourol Urodyn 26:570–577

Tai C, Shen B, Wang J et al (2008) Inhibitory and excitatory perigenital-to-bladder spinal reflexes in the cat. Am J Physiol Renal Physiol 294:F591–602

Thor KB, Morgan C, Nadelhaft I et al (1989) Organization of afferent and efferent pathways in the pudendal nerve of the female cat. J Comp Neurol 288:263–279
Vanderhorst VG, Holstege G (1997) Nucleus retroambiguus projections to lumbosacral motoneuronal cell groups in the male cat. J Comp Neurol 382:77–88
Vanderhorst VG, Mouton LJ, Blok BF et al (1996) Distinct cell groups in the lumbosacral cord of the cat project to different areas in the periaqueductal gray. J Comp Neurol 376:361–385
Vaughan CW (1992) Hypogastric nerve section reveals a role for both afferent and efferent fibres in the feline continence process. J Auton Nerv Syst 41:197–207
Weaver FM, Follett K, Stern M et al (2009) Bilateral deep brain stimulation vs best medical therapy for patients with advanced Parkinson disease: a randomized controlled trial. JAMA 301:63–73
Yoo PB, Woock JP, Grill WM (2008) Bladder activation by selective stimulation of pudendal nerve afferents in the cat. Exp Neurol 212:218–225
Yoshimura N (1999) Bladder afferent pathway and spinal cord injury: possible mechanisms inducing hyperreflexia of the urinary bladder. Prog Neurobiol 57:583–606
Yoshimura N, de Groat WC (1997) Neural control of the lower urinary tract. Int J Urol 4:111–125
Yoshimura N, Sasa M, Ohno Y et al (1988) Contraction of urinary bladder by central norepinephrine originating in the locus coeruleus. J Urol 139:423–427
Yoshimura N, Sasa M, Yoshida O et al (1990a) Mediation of micturition reflex by central norepinephrine from the locus coeruleus in the cat. J Urol 143:840–843
Yoshimura N, Sasa M, Yoshida O et al (1990b) Alpha 1-adrenergic receptor-mediated excitation from the locus coeruleus of the sacral parasympathetic preganglionic neuron. Life Sci 47:789–797
Yoshimura N, Bennett NE, Hayashi Y et al (2006) Bladder overactivity and hyperexcitability of bladder afferent neurons after intrathecal delivery of nerve growth factor in rats. J Neurosci 26:10847–10855
Yoshiyama M, Roppolo JR, de Groat WC (1993a) Effects of MK-801 on the micturition reflex in the rat–possible sites of action. J Pharmacol Exp Ther 265:844–850
Yoshiyama M, Roppolo JR, Thor KB et al (1993b) Effects of LY274614, a competitive NMDA receptor antagonist, on the micturition reflex in the urethane-anaesthetized rat. Br J Pharmacol 110:77–86
Yoshiyama M, Roppolo JR, de Groat WC (1994) Interactions between glutamatergic and monoaminergic systems controlling the micturition reflex in the urethane-anesthetized rat. Brain Res 639:300–308
Yoshiyama M, Roppolo JR, De Groat WC (1995a) Interactions between NMDA and AMPA/kainate receptors in the control of micturition in the rat. Eur J Pharmacol 287:73–78
Yoshiyama M, Roppolo JR, de Groat WC (1995b) Effects of GYKI 52466 and CNQX, AMPA/kainate receptor antagonists, on the micturition reflex in the rat. Brain Res 691:185–194

Afferent Mechanism in the Urinary Tract

Anthony J. Kanai

Contents

Abstract Much of the current research on lower urinary tract dysfunction is focused on afferent mechanisms. The main goals are to define and modulate the signaling pathways by which afferent information is generated and conveyed to the central nervous system. Alterations in bladder afferent mechanisms are a potential source of voiding dysfunction and an emerging source of drug targets. Even some

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established drug therapies such as muscarinic receptor antagonists, as well as emerging therapies such as botulinum toxin type-A, may act partly through afferent mechanisms. This review presents up-to-date findings on the localization of afferent fiber types within the bladder wall, afferent receptors and transmitters, and how these may communicate with the urothelium, interstitial cells, and detrusor smooth muscle to regulate micturition in normal and pathological bladders. Peripheral and central mechanisms of afferent sensitization and myogenic mechanisms that lead to detrusor overactivity, overactive bladder symptoms, and urgency sensations are also covered as well as new therapeutic approaches and new and established methods of measuring afferent activity.

Keywords Afferent sensitization · Animal models · Boutlinum toxin type-A · Myogenic and neurogenic bladder overactivity · Optical mapping · Voltage-sensitive dyes

1 Introduction

Over the last 10 years, studies of lower urinary tract (LUT) dysfunction have provided a number of new concepts regarding central and peripheral nervous involvement (Andersson and Arner 2004; Tai et al. 2006; Yoshimura et al. 2008). This has led to an intensive search for new therapeutic targets aimed at micturition control through modulation of urothelial and afferent signaling mechanisms (Andersson 2002; Apodaca et al. 2007). Although our understanding of bladder function has increased during this period, the functional regulation of the detrusor and its innervation remains challenging. The detrusor muscle has for many years been the target for antimuscarinic drug therapy. However, even though antimuscarinics have proved effective at ameliorating symptoms of bladder overactivity, there is poor patient compliance due to significant side effects. Voiding is the result of the coordinated contraction of the detrusor and sphincter relaxation, made possible by the interaction of afferent and efferent nerves as well as the urothelium and interstitial cells in the bladder. The goal in treating detrusor overactivity (DO) and overactive bladder (OAB) symptoms is not to interfere with bladder emptying, but to eliminate involuntary bladder contractions.

The detrusor muscle in healthy bladders is not quiescent during the filling phase as the myocytes exhibit an uncoordinated low amplitude spontaneous activity. In detrusor overactivity, this spontaneous activity becomes coordinated, increases in amplitude, and can stimulate afferent nerves. This may contribute to uncontrolled activation of the micturition reflex and sensations of urgency. Accordingly, much of the current research on LUT function is focused on afferent mechanisms and how information is generated and conveyed to the central nervous system and the roles of the urothelium and interstitial cells. The present review focuses on these interactions along with peripheral and central afferent sensitization as it relates to the pathophysiology of DO and the OAB syndrome. It also discusses new and established methods for measuring afferent activity and new therapeutic approaches aimed at controlling it.

2 Afferent Recordings

2.1 Multi- and Single-Unit Recordings

A number of in vivo and in vitro approaches have been used to investigate the different classes and roles of afferent nerve fibers within the bladder. Many investigators record from afferent nerves using platinum or silver wire electrodes with the nerves electrically isolated from the bladder in a chamber containing oil. Dissected nerve bundles are placed on a recording electrode in close proximity to a reference/null electrode. In this way, multi- and single-unit recordings can be obtained depending upon the extent of nerve dissection (see Fig. 1). The decision to use multi- or single-unit recordings depends on the data desired. There are advantages and disadvantages to both approaches. Multiunit afferent recordings describe overall activity as it is not easy to distinguish between changes in the firing of a particular fiber, summation of a number of fibers, or recruitment of new fibers, although offline analysis can be performed in an attempt to distinguish individual fibers from the data collected. Suction electrodes can also be used in vivo or in vitro to record multiunit activity. This technique involves exposure of either the attached pelvic or hypogastric nerve, followed by careful splitting into smaller branches. A nerve branch is then sucked into a glass tube (~0.75 mm ID) with a small silver electrode (~0.2 mm dia). One benefit of this method is that all the tissues remain in physiological solution. The use of this approach has led to a number of important findings including the potential role of transient receptor potential vanilloid 1 (TRPV1) in normal bladder function (Daly et al. 2007) and the mechanosensory actions of $P2X_3$ receptors (Vlaskovska et al. 2001).

There are a number of benefits to single unit recordings: (1) The type of fiber being studied can be characterized based on responses to stimuli; (2) conduction

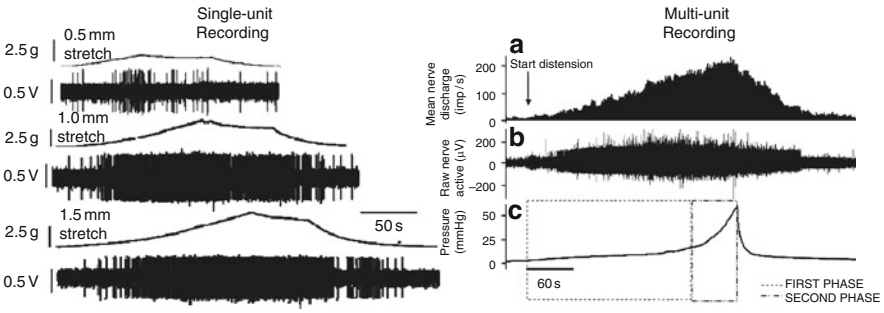

Fig. 1 This figure contrasts single unit afferent nerve recordings from mouse whole bladder sheets (left panel) with multiunit (right panel) recordings from intact mouse bladders during stretch and distension, respectively. In the single unit recording, it is clear that the frequency of firing increases as a function of tension. However, while the frequency of afferent firing can be seen to also increase in the multiunit recording, the response of individual units is difficult to interpret due to the simultaneous firing and recruitment of some afferents (summation) as well as the decrease in firing and loss of other fibers (adaptation). Left panel from McCarthy et al. (2009); right panel from Daly et al. (2007)

velocities can be measured to determine if the fiber is an Aδ- or C-fiber neuron; (3) thresholds can be investigated; and (4) variations in firing rates can easily be recognized. However, an extensive number of individual fibers have to be studied to give a representation of any overall effect on all the various types of fibers located in the bladder. Multiunit recordings have been used to show that the excitability of afferent nerves is increased by both P2X receptor activation and cyclophosphamide (CYP)-induced cystitis. Distension of the bladder resulted in spontaneous contractions, leading to multiple afferent unit firing, which increased in the presence of α,β-methyleneATP, even though the contraction amplitude remained the same (Yu and de Groat 2008). However, these results could not determine which fibers precisely increased in activity, just that there was an overall increase. In a number of other studies aimed at characterizing the afferent nerves that supply the bladder, single-unit activity was recorded. Sengupta and Gebhart demonstrated that of the mechanosensitive fibers studied, 80% were low threshold while 20% were high threshold (Sengupta and Gebhart 1994a). Zagorodnyuk et al. (2006) and Xu and Gebhart (2008) have tried to characterize and determine the properties of the types of fibers based on various stimuli including, stretching, probing, stroking, and chemicals.

2.2 *Patch Clamp Recording*

Patch clamp recording has been used to study the activity of bladder afferent neurons isolated from dorsal root ganglia (DRG) as shown in Fig. 2. For example, Yoshimura et al. (2003) used this approach to investigate the electrophysiological properties of afferent neurons innervating different regions of the LUT. While only a small proportion (1–2%) of the neurons in S_1–S_3 ganglia send projections to the pelvic viscera including the bladder (Janig and Morrison 1986; De Groat 1986), those innervating the bladder can be differentiated by retrograde axonal labeling using fluorescent dyes such a fast blue. The advantage of the patch clamp technique is that it allows for the analysis of ionic currents in discrete cells and their response to pharmacological agents. This approach has been used to identify receptors on the cell bodies with the assumption that these are also expressed at the terminals.

2.3 *Afferent Recording and Optical Mapping*

To better analyze afferent activity, many studies combine nerve recordings with complimentary techniques. For example, cystometry can be performed when afferent activity is recorded in vivo from intact bladders (De Groat and Lalley 1972; Shea et al. 2000). Also, pressure or tension measurements can be taken when afferent activity is recorded in vitro using intact bladders (Namasivayam et al. 1998; Rong et al. 2002) or bladder sheet-nerve preparations (Zagorodnyuk et al. 2006; Xu and Gebhart 2008; McCarthy et al. 2009), respectively. Intact bladders

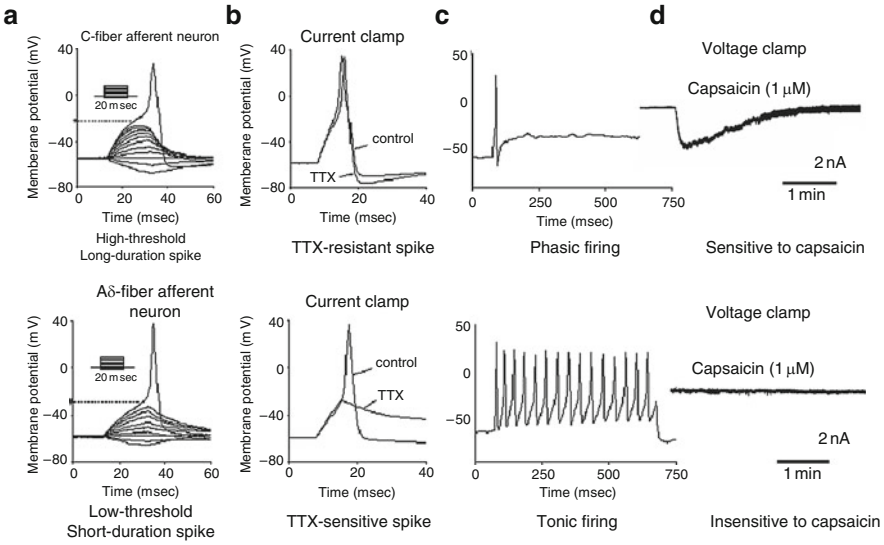

Fig. 2 Characteristics of a bladder afferent neuron (24 μm diameter, C-fiber afferent neuron, top records) exhibiting tetrodotoxin (TTX)-resistant action potentials and a bladder afferent neuron (33 μm diameter, Aδ-fiber afferent neuron, bottom records) exhibiting TTX-sensitive action potentials. (**a**) Voltage responses and action potentials evoked by 30 ms depolarizing current pulses injected through the patch pipette in current-clamp conditions. Asterisks with dashed lines indicate the thresholds for spike activation. (**b**) Effects of TTX application (1 μM) on action potentials. (**c**) Firing patterns during membrane depolarization (700 ms duration). (**d**) Responses to extracellular application of capsaicin (1 μM) in voltage-clamp conditions. Note that the TTX-resistant bladder afferent neuron exhibited phasic firing (i.e., 1–2 spikes during prolonged membrane depolarization) and an inward current in response to capsaicin while the TTX-sensitive afferent neuron exhibited tonic firing (i.e., repetitive firing during membrane depolarization) and no response to capsaicin. Adapted from Yoshimura et al. (2003)

allow for physiological multidirectional distension using saline with or without drugs. However, identification of specific receptive fields is not easy. Bladder sheets, on the other hand, permit the determination of receptive fields, but stretch can only be applied in one or two directions.

The chemosensitivity of bladder afferents can be tested by intravesical application of chemical mediators into intact bladders or on isolated areas on bladder sheets by placing solutions of inflammatory agents into small rings set on the bladder surface (Zagorodnyuk et al. 2007). A general problem with the application of chemical agents is that they may not penetrate to the afferent nerves in deeper layers of the bladder.

In order to demonstrate that multiple receptive fields can interact to encode mechanical information, spatiotemporal mapping has been tested. Indian ink carbon particles were used to mark the receptive fields in ileum (Brookes et al. 1999) and esophagus (Zagorodnyuk and Brookes 2000), following their identification using Von Frey stimulation. The deflections of the carbon particles by distension were video recorded and correlated with afferent firing. To demonstrate that

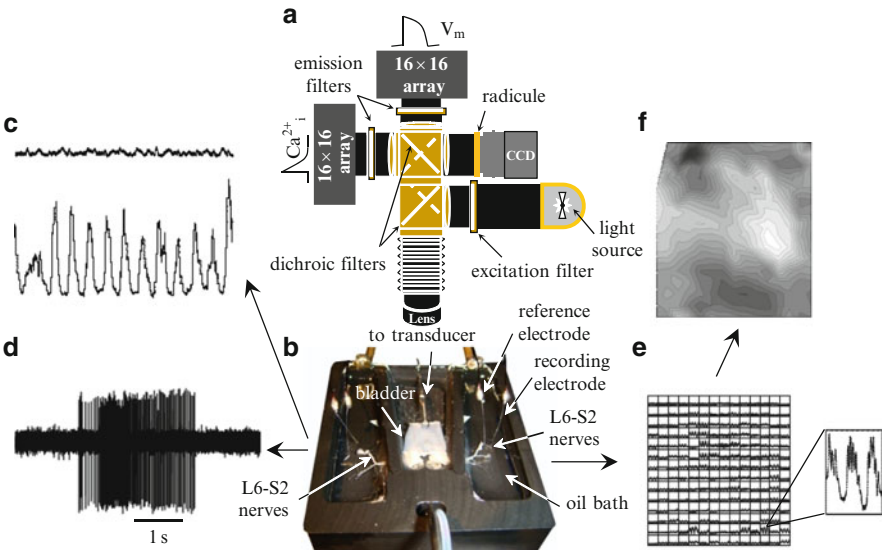

Fig. 3 Spontaneous optical, afferent nerve, and tension recordings. (**a**) Optical system showing the arrangement of the light source, filters, and photodiode array and CCD cameras. (**b**) Bladder-sheet-nerve preparation. (**c**) Tension recordings from normal (upper trace) and overactive (lower trace) mouse bladders. (**d**) Single unit afferent recordings. (**e**) Ca^{2+} signals recorded from the tissue using the photodiode array. (**f**) Isochronal map derived from the data in **e**. The initiation site corresponds to the imaged region of tissue with the earliest Ca^{2+} transients (*white regions*). Adapted from McCarthy et al. (2009)

spontaneous smooth muscle contractions can stimulate afferent firing within a receptive field, Ca^{2+} transients were mapped across the mucosal surface of a bladder sheet while simultaneously recording afferent activity and bladder tension (McCarthy et al. 2009) as shown in Figs. 3 and 4. In this way, the size of a receptive field can also be determined.

3 Afferent Nerve Localization Within the Bladder Wall

3.1 Peripheral and Central Pathways

The coordination between the urinary bladder and the outlet (the bladder neck, urethra, and urethral sphincters) is mediated by a complex neural control system located in the brain, spinal cord, and the peripheral ganglia as depicted in Fig. 5. This includes innervations through sympathetic (hypogastric), parasympathetic (pelvic), and somatic (pudendal) nerves. Sympathetic fibers originate in the T_{11}-L_2 segments of the spinal cord. They run through the ganglia in the inferior mesenteric plexus and then the hypogastric nerves to enter the pelvic plexus and

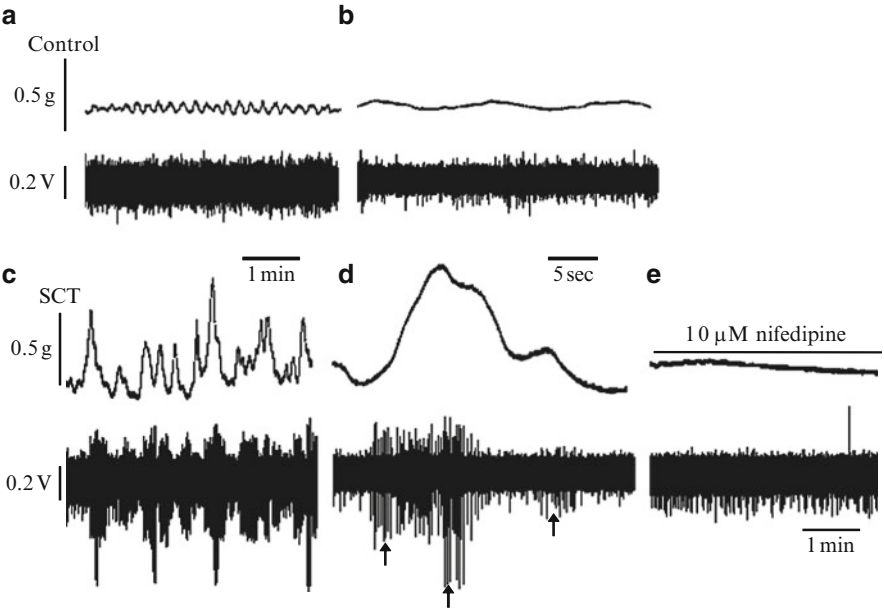

Fig. 4 Spontaneous contractions in a pathological bladder that drive afferent firing. Simultaneous recordings of spontaneous contractile activity and corresponding afferent firing from (**a**) the normal adult and (**c**) spinal cord transected (SCT, T8–T9 level) mouse bladders (expanded time base in panels **b** and **d**, respectively). (**e**) The effect of nifedipine (10 μM) on spontaneous contractions and afferent firing in a SCT mouse bladder. The *arrows* in **d** indicate action potentials of different amplitude recorded from three separate fibers that responded to the spontaneous contraction above. This figure is from McCarthy et al. (2009)

then the base of the bladder and the urethra (de Groat 1997). Parasympathetic fibers arise from the S_2–S_4 spinal segments and travel in the sacral roots and the pelvic nerves through ganglia in the pelvic plexus and into the bladder wall. Somatic nerves supplying the striated muscles of the external urethral sphincter arise from S_2–S_4 motor neurons in Onuf's nucleus and pass through the pudendal nerves.

The afferent components of these nerves consist of myelinated (Aδ-) and non-myelinated (C-) fibers. Sensory input from the bladder is conveyed to the spinal cord through the pelvic and hypogastric nerves (Morrison 1999; Weaver 1985), whereas sensations from the bladder neck and urethra are carried by the pudendal and hypogastric nerves (Fowler et al. 2008). The main function of afferent fibers in the hypogastric nerves is to carry painful impulses upon maximal distensions of the bladder and urethra (Talaat 1937). Aδ-fibers mediate mechanotransduction and volume-sensing functions, conveying information about bladder filling. These fibers are more sensitive, have thinly myelinated axons, are larger in the diameter, and conduct action potentials more rapidly than C-fibers (conduction velocities are < 2 m/s for C-fibers and 3–10 m/s for Aδ-fibers). The majority of C-fibers (~90%) (Habler et al. 1990) are insensitive under normal physiological conditions and are

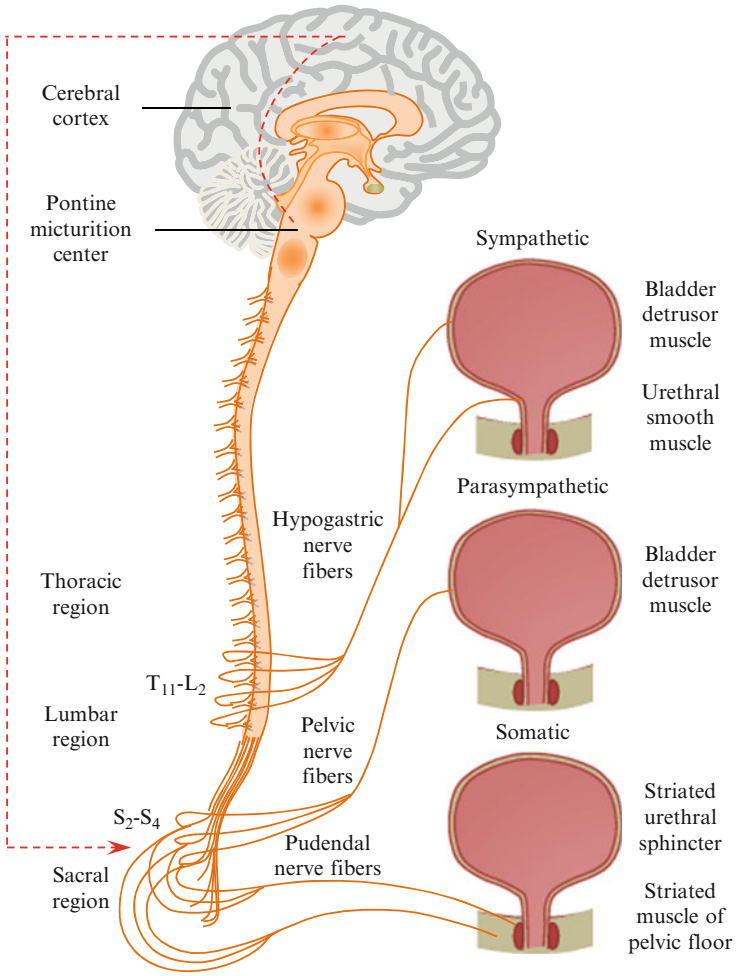

Fig. 5 Innervation of the LUT. The coordination between the urinary bladder and the outlet (the bladder neck, the urethra, and the urethral sphincters) is mediated by sympathetic (hypogastric), parasympathetic (pelvic), and somatic (pudendal) nerves. The primary cell bodies of Aδ- and C-fiber afferents of the pelvic and pudendal nerves are contained in lower lumbar and sacral dorsal root ganglia (DRGs); whereas afferent innervation in the hypogastric nerves arise in the rostral lumbar DRGs

termed "silent" responding primarily to noxious stimuli such as chemical irritation or inflammation.

The primary cell bodies of the Aδ- and C-fiber afferents that travel in the pelvic and pudendal nerves are contained in the lower lumbar and sacral dorsal root ganglia (DRG); the cell bodies of the afferents that travel in the hypogastric nerves arise in the rostral lumbar DRG. The central axons of the DRG neurons carry sensory information from the LUT to second order neurons in the spinal cord.

In the 1970s and 1980s, ventral afferents were reported to constitute an alternative pathway for nociception entering the spinal cord directly (Coggeshall et al. 1974; Coggeshall 1980). However, very few afferent fibers enter the central nervous system through the ventral roots and they now are believed to be of little importance (Risling et al. 1984). They can arise from: (1) specialized Ruffini-like endings within the ventral roots; (2) from free sensory nerve endings in the connective tissue of the pia mater on the ventral surface of the spinal cord; or (3) double back, in a hairpin-like loop, toward the distal end of the ventral roots, thus entering the spinal cord through the corresponding dorsal roots (Nilsson Remahl et al. 2008).

Parasympathetic and sympathetic central (preganglionic) neurons are located in the intermediate gray matter (laminae V–VII) of the sacral and lumbar segments of the spinal cord, respectively. Visceral afferent fibers of the pelvic nerves enter the cord and travel rostrocaudally within Lissauer's tract and transversely around the dorsal horn via the lateral collateral pathway (LCP) and medial collateral pathway (MCP) to reach the deeper layers of the spinal cord (Morgan et al. 1981). Within the spinal gray matter, the LCP and MCP provide a dense innervation to laminae I (superficial dorsal horn), V, and VII and the dorsal commissure. A similar axon collateral system has not been identified in sympathetic preganglionic neurons (Fowler et al. 2008). The somatic motor neurons innervating the external urethral sphincter are located in the ventral horn (lamina IX) in Onuf's nucleus (level S_2). They have similar arrangements of transverse dendrites, as parasympathetic neurons do, and an extensive system of longitudinal dendrites that travel within Onuf's nucleus (Thor et al. 1989). The overlap between parasympathetic and somatic interneurons and dendrites in the dorsal commissure and lateral dorsal horn indicates that these regions are probably important sites of coordination of bladder and sphincter activity.

Some of the lumbosacral spinal cord interneurons send long projections to the brain (Birder et al. 1999); others make local connections in the spinal cord and participate in segmental spinal reflexes (Araki and de Groat 1997). Brain neuron populations specific for micturition include the neurons of the periaqueductal gray, believed to receive bladder afferents from the cord (Duong et al. 1999); the insula, especially on the right, activation of which is linked to subjective visceral sensation ("feeling states"); and the dorsal anterior cingulate cortex, responsible for emotional responses and motivational behaviors. In addition, the prefrontal cortex, particularly the medial part, is believed to be responsible for deciding whether to void based on the social situation. Finally, if voluntary voiding occurs, there is activation of the anterior cingulated cortex and of the pontine micturition center (PMC, or Barrington's nucleus) that results in the coordination of urethral sphincter relaxation, detrusor contraction, and urination (Griffiths et al. 2009).

3.2 Myelinated Aδ-and Unmyelinated C-Fibers

Afferent fibers traveling in the pelvic, hypogastric (lumbar splanchnic), and pudendal nerves continually convey information from the bladder, urethra, and sphincters

to the spinal cord. The sensations transmitted (from fullness to pain) and the activation stimuli (e.g., stretch/volume/chemicals) of bladder afferents are wide ranging; consequently, the types of afferent fibers, their location, and properties are diverse. The characterization of afferent nerves has been studied in a number of species including cat (de Groat and Ryall 1969; Habler et al. 1993b; Yu and de Groat 2008; Jiang et al. 2009), guinea-pig (Zagorodnyuk et al. 2007), rat (Charrua et al. 2008, Yu and de Groat 2008; Cefalu et al. 2009), and mouse (Xu and Gebhart 2008; McCarthy et al. 2009; Walczak et al. 2009). The major afferent supply from the bladder travels in the pelvic nerves, which, along with the hypogastric nerves, have been more extensively studied.

Habler et al. (1990, 1993a, b) comprehensively studied both myelinated and unmyelinated axons within the cat bladder. Only a small subpopulation of unmyelinated afferents responded to high intravesical pressure and chemical irritants. When myelinated afferents were tested, all responded in a graded manner to bladder distension through slow intravesical filling. Habler et al. also showed the chemosensitive nature of the mechanoreceptive myelinated afferents.

Both Aδ- and C-fibers populations have been demonstrated to consist of low and high threshold fibers. The mechanosensitive fibers can be divided into 80% low threshold and 20% high threshold (>20 mmHg) (Sengupta and Gebhart 1994b). In the same study, it was shown that the majority of the fibers that responded to noxious stimuli were C-fibers (68%). Many of the low threshold fibers also responded to contraction. Given the role of afferent fibers in gaging the degree of bladder wall distension, the proportions of low and high threshold fibers are important for normal physiological filling. Studies have shown that the low threshold stretch-sensitive fibers fire in proportion to intravesical pressure and have therefore been described as in-series tension receptors (Shea et al. 2000). Separate from the in-series tension receptors are the proposed volume receptors that sense distension irrespective of bladder pressure. The volume receptors do not respond to increases in intravesical bladder pressure in the same manner as the tension receptors. Instead of responding linearly, they reach a threshold or even decrease in activity with increasing pressure (Shea et al. 2000). Morrison et al. (1999) reported that the volume receptors had a higher threshold than the in-series tension receptors. The high threshold fibers have been associated with the transduction of pain (de Groat 1997).

There is a class of "silent" C-fibers that are not excited by known physiological stimuli even at intensities that can damage the innervated tissue (Michaelis et al. 1996). These fibers are particularly numerous in the bladder and other viscera and may constitute up to 90% of the C-fibers (Habler et al. 1990). During inflammation induced by mustard oil, about 45% of these silent fibers become spontaneously active with 10% thereafter becoming sensitized to physiological stimuli. It is suggested that these fibers are nociceptors with their orthodromic activity summing spatially and temporally at second order neurons to contribute to different pain states (Michaelis et al. 1996). This correlates with the activity of C-fibers increasing in pathological conditions including bladder outlet obstruction (BOO) (Steers et al. 1991), spinal cord injury (Cheng and de Groat 2004), diabetes (Steers et al. 1994),

cystitis (Yoshimura and de Groat 1999), and colitis (Ustinova et al. 2007). C-fibers are also sensitive to overdistension. A model of afferent nerve activity being proportional to the bladder stress (pressure × volume) was proposed where bladder contraction is initiated by exceeding an afferent threshold due to increasing pressure and volume. The contraction then continues until afferent activity drops below threshold as a result of the decreasing volume (le Feber et al. 2004).

3.3 Chemo-, Mechano-, and Thermo-Sensitivity

Two main mechanisms of afferent activation have been described, direct and indirect. Direct refers to physical activation of afferent nerve endings via stimulation of mechanogated receptors such as ENaC, TREK-1, TRPA1, and the osmolarity receptor, TRPV4 (Fig. 6), whereas indirect describes activation of receptors on the nerve fibers by mediators that are released from surrounding non-neuronal cells

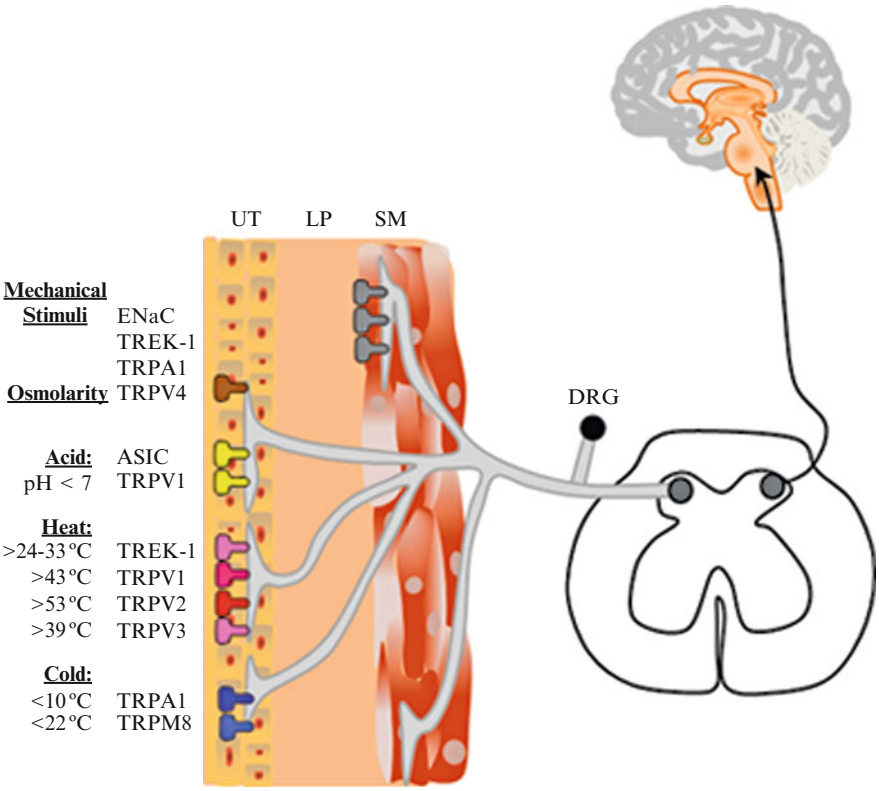

Fig. 6 The regional distribution of chemo-, mechano-, and thermosensitive afferent receptors within the bladder wall. UT urothelium, LP lamina propria, and SM smooth muscle

activated by mechanical stimuli. An example is ATP, which is released from the urothelium in response to stretch (Ferguson et al. 1997) and may activate purinoreceptors on afferent nerve endings. In fact, the use of antagonists such as PPADS and TNP–ATP reduces the mechanosensitivity of afferent nerves (Namasivayam et al. 1999; Rong et al. 2002). The bladder also displays reflex responses to acidity, heat, cold, and noxious cold. These are mediated, respectively, by the ASIC (pH < 7) and TRPV1 (pH < 7) receptors; TREK-1 (>24–33°C), TRPV1 (>43°C), TRPV2 (>53°C), and TRPV3 (>39°C) receptors; and the TRPM8 (<22°C) and TRPA1 (<10°C) receptors.

3.4 *Mucosal, Muscle, and Serosal Afferents*

The response of mechanosensitive afferent nerves in the mouse to stretching, probing, and stroking was used by one group to characterize the fibers tested as either mucosal, muscle-mucosal, muscle, or serosal (Xu and Gebhart 2008) as shown in Fig. 7. However, a second group using similar mechanical tests with the addition of chemical (α,β-methyleneATP and capsaicin) and hypertonic solution stimuli differentiated the fibers into four different functional populations in the guinea

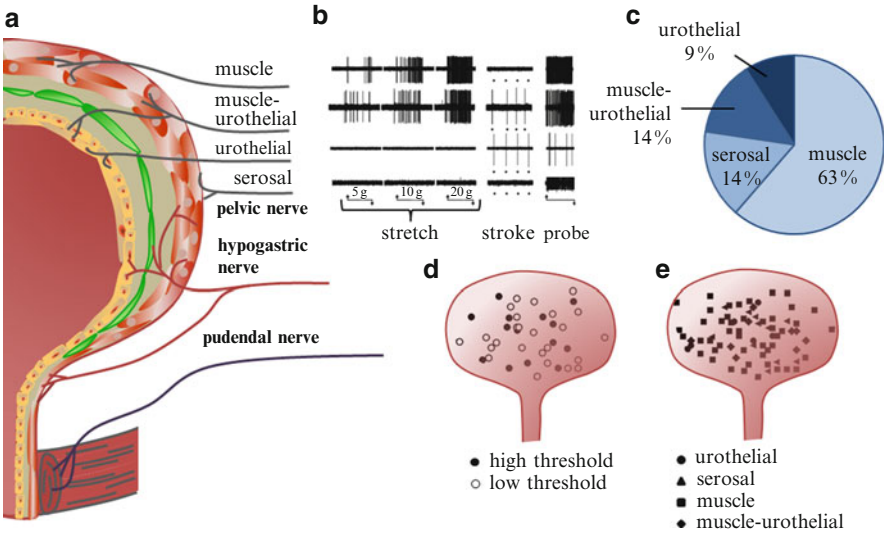

Fig. 7 Classes and distribution of afferent nerves in the LUT. (**a**) The distribution of the different classes of fibers in the bladder wall and urethra. (**b**) In the pelvic nerve, four types of mechanosensitive fibers were identified by stretch, stroke, and probe. (**c**) Proportions of afferent fiber types recorded in the pelvic nerve. (**d**) Distribution of low- and high-threshold receptive fields of pelvic nerve muscle fibers based on responses to stretch and (**e**) Distribution of receptive fields of the four classes of pelvic nerve fibers. The data and illustrations in **b–e** are adapted from Xu and Gebhart Xu and Gebhart (2008)

Table 1 Localization & Characterization of Afferent Nerves in the Bladder Wall

Xu et al.	Zagorodnyuk et al.	Stimulus response
Mucosal	Mucosal high Mucosal low	Stroke and probe
Muscle-mucosal	Muscle-mucosal	Stretch, stroke, and probe
Muscle Serosal	Muscle	Stretch and probe Probe

pig. They are muscle, muscle-mucosal, mucosal high responding, and mucosal low responding mechanoreceptors (Zagorodnyuk et al. 2007). Comparing the response of these different groups of fibers to the particular stimuli suggests overlap between the proposed fiber categories demonstrating consistency as shown in Table 1.

3.5 Pelvic Organ Cross Innervation

Afferent neurons typically innervate a single visceral organ, but there are DRG neurons with multiple or dichotomizing axons, which can innervate adjacent structures (Dawson et al. 1992; Yoshimura et al. 1994). For example, convergent neurons innervating both the bladder and the colon are predominantly found in T_{12}-L_2 and L_6-S_2 DRG (Christianson et al. 2007; Malykhina et al. 2006). Cross-sensitization between pelvic organs promotes the antidromic transmission of noxious stimuli from an irritated organ to an adjacent structure sensitizing the neurons via dichotomizing axons (Pezzone et al. 2005; Malykhina 2007).

There are several theories regarding the mechanism(s) underlying pelvic organ cross-sensitization as shown in Fig. 8. These include an antidromic axon reflex via a single DRG afferent neuron with several branches supplying different organs (Christianson et al. 2007) and afferent convergence in the spinal cord or the brain (Malykhina 2007). Studies in animals showing increased Na^+ currents in bladder afferent neurons in DRG (Malykhina et al. 2004), sensitization of bladder afferent neurons in the spinal dorsal horn by chemically induced colitis (Qin and Foreman 2004), and convergence of information from the bladder and colon to individual Barrington's nucleus neurons (Rouzade-Dominguez et al. 2003) support all three theories, respectively. Cross-sensitization is often implicated as one of the major contributing factors in studies of chronic pelvic pain and bladder overactivity, but it is not limited to the bladder and the bowel. Women with vulvar vestibulitis in many cases also report symptoms of interstitial cystitis (IC) (Fitzpatrick et al. 1993). Experimental studies showed increased uterine motility following bladder inflammation (Dmitrieva et al. 2001). The inflammation of the uterus and endometriosis resulted in increased vascular permeability of the bladder (Morrison et al. 2006). About half of all men with chronic prostatitis also exhibit painful bladder filling (Moldwin 2002).

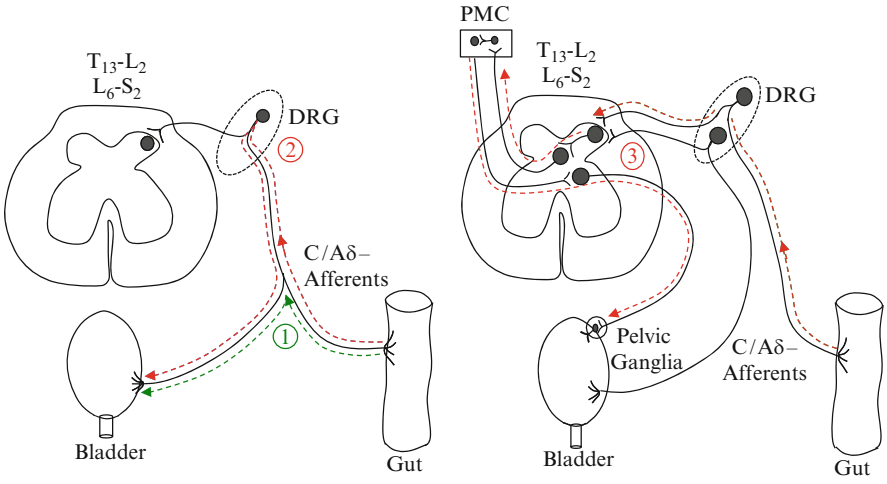

Fig. 8 Putative pathways for peripheral (1 and 2) and central (3) co-innervation of the colon and urinary bladder. (1 and 2) show a dorsal root ganglion (DRG) neuron with multiple axons or branches innervating the colon and the bladder and (3) depicts afferent converge in the spinal cord

During the last decade, the problem of pelvic organ cross-sensitization has attracted much interest. The more widely used model for investigation of the symptoms overlapping with gastrointestinal inflammation is colonic irritation induced by intrarectal instillation of inflammatory agents such as trinitrobenzene sulfonic acid (TNBS) and dextran sulfate sodium (DSS). Using TNBS-induced colitis, some groups of investigators reported increased sensitivity of convergent DRG neurons to mechanical and chemical (capsaicin, bradykinin and Substance P) stimuli 1 h after induction of colitis. This enhanced sensitivity persisted in rats (Ustinova et al. 2006) for 10–30 days (Malykhina et al. 2006) in the absence of obvious colonic inflammation. Studies using DSS-induced colitis showed increased sensitization of spinal lumbosacral neurons receiving input from the bladder (Qin et al. 2005). The instillation of 10% mustard oil intrarectally and into the uterine horn (in proestrus) resulted in protein extravasation in the urinary bladder within 2 h. Hypogastric neurectomy eliminated these effects (Winnard et al. 2006), suggesting that the mechanism involves neurogenic inflammation.

The consequences of bladder irritation on the colon, uterus, and cutaneous sites were studied by several groups. Bielefeldt et al. showed that CYP-induced cystitis and nerve growth factor (NGF) overexpression in the bladder caused enhanced responses to colorectal distention and mechanical and thermal hypersensitivity of the cutaneous referral sites (Bielefeldt et al. 2006). Bladder irritation with instilled protamine sulfate and KCl resulted in reduced thresholds to colorectal distention (Pezzone et al. 2005). Fifty percent turpentine (in olive oil)-induced bladder

inflammation resulted in increased uterine contractions where the hypogastric nerves were shown to be involved (Dmitrieva et al. 2001).

4 Receptors on Afferent Neurons

4.1 Vanilloids (Table 2)

Transient receptor potential vanilloids (TRPV) are nonspecific ion channels expressed predominantly on unmyelinated C-fibers, which are activated by heat, protons, and vanilloid compounds such as capsaicin. TRPV1 receptors are expressed on bladder afferents, detrusor smooth muscle, and urothelial cells, and appear to have a role in modulating bladder sensory function, particularly nociception (Avelino et al. 2002). TRPV1 has been implicated in the development of visceral pain conditions that are triggered by inflammation, e.g., cystitis. CYP- or acrolein-induced cystitis in TRPV1 knockout ($TRPV^{-/-}$) mice have demonstrated that the channel is necessary for the development of hypersensitivity to mechanical and thermal stimulation of the pelvic region (Wang et al. 2008). The sensitization mechanism is thought to involve upregulation of protease-activated receptors (PAR) that co-localize with TRPV1 receptors on afferent nerves in the bladder (Dattilio and Vizzard 2005; Shimizu et al. 2007). In addition, there is increased expression of PAR in patients with IC, which may enhance the sensitization of TRPV1 afferent nerves.

It has also been demonstrated that $TRPV1^{-/-}$ mice have impaired micturition, with increased bladder capacity and decreased force of voiding contractions (Birder et al. 2002). Pelvic nerve recordings from $TRPV1^{-/-}$ mice during voiding cystometry have demonstrated markedly reduced nerve firing in response to bladder distension, indicating that TRPV1 receptors also contribute to mechanosensory function during filling.

TRPA1 receptors have been described as responding to mechanical stimuli and noxious cold (see Fig. 6) and are expressed on sensory nerves in the bladder wall and in the DRG (Du et al. 2007). In recent studies, TRPA1 has been shown to specifically localize to TRPV1-expressing C-fibers (Streng et al. 2008), adding further evidence for their role in mediating nociceptive responses.

TRPV4 is also postulated to have a mechanosensory function in the bladder as well as in detecting changes in osmolality and pressure. This receptor is believed to act as a sensor in the urothelium, where it is abundantly expressed. $TRPV4^{-/-}$ mice also display symptoms of incontinence with spontaneous voiding and increases in micturition contraction intervals (Gevaert et al. 2007).

TRPM8 is believed to be the receptor responsible for detecting and eliciting bladder reflexes to cold. These receptors are expressed throughout the urothelium and on sensory nerve terminals. They may play a role in enhancing sensory responses in idiopathic DO as there is increased numbers of TRPM8-positive nerves in the suburothelium of these patients. However, there is no change in TRPM8 with DO as a result of BOO (Araki et al. 2008).

Table 2 Receptors Localized on Afferent Nerves

Receptor	Agonist/stimuli	Localization	Physiological role
TRPV1	Heat and protons, Capsaicin and resiniferatoxin	C-fibers (DRG and terminals) Kobayashi et al. (2005), Urothelium and detrusor	Nociception
TRPV4	Osmolarity and pressure, bisandrographolide A	C-fibers (DRG and terminals) Kobayashi et al. (2005), Urothelium	Osmolarity and pressure sensor
TRPA1	Noxious cold, *trans*-cinnamaldehyde	C-fibers (DRG and terminals) Kobayashi et al. (2005)	Mechano and noxious cold sensor
TRPM8	Cold, menthol	Aδ and C-fibers (DRG and terminals) Kobayashi et al. (2005)	Cold sensor
$P2X_2$	ATP	C-fibers (DRG and terminals), urothelium	Enhances afferent firing, nociception Cockayne et al. (2005)
$P2X_3$	ATP	C-fibers (DRG and terminals), urothelium	Enhances afferent firing Vlaskovska et al. (2001)
M_3	Acetylcholine	C-fibers (brain, DRG, and terminals), detrusor	Enhances afferent firing Kullmann et al. (2008)
M_2	Acetylcholine	C-fibers (Spinal cord)	Inhibitory effect on micturition Masuda et al. (2009a)
EP receptors	Prostaglandins	C-fibers (terminals)	Increases bladder afferent firing and sensitization through action on NaV1.9 channel Ritter et al. (2009)
Neurokinin (NK)-1, NK-2, NK-3 receptors	Tachykinins	C-fibers (DRG and terminals), Detrusor	NK1 – nociceptive responses Laird et al. (2000) NK2 – enhances detrusor contractions Lecci et al. (1998) NK3 – inhibition of micturition reflex Kamo et al. (2005)
TrkA1	Nerve growth factor (NGF)	Aδ and C-fibers (DRG and terminals)	Responsible for nerve survival, growth and differentiation. May be involved in development of chronic pain following bladder injury Guerios et al. (2006)
5-HT/ serotonin receptors	5-HT/serotonin	Aδ and C-fibers (DRG and terminals)	Inhibits relaxation of bladder neck/urethra through action on receptors in the spinal cord Thor et al. (2002)
Guanylyl cyclase	Nitric oxide	Afferents, urothelium interstitial cells	Inhibits afferent firing Yoshimura et al. (2001)

4.2 Purinergics

The role of purinergic receptors in bladder sensory function was demonstrated using animal cystometric studies, where desensitization of the P2X receptors with α,β-methylene ATP or blockade with a nonspecific antagonist such as suramin significantly reduced pelvic nerve firing. The receptor subtypes were later identified as $P2X_2$, $P2X_3$, and the heteromeric $P2X_{2/3}$, and their role in bladder function has since been extensively studied in knockout mice. In $P2X_3^{-/-}$ animals, it was found that there was increased bladder capacity and reduced afferent firing in response to distension during filling (Cockayne et al. 2005). It was suggested that a subpopulation of $P2X_{2/3}$ positive afferent nerves in the urothelial/suburothelial region are responsible for ATP-mediated firing. This promoted the hypothesis that ATP released endogenously in the bladder in response to distension (potentially from the urothelium) can affect bladder afferent nerve firing. Central and peripheral $P2X_3$ and $P2X_{2/3}$ receptors appear to mediate different sensory pathways, with $P2X_3$ predominantly regulating mechanosensitivity and $P2X_{2/3}$ regulating nociception following neurogenic or externally mediated inflammation.

The P2Y receptors are also believed to have a regulatory role in bladder sensory neurons. Immunohistochemical studies have shown that $P2Y_1$ and $P2Y_4$ subtypes are mostly expressed on sensory neurons in the DRG and that these neurons also express $P2X_3$ (Ruan and Burnstock 2003; Ruan et al. 2005). $P2Y_2$ and $P2Y_4$ receptors on the urothelium (Chopra et al. 2008) and $P2Y_6$ receptors on suburothelial interstitial cells (Sui et al. 2006) may also have a role in modulating the activity of afferent terminals.

4.3 Muscarinics

Muscarinic antagonists are the first-line pharmacological agents utilized for the treatment of OAB. They were originally thought to be acting by inhibiting the contractile activity of the detrusor smooth muscle; however, there is evidence that they have efficacy in the treatment of urgency symptoms in the absence of detrusor overactivity. This suggests that there are targets other than the detrusor that they are acting on to ameliorate these symptoms.

Afferent firing in the rat pelvic nerve was found to decrease in response to systemic administration of the M_3-selective antimuscarinic drug, darifenacin (Iijima et al. 2007). However, it is not known if antimuscarinic drugs directly act through afferent nerves as they may be acting up or downstream of the afferent firing. Alternative targets include the urothelium and IC, which both abundantly express muscarinic receptors. Intravesical administration of the nonspecific muscarinic antagonist, oxybutynin, in rats caused a reduction in C-fiber firing, presumably by acting on muscarinic receptors on the urothelium (De Wachter and Wyndaele 2003). Oxybutynin was also found to be effective in increasing intercontractile intervals in rats with bladder irritation, bringing the interval length closer to those

seen in control rats (Masuda et al. 2009b). Long-term administration of oxybutynin also decreased c-fos expression in the lumbosacral spinal cord in response to bladder distension (Haga et al. 2009). These studies suggest there are different levels at which muscarinic receptors may be acting to modulate bladder afferent nerves and that the detrusor is not the sole target.

4.4 Other Receptors

There are five classes of prostaglandins (PGD_2, PGE_2, PGF_2, PGI_2, and thromboxane A_2), with specific receptors for each type (DP, EP, FP, IP, and TP, respectively). Prostaglandins are produced by various cell types in the bladder and have been shown to increase in expression under conditions such as IC. Some clinical trials have demonstrated efficacy of cyclooxygenase inhibitors in ameliorating the symptoms of IC. Prostaglandins may have effects both centrally and peripherally. Intrathecal administration of EP3 receptor antagonists reduced intercontractile intervals and nociceptive responses to bladder distention (Su et al. 2008b). Similar responses were obtained with peripheral administration (Su et al. 2008a).

5-Hydroxytryptamine (5-HT) or serotonin releasing neurons innervate various regions of the bladder, but their interaction with bladder afferents is believed to occur predominantly at the spinal cord level. There are numerous 5-HT receptors expressed on visceral afferent DRG cells in the dorsal horn ($5\text{-}HT_{1A}$, $5\text{-}HT_2$ and $5\text{-}HT_3$) (Burgard et al. 2003). The action of each 5-HT subtype on afferent activity appears to be different. For example, $5\text{-}HT_2$ receptors appear to mediate an inhibitory effect on parasympathetic neurons innervating the bladder, potentially through activation of inhibitory interneurons. Nociceptive activity of bladder afferents appears to be modulated by $5\text{-}HT_1$ receptors, where activation of the receptor dampens nociceptive responses (Thor et al. 2002). 5HT-receptors also appear to modulate smooth muscle contractions, with an excitatory effect on the cells (Messori et al. 1995; Sellers et al. 2000; Darblade et al. 2005). There have also been clinical trials with serotonin/norepinephrine reuptake inhibitors as a potential treatment for stress urinary incontinence (Mariappan et al. 2007).

NGF is a survival factor that binds to the tyrosine receptor kinase A (trkA) receptor and has been shown to be essential for certain neuronal cells such as those in DRG and sympathetic neurons. It is also involved in regulating neuronal function and plasticity, and has been implicated in the development of visceral pain. Increased levels of NGF in the urine have been found in multiple bladder pathologies including patients with IC (Liu et al. 2009), BOO (Liu and Kuo 2008), and neurogenic and idiopathic bladder overactivity (Liu et al. 2008; Yokoyama et al. 2008), thus making it a potential target for the treatment of sensory disorders of the urinary tract. NGF levels increase in animal models following chemical irritation or inflammation in the bladder (Oddiah et al. 1998; Saban et al. 2002). Intravesical instillation of NGF can also cause symptoms of bladder overactivity (Guerios et al. 2006). Adenoviral vector-mediated overexpression of NGF in the urinary bladder

caused overactivity similar to that observed with acetic acid irritation. This suggests that along with inflammatory mediators, there is an additional contribution of NGF to peripheral afferent sensitization (Lamb et al. 2004).

Nitric oxide (NO) is believed to be an important modulator of bladder activity, including sensory nerves. Nitric oxide synthase (NOS) is expressed in afferent nerves that innervate all regions of the bladder. Their role in afferent sensitization was postulated by demonstration of increased expression of NOS in neurons from the upper lumbar ganglia (L_1–L_2) following inflammation of the bladder. However, an increase in NOS was not observed in the lumbarsacral (L_6-S_1) DRG (Vizzard et al. 1995a, b, 1996; Callsen-Cencic and Mense 1997). Increased release of NO from the bladder has also been demonstrated following chemical irritation. It was suggested that the increased production of NO by rostral lumbar sensory nerves enhances bladder overactivity by reducing sympathetic inhibition.

5 Afferent Adaptation

5.1 Peripheral and Central Afferent Sensitization

Animal models exhibiting myogenic and neurogenic bladder overactivity, respectively, include the established upper motor neuron lesioned mouse, spinal cord transected (SCT) between T_8 and T_9, and a new irradiation-induced colitis mouse model (developed in the author's laboratory) (Zabbarova et al. 2009). Both of these models exhibit bladder overactivity when studied using in vivo cystometry. However, when the bladders are excised and studied as whole sheets, only those from SCT mice exhibited overactivity, suggesting that the colitis-induced overactivity involves central sensitization (Fig. 9, left panel). Urinary diversion prior to upper motor neuron lesion prevented the development of overactivity, demonstrating the importance of urinary retention and bladder distension in the development of myogenic overactivity (Fig. 9, center panel).

Some afferent fibers not only serve sensory functions but also possess local efferent functions, which take place at the level of their peripheral endings. These fibers are thought to be mainly C-fibers, although Aδ-fibers can also be involved (Maggi 1995). They can be pharmacologically characterized by sensitivity to capsaicin, which can initially excite, but subsequently desensitize them (Holzer 1991). Their efferent functions are mediated by the local release of a number of agents including calcitonin gene related-peptide (CGRP), neurokinin A (NKA), substance P (SP), and vasoactive intestinal polypeptide (VIP) in response to noxious stimuli (see Fig. 10). These peptides are co-stored in the large dense core vesicles; probably co-released from sensory terminals and, on their peripheral targets, bind to CGRP, NK_2 (NKA), NK_1/NK_2 (SP), and $VPAC_1$/$VPAC_2$ (VIP) receptors, producing neurogenic inflammation either directly or through activation of mast cells (Andrews and Helme 1987; Pontari and Ruggieri 2008). The

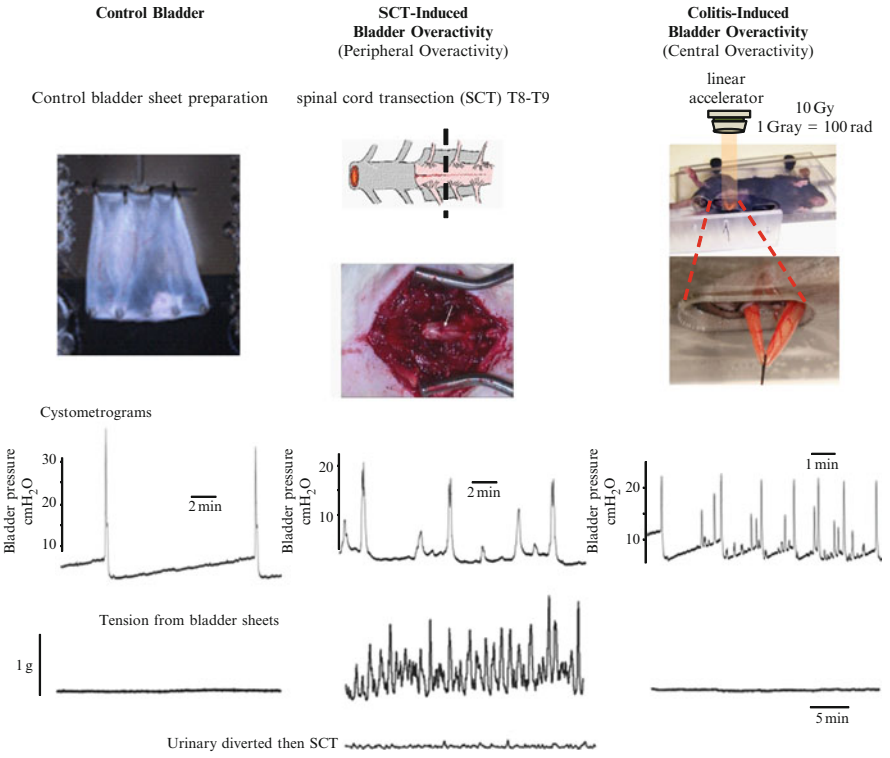

Fig. 9 Mouse models for myogenic (T8–T9 SCT; center panels) and neurogenic (radiation-induced colitis; right panels) bladder overactivity. Using in vivo cystometry, both pathological models exhibited bladder overactivity at 7–14 days. However, upon excision and in vitro sheet tension measurements, only bladders from SCT animals exhibited spontaneous contractions, demonstrating myogenic overactivity in the SCT model. Diversion of the ureters to the uterus prior to SCT prevented bladder distension, hypertrophy and the development of overactivity. The control bladder sheet preparation, cystometrogram, and tension measurements are shown in the left panels. Live animal photos by permission of the IACUC, University of Pittsburgh

neurogenic inflammation is characterized by vasodilatation and plasma extravagation (Wesselmann 2001). Glutamate (Glu) has been also recognized as a neurotransmitter. Many studies have focused on its release from central synapses in the dorsal horn (Yoshimura and Jessell 1990; King and Lopez-Garcia 1993). However, Glu receptors are also expressed on the peripheral terminals of many sensory neurons (Coggeshall and Carlton 1998; Zhou et al. 1996), and their expression is increased during inflammation (Carlton and Coggeshall 1999; Westlund et al. 1992; Keast and Stephensen 2000).

Another mechanism of afferent adaption involves neuronal remodeling and sprouting. Following a damaging insult to the bladder, there can be alterations in the function or architecture of the peripheral afferent terminals or within the spinal cord. It has been demonstrated recently in T_4-SCT rats that there is increased sprouting of unmyelinated CGRP containing nerves in the lumbosacral region of

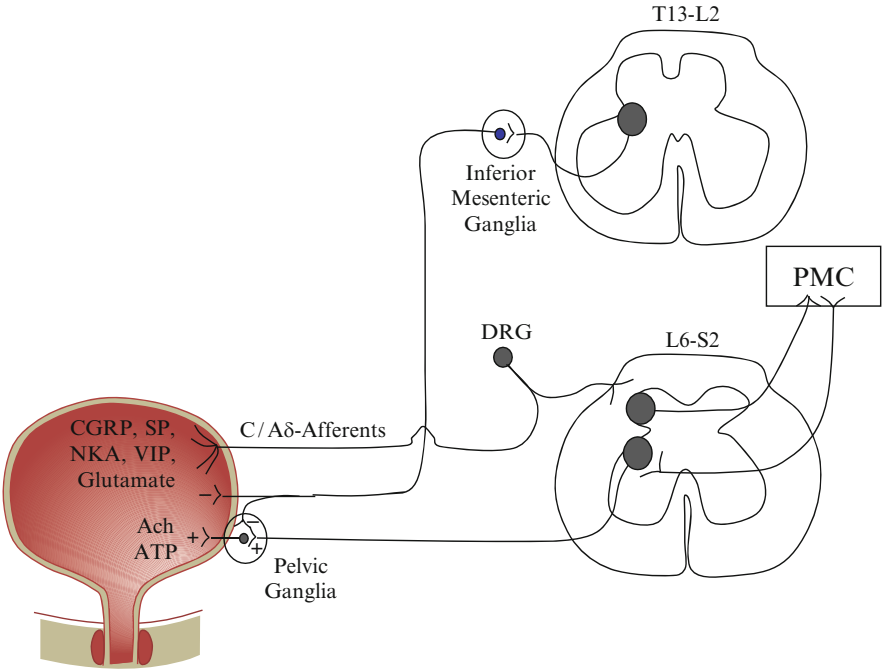

Fig. 10 Schematic depicting the antidromic release of excitatory neuropeptides (CGRP, neurokinin A, substance P, VIP, and glutamate) from afferent neurons

the spinal cord (Zinck et al. 2007; Hou et al. 2009). This regeneration and remodeling of the afferent nerves is believed to facilitate the emergence of reflex bladder activity as well as contribute to the development of visceral pain.

The functional remodeling of afferent nerves is driven by a variety of neurotrophic factors including NGF, not only at the spinal cord level but also in the periphery. There have been numerous studies suggesting that peripheral NGF contributes to the sensitization of sensory nerves and the development of chronic pain in various visceral organs.

An increase in the responsiveness of dorsal root neurons to noxious stimuli and changes in gene expression can cause a decrease in pain thresholds that characterize the phenomenon of central sensitization. Stimuli that can cause central sensitization include tissue inflammation and nerve injury where there is repeatable or intense C-fiber stimulation. Injury-induced central sensitization is thought to underlie allodynia and hyperalgesia in acute and chronic pain. Cellular changes associated with central sensitization are not restricted to neurons. Non-neuronal cell types, particularly immune cells, can play a role in the induction and maintenance of hyperalgesia. Primary afferents excited by a variety of strong noxious stimuli release neurotransmitters (including glutamate, tachykinins, etc.) antidromically from the peripheral terminals as well as in the spinal cord dorsal horn. Neurotransmitters released in the latter case can sensitize second and higher order sensory

Table 3 List of some of the excitatory neuropeptides that can be released antidromically from afferent neurons and their target receptor

Peptide	Target receptor
CGRP	CGRP
Neurokinin A	NK_2
Substance P	NK_1/NK_2
VIP	$VPAC_1/VPAC_2$
Glutamate	NMDA, AMPA, mGluRs1–8

neurons including projection neurons, leading to the transmission of noxious signals to the brain (Table 3).

A range of Ca^{2+} and K^+ ion channels also regulate the activation of afferent nerves. Changes to these channels following a sensitizing insult to the sensory nerves could explain alterations in their excitability. The majority of studies on afferent nerve ion channels have used isolated DRG cells and patch clamping methods. Capsaicin-sensitive bladder afferent C-fibers show high threshold Na^+ currents, with action potentials resistant to TTX. They also express slowly inactivating A-type K^+-currents that decrease the excitability of the nerves. Following CYP-induced cystitis, it was observed that there was hypertrophy and increased excitability of C-fiber neurons (Yoshimura and de Groat 1999). Therefore, K^+-channels on DRG cell bodies may be a potential therapeutic target for bladder overactivity. Several specific K^+-channel openers have been shown to decrease the excitability of these neurons (Sculptoreanu et al. 2004).

5.2 Myogenic Enhancement of Afferent Firing

In addition to the functional alterations that occur to sensory nerves, other structures in the bladder may contribute to or exacerbate afferent sensitization as shown in the models in Fig. 11. Isolated in vitro bladder preparations exhibit spontaneous contractile activity, which appear to be driven by structures intrinsic to the bladder (e.g., smooth muscle, intramural ganglia, interstitial cells or the urothelium). Using animal models, it has been demonstrated that BOO can cause significant remodeling of the bladder wall affecting all cell types. In addition, the detrusor smooth muscle from obstructed bladders displays enhanced spontaneous contractile activity, likely a consequence resulting from the hypertrophy of the smooth muscle. There is also a change in the pattern of spontaneous contractions in obstructed versus control bladders, where pathological bladders display an increase in the amplitudes of spontaneous contractions, a decrease in their frequency, and a regular periodicity. This suggests that there is a mechanism that organizes smooth muscle bundles to coordinate and drive spontaneous contractions. One possible mechanism would be the development of a syncytial interstitial cell network following an insult to the bladder, one that is similar to the network found in the digestive tract.

McCarthy et al. 2009 demonstrated that mechanosensitive afferent nerves are activated by enhanced spontaneous contractions in the bladders of SCT mice

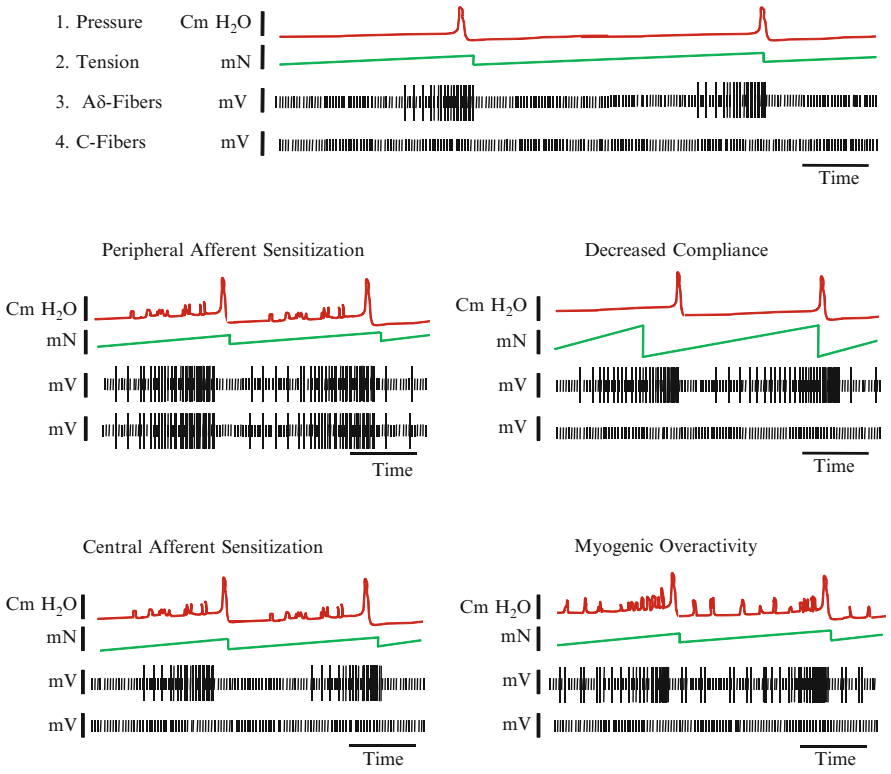

Fig. 11 Modeling of different types of afferent sensitization. In normal bladders, Aδ-fibers begin to fire as the bladder fills in order to initiate a micturition contraction. In different pathologies, this pattern of afferent activity can be altered. For example, following afferent sensitization, Aδ-fibers may begin to respond at lower levels of distention, and previously nonresponsive C-fibers may be recruited. Aδ-fibers also fire in response to increased contractile activity of the detrusor

(Figs. 3 and 4). Moreover, SCT causes inflammation, which may lead to the sensitization of C-fibers, which would not normally be mechanosensitive. This combination of large amplitude spontaneous contractions and sensitized C-fibers could result in increased sensation at lower filling volumes and potentially urgency.

The urinary bladders of many species, including man, normally exhibit low amplitude spontaneous contractions during bladder filling. This activity involves waves of contraction and localized stretches of the wall that are influenced by several factors including the myocytes themselves (Gillespie 2004). The role of spontaneous contractions in the normal bladder is still unclear. It has been suggested that they help to adjust muscle tone and keep the bladder taut against the developing bolus of urine as the bladder fills (Brading 2006). They may also have a role in delivering additional signals to mechanosensitive afferents regarding bladder fullness. Accordingly, changes in spontaneous activity may alter the ability of afferent nerves to correctly respond during the filling phase.

Spontaneous contractile activity has been shown to develop more often in muscle strips from overactive than normal bladders (Kinder and Mundy 1987; Brading 1997; Mills et al. 2000). In isolated detrusor smooth muscle preparations, the frequency of the spontaneous rhythmic contractions varies between species and is probably influenced by experimental factors (Andersson and Arner 2004; Gillespie 2004). These contractions are resistant to the Na^+ channel blocker, tetrodotoxin (TTX), hexamethonium, atropine, α-adrenoceptor blockers, β-adrenoceptor blockers, and the nonselective purinergic antagonist, suramin, excluding direct involvement of nerves and nerve-released transmitters (Andersson and Arner 2004). However, they can be effectively inhibited by L-type Ca^{2+} channel blockers, K^+ channel openers and agents that decrease K^+ permeability, supporting the important role of L-type Ca^{2+} channels for the activity.

There are reasons to believe that the spontaneous activity of the detrusor during filling can generate afferent input ("afferent noise") and that this activity may contribute to pathological conditions, e.g., in OAB/DO (Gillespie et al. 2009). The influence of spontaneous contractions on afferent excitability in normal and CYP treated rats, using an in vitro bladder pelvic nerve preparation and multiunit afferent recordings, was studied by Yu and de Groat (2008). In normal rat bladders, distension resulted in phasic and tonic afferent activity. Phasic firing mirrored spontaneous contractions, while tonic activity increased as a function of stretch, which is a consequence of fiber recruitment in multiunit recordings. CYP-induced cystitis increased bladder afferent nerve activity mimicking the sensitizing effect of purinergic agonists. Antagonists of purinergic receptors (TNP–ATP and PPADS) reduced these effects, suggesting the contribution of purinergic mechanisms to afferent sensitization induced by chemical cystitis.

The contribution of afferent hyperexcitability to the emergence of DO and OAB symptoms has been identified in clinical studies using botulinum toxin type-A (BTX-A) and resiniferatoxin (RTX). The upregulation of C-fiber in subjects with neurologic DO and BOO can be brought out using ice water cystometry, which elicits a C-fiber-dependent spinal micturition reflex (Hirayama et al. 2003). The use of BTX-A, which in effect produces chemical denervation and suppresses bladder afferent activity, mitigated urgency in both neurogenic and idiopathic DO and, with sustained therapy, reduced the expression of TRPV1 and $P2X_3$ receptors in C-fibers (Apostolidis et al. 2005a). In patients with DO induced by spinal cord injury, intravesical therapy with the C-fiber toxin, RTX, led to a decrease in the number of nerve fibers positively stained for the neuronal marker, PGP9.5, a decrease in the expression of TRPV1 receptors and an improvement in cystometric parameters (Brady et al. 2004). In patients with idiopathic DO, intravesical RTX delayed or suppressed involuntary detrusor contractions during filling cystometry (Silva et al. 2002). Overall, it is likely that the hyperexcitability of bladder afferent pathways, especially the C-fiber population, contributes to the emergence of OAB symptoms. Therefore, therapies targeting receptors and ion channels expressed in C-fibers may help to decrease DO and reduce OAB symptoms.

6 New Pharmacological Approaches

Anti-muscarinic drugs are currently the only orally administered pharmacological therapy approved for use in the treatment of OAB symptoms. They were initially designed to inhibit postsynaptic muscarinic receptors as it was assumed that they were acting on the detrusor muscle to inhibit contractile activity. However, detrusor overactivity is not always associated with OAB, suggesting that antimuscarinics may have effects on sensory mechanisms to ameliorate symptoms. M_3-receptors are expressed not only on the detrusor but also on the urothelium (Bielefeldt et al. 2006; Zarghooni et al. 2007) and interstitial cells (Grol et al. 2009). Muscarinic receptors may also act directly on sensory nerves as the M_3 selective antagonist, darifenacin, can inhibit afferent nerve firing during bladder filling in rats (Iijima et al. 2007). Therefore, the mechanisms and sites of action of antimuscarinics are varied. Nevertheless, although antimuscarinics have been effective in reducing OAB symptoms, there are significant sides effects that can affect patient compliance (Yu et al. 2005). These commonly include dry mouth, constipation, and blurred vision (Abrams and Andersson 2007). Therefore, there has been a drive to elucidate alternative pharmacological targets for the treatment of OAB as depicted in Fig. 12.

Other potential targets that have been suggested include β_3-receptors. It has been well established that activation of β_3-receptors can cause relaxation of detrusor smooth muscle (Yamanishi et al. 2002, 2006). However, there are recent studies suggesting that β_3-receptors may also be involved in urothelial signaling as they are highly expressed on the urothelium (Otsuka et al. 2008). It has been suggested that sensory nerves may contribute to the relaxation effect of β_3 receptor agonists (Tucci et al. 2002).

There has also been a focus on directly modulating the activity of bladder sensory nerves. The $P2X_3/P2X_2$ receptors have been suggested as potential targets as they increase in expression following bladder inflammation and contribute to enhanced afferent firing (Dang et al. 2008). Inhibition of these receptors has been shown to decrease bladder hyperactivity induced by inflammation potentially by affecting afferent activity (Ito et al. 2008). Therefore, $P2X_{2/3}$ receptor antagonists may aid in treating conditions such as IC or neurogenic overactivity.

Oral therapeutics is the most desired method for clinical treatment. Intravesical therapies, however, are still utilized as they may have less systemic effects. Intravesical capsaicin and RTX have been tested clinically to treat IC (Lazzeri et al. 1996; Payne et al. 2005), idiopathic (Yokoyama et al. 2005) and neurogenic bladder overactivity (Watanabe et al. 2004; Silva et al. 2005). Both these groups of patients showed improvement in bladder function and the quality of life scores. However, in patients with IC, there appeared to be mixed results on pain symptoms, with some studies reporting no effect (Payne et al. 2005) and others reporting significant improvements (Apostolidis et al. 2006).

It is hypothesized that suburothelial interstitial cells may be involved in modulating sensory nerve activity (Wiseman et al. 2003) (see Fig. 12). Similarly to intestinal interstitial cells, those in the bladder express the pro-oncogene tyrosine

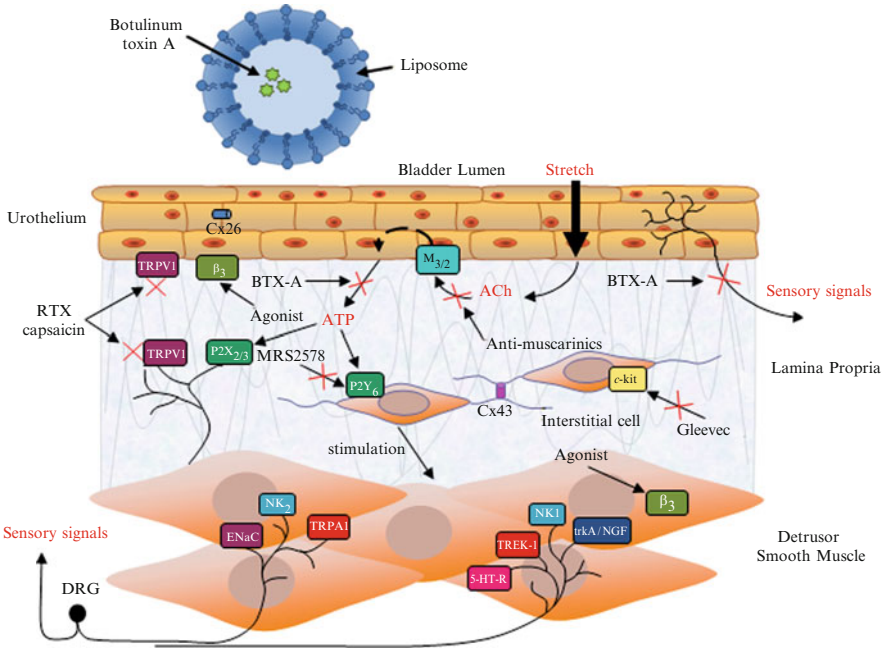

Fig. 12 There are multiple therapeutic targets within the bladder that can be selected to modulate afferent activity. These include receptors on the urothelium, interstitial cells, and afferent terminals. For example, stretch-mediated release of transmitters from the urothelium can be inhibited by desensitizing TRPV1 channels using capsaicin or RTX. Interstitial cell activity can be inhibited by blocking $P2Y_6$ receptor activation (e.g., MRS2578, a selective $P2Y_6$ antagonist) or *c-kit* (*e.g.*, Gleevec, a *c-kit* tyrosine kinase inhibitor). Afferent nerves can be modulated directly by targeting numerous receptors such as those shown in the diagram. Clinical studies have also demonstrated the efficacy of compounds such as botulinum toxin A (BTX-A) on sensory symptoms indicating an action on afferent nerves

kinase receptor, *c-kit*. In isolated bladder tissue, inhibition of *c-kit* receptors using imatinib myselate (known as Gleevec or Glivec) resulted in reduction of spontaneous contractile activity in the bladder (Kubota et al. 2006), which correlated with increased numbers of *c-kit* positive interstitial cells in OAB human bladder samples (Biers et al. 2006). However, a direct link between interstitial cell activity and afferent firing has yet to be established.

The use of BTX-A has received considerable attention recently for the treatment of a number of bladder disorders including OAB and IC. There is evidence that in addition to the known effect on neurotransmitter release from efferent nerves, BTX-A has a therapeutic effect on sensory nerves. For example, one study has shown that intravesical administration of BTX-A reduced the release of CGRP from sensory nerves (Chuang et al. 2004), which may improve IC symptoms. However, in order to enable the large molecular weight toxin (~150 kDa) to access the nerves, protamine sulfate was used to increase urothelial permeability. An alternative is to inject the toxin directly into the detrusor. Using this route of administration,

Apostolidis et al. demonstrated a decrease in sensory receptors on suburothelial nerves in patients with detrusor activity (Apostolidis et al. 2005b). Although these methods of delivery have proven to be successful, there are a number of potential complications such as leakage, pain, and uneven distribution. More recently, a study has demonstrated the feasibility of using liposomes as a vehicle to deliver the toxin without the need to disrupt the urothelium or inject the bladder (Chuang et al. 2009) (see Fig. 12). In this study, liposomal delivery of BTX-A in rats reduced acetic acid-induced bladder hyperactivity.

7 Conclusion

Current research on afferent mechanisms in bladder pathophysiology, focusing on afferent mechanisms initiated by the urothelial and the myogenic pathways, has revealed a number of peripheral mechanisms involved in the regulation of normal and dysfunctional micturition. While some of these mechanisms may be realistic drug targets, it is important to keep in mind the large number of steps between a preclinical target and a successful drug candidate.

References

Abrams P, Andersson KE (2007) Muscarinic receptor antagonists for overactive bladder. BJU Int 100:987–1006

Andersson KE (2002) Bladder activation: afferent mechanisms. Urology 59:43–50

Andersson KE, Arner A (2004) Urinary bladder contraction and relaxation: physiology and pathophysiology. Physiol Rev 84:935–986

Andrews PV, Helme RD (1987) Neurogenic plasma extravasation in response to mechanical, chemical and thermal stimuli. Clin Exp Neurol 23:95–100

Apodaca G, Balestreire E, Birder LA (2007) The uroepithelial-associated sensory web. Kidney Int 72:1057–1064

Apostolidis A, Brady CM, Yiangou Y, Davis J, Fowler CJ, Anand P (2005a) Capsaicin receptor TRPV1 in urothelium of neurogenic human bladders and effect of intravesical resiniferatoxin. Urology 65:400–405

Apostolidis A, Popat R, Yiangou Y, Cockayne D, Ford AP, Davis JB, Dasgupta P, Fowler CJ, Anand P (2005b) Decreased sensory receptors P2X3 and TRPV1 in suburothelial nerve fibers following intradetrusor injections of botulinum toxin for human detrusor overactivity. J Urol 174:977–982, discussion 82–83

Apostolidis A, Gonzales GE, Fowler CJ (2006) Effect of intravesical Resiniferatoxin (RTX) on lower urinary tract symptoms, urodynamic parameters, and quality of life of patients with urodynamic increased bladder sensation. Eur Urol 50:1299–1305

Araki I, de Groat WC (1997) Developmental synaptic depression underlying reorganization of visceral reflex pathways in the spinal cord. J Neurosci 17:8402–8407

Araki I, Du S, Kobayashi H, Sawada N, Mochizuki T, Zakoji H, Takeda M (2008) Roles of mechanosensitive ion channels in bladder sensory transduction and overactive bladder. Int J Urol 15:681–687

Avelino A, Cruz C, Nagy I, Cruz F (2002) Vanilloid receptor 1 expression in the rat urinary tract. Neuroscience 109:787–798

Bielefeldt K, Lamb K, Gebhart GF (2006) Convergence of sensory pathways in the development of somatic and visceral hypersensitivity. Am J Physiol Gastrointest Liver Physiol 291: G658–G665

Biers SM, Reynard JM, Doore T, Brading AF (2006) The functional effects of a c-kit tyrosine inhibitor on guinea-pig and human detrusor. BJU Int 97:612–616

Birder LA, Roppolo JR, Erickson VL, de Groat WC (1999) Increased c-fos expression in spinal lumbosacral projection neurons and preganglionic neurons after irritation of the lower urinary tract in the rat. Brain Res 834:55–65

Birder LA, Nakamura Y, Kiss S, Nealen ML, Barrick S, Kanai AJ, Wang E, Ruiz G, De Groat WC, Apodaca G, Watkins S, Caterina MJ (2002) Altered urinary bladder function in mice lacking the vanilloid receptor TRPV1. Nat Neurosci 5:856–860

Brading AF (1997) A myogenic basis for the overactive bladder. Urology 50:57–67, discussion 68–73

Brading AF (2006) Spontaneous activity of lower urinary tract smooth muscles: correlation between ion channels and tissue function. J Physiol 570:13–22

Brady CM, Apostolidis AN, Harper M, Yiangou Y, Beckett A, Jacques TS, Freeman A, Scaravilli F, Fowler CJ, Anand P (2004) Parallel changes in bladder suburothelial vanilloid receptor TRPV1 and pan-neuronal marker PGP9.5 immunoreactivity in patients with neurogenic detrusor overactivity after intravesical resiniferatoxin treatment. BJU Int 93:770–776

Brookes SJ, Chen BN, Costa M, Humphreys CM (1999) Initiation of peristalsis by circumferential stretch of flat sheets of guinea-pig ileum. J Physiol 516(Pt 2):525–538

Burgard EC, Fraser MO, Thor KB (2003) Serotonergic modulation of bladder afferent pathways. Urology 62:10–15

Callsen-Cencic P, Mense S (1997) Expression of neuropeptides and nitric oxide synthase in neurones innervating the inflamed rat urinary bladder. J Auton Nerv Syst 65:33–44

Carlton SM, Coggeshall RE (1999) Inflammation-induced changes in peripheral glutamate receptor populations. Brain Res 820:63–70

Cefalu JS, Guillon MA, Burbach LR, Zhu QM, Hu DQ, Ho MJ, Ford AP, Nunn PA, Cockayne DA (2009) Selective pharmacological blockade of the TRPV1 receptor suppresses sensory reflexes of the rodent bladder. J Urol 182:776–785

Charrua A, Reguenga C, Paule CC, Nagy I, Cruz F, Avelino A (2008) Cystitis is associated with TRPV1b-downregulation in rat dorsal root ganglia. Neuroreport 19:1469–1472

Cheng CL, de Groat WC (2004) The role of capsaicin-sensitive afferent fibers in the lower urinary tract dysfunction induced by chronic spinal cord injury in rats. Exp Neurol 187:445–454

Chopra B, Gever J, Barrick SR, Hanna-Mitchell AT, Beckel JM, Ford AP, Birder LA (2008) Expression and function of rat urothelial P2Y receptors. Am J Physiol Renal Physiol 294: F821–F829

Christianson JA, Liang R, Ustinova EE, Davis BM, Fraser MO, Pezzone MA (2007) Convergence of bladder and colon sensory innervation occurs at the primary afferent level. Pain 128:235–243

Chuang YC, Yoshimura N, Huang CC, Chiang PH, Chancellor MB (2004) Intravesical botulinum toxin a administration produces analgesia against acetic acid induced bladder pain responses in rats. J Urol 172:1529–1532

Chuang YC, Tyagi P, Huang CC, Yoshimura N, Wu M, Kaufman J, Chancellor MB (2009) Urodynamic and immunohistochemical evaluation of intravesical botulinum toxin A delivery using liposomes. J Urol 182:786–792

Cockayne DA, Dunn PM, Zhong Y, Rong W, Hamilton SG, Knight GE, Ruan H-Z, Ma B, Yip P, Nunn P, McMahon SB, Burnstock G, Ford APDW (2005) P2X2 knockout mice and P2X2/P2X3 double knockout mice reveal a role for the P2X2 receptor subunit in mediating multiple sensory effects of ATP. J Physiol 567:621–639

Coggeshall RE (1980) Law of separation of function of the spinal roots. Physiol Rev 60:716–755

Coggeshall RE, Carlton SM (1998) Ultrastructural analysis of NMDA, AMPA, and kainate receptors on unmyelinated and myelinated axons in the periphery. J Comp Neurol 391:78–86
Coggeshall RE, Coulter JD, Willis WD Jr (1974) Unmyelinated axons in the ventral roots of the cat lumbosacral enlargement. J Comp Neurol 153:39–58
Daly D, Rong W, Chess-Williams R, Chapple C, Grundy D (2007) Bladder afferent sensitivity in wild-type and TRPV1 knockout mice. J Physiol 583:663–674
Dang K, Lamb K, Cohen M, Bielefeldt K, Gebhart GF (2008) Cyclophosphamide-induced bladder inflammation sensitizes and enhances P2X receptor function in rat bladder sensory neurons. J Neurophysiol 99:49–59
Darblade B, Behr-Roussel D, Gorny D, Lebret T, Benoit G, Hieble JP, Brooks D, Alexandre L, Giuliano F (2005) Piboserod (SB 207266), a selective 5-HT4 receptor antagonist, reduces serotonin potentiation of neurally-mediated contractile responses of human detrusor muscle. World J Urol 23:147–151
Dattilio A, Vizzard MA (2005) Up-regulation of protease activated receptors in bladder after cyclophosphamide induced cystitis and colocalization with capsaicin receptor (VR1) in bladder nerve fibers. J Urol 173:635–639
Dawson NJ, Schmid H, Pierau FK (1992) Pre-spinal convergence between thoracic and visceral nerves of the rat. Neurosci Lett 138:149–152
De Groat WC (1986) Spinal cord projections and neuropeptides in visceral afferent neurons. Prog Brain Res 67:165–187
de Groat WC (1997) A neurologic basis for the overactive bladder. Urology 50:36–52, discussion 53–6
De Groat WC, Lalley PM (1972) Reflex firing in the lumbar sympathetic outflow to activation of vesical afferent fibres. J Physiol 226:289–309
de Groat WC, Ryall RW (1969) Reflexes to sacral parasympathetic neurones concerned with micturition in the cat. J Physiol 200:87–108
De Wachter S, Wyndaele JJ (2003) Intravesical oxybutynin: a local anesthetic effect on bladder C afferents. J Urol 169:1892–1895
Dmitrieva N, Johnson OL, Berkley KJ (2001) Bladder inflammation and hypogastric neurectomy influence uterine motility in the rat. Neurosci Lett 313:49–52
Du S, Araki I, Yoshiyama M, Nomura T, Takeda M (2007) Transient receptor potential channel A1 involved in sensory transduction of rat urinary bladder through C-fiber pathway. Urology 70:826–831
Duong M, Downie JW, Du HJ (1999) Transmission of afferent information from urinary bladder, urethra and perineum to periaqueductal gray of cat. Brain Res 819:108–119
Ferguson DR, Kennedy I, Burton TJ (1997) ATP is released from rabbit urinary bladder epithelial cells by hydrostatic pressure changes – a possible sensory mechanism? J Physiol 505 (Pt 2):503–511
Fitzpatrick CC, DeLancey JO, Elkins TE, McGuire EJ (1993) Vulvar vestibulitis and interstitial cystitis: a disorder of urogenital sinus-derived epithelium? Obstet Gynecol 81:860–862
Fowler CJ, Griffiths D, de Groat WC (2008) The neural control of micturition. Nat Rev Neurosci 9:453–466
Gevaert T, Vriens J, Segal A, Everaerts W, Roskams T, Talavera K, Owsianik G, Liedtke W, Daelemans D, Dewachter I, Van Leuven F, Voets T, De Ridder D, Nilius B (2007) Deletion of the transient receptor potential cation channel TRPV4 impairs murine bladder voiding. J Clin Invest 117:3453–3462
Gillespie JI (2004) The autonomous bladder: a view of the origin of bladder overactivity and sensory urge. BJU Int 93:478–483
Gillespie JI, van Koeveringe GA, de Wachter SG, de Vente J (2009) On the origins of the sensory output from the bladder: the concept of afferent noise. BJU Int 103:1324–1333
Griffiths DJ, Tadic SD, Schaefer W, Resnick NM (2009) Cerebral control of the lower urinary tract: How age-related changes might predispose to urge incontinence. Neuroimage 47(3):981–986

Grol S, Essers PB, van Koeveringe GA, Martinez-Martinez P, de Vente J, Gillespie JI (2009) M(3) muscarinic receptor expression on suburothelial interstitial cells. BJU Int 104:398–405
Guerios SD, Wang ZY, Bjorling DE (2006) Nerve growth factor mediates peripheral mechanical hypersensitivity that accompanies experimental cystitis in mice. Neurosci Lett 392:193–197
Habler HJ, Janig W, Koltzenburg M (1990) Activation of unmyelinated afferent fibres by mechanical stimuli and inflammation of the urinary bladder in the cat. J Physiol 425:545–562
Habler HJ, Janig W, Koltzenburg M (1993a) Myelinated primary afferents of the sacral spinal cord responding to slow filling and distension of the cat urinary bladder. J Physiol 463:449–460
Habler HJ, Janig W, Koltzenburg M (1993b) Receptive properties of myelinated primary afferents innervating the inflamed urinary bladder of the cat. J Neurophysiol 69:395–405
Haga N, Aikawa K, Shishido K, Takahashi N, Yanagida T, Yamaguchi O (2009) Effect of long-term oxybutynin administration on c-Fos expression in spinal neurons: inhibition of antimuscarinics on bladder afferents in conscious rats. Urology 73:200–204
Hirayama A, Fujimoto K, Matsumoto Y, Ozono S, Hirao Y (2003) Positive response to ice water test associated with high-grade bladder outlet obstruction in patients with benign prostatic hyperplasia. Urology 62:909–913
Holzer P (1991) Capsaicin: cellular targets, mechanisms of action, and selectivity for thin sensory neurons. Pharmacol Rev 43:143–201
Hou S, Duale H, Rabchevsky AG (2009) Intraspinal sprouting of unmyelinated pelvic afferents after complete spinal cord injury is correlated with autonomic dysreflexia induced by visceral pain. Neuroscience 159:369–379
Iijima K, De Wachter S, Wyndaele JJ (2007) Effects of the M3 receptor selective muscarinic antagonist darifenacin on bladder afferent activity of the rat pelvic nerve. Eur Urol 52:842–847
Ito K, Iwami A, Katsura H, Ikeda M (2008) Therapeutic effects of the putative P2X3/P2X2/3 antagonist A-317491 on cyclophosphamide-induced cystitis in rats. Naunyn Schmiedebergs Arch Pharmacol 377:483–490
Janig W, Morrison JF (1986) Functional properties of spinal visceral afferents supplying abdominal and pelvic organs, with special emphasis on visceral nociception. Prog Brain Res 67:87–114
Jiang CH, Mazieres L, Lindstrom S (2009) Gating of the micturition reflex by tonic activation of bladder cold receptors in the cat. Neurourol Urodyn 28(6):555–560
Kamo I, Chancellor MB, de Groat WC, Yoshimura N (2005) Differential effects of activation of peripheral and spinal tachykinin neurokinin(3) receptors on the micturition reflex in rats. J Urol 174:776–781
Keast JR, Stephensen TM (2000) Glutamate and aspartate immunoreactivity in dorsal root ganglion cells supplying visceral and somatic targets and evidence for peripheral axonal transport. J Comp Neurol 424:577–587
Kinder RB, Mundy AR (1987) Pathophysiology of idiopathic detrusor instability and detrusor hyper-reflexia. An in vitro study of human detrusor muscle. Br J Urol 60:509–515
King AE, Lopez-Garcia JA (1993) Excitatory amino acid receptor-mediated neurotransmission from cutaneous afferents in rat dorsal horn in vitro. J Physiol 472:443–457
Kobayashi K, Fukuoka T, Obata K, Yamanaka H, Dai Y, Tokunaga A, Noguchi K (2005) Distinct expression of TRPM8, TRPA1, and TRPV1 mRNAs in rat primary afferent neurons with adelta/c-fibers and colocalization with trk receptors. J Comp Neurol 493:596–606
Kubota Y, Biers SM, Kohri K, Brading AF (2006) Effects of imatinib mesylate (Glivec) as a c-kit tyrosine kinase inhibitor in the guinea-pig urinary bladder. Neurourol Urodyn 25:205–210
Kullmann FA, Artim DE, Birder LA, de Groat WC (2008) Activation of muscarinic receptors in rat bladder sensory pathways alters reflex bladder activity. J Neurosci 28:1977–1987
Laird JM, Olivar T, Roza C, De Felipe C, Hunt SP, Cervero F (2000) Deficits in visceral pain and hyperalgesia of mice with a disruption of the tachykinin NK1 receptor gene. Neuroscience 98:345–352
Lamb K, Gebhart GF, Bielefeldt K (2004) Increased nerve growth factor expression triggers bladder overactivity. J Pain 5:150–156

Lazzeri M, Beneforti P, Benaim G, Maggi CA, Lecci A, Turini D (1996) Intravesical capsaicin for treatment of severe bladder pain: a randomized placebo controlled study. J Urol 156:947–952

le Feber J, van Asselt E, van Mastrigt R (2004) Afferent bladder nerve activity in the rat: a mechanism for starting and stopping voiding contractions. Urol Res 32:395–405

Lecci A, Tramontana M, Giuliani S, Criscuoli M, Maggi CA (1998) Effect of tachykinin NK2 receptor blockade on detrusor hyperreflexia induced by bacterial toxin in rats. J Urol 160:206–209

Liu HT, Kuo HC (2008) Urinary nerve growth factor levels are increased in patients with bladder outlet obstruction with overactive bladder symptoms and reduced after successful medical treatment. Urology 72:104–108, discussion 08

Liu HT, Chancellor MB, Kuo HC (2008) Urinary nerve growth factor levels are elevated in patients with detrusor overactivity and decreased in responders to detrusor botulinum toxin-A injection. Eur Urol 56(4):700–706

Liu HT, Tyagi P, Chancellor MB, Kuo HC (2009) Urinary nerve growth factor level is increased in patients with interstitial cystitis/bladder pain syndrome and decreased in responders to treatment. BJU Int 104(10):1476–1481

Maggi CA (1995) Tachykinins and calcitonin gene-related peptide (CGRP) as co-transmitters released from peripheral endings of sensory nerves. Prog Neurobiol 45:1–98

Malykhina AP (2007) Neural mechanisms of pelvic organ cross-sensitization. Neuroscience 149:660–672

Malykhina AP, Qin C, Foreman RD, Akbarali HI (2004) Colonic inflammation increases Na + currents in bladder sensory neurons. Neuroreport 15:2601–2605

Malykhina AP, Qin C, Greenwood-van Meerveld B, Foreman RD, Lupu F, Akbarali HI (2006) Hyperexcitability of convergent colon and bladder dorsal root ganglion neurons after colonic inflammation: mechanism for pelvic organ cross-talk. Neurogastroenterol Motil 18:936–948

Mariappan P, Alhasso A, Ballantyne Z, Grant A, N'Dow J (2007) Duloxetine, a serotonin and noradrenaline reuptake inhibitor (SNRI) for the treatment of stress urinary incontinence: a systematic review. Eur Urol 51(1):67–74

Masuda H, Chancellor MB, Kihara K, Sakai Y, Koga F, Azuma H, de Groat WC, Yoshimura N (2009a) Effects of cholinesterase inhibition in supraspinal and spinal neural pathways on the micturition reflex in rats. BJU Int 104(8):1163–1169

Masuda H, Ichiyanagi N, Yokoyama M, Sakai Y, Kihara K, Chancellor MB, de Groat WC, Yoshimura N (2009b) Muscarinic receptor activation in the lumbosacral spinal cord ameliorates bladder irritation in rat cystitis models. BJU Int 104(10):1531–1537

McCarthy CJ, Zabbarova IV, Brumovsky PR, Roppolo JR, Gebhart GF, Kanai AJ (2009) Spontaneous contractions evoke afferent nerve firing in mouse bladders with detrusor overactivity. J Urol 181:1459–1466

Messori E, Rizzi CA, Candura SM, Lucchelli A, Balestra B, Tonini M (1995) 5-Hydroxytryptamine receptors that facilitate excitatory neuromuscular transmission in the guinea-pig isolated detrusor muscle. Br J Pharmacol 115:677–683

Michaelis M, Habler HJ, Jaenig W (1996) Silent afferents: a separate class of primary afferents? Clin Exp Pharmacol Physiol 23:99–105

Mills IW, Greenland JE, McMurray G, McCoy R, Ho KM, Noble JG, Brading AF (2000) Studies of the pathophysiology of idiopathic detrusor instability: the physiological properties of the detrusor smooth muscle and its pattern of innervation. J Urol 163:646–651

Moldwin RM (2002) Similarities between interstitial cystitis and male chronic pelvic pain syndrome. Curr Urol Rep 3:313–318

Morgan C, Nadelhaft I, de Groat WC (1981) The distribution of visceral primary afferents from the pelvic nerve to Lissauer's tract and the spinal gray matter and its relationship to the sacral parasympathetic nucleus. J Comp Neurol 201:415–440

Morrison J (1999) The activation of bladder wall afferent nerves. Exp Physiol 84:131–136

Morrison J, Wen J, Kibble A (1999) Activation of pelvic afferent nerves from the rat bladder during filling. Scand J Urol Nephrol Suppl 201:73–75

Morrison TC, Dmitrieva N, Winnard KP, Berkley KJ (2006) Opposing viscerovisceral effects of surgically induced endometriosis and a control abdominal surgery on the rat bladder. Fertil Steril 86:1067–1073

Namasivayam S, Eardley I, Morrison JF (1998) A novel in vitro bladder pelvic nerve afferent model in the rat. Br J Urol 82:902–905

Namasivayam S, Eardley I, Morrison JF (1999) Purinergic sensory neurotransmission in the urinary bladder: an in vitro study in the rat. BJU Int 84:854–860

Nilsson Remahl AI, Masterman T, Risling M (2008) Re-utilization of Schwann cells during ingrowth of ventral root afferents in perinatal kittens. J Anat 213:194–201

Oddiah D, Anand P, McMahon SB, Rattray M (1998) Rapid increase of NGF, BDNF and NT-3 mRNAs in inflamed bladder. Neuroreport 9:1455–1458

Otsuka A, Shinbo H, Matsumoto R, Kurita Y, Ozono S (2008) Expression and functional role of beta-adrenoceptors in the human urinary bladder urothelium. Naunyn Schmiedebergs Arch Pharmacol 377:473–481

Payne CK, Mosbaugh PG, Forrest JB, Evans RJ, Whitmore KE, Antoci JP, Perez-Marrero R, Jacoby K, Diokno AC, O'Reilly KJ, Griebling TL, Vasavada SP, Yu AS, Frumkin LR (2005) Intravesical resiniferatoxin for the treatment of interstitial cystitis: a randomized, double-blind, placebo controlled trial. J Urol 173:1590–1594

Pezzone MA, Liang R, Fraser MO (2005) A model of neural cross-talk and irritation in the pelvis: implications for the overlap of chronic pelvic pain disorders. Gastroenterology 128:1953–1964

Pontari MA, Ruggieri MR (2008) Mechanisms in prostatitis/chronic pelvic pain syndrome. J Urol 179:S61–S67

Qin C, Foreman RD (2004) Viscerovisceral convergence of urinary bladder and colorectal inputs to lumbosacral spinal neurons in rats. Neuroreport 15:467–471

Qin C, Malykhina AP, Akbarali HI, Foreman RD (2005) Cross-organ sensitization of lumbosacral spinal neurons receiving urinary bladder input in rats with inflamed colon. Gastroenterology 129:1967–1978

Risling M, Dalsgaard CJ, Cukierman A, Cuello AC (1984) Electron microscopic and immunohistochemical evidence that unmyelinated ventral root axons make u-turns or enter the spinal pia mater. J Comp Neurol 225:53–63

Ritter AM, Martin WJ, Thorneloe KS (2009) The voltage-gated sodium channel Nav1.9 is required for inflammation-based urinary bladder dysfunction. Neurosci Lett 452:28–32

Rong W, Spyer KM, Burnstock G (2002) Activation and sensitisation of low and high threshold afferent fibres mediated by P2X receptors in the mouse urinary bladder. J Physiol 541:591–600

Rouzade-Dominguez ML, Pernar L, Beck S, Valentino RJ (2003) Convergent responses of Barrington's nucleus neurons to pelvic visceral stimuli in the rat: a juxtacellular labelling study. Eur J Neurosci 18:3325–3334

Ruan HZ, Burnstock G (2003) Localisation of P2Y1 and P2Y4 receptors in dorsal root, nodose and trigeminal ganglia of the rat. Histochem Cell Biol 120:415–426

Ruan HZ, Birder LA, de Groat WC, Tai C, Roppolo J, Buffington CA, Burnstock G (2005) Localization of P2X and P2Y receptors in dorsal root ganglia of the cat. J Histochem Cytochem 53:1273–1282

Saban MR, Nguyen NB, Hammond TG, Saban R (2002) Gene expression profiling of mouse bladder inflammatory responses to LPS, substance P, and antigen-stimulation. Am J Pathol 160:2095–2110

Sculptoreanu A, Yoshimura N, de Groat WC (2004) KW-7158 [(2 S)-(+)-3, 3, 3-trifluoro-2-hydroxy-2-methyl-N-(5, 5, 10-trioxo-4, 10-dihydro thieno[3, 2-c][1]benzothiepin-9-yl)propanamide] enhances A-type K + currents in neurons of the dorsal root ganglion of the adult rat. J Pharmacol Exp Ther 310:159–168

Sellers DJ, Chess-Williams R, Chapple CR (2000) 5-Hydroxytryptamine-induced potentiation of cholinergic responses to electrical field stimulation in pig detrusor muscle. BJU Int 86:714–718

Sengupta JN, Gebhart GF (1994a) Characterization of mechanosensitive pelvic nerve afferent fibers innervating the colon of the rat. J Neurophysiol 71:2046–2060

Sengupta JN, Gebhart GF (1994b) Mechanosensitive properties of pelvic nerve afferent fibers innervating the urinary bladder of the rat. J Neurophysiol 72:2420–2430
Shea VK, Cai R, Crepps B, Mason JL, Perl ER (2000) Sensory fibers of the pelvic nerve innervating the Rat's urinary bladder. J Neurophysiol 84:1924–1933
Shimizu N, Nakahara T, Saito M, Ishii K (2007) Role of capsaicin-sensitive sensory nerves in protease-activated receptor-2-mediated contraction of rat urinary bladder. Eur J Pharmacol 569:145–148
Silva C, Ribeiro MJ, Cruz F (2002) The effect of intravesical resiniferatoxin in patients with idiopathic detrusor instability suggests that involuntary detrusor contractions are triggered by C-fiber input. J Urol 168:575–579
Silva C, Silva J, Ribeiro MJ, Avelino A, Cruz F (2005) Urodynamic effect of intravesical resiniferatoxin in patients with neurogenic detrusor overactivity of spinal origin: results of a double-blind randomized placebo-controlled trial. Eur Urol 48:650–655
Steers WD, Ciambotti J, Etzel B, Erdman S, de Groat WC (1991) Alterations in afferent pathways from the urinary bladder of the rat in response to partial urethral obstruction. J Comp Neurol 310:401–410
Steers WD, Mackway-Gerardi AM, Ciambotti J, de Groat WC (1994) Alterations in neural pathways to the urinary bladder of the rat in response to streptozotocin-induced diabetes. J Auton Nerv Syst 47:83–94
Streng T, Axelsson HE, Hedlund P, Andersson DA, Jordt SE, Bevan S, Andersson KE, Hogestatt ED, Zygmunt PM (2008) Distribution and function of the hydrogen sulfide-sensitive TRPA1 ion channel in rat urinary bladder. Eur Urol 53:391–399
Su X, Lashinger ES, Leon LA, Hoffman BE, Hieble JP, Gardner SD, Fries HE, Edwards RM, Li J, Laping NJ (2008a) An excitatory role for peripheral EP3 receptors in bladder afferent function. Am J Physiol Renal Physiol 295:F585–F594
Su X, Leon LA, Wu CW, Morrow DM, Jaworski JP, Hieble JP, Lashinger ES, Jin J, Edwards RM, Laping NJ (2008b) Modulation of bladder function by prostaglandin EP3 receptors in the central nervous system. Am J Physiol Renal Physiol 295:F984–F994
Sui GP, Wu C, Fry CH (2006) Characterization of the purinergic receptor subtype on guinea-pig suburothelial myofibroblasts. BJU Int 97:1327–1331
Tai C, Smerin SE, de Groat WC, Roppolo JR (2006) Pudendal-to-bladder reflex in chronic spinal-cord-injured cats. Exp Neurol 197:225–234
Talaat M (1937) Afferent impulses in the nerves supplying the urinary bladder. J Physiol 89:1–13
Thor KB, Morgan C, Nadelhaft I, Houston M, De Groat WC (1989) Organization of afferent and efferent pathways in the pudendal nerve of the female cat. J Comp Neurol 288:263–279
Thor KB, Katofiasc MA, Danuser H, Springer J, Schaus JM (2002) The role of 5-HT(1A) receptors in control of lower urinary tract function in cats. Brain Res 946:290–297
Tucci P, Bartocci C, Bolle P (2002) Cyclo-oxygenase- and capsaicin-sensitive afferent fibres affect Beta-adrenoceptor-evoked response in the rat urinary bladder. Pharmacology 64:57–62
Ustinova EE, Fraser MO, Pezzone MA (2006) Colonic irritation in the rat sensitizes urinary bladder afferents to mechanical and chemical stimuli: an afferent origin of pelvic organ cross-sensitization. Am J Physiol Renal Physiol 290:F1478–F1487
Ustinova EE, Gutkin DW, Pezzone MA (2007) Sensitization of pelvic nerve afferents and mast cell infiltration in the urinary bladder following chronic colonic irritation is mediated by neuropeptides. Am J Physiol Renal Physiol 292:F123–F130
Vizzard MA, Erdman SL, de Groat WC (1995a) Increased expression of neuronal nitric oxide synthase (NOS) in visceral neurons after nerve injury. J Neurosci 15:4033–4045
Vizzard MA, Erdman SL, de Groat WC (1995b) Increased expression of neuronal nitric oxide synthase in dorsal root ganglion neurons after systemic capsaicin administration. Neuroscience 67:1–5

Vizzard MA, Erdman SL, de Groat WC (1996) Increased expression of neuronal nitric oxide synthase in bladder afferent pathways following chronic bladder irritation. J Comp Neurol 370:191–202

Vlaskovska M, Kasakov L, Rong W, Bodin P, Bardini M, Cockayne DA, Ford AP, Burnstock G (2001) P2X3 knock-out mice reveal a major sensory role for urothelially released ATP. J Neurosci 21:5670–5677

Walczak JS, Price TJ, Cervero F (2009) Cannabinoid CB1 receptors are expressed in the mouse urinary bladder and their activation modulates afferent bladder activity. Neuroscience 159:1154–1163

Wang ZY, Wang P, Merriam FV, Bjorling DE (2008) Lack of TRPV1 inhibits cystitis-induced increased mechanical sensitivity in mice. Pain 139:158–167

Watanabe T, Yokoyama T, Sasaki K, Nozaki K, Ozawa H, Kumon H (2004) Intravesical resiniferatoxin for patients with neurogenic detrusor overactivity. Int J Urol 11:200–205

Weaver LC (1985) Organization of sympathetic responses to distension of urinary bladder. Am J Physiol Regul Integr Comp Physiol 248:R236–R240

Wesselmann U (2001) Neurogenic inflammation and chronic pelvic pain. World J Urol 19:180–185

Westlund KN, Sun YC, Sluka KA, Dougherty PM, Sorkin LS, Willis WD (1992) Neural changes in acute arthritis in monkeys. II. Increased glutamate immunoreactivity in the medial articular nerve. Brain Res Brain Res Rev 17:15–27

Winnard KP, Dmitrieva N, Berkley KJ (2006) Cross-organ interactions between reproductive, gastrointestinal, and urinary tracts: modulation by estrous stage and involvement of the hypogastric nerve. Am J Physiol Regul Integr Comp Physiol 291:R1592–R1601

Wiseman OJ, Fowler CJ, Landon DN (2003) The role of the human bladder lamina propria myofibroblast. BJU Int 91:89–93

Xu L, Gebhart GF (2008) Characterization of mouse lumbar splanchnic and pelvic nerve urinary bladder mechanosensory afferents. J Neurophysiol 99:244–253

Yamanishi T, Chapple CR, Yasuda K, Yoshida K, Chess-Williams R (2002) The role of beta(3)-adrenoceptors in mediating relaxation of porcine detrusor muscle. Br J Pharmacol 135:129–134

Yamanishi T, Yasuda K, Kitahara S, Nakai H, Yoshida K, Iizuka H (2006) Effects of 138-355, a beta3-adrenoceptor selective agonist, on relaxation of the human detrusor muscle in vitro. Neurourol Urodyn 25:815–819

Yokoyama O, Yusup A, Miwa Y, Oyama N, Aoki Y, Akino H (2005) Effects of tolterodine on an overactive bladder depend on suppression of C-fiber bladder afferent activity in rats. J Urol 174:2032–2036

Yokoyama T, Kumon H, Nagai A (2008) Correlation of urinary nerve growth factor level with pathogenesis of overactive bladder. Neurourol Urodyn 27:417–420

Yoshimura N, de Groat WC (1999) Increased excitability of afferent neurons innervating rat urinary bladder after chronic bladder inflammation. J Neurosci 19:4644–4653

Yoshimura M, Jessell T (1990) Amino acid-mediated EPSPs at primary afferent synapses with substantia gelatinosa neurones in the rat spinal cord. J Physiol 430:315–335

Yoshimura N, White G, Weight FF, de Groat WC (1994) Patch-clamp recordings from subpopulations of autonomic and afferent neurons identified by axonal tracing techniques. J Auton Nerv Syst 49:85–92

Yoshimura N, Seki S, de Groat WC (2001) Nitric oxide modulates Ca(2+) channels in dorsal root ganglion neurons innervating rat urinary bladder. J Neurophysiol 86:304–311

Yoshimura N, Seki S, Erickson KA, Erickson VL, Hancellor MB, de Groat WC (2003) Histological and electrical properties of rat dorsal root ganglion neurons innervating the lower urinary tract. J Neurosci 23:4355–4361

Yoshimura N, Kaiho Y, Miyazato M, Yunoki T, Tai C, Chancellor MB, Tyagi P (2008) Therapeutic receptor targets for lower urinary tract dysfunction. Naunyn Schmiedebergs Arch Pharmacol 377:437–448

Yu Y, de Groat WC (2008) Sensitization of pelvic afferent nerves in the in vitro rat urinary bladder-pelvic nerve preparation by purinergic agonists and cyclophosphamide pretreatment. Am J Physiol Renal Physiol 294:F1146–F1156

Yu YF, Nichol MB, Yu AP, Ahn J (2005) Persistence and adherence of medications for chronic overactive bladder/urinary incontinence in the california medicaid program. Value Health 8:495–505

Zabbarova I, Birder L, Roppolo J, de Groat W, Kanai A (2009) Bladder-colon cross-sensitization induced bladder overactivity. Neurourol Urodyn 28:914–915

Zagorodnyuk VP, Brookes SJ (2000) Transduction sites of vagal mechanoreceptors in the guinea pig esophagus. J Neurosci 20:6249–6255

Zagorodnyuk VP, Costa M, Brookes SJ (2006) Major classes of sensory neurons to the urinary bladder. Auton Neurosci 126–127:390–397

Zagorodnyuk VP, Gibbins IL, Costa M, Brookes SJ, Gregory SJ (2007) Properties of the major classes of mechanoreceptors in the guinea pig bladder. J Physiol 585:147–163

Zarghooni S, Wunsch J, Bodenbenner M, Bruggmann D, Grando SA, Schwantes U, Wess J, Kummer W, Lips KS (2007) Expression of muscarinic and nicotinic acetylcholine receptors in the mouse urothelium. Life Sci 80:2308–2313

Zhou S, Bonasera L, Carlton SM (1996) Peripheral administration of NMDA, AMPA or KA results in pain behaviors in rats. Neuroreport 7:895–900

Zinck ND, Rafuse VF, Downie JW (2007) Sprouting of CGRP primary afferents in lumbosacral spinal cord precedes emergence of bladder activity after spinal injury. Exp Neurol 204:777–790

Urothelial Signaling

Lori A. Birder

Contents

Abstract The urinary bladder "mucosa" or innermost portion of the bladder is composed of transitional epithelium, basement membrane, and the lamina propria. This chapter reviews the specialized anatomy of the bladder epithelium (urothelium) and speculates on possible communication mechanisms from urothelial cells to various cell types within the bladder wall. For example, beyond serving as a simple barrier, there is growing evidence that the urinary bladder urothelium exhibits specialized sensory properties and plays a key role in the detection and transmission of both physiological and nociceptive stimuli. Findings from a number of studies suggest that the urothelium exhibits both "sensor" (expressing receptors/

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ion channels capable of responding to thermal, mechanical, and chemical stimuli) and "transducer" (ability to release chemicals) properties. Thus, urothelial cells exhibit the ability to sense changes in their extracellular environment including the ability to respond to chemical, mechanical, and thermal stimuli that may communicate the state of the urothelial environment to the underlying nervous and muscular systems.

Keywords Barrier function · Sensory web · TRP channels · Urothelium

1 Anatomy and Barrier Function of the Urothelium

The urothelium forms the interface between the urinary space and the underlying vasculature, connective, nervous, and muscular tissues. Urothelium is composed of at least three layers: a basal cell layer attached to a basement membrane, an intermediate layer, and a superficial or apical layer composed of large hexagonal cells (diameters of 25–250 μm) known as "umbrella cells" (Lewis 2000; Apodaca 2004; Abraham et al. 2009; Khandelwal et al. 2009). Intermediate cells are pear shaped (pyriform; diameter 10–15 μm), while the basal cell layer is composed of mononucleate cells (diameter 10 μm) that are in contact with an underlying capillary bed. The umbrella cells are interconnected by tight junctions (which are composed of multiple proteins such as the claudins) and are covered on their apical surface (nearly 70–80%) by crystalline proteins called uroplakins that assemble into hexagonal plaques (Hicks 1975; Liang et al. 2001; Sun 2006). Interestingly, uroplakins and other urothelial cellular differentiation markers, such as cytokeratin 20, are not expressed in the stratified epithelium of the urethra. There have been reports of thin cytoplasmic extensions connecting the various cell layers to the basement membrane, suggesting a pseudostratified urothelium. More recent evidence supports a stratified urothelium with a relative lack of cytoplasmic extensions.

The ability of the bladder to maintain a barrier, despite large alterations in urine volume and increases in pressure during bladder filling and emptying, is dependent on several features of the umbrella cell layer. These features include tight junction complexes that reduce the movement of ions and solutes between cells and specialized lipid molecules and uroplakin proteins in the apical membrane, which reduce the permeability of the cells to small molecules (water, urea, and protons) (Acharya et al. 2004; Apodaca 2004; Abraham et al. 2009; Khandelwal et al. 2009). Mice lacking genes for uroplakins IIIa and II (UPIIIa and UPII) exhibit a number of abnormalities that lead to a compromised urothelial barrier. The apical surface of the urothelium is also covered with a sulfated polysaccharide glycosaminoglycan (GAG) or mucin layer that is thought to act as a nonspecific antiadherence factor and as a defense mechanism against infection (Parsons et al. 1977, 1990, 1991).

The size and shape of the umbrella cells is dependent upon the filling state of the urinary bladder. For example, umbrella cells are cuboidal in unfilled bladders, but

during bladder filling, the umbrella cells become flat and squamous and this shape change is accompanied by vesicular traffic (i.e., exocytosis/endocytosis), adding membrane to the apical surface and thereby increasing overall urinary bladder surface area (Hicks 1975; Wang et al. 2005; Balestreire and Apodaca 2007). There is evidence that this stretch-induced exocytosis is dependent on activation of epidermal growth factor receptor (EGFR) (Cheng and Zhang 2002; Balestreire and Apodaca 2007). These processes allow the bladder to accommodate increasing volumes of urine during filling without compromising barrier function. Exocytosis/endocytosis (vesicular recycling) may also play an important role in modulating the release of a number of neurotransmitters/mediators as well as regulating the function of many receptors and ion channels in urothelial cells (Apodaca 2004; Birder and de Groat 2007).

2 Urothelial Heterogeneity

Studies of different species have shown that the major part of the urinary tract is lined with a fully differentiated urothelium (Sun 2006). Findings in cultured cells reveal a distinct difference in morphology of ureteral and bladder urothelial cells, supporting a difference in cell lineage. There seems to be no apparent difference between the urothelium of the trigone compared to the detrusor. This is in contrast to the proximal urethra, a region in which a complete "barrier" is unnecessary, which transitions from urothelium to a stratified or columnar epithelium accompanied by a lack of urothelial-specific differentiation markers (Romih et al. 2005; Thomas et al. 2005; Sun 2006). There are also reports of microvilli on the apical surface of the urethral epithelium. The presence of microvilli may play a role in increasing the surface area of the cell, participation in both sensory and transducer functions, as well as in fluid transport. Taken together, present evidence suggests at least three urothelial lineages (1) those of the ureter/renal pelvis, (2) detrusor/trigone, and (3) bladder neck/proximal urethra (Liang et al. 2005). The functional significance of these findings has yet to be determined.

3 Evidence Suggesting a Role for Urothelial Cells in Visceral Sensation

While urothelial cells are often viewed as bystanders in the process of visceral sensation, recent evidence has supported the view that these cells function as primary transducers of some physical and chemical stimuli and are able to communicate with underlying cells including bladder nerves, smooth muscle, and even inflammatory cells (Fig. 1a).

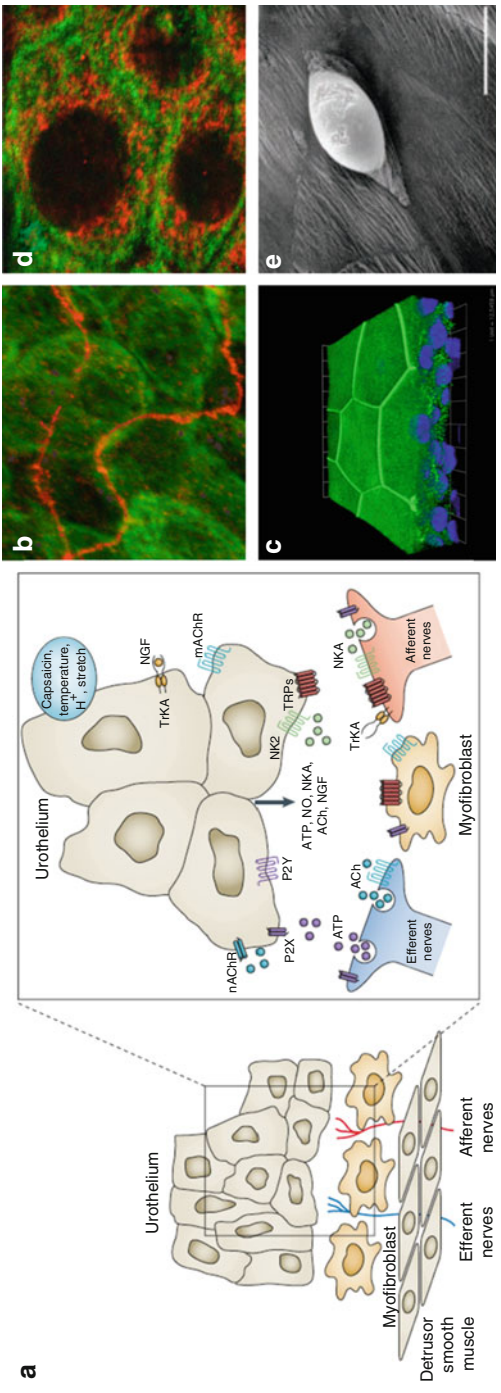

Fig. 1 (**a**) Hypothetical model depicting possible interactions between bladder afferent and efferent nerves, urothelial cells, smooth muscle, and myofibroblasts. Stimulation of urothelial receptors and channels can release mediators that target bladder nerves and other cell types; urothelial cells can also be targets for neurotransmitters released from nerves or other cell types. Urothelial cells can be activated by either autocrine (i.e., autoregulation) or paracrine (release from nearby nerves or other cells) mechanisms. Abbreviations: *ACh* acetylcholine, *AdR* adrenergic receptor, *BR* bradykinin receptor, H^+ proton, *MR* muscarinic receptor, *NE* norepinephrine, *NGF* nerve growth factor, *NR* neurokinin receptor, *NicR* nicotinic receptor, *NO* nitric oxide, *P2R* purinergic 2 receptor unidentified subtype, *P2X* and *P2Y* purinergic receptors, *PG* prostaglandin, *SP* substance P, *Trk-A* receptor tyrosine kinase A, high-affinity receptor for nerve growth factor, *TRPs* transient potential channels (Birder et al. 2007). (**b**) Confocal image of the urothelium depicts TRPV1-positive afferent nerve fibers (cy3, *red*) located in close proximity to basal (FITC, *green*) urothelial cells (Birder et al. 2001). (**c**) Image is a three-dimensional reconstruction (taken with a confocal microscope) depicting localization of P2X3 (*green*; nuclei, *blue*) in the urothelium (Wang et al. 2005). (**d**) TRPV1 expression detected within the rat urinary bladder urothelium. Image depicts basal cell TRPV1 (cy3, *red*) and cytokeratin 17 (FITC, *green*) immunoreactivity (Birder et al. 2001). (**e**) Image depicts an intracellular bacterial "pod" on the surface of a C3H/H3J mouse bladder infected with UT189 (Anderson et al. 2003)

There are at least three lines of evidence that suggest that urothelial cells participate in the detection of both physical and chemical stimuli. First, bladder nerves (afferent and efferent) are localized in close proximity, and some within the urothelium (Jen et al. 1995; Kunze et al. 2006; Birder and de Groat 2007). Ultrastructural studies of nerves in the human bladder have found only unmyelinated nerves in the urothelial and immediate suburothelial layer (Wiseman et al. 2002). Peptidergic, P2X-, and TRPV1-immunoreactive nerve fibers presumed to arise from afferent neurons in the lumbosacral dorsal root ganglia are distributed throughout the urinary bladder musculature as well as in a plexus beneath and extending into the urothelium (Birder et al. 2002a; Birder and de Groat 2007) (Fig. 1b). These axon collaterals are able to release neurotransmitters onto the various tissues in the bladder wall. In humans with neurogenic detrusor overactivity, intravesical administration of resiniferatoxin, a C-fiber afferent neurotoxin, reduces the density of TRPV1- and P2X3-immunoreactive suburothelial nerves, indicating that these are sensory nerves (Brady et al. 2004; Apostolidis et al. 2005). In addition, immunohistochemical studies have also revealed both adrenergic (tyrosine hydroxylase-positive) and cholinergic (choline acetyltransferase, ChAT-positive) nerves in close proximity to the urothelium (Jen et al. 1995). While this would commonly indicate the presence of efferent autonomic nerves, it has been reported in the guinea pig bladder that some ChAT immunoreactivity near the urothelium may be present in sensory rather than efferent nerves (Gillespie et al. 2006).

Additionally, there is also evidence that a suburothelial population of interstitial cells (IC) may play a relay role in the sensory response to bladder wall stimuli (Sui et al. 2004; Brading and McCloskey 2005; Ikeda et al. 2007; Grol et al. 2008). These cells may act as a variable amplifier of the sensory response, mediating signals between the urothelium and sensory afferents or the detrusor smooth muscle layer, either directly or via the activation of afferent nerve fibers (Fry et al. 2007). These suburothelial IC cells, which are extensively linked by gap junctions and have close contacts with nerves, can respond to neurotransmitters such as ATP released from nerves or urothelial cells, suggesting that they can mediate signals between the urothelium and sensory afferents or the detrusor smooth muscle layer (Sui et al. 2004; Brading and McCloskey 2005; Ikeda et al. 2007). In some types of cells, intercellular calcium "waves" may be a common way of translating extracellular stimuli into functional processes that can spread to nearby cells, ultimately leading to the release of neuroactive mediators. Studies using optical mapping techniques in both rodent and cat urinary bladder have revealed that both chemical and physical stimulation of cross-sections of the bladder can generate a series of calcium and membrane potential "waves," which propagate across the urothelial–suburothelial layers before invading the underlying smooth muscle (Ikeda et al. 2007, 2009; Ikeda and Kanai 2008). Thus, the flow of information between urothelium and other cell types within the bladder wall is not unidirectional but a complex chemical communication network between various cells including nerve fibers, IC cells, immune cells, and smooth muscle.

4 Involvement of the Urothelium in "Sensing" Chemical and Mechanical Stimuli

A second line of evidence suggesting that urothelial cells play a role in sensory function is the expression of numerous receptors/ion channels similar to that found in both nociceptors and mechanoreceptors. Examples of neuronal "sensor molecules" (receptors/ion channels) that have been identified in urothelium include receptors for purines ($P2X_{1-7}$ and $P2Y_{1,2,4}$) (Ferguson et al. 1997; Chopra et al. 2008; Burnstock 2001; Birder et al. 2003); adenosine (A_1, A_{2a}, A_{2b}, and A_3) (Yu et al. 2006); norepinephrine (α and β) (Birder et al. 2002b); acetylcholine (muscarinic and nicotinic) (Chess-Williams 2002; Kullmann et al. 2008; Beckel et al. 2006); protease-activated receptors (PARs) (Ossovskaya and Bunnett 2004); fractalkine receptors (Yuridullah et al. 2006); amiloride- and mechanosensitive epithelial sodium channels (ENaC) (Carattino et al. 2008); NSCC, TRAAK, and TREK-1 (Wang et al. 2003; Araki et al. 2008; Yu et al. 2009); bradykinin (B1 and B2) (Chopra et al. 2005); neurotrophins (p75, trkA) (Murray et al. 2004); epidermal growth factor (EGF family ErbB1-3) (Balestreire and Apodaca 2007); vascular endothelial growth factor (VEGF) and neuropilins (NRP) (Saban et al. 2008); pituitary adenylate cyclase-activating peptide (PACAP) (Girard et al. 2008); corticotrophin releasing factor (CRF1 and CRF2) (LeBerge et al. 2006); estrogen receptors (ERα and ERβ) (Taylor and Al-Azzawi 2000); cannabinoid (CB1) receptors (Walczak et al. 2009); ASIC-sensing ion channels and various TRP channels (TRPV1, TRPV2, TRPV4, TRPM8, and TRPA1) (Birder et al. 2002a; Birder and de Groat 2007; Kullmann et al. 2009). The expression of these various receptors enables the urothelium to respond to a number of "sensory inputs" from a variety of sources. These inputs include increased stretch during bladder filling; soluble factors (many found in the urine) such as EGF; or chemical mediators, peptides, and/or transmitters such as substance P, calcitonin gene-related peptide (CGRP), CRF, acetylcholine, adenosine, or norepinephrine released from nerves, inflammatory cells, and even nearby blood vessels (Apodaca 2004; LeBerge et al. 2006; Apodaca et al. 2007; Birder and de Groat 2007; Hanna-Mitchell et al. 2007).

The third line of evidence for a sensor role for urothelial cells is the secretion of transmitters or mediators such as neurotrophins, peptides, ATP, acetylcholine, prostaglandins, prostacyclin, nitric oxide (NO), and cytokines that are capable of modulating, activating, or inhibiting sensory nerves, smooth muscle, and/or cells of the immune and inflammatory systems (Apodaca et al. 2007; Birder and de Groat 2007). For example, the secretion of a diffusible factor from the urothelium (sonic hedgehog, Shh) has been proposed to induce smooth muscle differentiation (Shiroyanagi et al. 2007). In addition, the release of urothelial-derived factors in response to mechanical as well as chemical stimulation has been shown to either facilitate or inhibit the activity of bladder afferent nerves (Andersson and Persson 1995; Birder and de Groat 2007). There is evidence that intravesical administration of oxyhemoglobin (may act to scavenge NO) results in a bladder hyperactivity, demonstrating an inhibitory role of NO in the control of bladder reflexes

(Pandita et al. 2000). It has also been shown that removal of the urothelium using in vitro detrusor preparations can significantly increase the detrusor muscle contractile response evoked by acetylcholine. These findings suggest that the urothelium can release a factor that exerts an inhibitory effect on bladder smooth muscle contractility (Hawthorn et al. 2000). Though this "urothelial-derived inhibitory factor or UDIF" has not yet been identified, it is unlikely to be NO, adenosine, GABA, a cyclo-oxygenase product, or mediated by the small conductance Ca^{2+}-sensitive K^+ channel (Fovaeus et al. 1999; Hawthorn et al. 2000; Chaiyaprasithi et al. 2003; Murakami et al. 2007).

The mechanism underlying release of chemical mediators from the urothelium, including whether all sensory "inputs" stimulate membrane turnover (i.e., vesicular exocytosis), is not well understood. It is also not known whether different layers of the urothelium may secrete different mediators with differing effects. What little is known about the roles and dynamics of membrane-bound cytoplasmic vesicles in urothelial cell physiology is derived from measurements of membrane capacitance and microscopy of fixed tissues and cells. For example, there is evidence that once released, ATP can act as an important autocrine mediator, which can induce membrane turnover as well as enhance both stretch-induced exocytosis and endocytosis (Wang et al. 2005). Alterations in membrane turnover can not only increase apical surface area (as described earlier) but also regulate the number and function of receptors and channels at the cell surface.

5 Urothelial Sensor Targets and Mediators

5.1 ATP and Purinergic Receptors

Since the first report of distension-evoked ATP release from the urothelium, there has been abundant evidence supporting a role for urothelially derived release of ATP in autocrine and paracrine signaling within the lower urinary tract. ATP is released from both the apical and basolateral urothelial surfaces in response to bladder stretch and can act on P2X2 and P2X3 urothelial receptors to stimulate stretch-induced exocytosis (Wang et al. 2005) (Fig. 1c). It has been suggested that the amiloride-sensitive apical sodium channel or ENaC, expressed within the urothelium, may be involved in mechanotransduction by controlling basolateral release of ATP (Du et al. 2007). Additionally, the expression of both P2X and P2Y receptors in nerve fibers and suburothelial interstitial cells in close proximity to the bladder lumen and the sensitivity of these cells to ATP raises the possibility that basolateral ATP release from urothelial cells could influence not only adjacent urothelial cells, but also interstitial cells and nerves (Sui et al. 2006; Fry et al. 2007). The intercellular communication mediated by gap junctions in interstitial cells may provide a mechanism for long-distance spread of signals from the urothelium to the detrusor muscle (Ikeda et al. 2007).

Pathology results in augmented release of ATP from the bladder urothelium, which can cause painful sensations via excitation of purinergic receptors on nearby sensory fibers (Sun et al. 2001; Birder et al. 2003). This type of noncholinergic mechanism is likely to play an important role in a number of bladder pathologies such as bladder pain syndrome/interstitial cystitis (BPS/IC), a chronic clinical disease characterized by urgency, frequency, and bladder pain upon filling (Gillenwater and Wein 1998; Parsons et al. 1998; Nickel 2003; Parsons 2007). Studies of a comparable disease in cats (i.e., feline interstitial cystitis, FIC) revealed an augmented stretch-evoked release of urothelial-derived ATP and changes in purinergic receptor profiles in urothelial cells (Birder et al. 2003). This observation is consistent with similar findings in patients with BPS/IC and suggests that urothelial sensor molecules exhibit plasticity in pathologic conditions (Sun et al. 2001). ATP can also act in an autocrine manner to enhance its own release from urothelial cells. This autofeedback mechanism may be more important in pathological conditions. The enhanced release of ATP into the bladder lumen in response to bladder distension has also been observed in chronic spinal cord injured rats and in rats with chemically irritated bladders (Salas et al. 2007; Girard et al. 2008). ATP released from the urothelium can directly depolarize and initiate firing in sensory nerves by activating ionotropic P2X channels or by activating metabotropic P2Y receptors on afferent nerves (as well as the urothelium) to stimulate intracellular second messenger pathways that in turn modulate other ion channels. For example, it has been shown in sensory neurons that ATP can enhance TRPV1 currents by lowering the threshold for protons, capsaicin, and heat (Tominaga et al. 2001). This action, which is presumably mediated by activating intracellular protein kinases and phosphorylation of the TRPV1 channel, represents a mechanism by which large amounts of ATP released from damaged or sensitized cells in response to injury or inflammation may trigger the sensation of pain.

5.2 TRPV1 Channels

The ability of capsaicin to evoke NO release from rat urothelium, reported in 1998, provided the first, albeit indirect, demonstration that TRPV1 channels are expressed in urothelial cells and that urothelial cells and afferent nerves, which also express these channels, share a number of common properties (Birder et al. 1998). This ion channel protein is activated by capsaicin, as well as moderate heat, protons, and lipid metabolites such as anandamide (an endogenous ligand of both cannabinoid and vanilloids receptors) (Caterina et al. 1997).

TRPV1 is expressed not only by afferent nerves that form close contact with the bladder epithelium but also by nonneuronal cells such as urothelial cells and interstitial cells (Birder et al. 2001; Ost et al. 2002) (Fig. 1d). The activation of urothelial cells with capsaicin or resiniferatoxin can increase intracellular calcium, elicit transient currents, and evoke transmitter (NO or ATP) release. Similar to that in sensory neurons, urothelial response to vanilloids is enhanced by low pH, blocked

by TRPV1 antagonists, and eliminated in TRPV1-null mice (Birder et al. 2001, 2002a). In addition, in the presence of the TRPV1 antagonist capsazepine, changes in pH induced by HCl elicited ionic currents and increases in intracellular calcium (Kullmann et al. 2009). As these currents are sensitive to amilioride, these findings suggest the expression of ASIC-like channels within the urothelium. In afferent neurons, TRPV1 is thought to integrate/amplify the response to various stimuli and to play an essential role in the development of inflammation-induced hyperalgesia. It seems likely that urothelial-TRPV1 might participate in a similar manner, in the detection of irritant stimuli following bladder inflammation or infection.

Though TRPV1-null mice are anatomically normal, they exhibit a number of alterations in bladder function including a higher frequency of low-amplitude, nonvoiding bladder contractions, suggesting the possibility of a small but ongoing role for TRPV1 in normal urine storage function. In addition, TRPV1-null mice exhibit a reduction in stretch- and hypotonic-evoked ATP release from the urothelium and also an attenuated response of bladder afferents to distension (Birder et al. 2002a; Daly et al. 2007). These findings demonstrate that the functional significance of TRPV1 in the bladder extends beyond pain sensation to include participation in normal voiding function and is essential for mechanically evoked signaling by bladder afferents as well as the urothelium.

TRPV1 has attracted considerable attention as a potential contributor to bladder diseases. TRPV1 knockout mice also display differences in the response to chemically induced irritation or inflammation of the bladder as compared with their wild-type counterparts (Szallasi et al. 2006; Charrua et al. 2007; Wang et al. 2008). Furthermore, patients suffering from neurogenic detrusor overactivity exhibit significant increases in the number of TRPV1-expressing nerves as well as increased TRPV1 expression within the urothelium (Brady et al. 2004; Apostolidis et al. 2005). Intravesical instillation of vanilloids (capsaicin or resiniferatoxin) improves urodynamic parameters in patients with neurogenic detrusor overactivity and reduces pain in patients with hypersensitivity disorders, presumably by desensitizing bladder nerves (Fowler et al. 1992; Chancellor and de Groat 1999; Kalsi and Fowler 2005; Apostolidis et al. 2006). However, this treatment, which had some clinical success, could also target TRPV1 on urothelial cells, whereby a persistent activation might lead to receptor desensitization or depletion of urothelial transmitters.

5.3 Additional TRP Channels

Much less is known about the involvement of other TRPs in bladder function or disease. TRPV4, which is a nonselective cation channel activated by a number of stimuli including heat, shear stress, changes in osmolarity, and lipid ligands, is expressed mainly within the epithelium of the urinary bladder (Liedtke 2005; Birder et al. 2007; Gevaert et al. 2007). While a definitive role for TRPV4 in bladder function has not been established, there is evidence that null mice exhibit impaired voiding responses, and intravesical instillation of a TRPV4 agonist in the

rat triggers a novel voiding reflex, which could regulate the late phase of micturition (Birder and de Groat 2007; Gevaert et al. 2007). In addition, in the awake ewe, TRPV4 may also be involved in a urethra to bladder reflex, proposed to facilitate bladder emptying (Combrisson et al. 2007).

While little is known about the involvement of the cold-sensing TRP channels (TRPM8 and TRPA1) in bladder function, the effect of cold temperatures on lower urinary tract function has long been of interest (Cheng et al. 1997). The instillation of cold solutions (known as the ice water test) elicits involuntary detrusor contracts in patients with either chronic spinal cord lesions or following bladder outlet obstruction (Chai et al. 1998; Gotoh et al. 1999). This reflex may be mediated by activation of C-type bladder afferent nerves sensitive to cold temperatures. The finding that intravesical instillation of menthol facilitates the bladder cooling reflex in both cats and humans suggests that TRPM8, a cold- and menthol-sensing channel expressed in both nerves and the urothelium, may be involved in triggering the reflex (Jiang et al. 2002; Peier et al. 2002). Another member of the TRP family, TRPA1 (characterized as a thermoreceptor activated by noxious cold), is expressed in C-fiber afferents as well as urothelium and agonists to this channel induce bladder hyperreflexia (Du et al. 2007; Streng et al. 2008). In addition, hydrogen sulfide, which may be formed during infection/inflammation, is an activator of TRPA1 (Streng et al. 2008).

While the functional role of thermosensitive channels in the urothelium remains to be clarified, it seems likely that a primary role for these proteins may be to recognize noxious stimuli in the bladder. In lung epithelium, activation of a variant of TRPM8 results in secretion of mediators that are thought to play a role in airway inflammation (Sabnis et al. 2008). However, the diversity of stimuli that can activate these proteins suggests a much broader sensory and/or cellular role. For example, TRPM8 expression is increased in some epithelia in prostate tumors, suggesting a role in proliferating cells (Bidaux et al. 2005). Thus, further studies are needed to fully elucidate the role of TRP channels in urothelium and their influence on bladder function.

5.4 Nicotinic and Muscarinic Acetylcholine Receptors

The presence of muscarinic and nicotinic receptors in the urothelium has attracted interest in the role of acetylcholine as a chemical mediator of neural–urothelial interactions (Hawthorn et al. 2000; Templeman et al. 2002; Beckel et al. 2006; Hanna-Mitchell et al. 2007). These receptors could be stimulated by acetylcholine released from urothelial cells as well as by cholinergic nerves that have been detected in close proximity to the urothelial cells in the rat bladder (Hanna-Mitchell et al. 2007; Kullmann et al. 2008). Exogenous muscarinic and nicotinic cholinergic agonists applied to cultured urothelial cells can elicit an increase in intracellular calcium concentration and evoke the release of NO and ATP (Beckel et al. 2006; Kullmann et al. 2008). In bladder strips or whole bladder preparations, muscarinic

agonists also stimulate the release of a smooth muscle inhibitory factor from the urothelium (Hawthorn et al. 2000; Templeman et al. 2002). Electrical stimulation of the pelvic nerve or reflex activation of the autonomic nervous system by spinal cord injury (SCI) (Apodaca et al. 2003) can elicit changes in urothelial permeability as well as changes in the morphology of the urothelium in the rat, raising the possibility that autonomic or sensory nerves make "synaptic connections" with urothelial cells.

There is evidence that the urothelium expresses the full complement of muscarinic receptors as well as enzymes necessary for the synthesis and release (except vesicular choline transporter) of acetylcholine (Hanna-Mitchell et al. 2007; Lips et al. 2007; Zarghooni et al. 2007; Kullmann et al. 2008). Furthermore, the urothelium is able to release acetylcholine following both chemical and mechanical stimulation. Once released, urothelial-derived acetylcholine is likely to exert effects via a number of sites including smooth muscle, nerves, as well as urothelial-associated muscarinic or nicotinic receptors, the latter that could contribute to feedback mechanisms modifying urothelial function. Recent studies have demonstrated alterations in muscarinic receptor expression or sensitivity of urothelial muscarinic receptors to cholinergic agonists in a number of bladder pathologies (Cheng et al. 2007; Gupta et al. 2009) Thus, stimulation of urothelial–cholinergic receptors elicits release of mediators such as nitric oxide and ATP, which could alter bladder sensation by stimulating nearby sensory afferent nerves.

It has been speculated that the urothelium could be affected by antimuscarinics via the bloodstream as well as affecting luminal muscarinic receptors (since some of these agents and/or metabolites are likely to be excreted in urine) (Andersson et al. 2008). Thus, targeting muscarinic receptors and/or urothelial release mechanisms may play an important role in the treatment for a number of bladder disorders. However, the mechanism of release is uncertain. Recently, botulinum toxin type A (BoNTA) has been used to evaluate the release mechanism. Botulinum toxin type A is currently undergoing clinical investigation to treat a number of bladder disorders including neurogenic detrusor overactivity, detrusor-sphincter dyssynergia, as well as BPS/IC (Chancellor 2005; Tiwari and Naruganahalli 2006). Studies have shown that this treatment may inhibit detrusor muscle contractions by suppressing the release of transmitters such as ATP and acetylcholine from bladder nerves by blocking exocytosis. Recent evidence suggests that botulinum toxins prevent the release of transmitters such as ATP from the urothelium, which may suggest that urothelial-released mediators contribute to sensory urgency (Khera et al. 2004; Smith et al. 2005).

5.5 Estrogen and Estrogen Receptors

While not well studied, it has been suggested that age or hormonal changes could influence urinary bladder function. A number of dysfunctions of the lower urinary tract, such as urgency, incontinence, and nocturia, are altered in the aging

population and also following menopause (Fanti 1994; Robinson and Cardozo 2002, 2003). The steroid hormone 17β-estradiol (E2) is a key regulator of growth, differentiation, and function in a wide array of target tissues. Its predominant biological effects are mediated through two distinct estrogen receptors (ER): ERα and ERβ (Hall et al. 2001). The urothelium expresses both subtypes of estrogen receptors, ERα and ERβ, which may mediate estrogen-mediated proliferation in urothelial cells (Teng et al. 2008). Studies in estrogen-deficient animals have revealed a loss of mucosal barrier function and alterations in prostaglandin levels; the latter has been associated with preserving mucosal integrity (Hass et al. 2009). In addition, it has been shown that female ERβ$^{-/-}$ mice exhibit ulcerations and atrophy of the urinary bladder urothelium, which resembles changes in the urothelium in patients diagnosed with BPS/IC (Imamov et al. 2007). Thus, alterations in estrogen or ER signaling can alter the structure of the urothelium in addition to maintenance of barrier function.

In addition to the well-established classical ("genomic") pathway, which involves interaction with an estrogen response element on the promoter region of the target gene, E2 can exert rapid "nongenomic" effects (Seval et al. 2006), which involve putative estrogen-binding proteins in the cell membrane and cytoplasm (Song and Santen 2006). The rapid effects of estrogen involve activation of distinct signal transduction cascades, such as the mitogen-activated protein kinase (MAPK) pathways. The p38 MAPK pathway can be activated in response to chemical and physical stresses and have therefore been termed "stress-activated kinases." In addition, p38 MAP is thought to play an important role in a number of cellular processes including cellular proliferation (Chang and Karin 2001). Studies have shown that E2 can trigger an estrus-modifiable activation of p38 MAP in endometrial cells as well as the urinary bladder urothelium (Birder et al. 2009). Further studies are needed to elucidate the full range of the influences of alterations in ovarian hormones on lower urinary tract structure and function, which may be important in a number of bladder dysfunctions such as urethra and pelvic floor weakness, detrusor instability, BPS/IC, and even underactive detrusor.

5.6 Adrenergic Receptors

The activation of sympathetic nerves promotes bladder relaxation during the storage phase, via the activation of β-adrenergic receptors (Yamaguchi and Chapple 2007; Fowler et al. 2008). In humans, it has been shown that this relaxation is mediated through the activation of a β_3 subtype adrenergic receptor. Recent reports have identified all three (β_1, β_2, and β_3) adrenergic subtypes within the urinary bladder urothelium (Tyagi et al. 2009). The activation of urothelial β_3-receptors could, via release of mediators including NO, alter afferent excitability and, in turn, bladder sensation. These findings suggest that the activation of urothelial β-receptors may be a potential target in the treatment of overactive bladder as well as other bladder sensory disorders.

Patients with a variety of lower urinary tract disorders including overactive bladder, benign prostatic hyperplasia, and even BPS/IC are often treated with selective α_1-adrenergic antagonists (Toh and Ng 2006; Yassin et al. 2006). Studies in mice that lack the α_{1D}-adrenergic receptor have indicated that there is an important role of these receptors in the regulation of bladder function (Chen et al. 2005). The α_{1D} subtype adrenergic receptor is expressed within the bladder urothelium, and it seems that tonic activation of urothelial α_{1D}-receptors by catecholamines might be involved in bladder sensory mechanisms (Ishihama et al. 2006). For example, catecholamines could be released from nerves adjacent to the urothelium or from nerves that innervate nearby blood vessels, as the suburothelial region receives a rich blood supply (Inoue and Gabella 1991; Jen et al. 1995). In turn, stimulation of both urothelial α- and β-adrenergic receptors could lead to the release of a number of transmitters including ATP and NO (Birder et al. 1998, 2002b). Cats diagnosed with FIC exhibit an increase in norepinephrine content in the urinary bladder (Buffington et al. 2002) and an augmented release of mediators including ATP and NO, compared with healthy cats (Birder et al. 2003, 2005). It is not known whether catecholamines are involved in the increased release of these mediators, or whether a tonic activation of the urothelium via circulating or other sources of catecholamines has a role in the sensory abnormalities of FIC.

5.7 Nitric Oxide

Nitric oxide (NO) is thought to be involved in many functions of the lower urinary tract, ranging from inhibition of neurotransmission in the urethra to modulation of bladder afferent nerves and bladder reflex pathways in the spinal cord (Andersson and Persson 1995). The localization of neuronal nitric oxide synthase (NOS) and/or NADPH diaphorase (a marker for neuronal NOS) in efferent and afferent nerve fibers and the expression of multiple NOS isoforms (neuronal, endothelial, and inducible) within the urothelium indicate that NO has a role in the micturition reflex pathway (Andersson and Persson 1995; Birder et al. 2002b). The release of NO from the urothelium or other cells is thought to either facilitate or inhibit the activity of bladder afferent nerves. For example, a reduction in NO levels adjacent to the urothelium (caused by intravesical administration of oxyhemoglobin) results in bladder hyperactivity, which suggests that there is an inhibitory role of NO in the control of bladder function (Pandita et al. 2000).

Injury or chronic inflammation has been shown to alter the expression of NOS, which raises the possibility that the neurotransmitter function of NO is plastic and can be altered by chronic pathological conditions (de Groat et al. 1997). Alterations in NO levels have been demonstrated in both patients with BPS/IC and cats diagnosed with FIC (Hosseini et al. 2004; Birder et al. 2005), both of which are associated with abnormalities in urothelial barrier function including increased urothelial permeability (Lavelle et al. 2000; Parsons 2007). It has been shown that a change in NO production or synthesis in intestinal epithelial cells can increase

their permeability to hydrophilic macromolecules (Kolios et al. 2004). In addition, aminoguanidine, an inhibitor of inducible nitric oxide synthase (iNOS), has been shown to prevent oxidative stress-induced changes in urinary bladder following cyclophosphamide treatment in rats (Abraham et al. 2009; Korkmaz et al. 2005). These findings suggest that alterations in NO levels may also have a role in the modulation of epithelial function, and this mechanism might contribute to the loss of membrane barrier integrity in many disease states.

6 Pathology-Induced Urothelial Plasticity and Effect on Barrier Function

Basal cells, which are thought to be precursors for other cell types, normally exhibit a low (3- to 6-month) turnover rate, in fact the slowest turnover of any mammalian epithelial cells (Hicks 1975; Apodaca 2004). It has been well described that the urothelium exhibits regenerative ability, which can occur within days following injury or insult to the urinary bladder. It has been shown that neither urine-derived factors nor cyclic mechanical changes contribute to urothelial proliferation and differentiation; however, accelerated proliferation can occur in pathology. For example, psychological stress, streptozotocin (STZ)-induced diabetes, and sucrose-induced diuresis in rodents can all result in significant urothelial remodeling (Lui and Daneshgari 2006; Chang et al. 2009). In addition, using protamine sulfate, an agent that selectively damages the umbrella cell layer, it has been shown that the urothelium rapidly undergoes both functional and structural changes in order to restore the barrier in response to injury (Lavelle et al. 2002). The initiation of urothelial proliferation is thought to involve upregulation of growth factors such as fibroblast growth factor and nerve growth factor (NGF) (de Boer et al. 1996; Bassuk et al. 2003).

In the periphery, NGF is produced and utilized by several nonneuronal cell types including immune inflammatory cells, epithelial cells, and smooth muscle cells; because of these functions, NGF is better described as a pleiotropic factor (Micera et al. 2007). A specific "epitheliotropic" (Botchkarev et al. 1999) role for NGF is seen in the epidermis, where it is endogenously synthesized and plays an important role in wound healing (Li et al. 1980; Matsuda et al. 1998). Numerous inflammatory cytokines including interleukin (IL)-1, tumor necrosis factor (TNF)-α, and IL-6 can induce NGF production in nonneuronal cell types such as fibroblasts, endothelial cells, and glial cells.

Increased NGF levels have been identified in urothelium in animals with bladder outlet obstruction, SCI, FIC, and cyclophosphamide-induced inflammation. In animals, increased target NGF expression has been linked with increased urinary frequency and unstable bladder contractions, and intravesical NGF produces a hyperreflexia, suggestive of inflammatory pain, likely by sensitizing bladder afferents (Chuang et al. 2001; Seki et al. 2002). The increased target NGF may be linked

with alterations in urinary frequency and unstable bladder contractions (Vizzard 2000; Kim et al. 2004; Steers and Tuttle 2006). The increased NGF has also been identified in urine of patients with overactivity, idiopathic sensory urgency, and those diagnosed with BPS/IC (Liu and Kuo 2007). In fact, NGF has been proposed as a potential biomarker for certain bladder disorders due to the possible link between elevated NGF levels in tissue and urine to overactivity and painful inflammatory conditions (Liu et al. 2008), and an antibody to NGF is currently in phase II clinical trial (ClinicalTrials.gov identifier NCT00601484) for BPS/IC-associated pain (Vastag 2006).

Though the urothelium maintains a tight barrier to ion and solute flux, a number of local factors such as tissue pH, mechanical or chemical trauma, or bacterial infection can modulate the urothelial barrier (Hicks 1975; Anderson et al. 2003). A number of pathologies including diabetes have been shown to increase the susceptibility of the urothelium to infections, in particular by *Escherichia coli*. Urinary tract infections produced by uropathogenic *E. coli* (UPEC) are initiated by bacterial adherence to uroplakin proteins on the apical surface of umbrella cells (Anderson et al. 2003) (Fig. 1e). The UPEC express filamentous adhesive organelles (type 1 pili) that mediate both bacterial attachment and invasion of the urothelial cells. Internalization of UPEC in the umbrella cells and formation of intracellular colonies (biofilm-like pods) of UPEC in umbrella cells have been implicated in the mechanism of recurrent urinary tract infections (Mulvey et al. 1998; Klumpp et al. 2006; Billips et al. 2008). Recent evidence suggests that induction of differentiation markers such as uroplakin III may play an important role in UPEC-induced cell death (Thumbikat et al. 2009).

Other conditions such as BPS/IC or SCI are also associated with changes in urothelial barrier. When the barrier is compromised, water, urea, and toxic substances can pass into the underlying tissue (neural/muscle layers), resulting in urgency, frequency, and pain during bladder filling and voiding. In some pathological conditions, the disruption of the urothelial barrier is associated with ultrastructural changes and alterations in the levels of chemical mediators such as nitric oxide and ATP that may alter epithelial function and/or integrity. The disruption of urothelial barrier integrity has also been linked to the expression of substances such as antiproliferative factor (APF), which also slows urothelial cell growth (Keay et al. 2004). APF, a frizzled eight protein detected in the urine of patients with BPS/IC, is secreted by bladder epithelial cells obtained from these patients. Treatment of urothelial cells from normal patients with purified APF decreases the expression of adhesion and tight junction proteins.

The disruption of urothelial function can also be induced by more remote pathological conditions that influence neural or hormonal mechanisms. For example, both physical and psychological stresses have been associated with alterations in epithelial permeability and even mucosal inflammation (Mayer and Fanselow 2003; van de Merwe 2007). Short-term ischemia can result in decreased blood flow and oxygenation and may occur with conditions that produce urinary retention or detrusor instability. The urothelium is highly sensitive to ischemic alterations, such that 1 h of ischemia in an in vivo animal model can produce a reversible loss of tight

junction integrity and increased permeability with detachment and desquamation of urothelial cells (Korosec and Jezernik 2000). It has also been shown that estrogen deficiency is linked to urinary bladder dysfunctions including urgency, frequency, unstable bladder contractions, and even underactive bladder (Zhu et al. 2001; Robinson and Cardozo 2003). Some of these symptoms may be associated with alterations in the epithelial lining of the urinary bladder as estrogen deficiency can lead to epithelial shedding or mucosal atrophy (Imamov et al. 2007; Hass et al. 2009). Alteration in the mucosal barrier can be a result of spinal cord transection, which in rats has been shown to result in a rapid alteration in the urothelial barrier including ultrastructural changes and increased permeability (Apodaca et al. 2003). These changes are blocked by pretreatment with a ganglionic blocking agent, suggesting the involvement of the efferent autonomic pathways in the acute effects of SCI on bladder urothelium. Other types of urothelial–neural interactions are also likely, based on recent reports that various stimuli induce urothelial cells to release chemical mediators that can, in turn, modulate the activity of afferent nerves. This has raised the possibility that the urothelium may have a role in sensory mechanisms in the urinary tract.

7 Clinical Significance of the Sensory Web

Defects in urothelial sensor molecules and urothelial cell signaling are likely to contribute to the pathophysiology of bladder diseases. For example, a number of bladder conditions (e.g., BPS/IC, SCI, and chemically induced cystitis) are associated with augmented release of urothelial-derived ATP, which is likely to result in altered sensations and changes in bladder reflexes induced by excitation of purinergic receptors on nearby sensory fibers (Birder et al. 2003; Chopra et al. 2005; Salas et al. 2007; Girard et al. 2008). ATP can also act in an autocrine manner to facilitate its own release from urothelial cells (Wang et al. 2005), and once released, ATP can alter the threshold for activation of additional ion channels that could alter afferent excitability. Changes in epithelial signaling/barrier function are not unique to the urinary bladder. Like the urinary bladder urothelium, the epithelial cells lining both the airway and skin keratinocytes form a selective physical barrier between the external environment and internal tissues. It has also been shown that the airway epithelia in asthmatic patients as well as keratinocytes in certain types of skin diseases also exhibit a number of similar abnormalities and compromised repair processes (Holgate 2007, 2008; Bosse et al. 2008; Proksch et al. 2008). This is particularly relevant given the high incidence of associated diseases that can include both visceral and somatic conditions, many of which exhibit a shared loss of epithelial barrier function. Taken together, epithelial cells can respond to a number of challenges (including environmental pollutants and mediators released from nerves or nearby inflammatory cells) resulting in altered expression and/or sensitivity of various receptor/channels as well as changes in release of mediators, all of which could impact function.

8 Conclusions: Potential Clinical Implications

It is conceivable that the effectiveness of some agents currently used in the treatment of bladder disorders may involve urothelial receptors and/or release mechanisms. For example, intravesical instillation of vanilloids (capsaicin or resiniferatoxin) improves urodynamic parameters in patients with neurogenic detrusor overactivity and reduces bladder pain in patients with hypersensitivity disorders, presumably by desensitizing bladder nerves (Kim et al. 2003) This treatment could also target TRPV1 on urothelial cells, whereby a persistent activation might lead to receptor desensitization or depletion of urothelial transmitters. Recent studies have demonstrated that intradetrusor injection with botulinum neurotoxin type A (BoNTA) is an effective treatment for bladder hypersensitivity disorders including neurogenic detrusor overactivity (Chancellor et al. 2007). Following injection, the toxin binds to bladder cholinergic nerve terminals and cleaves the protein, SNAP25, necessary for exocytosis and release of acetylcholine (Chancellor et al. 2007). There is evidence that the BoNTA can suppress the release of a number of mediators (acetylcholine, ATP, and neuropeptides) from both neural and nonneural cells (Khera et al. 2004; Chancellor et al. 2007). Suppression of neurotransmitter release from urothelium would serve to blunt afferent activity driven by urothelial-derived release of mediators in a number of lower urinary tract dysfunctions. In addition, studies have shown that intravesical administration of liposomes, which have been shown to promote wound healing, may prove useful to improve barrier function in bladder pathologies such as BPS/IC (Fraser et al. 2003 ; Giannantoni et al. 2006). These findings suggest that urothelial cells exhibit specialized sensory and signaling properties that could allow them to respond to their chemical and physical environments and to engage in reciprocal communication with neighboring urothelial cells as well as nerves within the bladder well. Taken together, pharmacologic interventions aimed at targeting urothelial receptor/ion channel expression or release mechanisms may provide a new strategy for the clinical management of bladder disorders.

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References

Abraham P, Rabi S, Selvakumar D (2009) Protective effect of aminoguanidine against oxidative stress and bladder injury in cyclophosphamide-induced hemorrhagic cystitis in rat. Cell Biochem Funct 27:56–62

Acharya P, Beckel JM, Ruiz WG, Wang E, Rojas R, Birder LA, Apodaca G (2004) Distribution of the tight junction proteins Z0-1, occludin, and claudin-4, -8, and -12 in bladder epithelium. Am J Physiol Renal Physiol 287:F305–F318

Anderson G, Palermo J, Schilling J, Roth R, Heuser J, Hultgren S (2003) Intracellular bacterial biofilm-like pods in urinary tract infections. Science 301:105–107

Andersson KE, Persson K (1995) Nitric oxide synthase and the lower urinary tract: possible implications for physiology and pathophysiology. Scand J Urol Nephrol 175:43–53
Andersson KE, Fulhase C, Soler R (2008) Urothelial effects of oral agents for overactive bladder. Curr Urol Rep 9:459–464
Apodaca G (2004) The uroepithelium: not just a passive barrier. Traffic 5:117–128
Apodaca G, Kiss S, Ruiz WG, Meyers S, Zeidel M, Birder LA (2003) Disruption of bladder epithelium barrier function after spinal cord injury. Am J Physiol 284:F966–F976
Apodaca G, Balestreire E, Birder LA (2007) The uroepithelial-associated sensory web. Kidney Int 72:1057–1064
Apostolidis A, Brady CM, Yiangou Y, Davis J, Fowler CJ, Anand P (2005) Capsaicin receptor TRPV1 in urothelium of neurogenic human bladders and effect of intravesical resiniferatoxin. Urology 65:400–405
Apostolidis A, Gonzales GE, Fowler CJ (2006) Effect of intravesical resiniferatoxin (RTX) on lower urinary tract symptoms, urodynamic parameters, and quality of life of patients with urodynamic increased bladder sensation. Eur Urol 50:1299–1305
Araki I, Du S, Kobayashi H, Sawada N, Mochizuki T, Zakoji H, Takeda M (2008) Roles of mechanosensitive ion channels in bladder sensory transduction and overactive bladder. Int J Urol 15:681–687
Balestreire EM, Apodaca G (2007) Apical EGF receptor signaling: regulation of stretch-dependent exocytosis in bladder umbrella cells. Mol Biol Cell 18:1312–1323
Bassuk JA, Cochrane K, Mitchell ME (2003) Induction of urothelial cell proliferation by fibroblast growth factor-7 in RAG1-deficient mice. Adv Exp Med Biol 539:623–633
Beckel JM, Kanai AJ, Lee SJ, DeGroat WC, Birder LA (2006) Expression of functional nicotinic acetylcholine receptors in rat urinary bladder epithelial cells. Am J Physiol 290:F103–F110
Bidaux G, Roudbaraki M, Merle C, Crepin A, Delcourt P, Slomianny C, Thebault S, Bonnal JL, Benahmed M, Cabon F, Mauroy B, Prevarskaya N (2005) Evidence for specific TRPM8 expression in human prostate secretory epithelial cells: functional androgen receptor requirement. Endocr Relat Cancer 12:367–382
Billips BK, Schaeffer AJ, Klumpp DJ (2008) Molecular basis of uropathogenic *Escherichia coli* evasion of the innate immune response in the bladder. Infect Immun 76:3891–3900
Birder LA, de Groat WC (2007) Mechanisms of disease: involvement of the urothelium in bladder dysfunction. Nat Clin Pract 4:46–54
Birder LA, Apodaca G, de Groat WC, Kanai AJ (1998) Adrenergic and capsaicin-evoked nitric oxide release from urothelium and afferent nerves in urinary bladder. Am J Physiol 275: F226–F229
Birder LA, Kanai AJ, de Groat WC, Kiss S, Nealen ML, Burke NE, Dineley KE, Watkins S, Reynolds IJ, Caterina MJ (2001) Vanilloid receptor expression suggests a sensory role for urinary bladder epithelial cells. Proc Natl Acad Sci 98:13396–13401
Birder LA, Nakamura Y, Kiss S, Nealen ML, Barrick SR, Kanai AJ, Wang E, Ruiz WG, de Groat WC, Apodaca G, Watkins S, Caterina MJ (2002a) Altered urinary bladder function in mice lacking the vanilloid receptor TRPV1. Nat Neurosci 5:856–860
Birder LA, Nealen ML, Kiss S, de Groat WC, Caterina MJ, Wang E, Apodaca G, Kanai AJ (2002b) Beta-adrenoceptor agonists stimulate endothelial nitric oxide synthase in rat urinary bladder urothelial cells. J Neurosci 22:8063–8070
Birder LA, Barrick SR, Roppolo JR, Kanai AJ, de Groat WC, Kiss S, Buffington CAT (2003) Feline interstitial cystitis results in mechanical hypersensitivity and altered ATP release from bladder urothelium. Am J Physiol 285:F423–F429
Birder LA, Wolf-Johnton A, Buffington CAT, Roppolo JR, de Groat WC, Kanai AJ (2005) Altered inducible nitric oxide synthase expression and nitric oxide production in the bladder of cats with feline interstitial cystitis. J Urol 173:625–629
Birder L, Kullmann FA, Lee H, Barrick S, de Groat W, Kanai A, Caterina M (2007) Activation of urothelial transient receptor potential vanilloid 4 by 4alpha-phorbol 12, 13-didecanoate contributes to altered bladder reflexes in the rat. J Pharmacol Exp Ther 323:227–235

Birder LA, Wolf-Johnston AS, Chib MK, Buffington CA, Roppolo JR, Hanna-Mitchell AT (2009) Beyond neurons: involvement of urothelial and glial cells in bladder function. Neurourol Urodyn 29:88–96

Bosse Y, Pare PD, Seow CY (2008) Airway wall remodeling in asthma: from the epithelial layer to the adventitia. Curr Allergy Asthma Rep 8:357–366

Botchkarev VA, Metz M, Botchkareva NV, Welker P, Lommatzsch M, Renz H (1999) Brain-derived neurotrophic factor, neurotrophin-3, and neurotrophin-4 act as "epitheliotrophins" in murine skin. Lab Invest 79:557–572

Brading AF, McCloskey DD (2005) Mechanisms of disease: specialized interstitial cells of the urothelium – an assessment of current knowledge. Nat Clin Pract Urol 2:546–554

Brady CM, Apostolidis AN, Harper M, Yiangou Y, Beckett A, Jacques TS, Freeman A, Scaravilli F, Fowler CJ, Anand P (2004) Parallel changes in bladder suburothelial vanilloid receptor TRPV1 and pan-neuronal marker PGP9.5 immunoreactivity in patients with neurogenic detrusor overactivity after intravesical resiniferatoxin treatment. BJU Int 93:770–776

Buffington CA, Teng B, Somogyi GT (2002) Norepinephrine content and adrenoceptor function in the bladder of cats with feline interstitial cystitis. J Urol 167:1876–1880

Burnstock G (2001) Purine-mediated signalling in pain and visceral perception. Trends Pharmacol Sci 22:182–188

Carattino MD, Passero CJ, Steren CA, Maarouf AB, Pilewski JM, Myerburg MM, Hughey RP, Kleyman TR (2008) Defining an inhibitory domain in the alpha-subunit of the epithelial sodium channel. Am J Physiol 294:F47–F52

Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D (1997) The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 389:816–824

Chai TC, Gray J, Steers W (1998) The incidence of a positive ice water test in bladder outlet obstructed patients: evidence for bladder neural plasticity. J Urol 160:34–38

Chaiyaprasithi B, Mang CF, Kilbinger H, Hohenfellner M (2003) Inhibition of human detrusor contraction by a urothelium derived factor. J Urol 170:1897–1900

Chancellor MB (2005) Urgency, botulinum toxin and how botulinum toxin can help urgency. J Urol 174:818

Chancellor MB, de Groat WC (1999) Intravesical capsaicin and resiniferatoxin therapy: spicing up the ways to treat the overactive bladder. J Urol 162:3–11

Chancellor MB, Fowler CJ, Apostolidis A, de Groat WC, Smith CP, Somogyi GT, Aoki KR (2007) Drug insight: biological effects of botulinum toxin A in the lower urinary tract. Nat Clin Pract Urol 5:319–328

Chang L, Karin M (2001) Mammalian MAP kinase signalling cascades. Nature 410:37–40

Chang A, Butler S, Sliwoski J, Vanentino RJ, Canning DA, Zderic SA (2009) Social stress in mice induces voiding dysfunction and bladder wall remodeling. Am J Physiol 297:F1101–F1108

Charrua A, Cruz CD, Cruz F, Avelino A (2007) Transient receptor potential vanilloid subfamily 1 is essential for the generation of noxious bladder input and bladder overactivity in cystitis. J Urol 177:1537–1541

Chen Q, Takahashi S, Zhong S, Hosoda C, Zheng HY, Ogushi T, Fujimura T, Ohta N, Tanoue A, Tsujimoto G, Kitamura T (2005) Function of the lower urinary tract in mice lacking alpha1d-adrenoceptor. J Urol 174:370–374

Cheng JH, Zhang ZT (2002) Overexpression of epidermal growth factor receptor in urothelium elicits urothelial hyperplasia and promotes bladder tumor growth. Cancer Res 62:4157–4163

Cheng CL, Chai CY, de Groat WC (1997) Detrusor-sphincter dyssynergia induced by cold stimulation of the urinary bladder of rats. Am J Physiol 41:R1271–R1282

Cheng JT, Yu BC, Tong YC (2007) Changes of M3-muscarinic receptor protein and mRNA expressions in the bladder urothelium and muscle layer of streptozotocin-induced diabetic rats. Neurosci Lett 423:1

Chess-Williams R (2002) Muscarinic receptors of the urinary bladder: detrusor, urothelial and prejunctional. Auton Autacoid Pharmacol 22:133–145

Chopra B, Barrick SR, Meyers S, Beckel JM, Zeidel ML, Ford AP, de Groat WC, Birder LA (2005) Expression and function of bradykinin B1 and B2 receptors in normal and inflamed rat urinary bladder urothelium. J Physiol 562:859–871

Chopra B, Gever J, Barrick SR, Hanna-Mitchell AT, Beckel JM, Ford AP, Birder LA (2008) Expression and function of rat urothelial P2Y receptors. Am J Physiol 294:F821–F829

Chuang YC, Fraser MO, Yu Y, Chancellor MB, de Groat WC, Yoshimura N (2001) The role of bladder afferent pathways in bladder hyperactivity induced by the intravesical administration of nerve growth factor. J Urol 165:975–979

Combrisson H, Allix S, Robain G (2007) Influence of temperature on urethra to bladder micturition reflex in the awake ewe. Neurourol Urodyn 26:290–295

Daly D, Rong W, Chess-Williams R, Chapple C, Grundy D (2007) Bladder afferent sensitivity in wild-type and TRPV1 knockout mice. J Physiol 583:663–674

de Boer WI, Vermeij M, Diez de Medina SG, Bindels E, Radvanyi F, van der Kwast T, Chopin D (1996) Functions of fibroblast and transforming growth factors in primary organoid-like cultures of normal human urothelium. Lab Invest 75:147–156

de Groat WC, Kruse MN, Vizzard MA, Cheng CL, Araki I, Yoshimura N (1997) Modification of urinary bladder function after spinal cord injury. Adv Neurol 72:347–364

Du S, Araki I, Yoshiyama M, Nomura T, Takeda M (2007) Transient receptor potential channel A1 involved in sensory transduction of rat urinary bladder through C-fiber pathway. Urology 70:826–831

Fanti JA (1994) The lower urinary tract in women-effect of aging and menopause on continence. Exp Gerontol 29:417–422

Ferguson DR, Kennedy I, Burton TJ (1997) ATP is released from rat urinary bladder epithelial cells by hydrostatic pressure changes-a possible sensory mechanism? J Physiol 505:503–511

Fovaeus M, Fujiwara M, Hogestatt ED, Persson K, Andersson KE (1999) A non-nitreregic smooth muscle relaxant factor released from rat urinary bladder by muscarinic receptor stimulation. J Urol 161:649–653

Fowler CJ, Jewkes D, McDonald WI, Lynn B, de Groat WC (1992) Intravesical capsaicin for neurogenic bladder dysfunction. Lancet 339:1239

Fowler CJ, Griffiths D, de Groat WC (2008) The neural control of micturition. Nat Rev Neurosci 9:453–466

Fraser MO, Chuang YC, Tyagi P, Yokoyama T, Yoshimura N, Huang L, de Groat WC, Chancellor MB (2003) Intravesical liposome administration-a novel treatment for hyperactive bladder in the rat. Urology 61:656–663

Fry C, Sui GP, Kanai AJ, Wu C (2007) The function of suburothelial myfibroblasts in the bladder. Neurourol Urodyn 26:914–919

Gevaert T, Vriens J, Segal A, Everaerts W, Roskams T, Talavera K, Owsianik G, Liedtke W, Daelemans D, Dewachter I, Van Leuven F, Voets T, De Ridder D, Nilius B (2007) Deletion of the transient receptor potential cation channel TRPV4 impairs murine bladder voiding. J Clin Invest 117:3453–3462

Giannantoni A, Di Stasi SM, Chancellor MB (2006) New frontiers in intravesical therapies and drug therapy. Eur Urol 50:1183–1193

Gillenwater JY, Wein AJ (1998) Summary of the national institute of arthritis, diabetes, and kidney diseases workshop on interstitial cystitis. J Urol 140:205

Gillespie JI, Markerink-van Ittersum M, de Vente J (2006) Sensory collaterals, intramural ganglia and motor nerves in the guinea-pig bladder: evidence for intramural neural circuits. Cell Tissue Res 325:33–45

Girard BM, Wolf-Johnston A, Braas KM, Birder LA, May V, Vizzard MA (2008) PACAP-mediated ATP release from rat urothelium and regulation of PACAP/VIP and receptor mRNA in micturition pathways after cyclophosphamide (CYP)-induced cystitis. J Mol Neurosci 36:310–320

Gotoh M, Yoshikawa Y, Kondo AS, Kondo A, Ono Y, Ohshima S (1999) Positive bladder cooling reflex in patients with bladder outlet obstruction due to benign prostatic hyperplasia. World J Urol 17:126–130

Grol S, van Koeveringe GA, de Vente J, van Kerrebroeck PE, Gillespie JI (2008) Regional differences in sensory innervation and suburothelial interstitial cells in the bladder neck and urethra. BJU Int 102:870–877

Gupta GN, Lu SG, Gold MS, Chai TC (2009) Bladder urothelial cells from patients with interstitial cystitis have an increased sensitivity to carbachol. Neurourol Urodyn 28:1022–1027

Hall JM, Couse JF, Korach KS (2001) The multifaceted mechanisms of estradiol and estrogen receptor signaling. J Biol Chem 276:36869–36872

Hanna-Mitchell AT, Beckel JM, Barbadora S, Kanai AJ, de Groat WC, Birder LA (2007) Non-neuronal acetylcholine and urinary bladder urothelium. Life Sci 80:2298–2302

Hass MA, Nichol P, Lee L, Levin RM (2009) Estrogen modulates permeability and prostaglandin levels in the rabbit urinary bladder. Prostaglandins Leukot Essent Fatty Acids 80:125–129

Hawthorn MH, Chapple CR, Cock M, Chess-Williams R (2000) Urothelium-derived inhibitory factor(s) influences on detrusor muscle contractility. BJU Int 129:416–419

Hicks M (1975) The mammalian urinary bladder: an accommodating organ. Biol Rev 50:215–246

Holgate S (2007) Epithelium dysfunction in asthma. J Allergy Clin Immunol 120:1233–1244

Holgate ST (2008) The airway epithelium is central to the pathogenesis of asthma. Allergol Int 57:1–10

Hosseini A, Ehren I, Wiklund NP (2004) Nitric oxide as an objective marker for evaluation of treatment response in patients with classic interstitial cystitis. J Urol 172:2261–2265

Ikeda Y, Kanai A (2008) Urotheliogenic modulation of intrinsic activity in spinal cord-transected rat bladders: role of mucosal muscarinic receptors. Am J Physiol 295:F454–F461

Ikeda Y, Fry C, Hayashi F, Stolz D, Griffiths D, Kanai AJ (2007) Role of gap junctions in spontaneous activity of the rat bladder. Am J Physiol 293:F1018–F1025

Ikeda Y, Birder L, Buffington C, Roppolo J, Kanai A (2009) Mucosal muscarinic receptors enhance bladder activity in cats with feline interstitial cystitis. J Urol 181:1415–1422

Imamov O, Yakimchuk K, Morani A, Schwend T, Wada-Hiraike O, Razumov S, Warner M, Gustafsson JA (2007) Estrogen receptor beta-deficient female mice develop a bladder phenotype resembling human interstitial cystitis. Proc Natl Acad Sci 104:9806–9809

Inoue T, Gabella G (1991) A vascular network closely linked to the epithelium of the urinary bladder of the rat. Cell Tissue Res 263:137–143

Ishihama H, Momota Y, Yanase H, Wang X, de Groat WC, Kawatani M (2006) Activation of alpha1D adrenergic receptors in rat urothelium facilitates the micturition reflex. J Urol 175:358–364

Jen PY, Dixon JS, Gosling JA (1995) Immunohistochemical localization of neuromarkers and neuropeptides in human fetal and neonatal urinary bladder. Br J Urol 75:230–235

Jiang CH, Mazieres L, Lindstrom S (2002) Cold- and menthol-sensitive C afferents of cat urinary bladder. J Physiol 543:211–220

Kalsi V, Fowler CJ (2005) Therapy insight: bladder dysfunction associated with multiple sclerosis. Nat Clin Pract Urol 2:492–501

Keay SK, Szekely Z, Conrads TP, Veenstra TD, Barchi JJ, Zhang CO, Koch KR, Michejda CJ (2004) An antiproliferative factor from interstitial cystitis patients is a frizzled 8 protein-related sialoglycopeptide. Proc Natl Acad Sci 101:11803–11808

Khandelwal P, Abraham SN, Apodaca G (2009) Cell biology and physiology of the uroepithelium. Am J Physiol 297:F1477–F1501

Khera M, Somogyi GT, Kiss S, Boone TB, Smith CP (2004) Botulinum toxin A inhibits ATP release from bladder urothelium after chronic spinal cord injury. Neurochem Int 45:987–993

Kim JH, Rivas DA, Shenot PJ, Green B, Kennelly M, Erickson JR, O'Leary M, Yoshimura N, Chancellor MB (2003) Intravesical resiniferatoxin for refractory detrusor hyperreflexia: a multicenter, blinded, randomized, placebo-controlled trial. J Spinal Cord Med 26: 358–363

Kim JC, Kim DB, Seo S, Park YH, Hwang TK (2004) Nerve growth factor and vanilloid receptor expression, and detrusor instability, after relieving bladder outlet obstruction in rats. BJU Int 94:915–918

Klumpp DJ, Rycyk MT, Chen MC, Thumbikat P, Sengupta S, Schaeffer AJ (2006) Uropathogenic *Escherichia coli* induces extrinsic and intrinsic cascades to initiate urothelial apoptosis. Infect Immun 74:5106–5113

Kolios G, Valatas V, Ward SG (2004) Nitric oxide in inflammatory bowel disease: a universal messenger in an unsolved puzzle. Immunology 113:427–437

Korkmaz A, Oter S, Sadir S, Coskun O, Topal T, Ozler M, Bilgic H (2005) Peroxynitrite may be involved in bladder damage caused by cyclophosphamide in rats. J Urol 173:1793–1796

Korosec P, Jezernik K (2000) Early cellular and ultrastructural response of the mouse urinary bladder urothelium to ischemia. Virchows Arch 436:377–383

Kullmann FA, Artim D, Beckel J, Barrick S, de Groat WC, Birder LA (2008) Heterogeneity of muscarinic receptor-mediated calcium responses in cultured urothelial cells from rat. Am J Physiol 294:F971–F981

Kullmann FA, Shah MA, Birder LA, de Groat WC (2009) Functional TRP and ASIC-like channels in cultured urothelial cells from the rat. Am J Physiol 296:F892–F901

Kunze A, Neuhaus J, Stolzenburg JU (2006) Quantitative immunohistochemical study of the innervation of the guinea-pig lower urinary tract. BJU Int 98:424–429

Lavelle J, Meyers S, Ruiz WG, Buffington CA, Zeidel ML, Apodaca G (2000) Urothelial pathophysiological changes in feline interstitial cystitis: a human model. Am J Physiol 278: F540–F553

Lavelle J, Meyers S, Ramage R, Bastacky S, Doty D, Apodaca G, Zeidel ML (2002) Bladder permeability barrier: recovery from selective injury of surface epithelial cells. Am J Physiol 283:F242–F253

LeBerge J, Malley SE, Zvarova K, Vizzard MA (2006) Expression of corticotropin-releasing factor and CRF receptors in micturition pathways after cyclophosphamide-induced cystitis. Am J Physiol 291:R692–R703

Lewis SA (2000) Everything you wanted to know about the bladder epithelium but were afraid to ask. Am J Physiol 278:F867–F874

Li AK, Koroly MJ, Schattenkerk ME, Malt RA, Yong M (1980) Nerve growth factor: acceleration of the rate of wound healing in mice. Proc Natl Acad Sci 77:4379–4381

Liang FX, Riedel I, Deng FM, Zhou G, Wu C, Wu WR, Kon WP, Moll R, Sun TT (2001) Organization of uroplakin subunits: transmembrane topology, pair formation and plaque composition. J Biochem 355:13–18

Liang FX, Bosland MC, Huang H, Romih R, Baptiste S, Deng FM, Wu XR, Shapiro E, Sun TT (2005) Cellular basis of urothelial squamous metaplasia: roles of lineage heterogeneity and cell replacement. J Cell Biol 171:835–844

Liedtke W (2005) TRPV4 plays an evolutionary conserved role in the transduction of osmotic and mechanical stimuli in live animals. Pflugers Arch 451:176–180

Lips KS, Wunsch J, Zarghooni S, Bschleipfer T, Schukowski K, Weidner W, Wessler I, Schwantes U, Koepsell H, Kummer W (2007) Acetylcholine and molecular components of its synthesis and release machinery in the urothelium. Eur Urol 51:1042–1053

Liu HT, Kuo HC (2007) Intravesical botulinum toxin A injections plus hydrodistension can reduce nerve growth factor production and control bladder pain in interstitial cystitis. Urology 70:463–468

Liu HT, Chancellor MB, Kuo HC (2008) Urinary nerve growth factor level could be a biomarker in the differential diagnosis of mixed urinary incontinence in women. BJU Int 102:1440–1444

Lui G, Daneshgari F (2006) Temporal diabetes- and diuresis-induced remodeling of the urinary bladder of the rat. Am J Physiol 291:R837–R843

Matsuda H, Koyama H, Sato H, Sawada J, Itakura A, Tanaka A (1998) Role of nerve growth factor in cutaneous wound healing: accelerating effects in normal and healing-impaired diabetic mice. J Exp Med 187:297–306

Mayer EA, Fanselow MS (2003) Dissecting the components of the central response to stress. Nat Neurosci 6:1011–1012

Micera A, Lambiase A, Stampachiacchiere B, Bonini S, Levi-Schaffer F (2007) Nerve growth factor and tissue repair remodeling: trkA (NGFR) and p75 (NTR), two receptors one fate. Cytokine Growth Factor Rev 18:245–256

Mulvey MA, Lopez-Boado YS, Wilson CL, Roth R, Parks WC, Heuser J, Hultgren SJ (1998) Induction and evasion of host defenses by type 1-pillated uropathogenic *Escherichia coli*. Science 282:1494–1497

Murakami S, Chapple CR, Akino H, Seller DJ, Chess-Williams R (2007) The role of the urothelium in mediating bladder responses to isoprenaline. BJU Int 99:669–673

Murray E, Malley SE, Qiao LY, Hu VY, Vizzard MA (2004) Cyclophosphamide induced cystitis alters neurotropin and receptor tyrosine kinase expression in pelvic ganglia and bladder. J Urol 172:2434–2439

Nickel JC (2003) Interstital cystitis: an elusive clincal target? J Urol 170:816–817

Ossovskaya VS, Bunnett NW (2004) Protease-activated receptors: contribution to physiology and disease. Physiol Rev 84:579–621

Ost D, Roskams T, Van de Aa F, de Ridder D (2002) Topography of the vanilloid receptor in the human bladder: more than just the nerve fibers. J Urol 168:293–297

Pandita RK, Mizusawa H, Andersson KE (2000) Intravesical oxyhemoglobin initiates bladder overactivity in consious, normal rats. J Urol 164:545–550

Parsons CL (2007) The role of the urinary epithelium in the pathogenesis of interstitial cystitis/prostatitis/urethritis. Urology 69:9–16

Parsons CL, Greenspan C, Moore SW, Mulholland SG (1977) Role of surface mucin in primary antibacterial defense of bladder. Urology 9:48–52

Parsons CL, Boychuk D, Jones S, Hurst R, Callahan H (1990) Bladder surface glycosaminoglycans: an epithelial permeability barrier. J Urol 143:139–142

Parsons CL, Lilly JD, Stein P (1991) Epithelial dysfunction in nonbacterial cystitis (interstitial cystitis). J Urol 145:732–735

Parsons CL, Greenberger M, Gabal L, Bidair M, Barme G (1998) The role of urinary potassium in the pathogenesis and diagnosis of interstitial cystitis. J Urol 159:1862–1867

Peier AM, Moqrich A, Hergarden AC, Reeve AJ, Andersson DA, Story GM, Earley TJ, Dragoni I, McIntyre P, Bevan S, Patapoutian A (2002) A TRP channel that senses cold stimuli and menthol. Cell 108:705–715

Proksch E, Brandner JM, Jensen JM (2008) The skin: an indispensable barrier. Exp Dermatol 17:1063–1072

Robinson D, Cardozo LD (2002) Overactive bladder in the female patient: the role of estrogens. Curr Urol Rep 3:452–457

Robinson D, Cardozo LD (2003) The role of estrogens in female lower urinary tract dysfunction. Urology 62:45–51

Romih R, Korosec P, de Mello WJ, Jezernik K (2005) Differentiation of epithelial cells in the urinary tract. Cell Tissue Res 320:259–268

Saban MR, Backer J, Backer MV, Maier J, Fowler B, Davis CA, Simpson C, Wu XR, Birder L, Freeman MR, Soker S, Hurst RE, Saban R (2008) VEGF receptors and neuropilins are expressed in the urothelial and neuronal cells in normal mouse urinary bladder and are upregulated in inflammation. Am J Physiol 295:F60–F72

Sabnis AS, Reilly CA, Veranth JM, Yost GS (2008) Increased transcription of cytokine genes in human lung epithelial cells through activation of a TRPM8 variant by cold temperatures. Am J Physiol 295:L194–L200

Salas NA, Somogyi GT, Gangitano DA, Boone TB, Smith CP (2007) Receptor activated bladder and spinal ATP release in neurally intact and spinal cord injured rats. Neurochem Int 50:345–350

Seki S, Sasaki K, Fraser MO, Igawa Y, Nishizawa O, Chancellor MB, de Groat WC, Yoshimura N (2002) Immunoneutralization of nerve growth factor in lumbosacral spinal cord reduces bladder hyperreflexia in spinal cord injured rats. J Urol 168:2269–2274

Seval Y, Cakmak H, Kayisli UA, Arici A (2006) Estrogen-mediated regulation of p38 mitogen-activated protein kinase in human endometrium. J Clin Endocrinol Metab 91:2349–2357
Shiroyanagi Y, Liu B, Cao M, Agras K, Li J, Hsieh MH, Willingham EJ, Baskin LS (2007) Urothelial sonic hedgehog signaling plays an important role in bladder smooth muscle formation. Differentiation 75:968–977
Smith CP, Vemulkonda VM, Kiss S, Boone TB, Somogyi GT (2005) Enhanced ATP release from bladder urothelium during chronic inflammation: effect of botulinum toxin A. Neurochem Int 47:291–297
Song RX, Santen RJ (2006) Membrane initiated estrogen signaling in breast cancer. Biol Reprod 75:9–16
Steers WD, Tuttle JB (2006) Mechanisms of disease: the role of nerve growth factor in the pathophysiology of bladder disorders. Nat Clin Pract Urol 3:101–110
Streng T, Axelsson HE, Hedlund P, Andersson DA, Jordt SE, Bevan S, Andersson KE, Hogestatt ED, Zygmunt PM (2008) Distribution and function of the hydrogen sulfide-sensitive TRPA1 ion channel in rat urinary bladder. Eur Urol 53:391–399
Sui GP, Wu C, Fry CH (2004) Electrical characteristics of suburothelial cells isolated from the human bladder. J Urol 171:938–943
Sui GP, Wu C, Fry CH (2006) Characterization of the purinergic receptor subtype on guinea-pig suburothelial myofibroblasts. BJU Int 97:914–919
Sun TT (2006) Altered phenotype of cultured urothelial and other stratified epithelial cells: implications for wound healing. Am J Physiol 291:F9–F21
Sun Y, Keay S, DeDeyne P, Chai TC (2001) Augmented stretch activated adenosine triphosphate release from bladder uroepithelial cells in patients with interstitial cystitis. J Urol 166:1951–1956
Szallasi A, Cruz F, Geppetti P (2006) TRPV1: a therapeutic target for novel analgesic drugs? Trends Mol Med 12:545–554
Taylor AH, Al-Azzawi F (2000) Immunolocalization of oestrogen receptor beta in human tissues. J Mol Endocrinol 24:145–155
Templeman L, Chapple CR, Chess-Williams R (2002) Urothelium derived inhibitory factor and cross-talk among receptors in the trigone of the bladder of the pig. J Urol 167:742–745
Teng J, Wang ZY, Jarrard DF, Bjorling DE (2008) Roles of estrogen receptor alpha and beta in modulating urothelial cell proliferation. Endocr Relat Cancer 15:351–364
Thomas JC, DeMarco RT, Pope JC (2005) Molecular biology of ureteral bud and trigonal development. Curr Urol Rep 6:146–151
Thumbikat P, Berry RE, Schaeffer AJ, Klumpp DJ (2009) Differentiation-induced uroplakin III expression promotes urothelial cell death in response to uropathogenic *E. coli*. Microbes Infect 11:57–65
Tiwari A, Naruganahalli KS (2006) Current and emerging investigational medical therapies for the treatment of overactive bladder. Expert Opin Investig Drugs 15:1017–1037
Toh KL, Ng CK (2006) Urodynamic studies in the evaluation of young men presenting with lower urinary tract symptoms. Int J Urol 13:521–523
Tominaga M, Wada M, Masu M (2001) Potentiation of capsaicin receptor activity by metabotropic ATP receptors as a possible mechanism for ATP-evoked hyperalgesia. Proc Natl Acad Sci 98:6951–6956
Tyagi P, Thomas CA, Yoshimura N, Chancellor MB (2009) Investigations into the presence of functional beta1, beta2 and beta3-adrenoceptors in urothelium and detrusor of human bladder. Int Braz J Urol 35:76–83
van de Merwe J (2007) Interstitial cystitis and systemic autoimmune diseases. Nat Clin Pract Urol 4:8
Vastag B (2006) Monoclonals expand into neural disorders. Nat Biotechnol 24:595–596
Vizzard MA (2000) Changes in urinary bladder neurotrophic factor mRNA and NGF protein following urinary bladder dysfunction. Exp Neurol 161:273–284

Walczak JS, Price TJ, Cervero F (2009) Cannabinoid CB2 receptors are expressed in the mouse urinary bladder and their activation modulates afferent bladder activity. Neuroscience 159:1154–1163

Wang ECY, Lee JM, Johnson JP, Kleyman TR, Bridges R, Apodaca G (2003) Hydrostatic pressure-regulated ion transport in bladder uroepithelium. Am J Physiol 285:F651–F663

Wang EC, Lee JM, Ruiz WG, Balestreire EM, von Bodungen M, Barrick SR, Cockayne DA, Birder LA, Apodaca G (2005) ATP and purinergic receptor-dependent membrane traffic in bladder umbrella cells. J Clin Invest 115:2412–2422

Wang ZY, Wang P, Merriam FV, Bjorling DE (2008) Lack of TRPV1 inhibits cystitis-induced increased mechanical sensitivity in mice. Pain 139:158–167

Wiseman OJ, Brady CM, Hussain IF, Dasgupta P, Watt H, Fowler CJ (2002) The ultrastructure of bladder lamina propria nerves in healthy subjects and patients with detrusor hyperreflexia. J Urol 168:2040–2045

Yamaguchi O, Chapple CR (2007) Beta3-adrenoceptors in urinary bladder. Neurourol Urodyn 26:752–756

Yassin A, Saad F, Hoesl CE, Traish AM, Hammadeh M, Shabsigh R (2006) Alpha-adrenoceptors are a common denominator in the pathophysiology of erectile function and BPH/LUTS–implications for clinical practice. Andrologia 38:1–12

Yu W, Zarharia LC, Jackson EK, Apodaca G (2006) Adenosine receptor expression and function in bladder uroepithelium. Am J Physiol 291:C254–C265

Yu W, Khandelwal P, Apodaca G (2009) Distinct apical and basolateral membrane requirements for stretch-induced membrane traffic at the apical surface of bladder umbrella cells. Mol Biol Cell 20:282–295

Yuridullah R, Corrow KA, Malley SE, Vizzard MA (2006) Expression of fractalkine and fractalkine receptor in urinary bladder after cyclophosphamide (CYP)-induced cystitis. Auton Neurosci 126:380–389

Zarghooni S, Wunsch J, Bodenbenner M, Bruggmann D, Grando SA, Schwantes U, Wess J, Kummer W, Lips KS (2007) Expression of muscarinic and nicotinic acetylcholine receptors in the mouse urothelium. Life Sci 80:2308–2313

Zhu Q, Ritchie J, Marouf N, Dion SB, Resnick NM, Elbadawi A, Kuchel GA (2001) Role of ovarian hormones in the pathogenesis of impaired detrusor contractility: evidence in ovariectomized rodents. J Urol 166:1136–1141

Interstitial Cells of Cajal in the Urinary Tract

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Contents

Abstract The study of novel interstitial cells in the tissues of the urinary tract has defined advances in the field in the last decade. These intriguing cells belong to the same family as the better known interstitial cells of Cajal (ICC) of the gastrointestinal tract, and their discovery has been interpreted to suggest that pacemaker cells may be present in the urinary tract, driving the spontaneous or myogenic activity of the neighboring smooth muscle. This scenario may be true for the urethra where ICC have been described as "loose pacemakers" providing multiple, random inputs to modulate urethral smooth muscle activity. However, there is a paucity of direct evidence available to support this hypothesis in the bladder (where the smooth

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muscle cells are spontaneously active) or the renal pelvis (where atypical smooth muscle cells are the pacemakers), and it now seems more likely that urinary tract ICC act as modulators of smooth muscle activity.

Interestingly, the literature suggests that the role of urinary tract ICC may be more apparent in pathophysiological conditions such as the overactive bladder. Several reports have indicated that the numbers of ICC present in overactive bladder tissues are greater than those from normal tissues; moreover, the contractility of tissues from overactive bladders in vitro appears to be more sensitive to the Kit antagonist, glivec, than those from normal bladder. Future research on urinary tract ICC in the short to medium term is likely to be dynamic and exciting and will lead to increasing our understanding of the roles of these cells in both normal and dysfunctional bladder.

Keywords c-Kit · Interstitial cells of Cajal · Smooth muscle · Ureter · Urethra · Urinary bladder

The study of interstitial cells of Cajal (ICC) in the gastrointestinal tract has revolutionized the way that researchers understand gut motility and neurotransmission. ICC were discovered by the Spanish neuroanatomist, Ramon and Cajal, one century ago (Cajal 1911) and are now known to be a specialized class of cells who act as pacemakers, driving peristaltic activity throughout the gut and who play a key role in the transmission of signals from nerves to smooth muscle (Sanders 1996; Horowitz et al. 1999). Outside the gastrointestinal field, ICC have been most widely studied in tissues of the urinary tract with numerous independent laboratories publishing research which points to the seemingly ubiquitous presence of ICC in the urinary tract. The discovery of cells with morphological and physiological properties of ICC in renal pelvis, ureter, bladder, and urethra not only provides new opportunities to advance our knowledge of cellular interactions within these tissues but is also of significant clinical impact in the development of new therapies to treat urinary tract disorders.

1 ICC in the Urinary Tract

The established role of ICC as pacemakers in the gut naturally led to the suspicion that other smooth muscle preparations with properties of spontaneous activity might contain similar cells. While gut ICC provide a useful point of reference, it is unwise to consider that ICC may play similar roles in other tissues in the absence of direct experimental evidence. The urinary bladder has long been shown to display myogenic, low-level, nonvoiding, “background” spontaneous contractions which are thought to underpin bladder tone and shape during filling (Turner and

Brading 1997). In vitro tension recordings from renal pelvis preparations showed rhythmic activity in the smooth muscle, implying the existence of a pacemaker mechanism (Lang et al. 1998), although activity in the ureter was less frequently encountered. The ability of urethral strips to develop tone in similar experiments also hinted at a specialized means of modulating contractility of the urethral smooth muscle (Brading 1999).

Transmission electron microscopy (TEM) has been the gold-standard tool for identifying ICC, and there are accepted ultrastructural criteria that a candidate cell must satisfy before being termed an ICC (Komuro 1999; Komuro et al. 1999). While there is some ultrastructural heterogeneity between ICC subtypes in the gut, ICC typically have thin (5 nm) and intermediate filaments (10 nm), abundant mitochondria, caveolae, rough and smooth endoplasmic reticulum, Golgi complexes, a basal lamina which may be discontinuous but do not tend to have thick filaments (15 nm), dense bodies or dense bands which are characteristic of smooth muscle cells (SMC). In TEM, an electron beam is passed through ultrathin sections of tissue, typically 70 nm thickness which enables investigation of the ultrastructure of the cells present within the section at high magnifications (up to 80,000×). The advantage of TEM is that one can establish that ICC are present within the tissue of interest, and with meticulous (and time consuming) serial sectioning, it is possible to study interactions between the cell of interest and neighboring cells. Confocal fluorescent microscopy is perhaps the method of choice to image interactions between cells as this allows the acquisition of optical sections from a "thicker" specimen, up to a volume of 50 μm. Reconstruction of the optical sections in three-dimensions (3D) enables one to analyze the 3D arrangement of a cellular network, and if the sample has been labeled with antibodies and fluorophores of different wavelengths to label, e.g., ICC and nerves, it is possible to visualize relationships between several cell populations. Both of these techniques have been successfully exploited in the study of ICC in the urinary tract and have provided morphological evidence that specialized cells are indeed present.

2 Nomenclature

There has been much debate on the correct nomenclature that should be adopted when describing these novel cells in tissues outside of the gastrointestinal tract, and the literature contains many papers which refers to them as: interstitial cells (IC); ICC; ICC-like cells or myofibroblasts. This issue was debated at the "Vth International Symposium on ICC" held in Ireland, July 2007 and a consensus was reached that these cells should be termed "ICC." This terminology describes a *family* of cells found in many disparate tissues including bladder, urethra, ureters, renal pelvis, other genitourinary tissues and blood vessels which possess morphological, ultrastructural, and physiological properties of ICC while not implying that physiological functions of gut ICC would be universal. This consensus was timely and served

to consolidate the work of many groups working on similar cells in many smooth muscle preparations. It should be noted, however, that this debate is ongoing and as knowledge in the field continues to advance, there may be changes to this nomenclature in the future. In this chapter, the term ICC will be used, and no disrespect is intended to the original authors who may have chosen an alternative description.

3 Urinary Bladder

3.1 Location and Morphology of Bladder ICC

The traditional view of the bladder considered the organ to be comprised of mucosa, including urothelium and lamina propria, and underlying muscularis of the detrusor, containing smooth muscle. A rich microvasculature was known to be in the lamina propria, along with sensory nerves and connective tissue, and the detrusor's rich innervation was widely demonstrated with many species exhibiting intramural ganglia. Bladder filling and emptying is well described in text books in terms of nervous control and smooth muscle contractility; however, the field has moved considerably in the last decade with the discovery that previously unknown cell types are also located throughout the bladder wall.

A study of the targets of cGMP signaling in guinea-pig and human bladder (Smet et al. 1996, later confirmed by Gillespie et al. 2004), after nitric oxide stimulation, first indicated that the bladder contained cells which were reminiscent of gut ICC by their morphological appearance (i.e., the cells had lateral processes or branches). The same study also showed a population of cells which were immunopositive for the intermediate filament, vimentin, which is typically found in ICC and other cells of mesenchymal origin, but not smooth muscle, leading the authors to speculate that the bladder may contain cells resembling ICC. Although not a selective ICC marker, vimentin antibodies provide a helpful means of visualizing cell types which may include ICC, within a tissue preparation without labeling SMC. McCloskey and Gurney (2002) later used antibodies to the established ICC marker, *c-Kit* to demonstrate that the guinea-pig bladder did indeed contain ICC, and this work has since been confirmed by several independent laboratories (Hashitani et al. 2004; Biers et al. 2006; Shafik et al. 2004; Piaseczna Piotrowska et al. 2004; Roosen et al. 2009). *c-Kit* is a proto-oncogene that encodes the tyrosine kinase receptor, *Kit* which is expressed by ICC and mast cells but not SMC or fibroblasts (Maeda et al. 1992). The discovery that gastrointestinal ICC could be labeled with anti-*c-Kit* was a milestone for ICC research, providing a reliable tool for identifying ICC in smooth muscle tissues with light microscopy, moreover, presenting the opportunity to manipulate the function of ICC in tissue preparations or animal models using pharmacological tools or neutralizing Kit antibodies (Sanders et al. 2002; Sanders and Ward 2007).

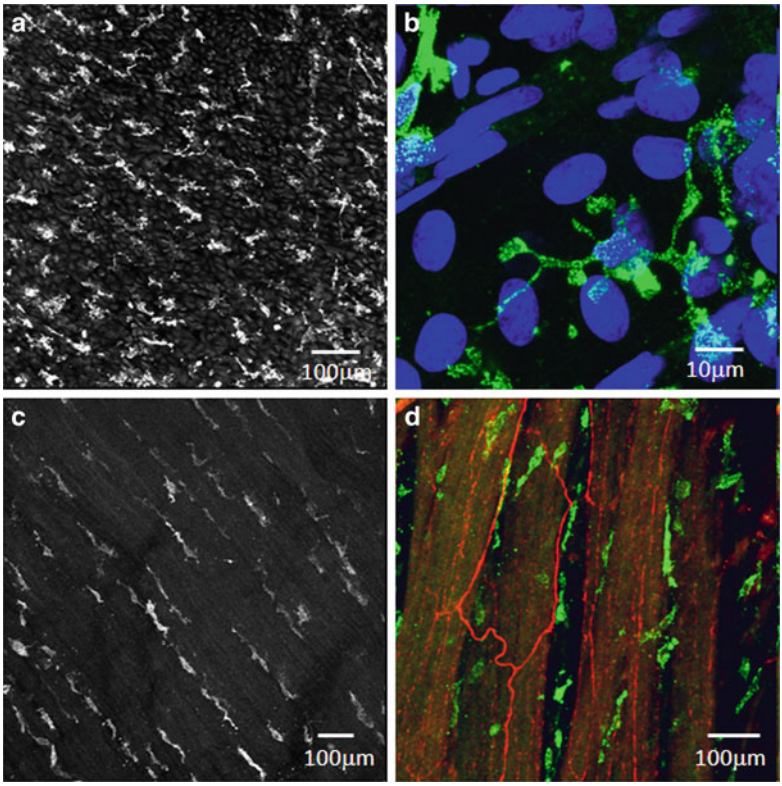

Fig. 1 Kit-positive ICC in guinea-pig and mouse bladder. ICC labeled with anti-*c-Kit* in guinea-pig bladder lamina propria at low magnification (**a**) and at higher magnification (**b**) where nuclei have been counterstained with DAPI (*blue*). Mouse detrusor Kit-positive ICC are elongated, branched cells orientated in parallel with the muscularis (**c**). Guinea-pig (**d**) detrusor ICC (*green*) are associated with nerves (*red*) and have similar arrangement to those in mouse detrusor. Images courtesy of Dr RA Davidson and Dr KD McCloskey

3.1.1 ICC in the Lamina Propria

A population of ICC has been identified with *c-Kit* and vimentin antibodies in the lamina propria region (ICC-LP) between the urothelium and the detrusor muscularis (Sui et al. 2002; Davidson and McCloskey 2005; see Figs. 1 and 2). These ICC-LP have a stellate-shaped morphology with several branches emanating from a central cell body (Davidson and McCloskey 2005) and make connections with neighboring ICC-LP to form an interconnected network. Immunohistochemistry and TEM have shown that this network is connected by connexin 43 gap junctions (Sui et al. 2002; Wiseman et al. 2003). The ICC-LP network is closely associated with mucosal nerves as shown by confocal imaging where Kit-positive cells made contacts with anti-PGP9.5 labeled nerves

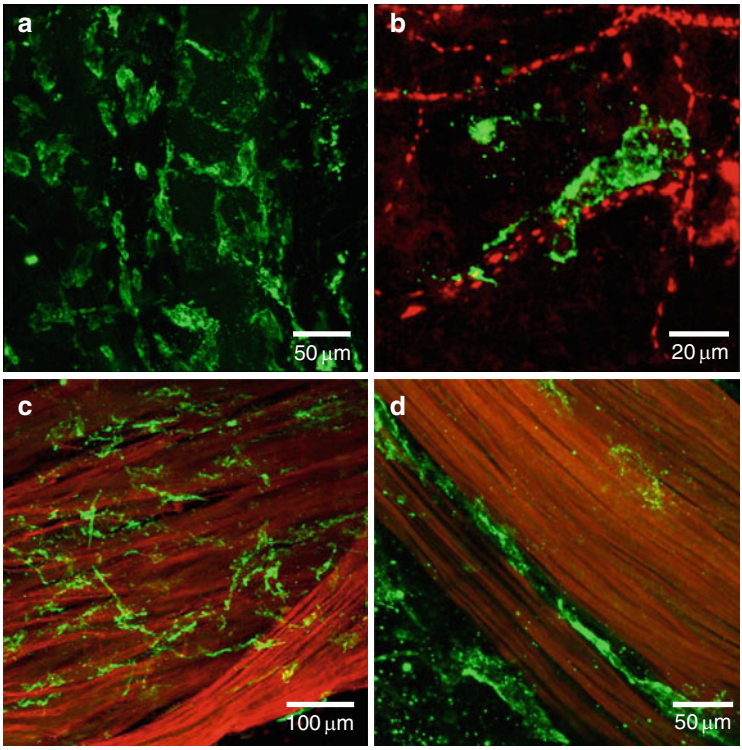

Fig. 2 Kit-positive ICC in human bladder. ICC labeled with anti-*c-Kit* (*green*) in human bladder lamina propria (**a**) forming a loose network between urothelium and detrusor and making connections with cholinergic nerves (*red*), labeled with antivesicular acetylcholine transferase. Kit-positive ICC in the human detrusor (**c**, **d**) have an elongated, branched morphology and are associated with smooth muscle bundles (*red*). Images courtesy of Dr L Johnston and Dr KD McCloskey

(Davidson and McCloskey 2005) and by TEM which demonstrated close contacts between ICC-LP and nerve endings (Wiseman et al. 2003). Recent work has shown that human bladder ICC-LP make frequent structural associations with a mucosal cholinergic plexus (Johnston et al. 2008; submitted), consistent with the finding that ICC-LP express M2 and M3 muscarinic receptors (Mukerji et al. 2006; Grol et al. 2009). de Jongh et al. (2007, 2009) suggested that cells resembling lCC-LP were immunopositive for cyclooxygenase 1; furthermore, Ost et al. (2002) reported vanilloid receptor immunoreactivity giving further insight into the pharmacological profile of these cells. The finding that purinergic receptors including P2X3, P2Y2, and P2Y4 but predominantly P2Y6 are expressed on ICC-LP (Sui et al. 2006) is particularly interesting as there is now substantial functional evidence for physiological responses to ATP in this cell type (see below).

3.1.2 ICC and the Bladder Microvasculature

A current study has demonstrated a new class of bladder Kit-positive cell, associated with blood vessels in the lamina propria of human bladder (Johnston et al. 2010). These cells are located on the outer surface of the microvessels with the branches of individual cells contacting up to six vascular SMC and may represent pericytes. Cells resembling ICC, associated with vascular smooth muscle, have been reported in guinea-pig gall-bladder (Lavoie et al. 2007) and may represent a local control of perfusion within the tissue in response to metabolic needs. The vascular perfusion of the bladder wall is a key determinant of normal bladder contractility as in vivo ischemia in animal models has been shown to induce bladder overactivity (Azadzoi et al. 1999). Further work is needed to determine whether Kit-positive cells on the bladder microvessels have any physiological role in the regulation of bladder blood flow.

3.1.3 ICC in the Detrusor

The arrangement of ICC in the detrusor region of guinea-pig, mouse, and human bladder is distinctively different from that of the mucosa. Confocal imaging of detrusor whole-mount, flat-sheet preparations, and subsequent 3D reconstruction has revealed that Kit-positive ICC are located on the boundary of detrusor smooth muscle bundles apparently tracking them (McCloskey and Gurney 2002; Hashitani et al. 2004; Davidson and McCloskey 2005; McCloskey et al. 2009; Johnston et al. 2010). These ICC have a distinctive elongated morphology with several lateral branches and appear to be placed as discrete cells with little evidence that they form complex networks (see Figs. 1 and 2). They have previously been termed "intramuscular ICC" (ICC-IM; Brading and McCloskey 2005) and have also been reported to be present within the smooth muscle bundles (Hashitani et al. 2004). These ICC-IM are associated with detrusor nerves as shown in double-labeling experiments with anti-*c-Kit* and the general neuronal marker, anti-PGP9.5 (Davidson and McCloskey 2005) and cholinergic nerves in particular with anti-vAChT (vesicular acetylcholine transferase, Johnston et al. 2008). Like the ICC-LP, Kit-positive ICC-IM also contain vimentin filaments (Davidson and McCloskey 2005).

Ultrastructurally, ICC-IM are very similar to gut ICC as shown by Kubota et al. (2008), Rasmussen et al. (2009) and Cunningham et al. (2009). These elongated, branched cells on the boundary of smooth muscle bundles have a basal lamina (or membrane dense bands), extensive rough and smooth endoplasmic reticulum, Golgi complexes, caveolae, mitochondria, thin and intermediate filaments, and a centrally placed nucleus. They are distinct from SMC by the absence of thick filaments and dense bodies and differ from fibroblasts by the absence of dilated rough endoplasmic reticulum which is a defining characteristic of fibroblasts and the presence of a basal lamina. Two studies reported the interesting finding that detrusor ICC contained vesicles or coated pits, perhaps indicative of a secretory function (Rasmussen et al. 2009; Cunningham et al. 2009).

A further ICC subtype is also present in the detrusor which has a stellate morphology and more closely resembles the ICC-LP (Davidson and McCloskey 2005). These so-called ICC-IB (interbundle ICC; Brading and McCloskey 2005) are *c-Kit*- and vimentin-positive and make connections with each other in the spaces between the detrusor smooth muscle bundles. The TEM work of Rasmussen et al. (2009) demonstrated detrusor ICC–ICC contacts via gap junctions and peg and socket junctions, supporting the existence of interconnected ICC occupying the space between the smooth muscle bundles.

Davidson and McCloskey (2005) proposed that bladder ICC could form a conduit for the relay of information from urothelium to detrusor, incorporating ICC-LP, ICC-IB, and ICC-IM. This view has been shared by others and is consistent with findings from studies of bladder ICC from mice, guinea-pigs, and humans. The morphological evidence suggests that ICC-LP form a network below the urothelium which presumably could respond to chemical transmitters released by urothelial cells, communicate with mucosal nerves, and/or relay information directly to underlying detrusor ICC and/or smooth muscle. Alternatively, ICC-LP could act as stretch-sensors, as proposed by Sui et al. (2004) capable of sensing bladder fullness and relaying information to mucosal sensory afferents.

3.2 Physiological Properties of Bladder ICC

The study of bladder ICC with traditional physiological techniques has been both intriguing and productive, generating a significant body of literature in less than a decade. Several laboratories have used the patch-clamp technique and real-time fluorescent Ca^{2+}-imaging to characterize the physiological properties of bladder ICC, and the overall picture is rather different from urethral ICC or their counterparts in the gastrointestinal tract. Furthermore, ICC-LP and detrusor ICC have been shown to have idiosyncratic differences which may hint at their uniquely different roles in bladder function.

3.2.1 ICC-LP Physiological Properties

Patch-clamp studies of enzymatically dispersed ICC-LP have shown the presence of voltage-dependent Ca^{2+} currents and TEA-sensitive K^+ currents (Sui et al. 2004). Wu et al. (2004) reported spontaneous transient inward currents (STICs) in 45% of cells tested, which reversed close to the chloride equilibrium potential, were associated with increases in intracellular Ca^{2+}-concentration $[Ca^{2+}]_i$ and were reduced by the Cl^- channel blocker, DIDS. Inward currents, generated in response to ATP application (Sui et al. 2004; Wu et al. 2004), had a similar profile, indicative of a Ca^{2+}-activated Cl^- current. The ATP-generated conductance was attenuated by capsaicin (Sui et al. 2008) in keeping with reports that ICC-LP possess vanilloid or TRPV1 receptors (Ost et al. 2002). The original authors recently questioned the reliability of

the TRPV1 antibody as nonspecific cellular TRPV1-immunoreactivity was observed in bladders from TRPV1 knockout mice (Everaerts et al. 2009); however, further work with more selective antibodies or molecular techniques should clarify this issue. ICC-LP also fired Ca^{2+}-activated Cl^- currents and Ca^{2+}-transients in response to reduction of extracellular pH (Sui et al. 2008) which were similar to those evoked by ATP application.

The mean resting membrane potential (RMP) of ICC-LP from current-clamp studies was found to be around −60 mV (Sui et al. 2004; Wu et al. 2004), and spontaneous depolarizing fluctuations were recorded, demonstrating that ICC-LP are electrically active. The RMP was shown to lie between E_K and E_{Cl} as recordings with K^+ filled pipettes gave a RMP of −60 mV, whereas recordings with Cs^+-filled pipettes gave RMPs of −30 mV. The spontaneous depolarizations were supported by Ca^{2+}-signaling studies of isolated ICC-LP which demonstrated the ability of ICC-LP to undergo spontaneous changes in $[Ca^{2+}]_i$ (Sui et al. 2004; Wu et al. 2004).

3.2.2 Detrusor ICC Physiological Properties

Detrusor ICC have also been studied with patch clamp and been shown to possess several ion channels including L-type Ca^{2+} currents, a nickel-sensitive Ca^{2+} current which was not a T-type conductance (McCloskey 2006); Ca^{2+}-activated K^+ currents (BK) and voltage-dependent K^+ currents (McCloskey 2005) including a KCNQ component (Anderson et al. 2009). The urethral pacemaker conductance, a Ca^{2+}-activated Cl^- current (Sergeant et al. 2000), similar to that found in bladder ICC-LP (see above), has not yet been clearly demonstrated in detrusor ICC, although spontaneous transient depolarizations (STDs) (of an uncharacterized ionic basis) have been recorded from detrusor ICC in current-clamp mode (Anderson et al. 2009), implying rhythmic electrical firing, consistent with pacemaker-like behavior.

Spontaneous activity was also seen in Ca^{2+}-imaging experiments from detrusor ICC which fired long-duration Ca^{2+}-transients at a rate of approximately three per minute both in isolated cells and in tissue sheets (McCloskey and Gurney 2002; Hashitani et al. 2004; Johnston et al. 2008). This pattern of spontaneous activity was clearly different from SMC which fired Ca^{2+}-transients of comparatively greater frequency and shorter duration. The relationship between ICC and SMC Ca^{2+}-signaling remains rather elusive and is not easily explained by either the views that ICC act as pacemakers or indeed the view that ICC have no meaningful role in bladder spontaneous activity. The observation that the SMC never fire at a rate less than the ICC may suggest that ICC provide a baseline input; moreover, multiple ICC may "pace" a smooth muscle bundle in response to local needs, providing a fine-control on smooth muscle myogenic activity. The situation has similarities to urethral ICC and urethral SMC (see below), where urethral ICC are considered to randomly enhance the activity of neighboring SMC (Hashitani and Suzuki 2007), rather than act as a coordinated pacemaker cellular network.

Muscarinic stimulation by application of carbachol to whole-sheet "in situ" preparations increased the frequency of detrusor ICC Ca^{2+}-transients (Johnston

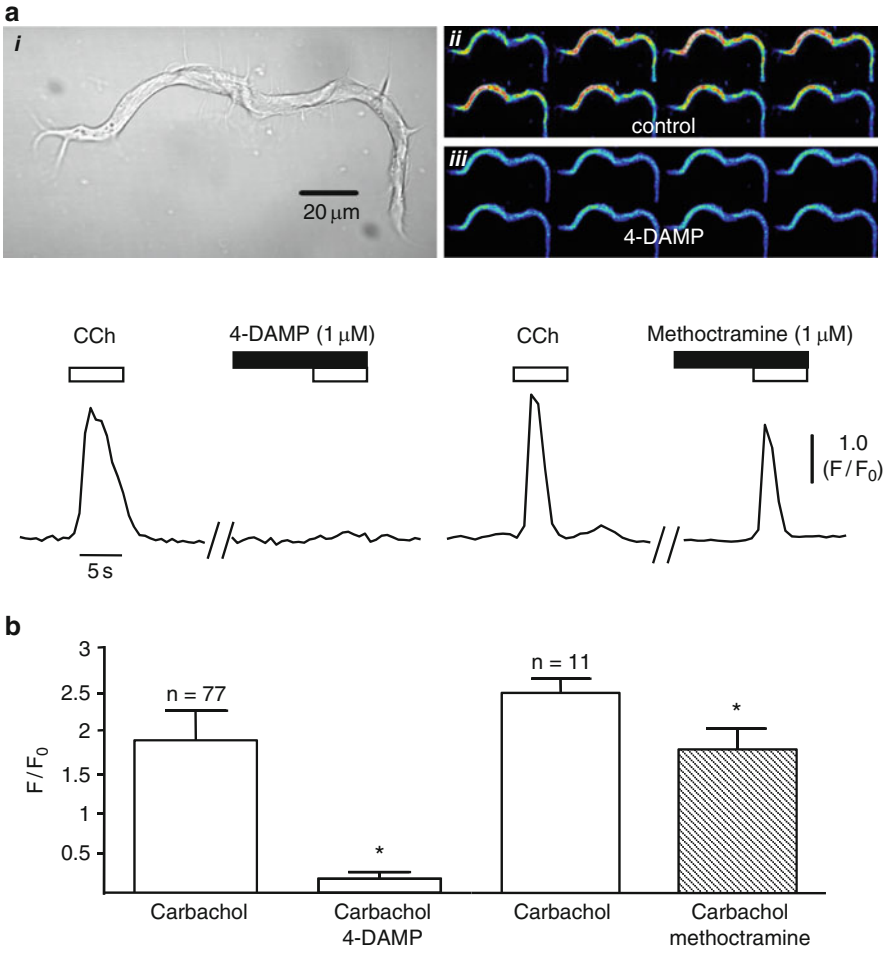

Fig. 3 Bladder detrusor ICC physiology. (**a**i) Typical morphology of bladder ICC. (**a**ii–iii) Time series micrograph showing Ca^{2+} response to 1 μM carbachol which was blocked by the M3 antagonist 4-DAMP (1 μM). Traces show intensity time series of responses to carbachol and the effects of 4-DAMP and the M2 antagonist methoctramine (1 μM). Fluorescence (F) of an event is expressed as a ratio of background (F0). *CCh* carbachol. (**b**) Graph summarizing the effect of 4-DAMP and methoctramine on 7 and 11 cells, respectively. *Asterisk* denotes statistical significance. Figure taken from Johnston et al. (2008)

et al. 2008; see Fig. 3), suggesting that detrusor ICC activity can be modulated by parasympathetic nerves. Application of carbachol to enzymatically dispersed detrusor ICC induced an intracellular Ca^{2+}-transient which was not associated with contraction, in contrast to detrusor SMC which were vigorously contractile under identical experimental conditions. The cholinergic signaling pathway in these cells has been shown to be mediated largely via M_3 muscarinic receptors and an IP_3 and ryanodine receptor-dependent release of Ca^{2+} from intracellular stores (Johnston

et al. 2008). It is interesting that while carbachol generates an increase in $[Ca^{2+}]_i$ in detrusor ICC, there is no such response in ICC-LP (Wu et al. 2004) and this difference may represent an important division of labor between two ICC subtypes in the bladder. The physiological consequence of the detrusor ICCs' Ca^{2+}-response to cholinergic stimulation has not yet been ascertained, although Johnston et al. (2008) suggested that release of transmitter substances may result, consistent with the observation of vesicles in ICC from TEM studies (Rasmussen et al. 2009; Cunningham et al. 2009). de Jongh et al. (2007) suggested that ICC may release prostaglandins and further work is necessary to determine whether detrusor ICC actually exhibit a secretory-type function.

3.3 Clinical Significance of Bladder ICC

The morphological and physiological studies of the various subtypes of bladder ICC indicate that these are novel cells, with many properties of classical gut ICC which are ideal candidates to contribute to normal bladder function. However, current research does not adequately resolve the issue of what the actual roles of ICC are in bladder filling and emptying. When these cells were first discovered, several groups considered that ICC could fulfill a pacemaking role, responsible for the origin and propagation of spontaneous activity in the bladder wall during filling. While both ICC-LP and detrusor ICC have been shown to exhibit spontaneous electrical and Ca^{2+}-signaling, a profile consistent with a pacemaking phenotype, direct evidence that this signaling acts to modulate the activity of detrusor smooth muscle has not yet been published.

Pharmacological and animal model-based experimental approaches have been used in the study of ICC function in bladder. The fact that ICC express the tyrosine kinase receptor, Kit, has been exploited using the drug imatinib mesylate (Glivec) to block the Kit receptor in in vitro and in vivo studies. Glivec is used clinically in the treatment of chronic myeloid leukemia and gastrointestinal stromal tumors and targets the tyrosine kinases PDGF receptor, bcr–abl, and c-Kit. Biers et al. (2006) found Glivec to improve capacity, compliance, urinary frequency and to reduce spontaneous activity in cystometric studies in guinea-pigs. This was consistent with the finding that Glivec reduced spontaneous electrical and mechanical activity in isolated guinea-pig detrusor tissues (Kubota et al. 2004, 2006). The study of a mutant mouse strain which had been pivotal in revealing the role of ICC in the gut (Sanders et al. 1999, 2006; Sanders and Ward 2007) was less promising for bladder ICC research. The *W*/*Wv* mouse has defects in the W locus which encodes the Kit receptor and does not contain several populations of gut ICC; however, detrusor ICC were apparently little affected by the mutation and were present in comparable numbers and localization to bladders from wild-type animals (McCloskey et al. 2009).

The activity and presence of ICC seems to be less prominent in normal, healthy bladders compared with pathological conditions, and studies of "abnormal" bladder have enhanced our understanding of the implications of ICC in a clinical setting.

Biers et al. (2006) demonstrated increased numbers of Kit-positive ICC in overactive human bladder samples compared with normal tissues and interestingly demonstrated a greater inhibitory effect of Glivec on detrusor contractions in samples from overactive bladder. Kubota et al. (2008) reported an increase in the population of ICC in guinea-pig bladder after outlet obstruction. Increased expression of connexin 43 in the bladder lamina propria of rats after spinal cord transection (SCT) was associated with increased coordination of spontaneous activity compared with normal adult rats (Ikeda et al. 2007). Moreover, Glivec was found to have a marked inhibitory effect on the enhanced spontaneous contractions of whole bladders from spinal cord transected rats, whereas there was little effect on control bladders (Sui et al. 2008). Roosen et al. (2009) found increased lamina propria connexin 43-immunostaining in human overactive bladder but noted little change in *c-Kit* expression perhaps indicating that gap junctions numbers were upregulated rather than the actual numbers of ICC. Piaseczna Piotrowska et al. (2004) compared the presence of ICC in normal bladders and samples from patients with megacystis-microcolon intestinal hypoperistalsis syndrome and demonstrated marked lack of ICC in the MMIHS sample set. This is particularly interesting as the MMIHS bladder is distended, unobstructed, and dysfunctional and supports the finding of increased numbers of ICC in obstructed, overactive bladders.

The literature currently seems to support a more prominent role for ICC in diseased or abnormal bladders, largely explained by an increase in their populations and/or the gap junctions connecting the network. The question remains for the normal bladder, do ICC simply act as bystanders, capable of fine-tuning and regulating SMC activity in response to the needs of the tissue or do they have a primary role as communicators, sensing and relaying information between the complex system of heterogeneous cells (SMC, ICC, nerves, microvessels, and urothelial cells) that make up the bladder wall? The existing body of evidence points to multiple roles for ICC in the bladder, dependent on their location, structural connections with neighboring cells, expression of membrane receptors, and ion channels and appears to be tightly controlled by the physiological/pathophysiological state of the organ. This area of research is dynamic and exciting and is contributing to many areas of inquiry. For example, the multiple and complex functions of the urothelium is a rapidly progressing field in which ICC are clearly involved. The areas of painful bladder syndrome (PBS) and interstitial cystitis are also likely to reveal ICC participation. The work of Mukerji et al. (2006) correlated ICC and M2/M3 receptors to urgency scores in patients with PBS and idiopathic detrusor overactivity. Furthermore, we do not yet know the fate of ICC in diabetic or age-related lower urinary tract symptoms.

Further research should be directed to address the current gaps in our knowledge of bladder ICC with the full complement of techniques available at the level of the gene, protein, cell, tissue, organ, animal model, and translational research in patients. This area has attracted attention from many reputable research groups but remains largely unexploited. Given that present therapies for the treatment of urgency and many of the types of incontinence are effective in only a subset of patients, ICC may present novel opportunities for the development of better treatments.

4 Urethra

The study of urethral ICC is arguably more advanced than our knowledge of ICC in other tissues of the urinary tract, particularly in terms of cellular physiology. Smet et al. (1996) first demonstrated cells morphologically resembling ICC in the guinea-pig and human urethra using cGMP immunohistochemistry. A study of the electrical activity of rabbit urethral smooth muscle with intracellular microelectrode recordings demonstrated STDs which were reminiscent of gut slow waves, normally generated by ICC (Callahan and Creed, 1981; Hashitani et al. 1996). However, the first direct evidence that the urethra contained ICC-like cells was reported by Sergeant et al. (2000), who observed a mixed population of enzymatically dispersed cells from the rabbit urethra muscularis including majority spindle-shaped SMC and a smaller population of branched stellate-shaped cells and elongated cells with lateral branches, both of which were morphologically reminiscent of gut ICC.

4.1 Location and Morphology of ICC in the Urethral Wall

Sergeant et al. (2000) used vimentin immunohistochemistry to distinguish the branched cells from the SMC which contained myosin filaments but not vimentin. In addition, they demonstrated with TEM that the cell dispersal contained branched cells with the defining ultrastructural characteristics of ICC, i.e., abundant mitochondria, intermediate filaments, Golgi complexes, rough and smooth endoplasmic reticulum, caveolae, and a basal lamina. These ICC were clearly distinct from the SMC ultrastructural phenotype, adding support to the hypothesis that the urethra contained specialized ICC. The ICC identified in enzymatic cell dispersals were derived from the muscularis layers and this work was later advanced by Lyons et al. (2007) who carried out a morphological characterization of ICC in whole-mount preparations of rabbit urethra with confocal microscopy (see Fig. 4). Immunohistochemical labeling with anti-*c-Kit* and anti-vimentin showed that ICC were located within the circular and longitudinal layers of the muscularis and were arranged in parallel with the SMC. Moreover, reconstruction of optical sections demonstrated that ICC were in close proximity to the SMC, consistent with the idea that urethral ICC may have a pacemaker type role. The morphological profiles of the Kit-positive cells described by Lyons et al. (2007) were consistent with those found in the cell dispersals by Sergeant and clearly comprised several subtypes; unipolar, bipolar, stellate, and elongated with several lateral branches. Similar to bladder, several subpopulations of ICC have been reported in the urethral wall; lamina propria ICC, ICC in the muscularis, and ICC associated with the serosa (García-Pascual et al. 2008).

Investigation of the relationships between intramural nerves and ICC in the urethra in double-labeling experiments with anti-*c-Kit* and antineurofilament (or anti-PGP 9.5) showed a close association between ICC and nerves within the

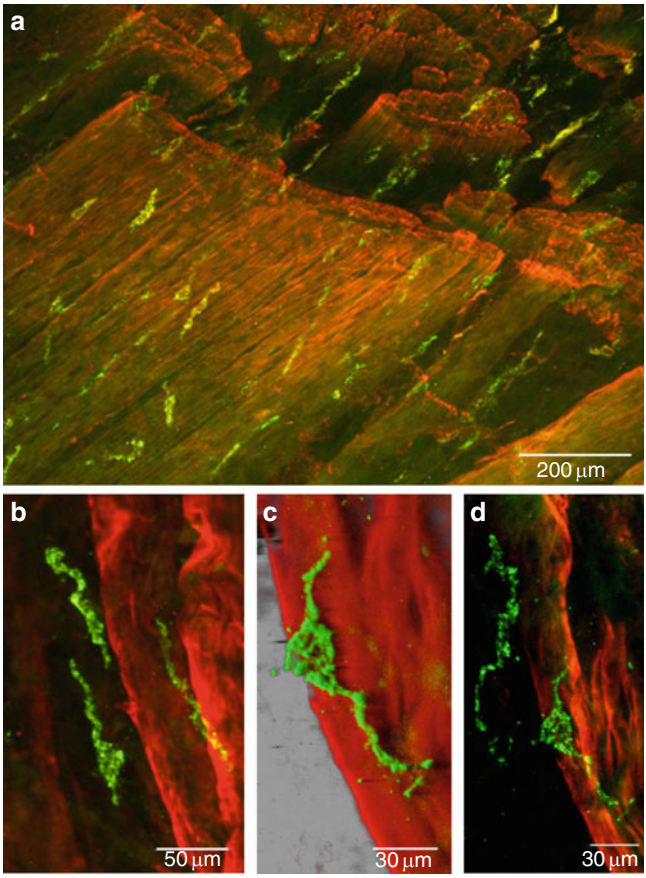

Fig. 4 Kit-positive ICC in the rabbit urethra. ICC in rabbit urethra labeled with anti-*c-Kit* (*green*). Smooth muscle is labeled with antismooth muscle myosin (*red*). Urethral ICC are located on the both the edge of and between the smooth muscle bundles, running in parallel with the bundle orientation. Figure from Lyons et al. (2007)

muscularis layer. This suggested that the activity of ICC could be controlled by neurotransmitters released by adjacent urethral nerves perhaps providing a means of pacemaker regulation. More specifically, close relationships have been reported between urethral ICC and nitergic nerves labeled with antinitric oxide synthase (Lyons et al. 2007; García-Pascual et al. 2008).

4.2 Physiological Properties of Urethral ICC

Investigation of urethral ICC with patch-clamp electrophysiology and fluorescent Ca^{2+}-imaging has established that these cells possess properties expected of

pacemaker cells. The initial study of Sergeant et al. (2000) demonstrated that non-contractile cells morphologically resembling ICC fired STDs, larger slow waves and STICs. The ionic basis of the pacemaking conductance was a depolarizing Ca^{2+}-activated Cl^- current which had previously been found in sheep urethral SMC (Cotton et al. 1997; Sergeant et al. 2001) and which depolarized the RMP until L-type Ca^{2+}-currents were activated, carrying the "upstroke" of the slow wave. The finding that in rabbit urethra, the pacemaking current was exclusively found in ICC and notably absent in SMC attributed a pacemaking function to rabbit urethral ICC. This work defined the earlier findings of Hashitani et al. (1996) at the cellular level who had demonstrated Cl^--dependent STDs in rabbit urethral tissue preparations.

The pacemaker current was found to be regulated by exogenously applied noradrenaline via α1 adrenergic receptors or ATP mediated by purinergic P_{2Y} receptors, as demonstrated by an increase in the frequency of firing (Sergeant et al. 2002, 2009), consistent with the morphological studies which showed structural relationships between ICC and nerves. The source of Ca^{2+} to activate the current was shown to be largely via release from the IP_3 sensitive intracellular Ca^{2+}-stores (Sergeant et al. 2001). Rhythmic Ca^{2+}-waves were initiated by ryanodine-mediated release of Ca^{2+} and wave propagation was controlled by the IP_3-sensitive stores and also found to be highly sensitive to the external Ca^{2+} concentration (Johnston et al. 2005). These Ca^{2+} events are the primary signal which activates Ca^{2+}-activated Cl^- channels, leading to depolarization of the cell membrane and subsequent opening of L-type Ca^{2+}-channels and slow wave firing. Interestingly, the frequency of spontaneous Ca^{2+}-waves was increased by noradrenaline or ATP (Sergeant et al. 2009), as suggested by the finding of adrenergic and purinergic modulation of the pacemaker conductance. Further characterization of the pacemaking mechanism has revealed the role of the membrane Na^+/Ca^{2+} exchanger in regulating the frequency of the pacemaker conductance (Bradley et al. 2006); moreover, mitochondrial buffering of $[Ca^{2+}]_i$ has also been demonstrated to regulate urethral ICC Ca^{2+}-signaling (Sergeant et al. 2008).

4.3 Functional Role of Urethral ICC

The work described above from isolated cells has been furthered by Ca^{2+}-imaging experiments from whole-sheet preparations of rabbit urethra Hashitani and Suzuki (2007). Spontaneous Ca^{2+}-signals were recorded from both ICC and SMC, which like the bladder were different in frequency and duration, with the ICC firing events at a mean rate of three per minute of longer duration than SMC events. While signals from neighboring ICC were often synchronized, there was little evidence of correlation with the SMC events. The hypothesis that urethral ICC act as pacemakers, regulating the activity of the smooth muscle was not necessarily supported; however, the authors speculated that the ICC could act as a "loose" pacemaker, providing multiple random depolarizing inputs to the smooth muscle to maintain activity at the optimum level to generate appropriate urethral tone.

The physiological characteristics of urethral ICC have been convincingly established by the McHale laboratory and clearly, these novel cells are among the most promising areas in the study of urethral function. However, much work needs to be done in order to consolidate any clinical implications of ICC in the urethra, not least the need to develop robust animal models in which the functional roles of urethral ICC can be deduced and the necessary translation of work from animal cells to human urethral ICC. The limited availability of "normal" human urethral tissue has undoubtedly been a major limiting factor; however, van der Aa et al. (2004) demonstrated kit-positive cells resembling ICC in unfixed, frozen sections of human urethra. Urethral smooth muscle tone is known to contribute to urinary continence and if this tone is indeed regulated by ICC activity, it remains to be shown whether aspects of LUTS are attributable to defects in either the quantities or function of urethral ICC.

5 Upper Urinary Tract: Renal Pelvis and Ureter

The upper urinary tract has long been known to display spontaneous peristaltic activity which propels urine from the kidneys to the bladder via the ureters. This activity is predominant in the renal pelvis and lessens along the tract to the distal ureter which is comparatively quiescent in the majority of mammalian species, with the exception of human and pig (Constantinou et al. 1978; Constantinou 1977). For several decades, it has been known that the pacemaker region, driving the spontaneous electrical and mechanical activity is located in a group of specialized cells, termed atypical smooth muscle cells (ASMC; Gosling and Dixon 1971, 1974). These cells are dominantly present in the proximal renal pelvis but absent in the ureter and are morphologically and ultrastructurally distinct from the SMC (Klemm et al. 1999). A defining feature of ASMC was the fact that 40% of their cellular sectional area contained contractile filaments, compared with the SMC which had more than 60%. ASMC possessed branched processes, formed a cellular network and were not immunopositive for *c-Kit*. The high-frequency pacemaking properties of the ASMC were elegantly demonstrated in intracellular microelectrode recordings in which the cell of interest was filled with neurobiotin so that its morphology could be visualized (Klemm et al. 1999).

5.1 Location and Morphology of ICC in the Upper Urinary Tract

Klemm et al. (1999) first described a new cell type in guinea-pig renal pelvis which was absent from ureter, morphologically resembling ICC. These cells differed from fibroblasts by the presence of numerous membrane-associated caveolae and an incomplete basal lamina. Like the ASMC, the ICC also had many processes and

formed junctional contacts with similar cells or SMC, but differed from both ASMC and SMC by their distinct lack of contractile filaments. While the initial study in guinea-pig did not find the ICC to be immunopositive for *c-kit*, others have since reported *c-Kit*-positive ICC in upper urinary tract tissues, indicating that as in bladder and urethra, detection of *c-Kit* positivity depends on the use of a panel of *c-Kit* antibodies and perhaps interspecies differences. Kit-positive ICC have been reported to be located between the smooth muscle bundles in uteropelvic junction samples (Solari et al. 2003; Lang and Klemm 2005) and in lamina propria and muscle layers in ureter from mouse (Pezzone et al. 2003; David et al. 2005), human (Metzger et al. 2004) and other mammalian species (Metzger et al. 2005, 2008). Typically, the presence of ICC is reported to decrease from proximal to distal segments.

5.2 Physiological Properties of Upper Urinary Tract ICC

Neurobiotin experiments, similar to those described above, revealed that the ICC cell population fired intermediate-type action potentials (Klemm et al. 1999) characterized by a single spike, quiescent plateau phase, and abrupt repolarization. Interestingly, ICC were found in distal regions of renal pelvis where pacemaker type activity was absent, leading the authors to speculate that ICC in the upper urinary tract were not primary pacemakers but could form a conduit for transmission of signals from the ASMC pacemakers to the SMC. Further study of the three cell types in the mouse renal pelvis examined the electrical and Ca^{2+}-signaling basis of their spontaneous activity. Again, the ASMC were found to be the main pacemakers, whereas the ICC had more of a supportive role, firing less frequent Ca^{2+}-transients, and long duration action potentials (Lang et al. 2007a and b). Spontaneous Ca^{2+} signals in ICC were sensitive to blockade of Ca^{2+} release from IP3 or ryanodine-dependent intracellular stores (Lang et al. 2010). Study of *W/Wv* transgenic mice unfortunately showed no change in ICC Ca^{2+}-signaling (Lang et al. 2009), suggesting that the renal pelvis, like the urinary bladder, is not significantly affected by the *c-Kit* mutation (McCloskey et al. 2009).

Lang et al. (2007b) studied isolated ICC from mouse UPJ under voltage-clamp and found high-frequency STICs and long-lasting large inward currents (LICs). These currents were relatively insensitive to Cl^- channel blockers and were considered to represent cationic-selective currents. This direct evidence of depolarizing spontaneous electrical activity in the ICC suggested that these cells could perhaps provide a type of pacemaking or modulatory input to adjacent smooth muscle bundles, especially if activity from ASMC was not present.

5.3 Functional Implications of ICC in the Upper Urinary Tract

As seems to be the case for bladder, the putative role of upper urinary tract ICC has been revealed in studies outside of the normal adult physiological situation.

Solari et al. (2003) noted a decrease in the density of Kit-positive ICC in obstructed human UPJ specimens. Incubation of murine ureter tissues with neutralizing Kit antibodies under tissue culture conditions not only altered ureter morphology but also disrupted peristalsis leading the authors to suggest that Kit was required for the spontaneous activity (David et al. 2005). Consistent with this, the same study investigated the embryonic development of ureter ICC and contractility and reported a correlation between the ability of isolated ureter preparations to exhibit unidirectional contractions and an upregulation of *c-Kit* expression. To date, the literature supports pacemaking in the upper urinary tract coming primarily from ASMC but also with a convincing, although less dominant input from ICC.

6 Future Perspectives for Urinary Tract ICC

The last decade has seen significant advances in our knowledge of the complexity of cells present in the tissues of the urinary tract and their morphological and physiological characteristics. Cells with properties typical of ICC are expressed in renal pelvis, ureter, urinary bladder, and the outlet urethra in many species, including human. What has become clear is that these cells have a unique arrangement in urinary tract tissues compared with the gastrointestinal tract. The extensive and dense networks of ICC typical of the pacemaking myenteric plexus regions of the GI tract have not yet been shown in any of the urinary tract tissues. Whether this is a true reflection of the actual arrangement of ICC or represents limitations in currently available detection methods remains to be seen. However, the comparatively less-dense networks of ICC in the lamina propria regions of the tissues are clearly distinct from the ICC present in the muscularis regions, which do not appear to be widely networked but are placed along the boundary of smooth muscle bundles. Another common finding in the urinary tract is that the ICC do not appear to be the main pacemaker or "driver" for the adjacent smooth muscle. This is perhaps most evident in the renal pelvis and ureter where the ASMC population have already been directly demonstrated to be the primary pacemakers. In addition, in the bladder and urethra, the smooth muscle activity appears to be myogenic in origin, with the ICC perhaps providing positive or negative signals to regulate the activity.

For those who have perhaps lost faith in the urinary tract ICC having clearly defined physiological roles, the answer may lie in the study of pathological conditions. There is good agreement, for example, that numbers of ICC and spontaneous activity are increased in obstructed bladder, and the Kit-blocking drug Glivec appears to have a more convincing effect in those tissues compared with normal controls. Similar findings in spinal cord injury bladders add support to these findings. The studies of patient samples with megacystic-microcolon intestinal hypoperistalsis are especially relevant as the dysfunctional, distended, and unobstructed bladders were correlated with a clear lack of ICC. Future work should be directed to the study of ICC in patient samples with clinically defined lower urinary tract symptoms with the specific aim of assessing the presence of ICC and their activity in each condition.

Furthermore, work on animal models will perhaps enable the field to progress more rapidly providing the opportunity to correlate urinary tract activity with the absence, presence, or increased numbers of ICC.

While there are many unknowns relating to urinary tract ICC and the field lags behind knowledge of gut ICC, the fact that urinary tract ICC persist under normal adult physiological conditions strongly suggests that they are important players in the concert type of activity from all the cells present within these complex tissues. Their roles may not be dominant in primary pacemaking as initially anticipated, yet they are unlikely to be maintained as uninvolved bystanders. To use an orchestral analogy, which is the most important instrument (or cell)? ICC may not be the applauded classical solo instruments such as violin, clarinet or flute, but even the percussionist's humble triangle has its own part to play in the most wonderful of concertos. I have no doubt that the roles of ICC in the normal and pathophysiological urinary tract will ultimately be discovered and may even surprise us.

References

Anderson UA, Carson C, McCloskey KD (2009) KCNQ currents and their contribution to resting membrane potential and the excitability of interstitial cells of Cajal from the guinea pig bladder. J Urol 182:330–336

Azadzoi KM, Tarcan T, Kozlowski R, Krane RJ, Siroky MB (1999) Overactivity and structural changes in the chronically ischemic bladder. J Urol 162:1768–1778

Biers SM, Reynard JM, Doore T, Brading AF (2006) The functional effects of a c-kit tyrosine inhibitor on guinea-pig and human detrusor. BJU Int 97(3):612–616

Brading AF (1999) The physiology of the mammalian urinary outflow tract. Exp Physiol 84(1):215–221

Brading AF, McCloskey KD (2005) Mechanisms of disease: specialized interstitial cells of the urinary tract-an assessment of current knowledge. Nat Clin Pract Urol 2:546–554

Bradley E, Hollywood MA, Johnston L, Large RJ, Matsuda T, Baba A, McHale NG, Thornbury KD, Sergeant GP (2006) Contribution of reverse Na+-Ca2+ exchange to spontaneous activity in interstitial cells of Cajal in the rabbit urethra. J Physiol 574:651–661

Cajal SR (1911) Histologie du système nerveux de l'homme et des vertébrés, vol 2. Maloine, Paris, pp 891–942

Callahan SM, Creed KE (1981) Electrical and mechanical activity of the isolated lower urinary tract of the guinea-pig. Br J Pharmacol 74:353–358

Constantinou CE, Silvert MA, Gosling J (1977) Pacemaker system in the control of ureteral peristaltic rate in the multicalyceal kidney of the pig. Invest Urol 14:440–441

Constantinou CE, Neubarth JL, Mensah-Dwumah M (1978) Frequency gradient in the autorhythmicity of the pyeloureteral pacemaker system. Experientia 34:614–615

Cotton KD, Hollywood MA, McHale NG, Thornbury KD (1997) Ca^{2+} current and Ca^{2+}-activated chloride current in isolated smooth muscle cells of the sheep urethra. J Physiol 505:121–131

Cunningham RMJ, Larkin P, McCloskey KD (2011) Ultrastructural Properties of Interstitial Cells of Cajal in the Guinea Pig Bladder. J Urol (in press)

David SG, Cebrian C, Vaughan ED Jr, Herzlinger D (2005) c-kit and ureteral peristalsis. J Urol 173:292–295

Davidson RA, McCloskey KD (2005) Morphology and localization of interstitial cells in the guinea-pig bladder: structural relationships with smooth muscle and neurons. J Urol 173: 1385–1390

Everaerts W, Sepúlveda MR, Gevaert T, Roskams T, Nilius B, De Ridder D (2009) Where is TRPV1 expressed in the bladder, do we see the real channel? Naunyn Schmiedebergs Arch Pharmacol 379:421–425

García-Pascual A, Sancho M, Costa G, Triguero D (2008) Interstitial cells of Cajal in the urethra are cGMP-mediated targets of nitrergic neurotransmission. Am J Physiol Renal Physiol 295: F971–F983

Gillespie JI, Markerink-van Ittersum M, de Vente J (2004) cGMP-generating cells in the bladder wall: identification of distinct networks of interstitial cells. BJU Int 94(7):1114–1124

Gosling JA, Dixon JS (1971) Morphologic evidence that rhe renal calyx and pelvis control ureteric activity in the rabbit. Am J Anat 130:393–408

Gosling JA, Dixon JS (1974) Species variation in the location of upper urinary tract pacemaker cells. Invest Urol 11:418–423

Grol S, Essers PB, van Koeveringe GA, Martinez-Martinez P, de Vente J, Gillespie JI (2009) M(3) muscarinic receptor expression on suburothelial interstitial cells. BJU Int 104:398–405

Hashitani H, Suzuki H (2007) Properties of spontaneous Ca^{2+} transients recorded from interstitial cells of Cajal-like cells of the rabbit urethra in situ. J Physiol 583:505–519

Hashitani H, Van Helden DF, Suzuki H (1996) Properties of spontaneous depolarizations in circular smooth muscle cells of rabbit urethra. Br J Pharmacol 118:1627–1632

Hashitani H, Yanai Y, Suzuki H (2004) Role of interstitial cells and gap junctions in the transmission of spontaneous Ca^{2+} signals in detrusor smooth muscles of the guinea-pig urinary bladder. J Physiol 559:567–581

Horowitz B, Ward SM, Sanders KM (1999) Cellular and molecular basis for electrical rhythmicity in gastrointestinal muscles. Annu Rev Physiol 61:19–43

Ikeda Y, Fry C, Hayashi F, Stolz D, Griffiths D, Kanai A (2007) Role of gap junctions in spontaneous activity of the rat bladder. Am J Physiol Renal Physiol 293:F1018–F1025

Johnston L, Sergeant GP, Hollywood MA, Thornbury KD, McHale NG (2005) Calcium oscillations in interstitial cells of the rabbit urethra. J Physiol 565:449–461

Johnston L, Carson C, Lyons AD, Davidson RA, McCloskey KD (2008) Cholinergic-induced Ca^{2+} signaling in interstitial cells of Cajal from the guinea pig bladder. Am J Physiol Renal Physiol 294:F645–F655

Johnston L, Woolsey S, O'Kane H, Duggan B, Keane P, McCloskey KD (2010) Expression of kit-positive interstitial cells of cajal in human bladder. J Urol 184:370–377

de Jongh R, van Koeveringe GA, van Kerrebroeck PE, Markerink-van Ittersum M, de Vente J, Gillespie JI (2007) The effects of exogenous prostaglandins and the identification of constitutive cyclooxygenase I and II immunoreactivity in the normal guinea pig bladder. BJU Int 100:419–429

de Jongh R, Grol S, van Koeveringe G, van Kerrebroeck P, de Vente J, Gillespie J (2009) The localisation of cyclo-oxygenase immuno-reactivity (COX I-IR) to the urothelium and to interstitial cells in the bladder wall. J Cell Mol Med 13:3069–3081

Klemm MF, Exintaris B, Lang RJ (1999) Identification of the cells underlying pacemaker activity in the guinea-pig upper urinary tract. J Physiol 519:867–884

Komuro T (1999) Comparative morphology of interstitial cells of Cajal: ultrastructural characterization. Microsc Res Tech 47(4):267–285

Komuro T, Seki K, Horiguchi K (1999) Ultrastructural characterization of the interstitial cells of Cajal. Arch Histol Cytol 62:295–316

Kubota Y, Kajioka S, Biers SM, Yokota E, Kohri K, Brading AF (2004) Investigation of the effect of the c-kit inhibitor Glivec on isolated guinea-pig detrusor preparations. Auton Neurosci 30 (115):64–73

Kubota Y, Biers SM, Kohri K, Brading AF (2006) Effects of imatinib mesylate (Glivec) as a c-kit tyrosine kinase inhibitor in the guinea-pig urinary bladder. Neurourol Urodyn 25:205–210

Kubota Y, Hashitani H, Shirasawa N, Kojima Y, Sasaki S, Mabuchi Y, Soji T, Suzuki H, Kohri K (2008) Altered distribution of interstitial cells in the guinea pig bladder following bladder outlet obstruction. Neurourol Urodyn 27:330–340

Lang RJ, Exintaris B, Teele ME, Harvey J, Klemm MF (1998) Electrical basis of peristalsis in the mammalian upper urinary tract. Clin Exp Pharmacol Physiol 25(5):310–321

Lang RJ, Klemm MF (2005) Interstitial cell of Cajal-like cells in the upper urinary tract. J Cell Mol Med 9:543–556

Lang RJ, Hashitani H, Tonta MA, Parkington HC, Suzuki H (2007a) Spontaneous electrical and Ca^{2+} signals in typical and atypical smooth muscle cells and interstitial cell of Cajal-like cells of mouse renal pelvis. J Physiol 583:1049–1068

Lang RJ, Zoltkowski BZ, Hammer JM, Meeker WF, Wendt I (2007b) Electrical characterization of interstitial cells of Cajal-like cells and smooth muscle cells isolated from the mouse ureteropelvic junction. J Urol 177:1573–1580

Lang RJ, Hashitani H, Tonta MA, Bourke JL, Parkington HC, Suzuki H (2010) Spontaneous electrical and Ca^{2+} signals in the mouse renal pelvis that drive pyeloureteric peristalsis. Clin Exp Pharmacol Physiol 37:509–515

Lavoie B, Balemba OB, Nelson MT, Ward SM, Mawe GM (2007) Morphological and physiological evidence for interstitial cell of Cajal-like cells in the guinea pig gallbladder. J Physiol 579:487–501

Lyons AD, Gardiner TA, McCloskey KD (2007) Kit-positive interstitial cells in the rabbit urethra: structural relationships with nerves and smooth muscle. BJU Int 99:687–694

Maeda H, Yamagata A, Nishikawa S, Yoshinaga K, Kobayashi S, Nishi K, Nishikawa S (1992) Requirement of c-kit for development of intestinal pacemaker system. Development 116: 369–375

McCloskey KD (2005) Characterization of outward currents in interstitial cells from the guinea pig bladder. J Urol 173:296–301

McCloskey KD (2006) Calcium currents in interstitial cells from the guinea-pig bladder. BJU Int 97:1338–1343

McCloskey KD, Gurney AM (2002) Kit-positive cells in the guinea pig bladder. J Urol 168: 832–836

McCloskey KD, Anderson UA, Davidson RA, Bayguinov YR, Sanders KM, Ward SM (2009) Comparison of mechanical and electrical activity and interstitial cells of Cajal in urinary bladders from wild-type and W/W^v mice. Br J Pharmacol 156(2):273–283

Metzger R, Schuster T, Till H, Stehr M, Franke FE, Dietz HG (2004) Cajal-like cells in the human upper urinary tract. J Urol 172:769–772

Metzger R, Schuster T, Till H, Franke FE, Dietz HG (2005) Cajal-like cells in the upper urinary tract: comparative study in various species. Pediatr Surg Int 21:169–174

Metzger R, Neugebauer A, Rolle U, Böhlig L, Till H (2008) c-Kit receptor (CD117) in the porcine urinary tract. Pediatr Surg Int 24:67–76

Mukerji G, Yiangou Y, Grogono J, Underwood J, Agarwal SK, Khullar V, Anand P (2006) Localization of M2 and M3 muscarinic receptors in human bladder disorders and their clinical correlations. J Urol 176:367–373

Ost D, Roskams T, Van Der Aa F, De Ridder D (2002) Topography of the vanilloid receptor in the human bladder: more than just the nerve fibers. J Urol 168:293–297

Pezzone MA, Watkins SC, Alber SM, King WE, de Groat WC, Chancellor MB, Fraser MO (2003) Identification of c-kit-positive cells in the mouse ureter: the interstitial cells of Cajal of the urinary tract. Am J Physiol Renal Physiol 284(5):F925–F929

Piaseczna Piotrowska A, Rolle U, Solari V, Puri P (2004) Interstitial cells of Cajal in the human normal urinary bladder and in the bladder of patients with megacystis-microcolon intestinal hypoperistalsis syndrome. BJU Int 94:143–146

Rasmussen H, Rumessen JJ, Hansen A, Smedts F, Horn T (2009) Ultrastructure of Cajal-like interstitial cells in the human detrusor. Cell Tissue Res 335:517–527

Roosen A, Datta SN, Chowdhury RA, Patel PM, Kalsi V, Elneil S, Dasgupta P, Kessler TM, Khan S, Panicker J, Fry CH, Brandner S, Fowler CJ, Apostolidis A (2009) Suburothelial myofibroblasts in the human overactive bladder and the effect of botulinum neurotoxin Type A treatment. Eur Urol 55:1440–1448

Sanders KM (1996) A case for interstitial cells of Cajal as pacemakers and mediators of neurotransmission in the gastrointestinal tract. Gastroenterology 111:492–515
Sanders KM, Koh SD, Ward SM (2006) Interstitial cells of Cajal as pacemakers in the gastrointestinal tract. Annu Rev Physiol 68:307–343
Sanders KM, Ordög T, Ward SM (2002) Physiology and pathophysiology of the interstitial cells of Cajal: from bench to bedside. IV. Genetic and animal models of GI motility disorders caused by loss of interstitial cells of Cajal. Am J Physiol Gastrointest Liver Physiol 282(5):G747–G756
Sanders KM, Ordög T, Koh SD, Torihashi S, Ward SM (1999) Development and plasticity of interstitial cells of Cajal. Neurogastroenterol Motil 5:311–338
Sanders KM, Ward SM (2007) Kit mutants and gastrointestinal physiology. J Physiol 578:33–42
Sergeant GP, Hollywood MA, McCloskey KD, Thornbury KD, McHale NG (2000) Specialized pacemaking cells in the rabbit urethra. J Physiol 526:359–366
Sergeant GP, Hollywood MA, McCloskey KD, McHale NG, Thornbury KD (2001) Role of IP(3) in modulation of spontaneous activity in pacemaker cells of rabbit urethra. Am J Physiol Cell Physiol 280:C1349–C1356
Sergeant GP, Thornbury KD, McHale NG, Hollywood MA (2002) Characterization of norepinephrine-evoked inward currents in interstitial cells isolated from the rabbit urethra. Am J Physiol Cell Physiol 283:C885–C894
Sergeant GP, Bradley E, Thornbury KD, McHale NG, Hollywood MA (2008) Role of mitochondria in modulation of spontaneous Ca2+ waves in freshly dispersed interstitial cells of Cajal from the rabbit urethra. J Physiol 586:4631–4642
Sergeant GP, Bradley E, Drumm B, Hollywood MA, McHale NG, Thornbury KD (2009) Regulation of spontaneous activity in interstitial cells of Cajal of the rabbit urethra by ATP. Proc Physiol Soc 15:C139
Shafik A, El-Sibai O, Shafik AA, Shafik I (2004) Identification of interstitial cells of Cajal in human urinary bladder: concept of vesical pacemaker. Urology 64:809–813
Smet PJ, Jonavicius J, Marshall VR, de Vente J (1996) Distribution of nitric oxide synthase-immunoreactive nerves and identification of the cellular targets of nitric oxide in guinea-pig and human urinary bladder by cGMP immunohistochemistry. Neuroscience 71:337–348
Solari V, Piotrowska AP, Puri P (2003) Altered expression of interstitial cells of Cajal in congenital ureteropelvic junction obstruction. J Urol 170:2420–2422
Sui GP, Rothery S, Dupont E, Fry CH, Severs NJ (2002) Gap junctions and connexin expression in human suburothelial interstitial cells. BJU Int 90:118–129
Sui GP, Wu C, Fry CH (2004) Electrical characteristics of suburothelial cells isolated from the human bladder. J Urol 171:938–943
Sui GP, Wu C, Fry CH (2006) Characterization of the purinergic receptor subtype on guinea-pig suburothelial myofibroblasts. BJU Int 97:1327–1331
Sui GP, Wu C, Roosen A, Ikeda Y, Kanai AJ, Fry CH (2008) Modulation of bladder myofibroblast activity: implications for bladder function. Am J Physiol Renal Physiol 295:F688–F697
Turner WH, Brading AF (1997) Smooth muscle of the bladder in the normal and the diseased state: pathophysiology, diagnosis and treatment. Pharmacol Ther 75:77–110
Wu C, Sui GP, Fry CH (2004) Purinergic regulation of guinea pig suburothelial myofibroblasts. J Physiol 559:231–243
Wiseman OJ, Fowler CJ, Landon DN (2003) The role of the human bladder lamina propria myofibroblast. BJU Int 91:89–93

Potential Applications of Gene Therapy/ Transfer to the Treatment of Lower Urinary Tract Diseases/Disorders

George J. Christ

Contents

Abstract Identification of molecular targets for novel therapeutics is a natural consequence of the age of molecular and personalized medicine. How this information is leveraged and applied to the treatment of functional diseases/disorders of the lower urinary tract will determine if this field of medicine can keep pace with technological developments and patient expectations for improved therapies. In this regard, therapeutic improvements for the treatment of lower urinary tract diseases and disorders have been largely incremental over the past 30 years. The goal of this report is to review the evidence pointing toward the enormous potential of gene therapy/transfer to provide a paradigm shift from palliative to curative therapeutic solutions for lower urinary tract diseases/disorders. In fact, it seems clear that gene therapy represents a biotechnology approach particularly suitable to applications in the lower urinary tract. Although much more research is required, ample preclinical evidence already indicates that, for example, gene therapy can favorably impact/alter virtually every aspect of bladder physiology/function. In short, further

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investigations and continued applications of gene therapy to the treatment of lower urinary tract diseases/disorders seems a prudent step toward potentially marked and more durable therapeutic improvements.

Keywords Benign disease · Bladder · Gap junctions · Gene therapy · Gene transfer · Localized delivery · Quality of life · Smooth muscle · Ureter · Urethra · Urothelium

1 Introduction to Gene Therapy

The age of molecular and personalized medicine has arrived (Hamburg and Collins 2010), bringing with it the promise of using biotechnology approaches to create novel and exciting therapies for the treatment of a diversity of human diseases and disorders, including the various bladder-related dysfunctions that are the subject of this report. Gene therapy or gene transfer is one particularly attractive approach, and further, one that offers the potential to provide curative rather than palliative or symptomatic relief of cell, tissue, and organ disease and pathology of the lower urinary tract. According to the American Society of Gene and Cell Therapy (ASGCT; http://www.asgct.org/the_society/history.php), gene therapy can be defined as the use of genetic material to modify a patient's cells for the treatment of an inherited or acquired disease. As pointed out by Morgan and Anderson (1993), the technology originally derives from work in the early 1970s on RNA and DNA tumor viruses and the recognition that the mechanism(s) of cellular transformation was related to the transfer of genetic material – leading to the seminal concept that the virus itself could be used as a vector to shuttle genes of interest into target cells. Certainly, the gene therapy field has developed tremendously in the past 20 years, but nonetheless, the therapeutic use of viral vectors is not without risk.

2 Current Clinical Applications of Gene Therapy

In fact, it is because of the inherent risks and uncertainties concerning the long-term impact of manipulation of genetic material on cell, tissue, and organismal biology that gene therapy has most commonly been applied to the treatment of life-threatening diseases such as cancer and other acquired and hereditary disorders where few effective treatment options are available. In the case of such life-threatening scenarios, the risk/benefit ratio intrinsic to gene transfer is considered to be acceptable. Unfortunately, the wisdom and scientific rationale for this conservative approach has become clear on at least a few occasions. The first widely reported gene therapy tragedy occurred in 1999 for a patient being treated in order to correct a metabolic disorder due to a deficiency of ornithine transcarbamylase (OTC). That patient, 18-year-old Jessie Gelsinger, suffered multiple organ failure and died within 48 h of the intrahepatic administration of a recombinant adenoviral vector containing the OTC gene. On another occasion, in 2002, two children in Paris developed leukemia after treatment

to modify stem cells with a retroviral vector for the treatment of severe combined immunodeficiency syndrome (SCID). More recently, in 2005, a 36-year-old woman, Jolee Mohr, died following use of an adeno-associated viral vector for the treatment of chronic painful rheumatoid arthritis (Johnston and Baylis 2004; Kay and High 1999; Kay et al. 2000; Marshall 2001; Pattee 2008). These tragic deaths have created a legitimate atmosphere of concern that has had a significant impact on the progress of the entire field, especially for those clinical applications for nonlife-threatening conditions such as that proposed herein.

Perhaps it is not surprising then that although the first clinical trial for gene therapy occurred in 1990 (Morgan and French), there are still no FDA-approved gene therapy treatments. To put this into proper perspective with respect to urological application(s) of gene therapy, there are currently 1579 ongoing clinical trials worldwide (see http://www.wiley.co.uk/genetherapy/clinical/; this database was queried in June, 2010 – but is updated regularly), with roughly 63% of these in the USA alone. The majority of the indications are for cancer (64.5%; $n = 1{,}019$), cardiovascular (8.7%; $n = 138$), and monogenic diseases (7.9%; $n = 125$), which together account for more than 80% of all ongoing trials. Trials for infectious (8%; $n = 12$), neurological (1.9%; $n = 30$), and ocular diseases (1.1%; $n = 18$), as well as gene marking (3.3%; $n = 50$) push the total to over 90%. In fact, even trials on healthy volunteers (2.3%; $n = 37$) exceed the number of trials on ALL other diseases (not listed above), which account for only 2.2% of clinical trials ($n = 33$). From the information available, it seems that at present only two clinical trials have been conducted related to the lower urinary tract for nonlife-threatening diseases (0.1%). One of these trials is for ED (Melman et al. 2005) and the other is for the treatment of overactive bladder in postmenopausal women [see GeMCRIS (Genetic Modification Clinical Research Information System); http://www.gemcris.od.nih.gov for more details]. Both of these trials were sponsored by the same company, Ion Channel Innovations, LLC.

The published literature database on gene therapy is of course correspondingly large. For example, a Medline search using the key word "gene therapy" yields more than 110,000 hits, including nearly 29,000 review articles alone. Clearly, this is an expansive field of translational research with numerous potential applications. The goal of this report is to focus on potential applications of gene therapy to the ureter, bladder, and urethra. The rationale for this approach is related to the fact that proper and coordinated function of these three components is an absolute prerequisite for the bladder to be able to fill, store, and empty as required, and moreover, each of these components can be accessed individually to ensure local gene delivery.

3 The Current Status of Gene Therapy for the Lower Urinary Tract

So, what is the state of gene therapy for treatment of benign diseases/disorders of the lower urinary tract? To begin to address this question, the medline database was queried again, and this time, "gene therapy" was crossed with "bladder," and 961

hits were found, although many of these were related to cancer therapeutics. To further refine the query, a text search for "gene therapy" and "bladder dysfunction" was conducted, and that search provided a much better approximation of the applications of gene transfer to benign bladder disease (91 hits). However, even so, only a fraction of these "hits" are truly related to gene therapy for the lower urinary tract, and most of those will be discussed herein. Similar searches crossing "gene therapy" with "ureter" (30 hits) or "urethra" (24 hits) yielded very few publications, and of those, only two were truly relevant to the former and only one to the latter. Moreover, all of the aforementioned "hits" describe preclinical work. The bottom line is that to date there is still a relative paucity of information available concerning the utility of gene therapy for the treatment of benign disease in the lower urinary tract. Again, of particular interest to this report is bladder disease/dysfunction.

In light of the fact that there have been no FDA-approved therapies, and furthermore, that there has been relatively little exploration of the utility of gene therapy/transfer to the lower urinary tract, what is the rationale for pursuing this avenue of research for clinical development? There are at least two major reasons for doing so. First, bladder dysfunction is one of the most common (and costly) urologic conditions. In this regard, despite progress, even the most recently developed pharmacologic therapies for urinary incontinence and lower urinary tract symptoms (LUTS) represent incremental improvements that have contraindications as well as untoward side effects and/or limited efficacy in certain patient populations (Andersson 2009; Andersson et al. 2005, 2009). Moreover, the goals of treatment have shifted. That is, the therapeutic focus is no longer to merely relieve symptoms but also to delay or prevent disease progression, and further, to provide amelioration of disease-related morbidities. Taken together, such improvements would significantly increase the patient's quality of life. These facts clearly emphasize the importance of developing new lines of investigation for improved therapeutics, and the thesis of this short report is that gene therapy is a very viable solution. A second reason for pursuing gene therapy/transfer for the treatment of lower urinary tract disease/disorder is that the ureters, bladder, and urethra are all individually accessible on a routine basis by urologists. In fact, the utility of localized delivery in the treatment of bladder disease/dysfunction has been previously described (Christ et al. 2006, 2007; Christ 2004; Fraser et al. 2002; Tyagi et al. 2006; Yokoyama et al. 2001). This has important implications to the applicability and clinical translation of gene therapy, as it will provide for localized delivery of the gene therapy product, thus ensuring minimal systemic distribution of therapeutic genes of interest.

In summary, the current environment undoubtedly signals the need for a paradigm shift in the direction of research in this important field of medicine. More specifically, improvements in the treatment of lower urinary tract diseases/disorders is dependent on the identification of more rational therapeutic targets coupled to the advent of novel therapeutics. In this regard, gene therapy is a biotechnology that provides an opportunity for the treatment of lower urinary tract diseases/disorders that will allow urologists to keep pace with technologic advances and patient

expectations. Presented below are some key examples that highlight the enormous possibilities of this technology.

4 Preclinical Investigations of Gene Therapy for the Lower Urinary Tract: A Variety of Targets and Possibilities

As described above, a review of the published literature reveals only a handful of articles relevant to gene therapy for benign lower urinary tract disease. The vast majority of these are squarely focused on the bladder, with only a few preliminary "proof of concept" studies available on gene therapy in the ureter and urethra. All of the available information is compiled in Table 1. Recent reviews on the subject have documented the numerous gene therapy strategies currently being contemplated for the treatment of bladder diseases/dysfunction (Christ et al. 2006, 2007; Goins et al. 2009). More specifically, gene therapy approaches exist for virtually all of the commonly observed clinical conditions. These include the following (1) use of muscle-derived cells and ex vivo gene therapy, as well as in vivo gene transfer (with the M_3 receptor) for the treatment of the hypocontractile bladder, (2) use of nerve growth factor for the treatment of neuropathy (i.e., diabetic neuropathy), (3) use of endogenous opioids to treat interstitial cystitis/painful bladder syndromes, (4) use of manganese superoxide dismutase for the treatment of irradiation-induced cystitis, (5) use of ion channels (i.e., K channels) for the treatment of detrusor overactivity and OAB, (6) use of glutamic acid decarboxylase for the amelioration of nonvoiding contractions due to spinal cord injury, and (7) Use of hepatocyte growth factor to reduce bladder wall fibrosis following outlet obstruction. As such, one of the most attractive characteristics of gene therapy is that it affords one the opportunity to affect virtually every aspect of the *micturition* process (at the end organ level) by selectively, altering the expression of relevant targets.

5 Tuning Gene Therapy for the Lower Urinary Tract

Despite the undeniable level of preclinical success that currently exists, the devil will be in the details of clinical translation and therapeutic durability. In this regard, tuning the gene therapy approach for each indication will be a key factor. In fact, the successful delivery of the gene of interest to the target cell or tissue depends entirely on the use of "vector," that is, the vehicle or package into which the gene is placed, so that the gene can be shuttled into the cell and survive without enzymatic breakdown in body fluids, cytoplasm, or nucleoplasm. For example, since gene therapy for malignant cancers requires a high transfection efficiency and genetic durability, viral vectors are required. However, in the case of common smooth

Table 1 Preclinical gene therapy studies for lower urinary tract dysfunction

Vector used	Gene target	Physiologic endpoint	Duration	Reference
Bladder gene therapy				
Muscle-derived stem cells	1. None (used as bulking agent or combined with matrix to form sling or bladder augment) 2. May be transfected with genes for growth factor, cytokine, hormone production	Provide bulk for sphincteric deficiency as injectable or engineered sling, improve bladder contractility	Up to 8 weeks in preclinical studies	Huard et al. (2002); Lu et al. (2003)
Electroporation-mediated plasmid injection	Muscarinic M3 receptor	Significantly increased carbachol and EFS induced maximal contractile response	2 days	Otani et al. (2004)
Herpes Simplex Virus (HSV-1)	β-NGF (neural growth factor)	Elevated NGF levels in bladder and lumbar DRG, reduced bladder capacity and postvoid residual volume in diabetic rats	4–8 weeks	Sasaki et al. (2004)
Gene gun cDNA delivery	POMC (pro-opiomelanocortin)	Bladder analgesia, improved bladder hypersensitivity induced by acetic acid infusion in anesthetized rats	3 days	Chuang et al. (2003)
Herpes simplex virus (HSV)	Preproenkephalin (PPE)	Increased transgene expression of PPE in bladder and DRG innervating the bladder, reduced bladder hyperactivity and nociceptive responses induced by intravesical capsaicin application	Transgene expression up to 30 days Reductions in bladder hyperactivity up to 1 week and nociceptive behavior up to 2 weeks	Yokoyama et al. (2009a, b); Yoshimura et al. 2001)
Herpes simplex virus (HSV)	Nerve Growth Factor (NGF)	Increased NGF levels in bladder tissue and DRG	Up to 21 days	Goins et al. (2001)
Herpes simplex virus (HSV)	Glutamic Acid Decarboxylase (GAD)	Reduced NVCs frequency and amplitude in SCI rodent model, increased GAD mRNA and protein levels	Up to 21 days	Miyazato et al. (2009)

Plasmid/ liposome complex	MnSOD (manganese superoxide dismutase)	Decreased radiation injury recovery time, detrusor pressure and voiding time as compared to controls	48 h (upregulated MnSOD expression), 6 months (improved detrusor function)	Kanai et al. (2002)
Naked DNA	*hSlo* (maxi-K channel)	Eliminated obstruction-associated bladder hyperactivity	3 days (later time points not examined)	Christ et al. (2001)
Naked DNA	Hepatocyte Growth Factor (HGF)	Increased HGF mRNA and reduced collagen content in bladder wall following partial urethral obstruction	2 weeks	Ku et al. (2006)
Ureteral gene therapy				
Adenovirus	Lac-Z gene	Proof of concept gene incorporation into strictured and infected porcine ureters using catheter	7 days	Anidjar et al. (1999)
Adenovirus	Lac-Z gene	Proof of concept gene incorporation into rodent ureters following direct injection into the rodent ureter wall	7 days	Kim et al. (1997)
Urethral gene therapy				
Adenovirus, retrovirus	Lac-Z gene	Proof of concept gene incorporation into strictured and infected rabbit urethra	5 days	Meria et al. (2000)

muscle disorders of the lower urinary tract, such as overactive bladder syndrome, the use of naked DNA, with its intrinsic limitations in transfection efficiency, appears to be effective nonetheless, without an absolute requirement for viral vectors (see Christ et al. 2001, 2006, 2007; Christ 2004). The major implication is that the frequency of incorporation of naked DNA into the cellular genome is vanishingly small, and thus, the risk of future malignancy as a result of gene therapy is correspondingly low. How is this possible? In the case of the bladder, specifically detrusor overactivity related to partial outlet obstruction in rodents, there appears to be a "physiological tolerance" for lower transfection efficiencies as a result of the presence of an intercellular network of connexin43-derived gap junction channels that allow rapid transmission of ions and second messengers between coupled smooth muscle cells (Christ et al. 2003a, b; Christ and Hodges 2006). More specifically, this mechanism reduces the need for a high transfection efficiency for the gene transfer target and provides an important distinction between naked DNA plasmid-based gene transfer for benign disorders such as bladder disorders, relative to the viral vectors required for the treatment of malignancies. In this scenario, the therapeutic goal of gene therapy for lower urinary tract dysfunction is based on the concept of achieving only the minimal cellular perturbation required to restore normal end organ function. That is, if subtle perturbations can create organ dysfunction, then it follows logically that relatively subtle modulation/restoration of cellular (i.e., smooth muscle tone) function would be sufficient to elicit therapeutic efficacy. Taken together, it is clear that this approach bodes well for the treatment of dysfunction of the lower urinary tract.

6 Summary and Conclusions

In summary, gene therapy is a potentially attractive treatment option for disease or dysfunction of the lower urinary tract. Preclinical gene therapy applications to bladder disease and dysfunction are particularly compelling at present, where, in fact, gene therapy offers the opportunity to virtually normalize bladder function, thus potentially eliminating the need for other forms of treatment. Moreover, the ability to locally deliver the gene therapy to the cell or tissue of interest minimizes the potential for systemic side effects, overcoming another major barrier to the eventual clinical translation of this technology. Since relatively subtle perturbations in a variety of modulators of bladder function can predispose to bladder dysfunction (i.e., urothelial, neural, smooth muscle, etc.), and, further, can be leveraged at the molecular level, there are a correspondingly large number of potential targets. In addition, given the known level of cellular and intercellular connectivity that exists throughout the bladder wall, there will undoubtedly be "physiological tolerance" for lower transfection efficiencies (see Christ et al. 2001, 2003a, b, 2006, 2007; Christ 2004; Christ and Hodges 2006 for more details), perhaps permitting the use of less invasive vectors. Finally, another positive attribute of gene therapy is that

when necessary it can be combined with other treatment options, for example, drug therapy, with the potential to reduce the dosage, and thereby, any dose-related side effects of available medications. As such, the application of gene therapy represents a natural progression in the treatment of urological disease whose time has finally arrived. Further investigations and applications of gene therapy to the lower urinary tract would seem a prudent step toward the therapeutic improvements that will be required to treat a seemingly ever-aging US and worldwide population in which the incidence of "quality of life" diseases/disorders will only continue to rise.

References

Andersson KE (2009) Pharmacotherapy of the overactive bladder. Discov Med 8:118–124

Andersson KE, Appell R, Awad S (2005) Pharmacological treatment of urinary incontinence. In: Abrams P, Khoury S, Wein A (eds) Incontinence, 3rd International Consultation on Incontinence. Helath, Paris, pp 811–854

Andersson KE, Chapple CR, Cardozo L, Cruz F, Hashim H, Michel MC, Tannenbaum C, Wein AJ (2009) Pharmacological treatment of overactive bladder: report from the International Consultation on Incontinence. Curr Opin Urol 19:380–394

Anidjar M, Mongiat-Artus P, Brouland JP, Cochand-Priollet B, Teillac P, Le Duc A, Berthon P, Cussenot O (1999) Ureteral gene transfer to porcine induced strictures using endourologic delivery of an adenoviral vector. J Urol 161:1636–1643

Christ GJ (2004) Gene therapy treatments for erectile and bladder dysfunction. Curr Urol Rep 5:52–60

Christ GJ, Hodges S (2006) Molecular mechanisms of detrusor and corporal myocyte contraction: identifying targets for pharmacotherapy of bladder and erectile dysfunction. Br J Pharmacol 147(Suppl 2):S41–S55

Christ GJ, Day NS, Day M, Santizo C, Zhao W, Sclafani T, Zinman J, Hsieh K, Venkateswarlu K, Valcic M, Melman A (2001) Bladder injection of "naked" hSlo/pcDNA3 ameliorates detrusor hyperactivity in obstructed rats in vivo. Am J Physiol Regul Integr Comp Physiol 281: R1699–R1709

Christ GJ, Day NS, Day M, Zhao W, Persson K, Pandita RK, Andersson KE (2003a) Increased connexin43-mediated intercellular communication in a rat model of bladder overactivity in vivo. Am J Physiol Regul Integr Comp Physiol 284:R1241–R1248

Christ GJ, Venkateswarlu K, Day NS, Valcic M, Santizo C, Zhao W, Wang HZ, Persson K, Andersson KE (2003b) Intercellular communication and bladder function. Adv Exp Med Biol 539:239–254

Christ GJ, Hodges S, Melman A (2006) An update on gene therapy/transfer treatments for bladder dysfunction. Curr Bladder Dysfunct Rep 1:119–125

Christ G, Andersson KE, Atala A (2007) The future of bladder research: molecular profiling, new drug targets, gene therapy, and tissue engineering. Curr Urol Rep 8:95–99

Chuang YC, Chou AK, Wu PC, Chiang PH, Yu TJ, Yang LC, Yoshimura N, Chancellor MB (2003) Gene therapy for bladder pain with gene gun particle encoding pro-opiomelanocortin cDNA. J Urol 170:2044–2048

Fraser MO, Lavelle JP, Sacks MS, Chancellor MB (2002) The future of bladder control-intravesical drug delivery, a pinch of pepper, and gene therapy. Rev Urol 4:1–11

Goins WF, Yoshimura N, Phelan MW, Yokoyama T, Fraser MO, Ozawa H, Bennett NJ, de Groat WC, Glorioso JC, Chancellor MB (2001) Herpes simplex virus mediated nerve growth factor

expression in bladder and afferent neurons: potential treatment for diabetic bladder dysfunction. J Urol 165:1748–1754

Goins WF, Goss JR, Chancellor MB, de Groat WC, Glorioso JC, Yoshimura N (2009) Herpes simplex virus vector-mediated gene delivery for the treatment of lower urinary tract pain. Gene Ther 16:558–569

Hamburg MA, Collins FS (2010) The path to personalized medicine. N Engl J Med 363:301–304

Huard J, Yokoyama T, Pruchnic R, Qu Z, Li Y, Lee JY, Somogyi GT, de Groat WC, Chancellor MB (2002) Muscle-derived cell-mediated ex vivo gene therapy for urological dysfunction. Gene Ther 9:1617–1626

Johnston J, Baylis F (2004) Gene therapy: two steps forward, one step back. CMAJ 170:1785–1786

Kanai AJ, Zeidel ML, Lavelle JP, Greenberger JS, Birder LA, de Groat WC, Apodaca GL, Meyers SA, Ramage R, Epperly MW (2002) Manganese superoxide dismutase gene therapy protects against irradiation-induced cystitis. Am J Physiol Renal Physiol 283:F1304–F1312

Kay MA, High K (1999) Gene therapy for the hemophilias. Proc Natl Acad Sci USA 96:9973–9975

Kay MA, Manno CS, Ragni MV, Larson PJ, Couto LB, McClelland A, Glader B, Chew AJ, Tai SJ, Herzog RW, Arruda V, Johnson F, Scallan C, Skarsgard E, Flake AW, High KA (2000) Evidence for gene transfer and expression of factor IX in haemophilia B patients treated with an AAV vector. Nat Genet 24:257–261

Kim S, Lin H, Barr E, Chu L, Leiden JM, Parmacek MS (1997) Transcriptional targeting of replication-defective adenovirus transgene expression to smooth muscle cells in vivo. J Clin Invest 100:1006–1014

Ku JH, Kim Y, Moon KC, Kim YS, Kim MS, Kim HH, Paick JS (2006) In vivo hepatocyte growth factor gene transfer to bladder smooth muscle after bladder outlet obstruction in the rat: a morphometric analysis. J Urol 176:1230–1235

Lu SH, Cannon TW, Chermanski C, Pruchnic R, Somogyi G, Sacks M, de Groat WC, Huard J, Chancellor MB (2003) Muscle-derived stem cells seeded into acellular scaffolds develop calcium-dependent contractile activity that is modulated by nicotinic receptors. Urology 61:1285–1291

Marshall E (2001) Gene therapy. Viral vectors still pack surprises. Science 294:1640

Melman A, Bar-Chama N, McCullough A, Davies K, Christ G (2005) The first human trial for gene transfer therapy for the treatment of erectile dysfunction: preliminary results. Eur Urol 48:314–318

Meria P, Anidjar M, Brouland JP, Teillac P, Berthon P, Cussenot O (2000) Gene transfer to urethral strictures in rabbits: a preliminary report. BJU Int 85:1120–1125

Miyazato M, Sugaya K, Goins WF, Wolfe D, Goss JR, Chancellor MB, de Groat WC, Glorioso JC, Yoshimura N (2009) Herpes simplex virus vector-mediated gene delivery of glutamic acid decarboxylase reduces detrusor overactivity in spinal cord-injured rats. Gene Ther 16:660–668

Morgan RA, Anderson WF (1993) Human gene therapy. Annu Rev Biochem 62:191–217

Otani M, Yoshida M, Iwashita H, Kawano Y, Miyamae K, Inadome A, Nishi T, Ueda S (2004) Electroporation-mediated muscarinic M3 receptor gene transfer into rat urinary bladder. Int J Urol 11:1001–1008

Pattee SR (2008) Protections for participants in gene therapy trials: a patient's perspective. Hum Gene Ther 19:9–10

Sasaki K, Chancellor MB, Goins WF, Phelan MW, Glorioso JC, de Groat WC, Yoshimura N (2004) Gene therapy using replication-defective herpes simplex virus vectors expressing nerve growth factor in a rat model of diabetic cystopathy. Diabetes 53:2723–2730

Tyagi S, Tyagi P, Van-le S, Yoshimura N, Chancellor MB, de Miguel F (2006) Qualitative and quantitative expression profile of muscarinic receptors in human urothelium and detrusor. J Urol 176:1673–1678

Yokoyama T, Chancellor MB, Yoshimura N, Huard J, Kumon H (2001) Gene therapy and tissue engineering for urologic dysfunction: status and prospects. Mol Urol 5:67–70

Yokoyama H, Sasaki K, Franks ME, Goins WF, Goss JR, de Groat WC, Glorioso JC, Chancellor MB, Yoshimura N (2009a) Gene therapy for bladder overactivity and nociception with herpes simplex virus vectors expressing preproenkephalin. Hum Gene Ther 20:63–71

Yokoyama H, Sasaki K, Franks ME, Goins WF, Goss JR, Degroat WC, Glorioso J, Chancellor MB, Yoshimura N (2009b) Gene therapy for bladder overactivity and nociception with herpes simplex virus vectors expressing preproenkephalin. Hum Gene Ther 20:63–71

Yoshimura N, Franks ME, Sasaki K, Goins WF, Goss J, Yokoyama T, Fraser MO, Seki S, Fink J, Glorioso J, de Groat WC, Chancellor MB (2001) Gene therapy of bladder pain with herpes simplex virus (HSV) vectors expressing preproenkephalin (PPE). Urology 57:116

Specific Pharmacokinetic Aspects of the Urinary Tract

Cees Korstanje and Walter Krauwinkel

Contents

Abstract This chapter reviews the evidence for "specific" pharmacokinetics playing a role in currently marketed drugs intended to treat lower urinary tract (LUT) symptoms. Principles of drug targeting include intrinsic properties of drugs or organs as well as drug formulations to modify drug release or to create confinement of drug presence. Prodrugs and specific formulations to deliver high drug concentrations at the site(s) of action as well as other ways to manipulate drug distribution to achieve enrichment in target tissues are considered. In overactive bladder (OAB), specific formulations for oxybutynin have been introduced to reduce the level of side effects of the active drug. Extended release tablet formulations and a topical gel formulation have been introduced, with efficacy similar to immediate release (IR) tablets, but with a reduction in anticholinergic adverse effects. However, these modifications have not led to outstanding performance parameters compared to other anticholinergic drugs marketed as IR formulations. Urinary excretion is discussed as potential mechanism for targeting LUT symptoms, but no strong indications appear to exist that this

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mechanism would contribute for currently available drugs. Intravesical administration of drugs is not a preferred option and only considered for drugs like botulinum toxin, where the inconvenient application compensates for a reasonable degree of long-term efficacy in severe refractory OAB. Alpha acid glycoprotein binding is discussed as a potential factor to influence drug tissue distribution, and it is concluded that there is reasonable evidence that for tamsulosin this mechanism is responsible for the difference in free fraction of the drug observed in plasma and prostate, which could contribute to its relative absence of blood pressure effects in patients with LUT symptoms related to benign prostate hyperplasia (LUTS-BPH). The principle of irreversible inhibition of type II 5α-reductase as a tool to develop drugs to reduce prostatic levels of dihydrotestosterone is employed by both dutasteride and finasteride for treatment of LUTS-BPH. Of the mechanisms discussed, the principles employed for the 5α-reductase blockers and tamsulosin in this respect can be considered relatively specific for its urological indication.

Keywords Alpha acid glycoprotein · Drug targeting · Drug tissue distribution · Irreversible enzyme inhibition · Prodrug · Prostate · Urinary bladder

1 Introduction

The question to address in this chapter is whether there is a place for specific pharmacokinetic approaches for optimal pharmacotherapy in the lower urinary tract (LUT). For this, currently available drugs and potential possibilities in the LUT segment are reviewed and the potential added value is discussed.

Although local application of a drug usually is the simplest way to get targeted drug delivery, this is only achievable for local diseases at accessible places (e.g., skin, teeth, and eyes), while suitable formulation devices allow local treatment in more interior places of the body, like bronchial-inhalers for treatment of asthma. Principles of drug targeting include intrinsic properties of drugs or organs as well as drug formulations to modify drug release or to create confinement of drug presence.

Probably the best known example to urologists is that of antibiotics such as nitrofurantoin for treatment of urinary tract infections. After oral dosing and absorption, nitrofurantoin is excreted in urine, thereby reaching adequate drug levels to kill pathogenic bacteria localized in urine, kidney, and in urothelium of the urether and bladder.

Pharmacokinetic properties that determine the concentration of a drug at its target after absorption are the distribution of the drug to the target and its clearance from the target. Distribution of a drug is to a large extent related to its molecular size, lipophilicity, ionic charge(s), and affinity for endogenic transporters, like P-glycoprotein (limiting brain penetration). Drug–protein binding in blood can limit or slow the free exchange of drug to tissues. Drug clearance is the process to remove drug from tissues via the blood. The main clearing organs are the liver (by metabolizing drugs), the

kidney (by transporting drug and metabolites into urine), and the lung (low metabolizing capacity and excretion of volatile substituents). The general principles that apply for this can be found in textbooks and will therefore not quantitatively be treated in this chapter. This chapter reviews the potential for "specific" pharmacokinetic aspects relevant for drugs intended to treat LUT symptoms.

The question is what LUT is different as a target from, for example, the heart or the lungs in the context of drug specificity in treating diseases affecting LUT? To start with, the problem starts with the word "target." For a medical doctor, the target may be the site of the disease and is often felt to be an organ. For the pharmacologist, the target usually is the molecular site where the drug should act at and this is often a receptor or an enzyme. For the pharmacokineticist, the target usually is a volume like the blood, total body fluid, or the spinal fluid. This differential interpretation of the word target should be realized when discussing ways for specificity in treatment based on pharmacokinetic properties of a drug.

Considering the LUT, some of it is piping: urether, urethra; some is an organ: prostate; and some is mixed: the bladder. Control/pharmacological manipulation of LUT function for some diseases is at the level of the organ/pipe, while for other diseases treatment is at the level of the control system, which can be in the brain (e.g., pontine micturition center), spine (e.g., Onuf's body), ganglia, or lining of the organs that convey the symptoms of the disease (e.g., the urothelium of the bladder). This implies that there is no unification of "urinary tract pharmacokinetics." It is important to know where the biological target of the pharmacological agent is situated and if its environment has specific characteristics that would have impact on the kinetics of the drug (e.g., is the target after the blood–brain barrier or not; how is the vascularisation and blood supply of the target; can the drug penetrate to the target from urine). To see what is "optimal" or "specific" in this respect, currently used drugs in LUT diseases will be reviewed for potential properties in this respect.

In the treatment of LUT symptoms, the treated targets in terms of tissues or organs may be heterogeneous (prostate, bladder) and the target for treatment may be different from the affected organ. Examples of treatment targets that are different from the organ suffering the symptoms are in stress urinary incontinence (SUI) where the target of treatment is Onuf's nucleus in the spinal cord, and potentially in the treatment of pelvic, prostatic, and bladder pain, where afferent projections to the spinal cord or the brain, or ganglia may be the drug target. In overactive bladder (OAB), drug targets are receptors at bladder smooth muscle as well as at endothelial sites in the bladder at the detrusor part.

Is there evidence for "specific" pharmacokinetics playing a role in currently marketed drugs for LUT diseases?

2 Mechanisms of Drug Targeting

Let us consider the mechanisms of drug targeting that are being used, or may be relevant for LUT diseases:

Prodrugs and specific formulations to deliver high drug concentrations at the site(s) of action.
Other ways to manipulate drug distribution to achieve enrichment in target tissues.

2.1 Prodrugs in OAB

Of the drugs used in OAB, tolterodine and fesoterodine have active metabolites and could therefore be considered prodrugs. Tolterodine is a nonselective antimuscarinic drug. It is primarily metabolized by CYP2D6, resulting in the active metabolite 5-hydroxymethyl tolterodine (5HMT), which is equipotent to tolterodine (Nilvebrant 2000). Consequently, in poor CYP2D6 metabolizers (about 7% of Caucasians), only negligible amounts of 5HMT are formed. So, pragmatically, tolterodine cannot be considered as a prodrug, because tolterodine itself is the dominant moiety that determines the activity. Fesoterodine is the isobutyric acid ester of 5HMT. During absorption, it is rapidly metabolized by nonspecific esterases into 5HMT (Cole 2004). Fesoterodine itself does not reach quantifiable plasma concentrations after oral administration (Simon and Malhotra 2009), and has lower affinity for the M1 to M5 muscarinic receptor subtypes compared with 5HMT. Therefore, it can be regarded as a prodrug. Although formation of 5HMT from fesoterodine is not dependent on CYP2D6, further metabolism of 5HMT does involve CYP2D6. After an oral dose of 8 mg fesoterodine, approximately 42% of the dose reaches the circulation as 5HMT. Once daily dosing of 4 mg fesoterodine for 3 days results in a mean ± SD: C_{max} of 2.12 ± 1.28 ng/ml, a C_{trough} of 0.37 ± 0.19 ng/ml, and an $AUC_{0–24\ h}$ of 20.16 ± 11.44 ng h/ml. In comparison, oral dosing of 4 mg tolterodine ER to extensive CYP2D6 metabolizers results in a mean (range) C_{max} of 7.3 (2.9–13) nmol/l, and an $AUC_{0–24\ h}$ of 94 (38–187) nmol h/l for 5HMT, which corresponds to approximately 2.5 (1.0–4.4) ng/ml and 32 (13–64) ng h/ml for C_{max} and $AUC_{0–24\ h}$, respectively (Olsson and Szamosi 2001). For tolterodine, a 100-fold range in C_{max} values of 5HMT has been reported; however, the sum of tolterodine and 5HMT appears to be less variable. For fesoterodine, the range appears to be about tenfold narrower, possibly because in case of tolterodine CYP2D6 is involved in the production and elimination of 5HMT, while in case of fesoterodine it only plays a role in the elimination of 5HMT.

2.2 Specific Formulations in OAB

Oxybutynin is one of the first anticholinergics used in OAB. It is a nonselective antimuscarinic drug. It has a higher withdrawal rate in immediate release (IR) formulations than other antimuscarinic drugs/pharmaceutical compositions (Chapple et al. 2008). Compared to more recently marketed anticholinergics, it has a short

*t*1/2 of about 2–3 h. Consequently, when administered as immediate release (IR) tablets, three times daily dosing of the IR tablet are necessary to maintain effective plasma concentrations. The bioavailability is low, around 6%, due to extensive first-pass metabolism. Metabolism proceeds mainly via CYP3A4, resulting in the formation of the active metabolite *N*-desethyloxybutynin. To overcome the disadvantage of frequent dosing, a number of alternative dosage forms have been developed. It is available as an extended release tablet (OROS®), which delivers oxybutynin at a controlled rate over a period of 24 h, allowing once daily dosing of 5–30 mg. Dosing frequency can be further reduced by using the transdermal patch formulation, which needs to be replaced every 3–4 days. It delivers 3.9 mg of oxybutynin per day. Finally, a topical gel formulation has been introduced, which should be applied once daily. The daily dose of the gel amounts to 100 mg.

Although both oxybutynin and its metabolite posses anticholinergic activity, there are indications for small differences in receptor and/or organ selectivity. It is suggested that *N*-desethyloxybutynin has a somewhat higher affinity for the receptors in the salivary gland than for those in the bladder (Waldeck et al. 1997), and occurrence of dry mouth is claimed to be related to *N*-desethyloxybutynin exposure (Sathayan et al. 2001). Consequently, reducing the exposure of the metabolite may help in reducing the commonly reported adverse event of dry mouth. Lowering peak concentrations may also be of benefit in reducing the occurrence of adverse events. Comparison of the IR tablet with the OROS formulation by Gupta and Sathyan (1999) in 13 healthy subjects showed that the OROS formulation is able to deliver oxybutynin with considerably reduced fluctuation in oxybutynin plasma concentration. Furthermore, a more favorable metabolite/parent drug ratio was achieved with the OROS formulation. On the fourth day of dosing of 5 mg oxybutyinin IR t.i.d. and once daily dosing of 15 mg oxybutynin as the OROS formulation, the mean ± SD AUC_{tau} of *N*-desethyloxybutynin amounted to 483 ± 281 ng/ml vs. 81 ± 43 ng h/ml for oxybutynin, while with the OROS formulation the respective AUC_{tau} amounted to 304 ± 145 and 109 ± 43 ng h/ml, respectively. Studies in patients in which the OROS formulation was compared with IR tablets showed a reduction in the frequency of dry mouth with the OROS formulation, but with a similar efficacy (Anderson et al. 1999; Versi et al. 2000). Unfortunately, no population pharmacokinetics was used in these studies, which would have helped to assess the exposure to oxybutynin and *N*-desethyloxybutynin in these patients.

Administration via a transdermal system prevents the formation of metabolites via first-pass metabolism completely, although it cannot prevent metabolism as part of systemic clearance. Administration of 3.9 mg/day resulted in a mean AUC_{inf} of oxybutynin varying between 284 and 324 ng h/ml when applied to the buttock, hip, and abdomen, while the AUC_{inf} of the *N*-desethyl metabolite amounted to 435–504 ng h/ml, indicating a further reduction in the exposure of *N*-desethyloxybutynin relative to the exposure to oxybutynin compared with the OROS tablet (Zobrist et al. 2003). This was confirmed in a study by Appell et al. (2003), showing that compared with the OROS formulation, the AUC ratio of *N*-desethyloxybutynin to oxybutynin was reduced from 4.1 to 1.2. Administration

via the transdermal system further reduced the occurrence of typical anticholinergic AEs, like dry mouth, compared with oral extended release tablets, but it was also associated with local application site reactions in part of the patients (Baldwin and Keating 2009). Application of the gel resulted in similar exposure to oxybutynin and *N*-desethyloxybutynin as with the transdermal patch system (Caramelli et al. 2008), or may even reduce the exposure to the metabolite further (MacDiarmid 2009).

Overall, compared with IR tablets, extended release formulations, whether oral or transdermal, result in a more constant exposure to oxybutynin and a reduced exposure to *N*-desethyloxybutynin. Efficacy appears to be similar to IR tablets, but with a reduction in anticholinergic AEs.

2.3 *Urinary Excretion as Targeting Mechanism in Bladder Diseases*

Excretion of pharmacologically active drug or metabolites into urine is potentially attractive for treating OAB, but is it feasible? Under normal physiological conditions, the urothelium covering the lining of the urinary tract is a good barrier for solutes, metabolites, and ions and the tight junctions of the urothelium secure the barrier function also under stretched conditions (Apodaca 2004; Lewis 2000). Only in the case of inflammation (bacterial or nonbacterial) or rupture of lining tissue (e.g., kidney stones), the urothelial barrier function may be diminished and can become permeable to drugs that pass via urine. However, OAB is not commonly associated with inflammation and loss of function of the endothelial barrier. In addition, active concentrations in the bladder for drugs currently used for OAB are expected to be low for most drugs based on urine elimination data (see Table 1), with perhaps the exception of trospium and solifenacin. Studies by Kim et al. (2005) and Chuang et al. (2008) showed that urine collected from human subjects treated orally with solifenacin and trospium, but not oxybutynin, tolterodine, or darifenacin instilled intravesically in carbachol-induced detrusor overactivity in rats showed a reduction of bladder contractions. The value of these data for the human situation cannot be predicted, however, since urinary bladder barrier differences and therefore drug penetration may differ for rat and human bladder and is not well studied. For fesoterodine, no data were found on this topic in the literature.

2.4 *Intravesical Administration*

Intravesical administration is being pursued as first-line treatment for superficial bladder cancer, but this falls outside of the scope of this chapter [see

Table 1 Selected pharmacokinetic properties of drugs used in OAB

Drug	F[a] (%)	Food effect	Vd[b] (L)	Plasma binding	Metabolism	Active metabolites	Fraction of drug dose + active metabolites in urine (%)	Absorption after intravesical administration	References
Oxybutynin (Ditropan ER[c] tablet)	≈10	No	193	AGP: > 99%	Cyp3A4	*N*-desethyloxybutynin	< 0.1	Yes	Drugdex (2009a), Guay (2003)
Trospium (XR capsule)	4–16	Yes	> 600		Esterase; conjugation; No P450	–	–	ND	Micromedex Healthcare series (2008)
Tolterodine (ER tablet)	10–70[d]		113		Cyp2D6; Cyp3A4	HMT[e]	≈5	ND	Drugdex (2009b), Hills et al. (1998)
Solifenacin	90	No	599–671	AGP: 98%	Cyp3A4	No	5–10	ND	Drugdex (2008b)
Darifenacin	15–25	No	163	AGP: 98%	Cyp2D6; Cyp3A4	No	3	ND	Micomedex Healthcare series (2008b)
Fesoterodine	52			AGP: ≈50% and HMT also ≈50%	Cyp2D6; Cyp3A4	HMT	≈16	ND	Drugdex (2008a)

[a] *F* Bioavailability
[b] Distribution volume
[c] *ER* Extended release
[d] High first-pass effect and metabolism to DD01
[e] *HMT* 5-hydroxymethyl tolterodine

(Malcowicz 2006) for further reading on this topic]. For LUTS indications, the intravesical administration route is not popular for reasons of convenience and because most patients respond to oral ways of applying therapeutics. However, for neurogenic OAB, oxybutynin is sometimes being used intravesically in adults and children. Administration of oxybutynin, usually as 5 mg/10–30 mL, twice/three times a day in saline or hydroxypropylcellulose solution is published as treatment for patients with neurogenic OAB who were not satisfied with oral anticholinergic agents (Lehtoranta et al. 2002; Saito et al. 2004), or in children with neurogenic bladders who could not tolerate or whose conditions were refractory to oral therapy (Kaplinsky 1996). The serum concentrations of oxybutynin after single 5 mg intravesical doses were found at least as high as those reported after oral drug intake, but the parent drug/metabolite ratio was much higher after intravesical administration. The elimination of oxybutynin as well as its metabolite was prolonged after intravesical administration compared with that reported after oral drug intake (Lehtoranta et al. 2002). It appears therefore that intravesically applied oxybutynin does penetrate the bladder wall from inside out, however, no published evidence exists showing the contribution of urinary excreted oxybutynin to the activity of the drug when taken orally. This may be related to the low percentage of urinary excreted drug after oral dosing (Table 1). The mechanism of action for intravesical oxybutynin may be different from systemically applied drug, however, and local anesthetic effect at these high local concentrations has been suggested, since in rats intravesical oxybutynin was found to temporarily desensitize C fiber afferents, while no measurable effect was found on Aδ-fibers (DeWachter and Wyndaele 2003).

The vanilloid TRPV1 antagonist resiniferatoxin (RTX) is being investigated intravesically for patients with intractable urgency and frequency related to detrusor overactivity and/or interstitial cystitis (IC). Capsaicin is a vanilloid TRPV1 receptor agonists that targets the same receptor, but acts by desensitizing it. Both drugs are used intravesically due to systemic toxicity, and RTX is less pungent than capsaicin and is the preferred compound to inhibit afferent sensations via bladder C-fibers (Silva et al. 2000). Instillations of RTX usually are made in hospital pharmacies “ex tempore” and consist of 100 ml of 50 nM in 10% ethanol in saline (Apostolidis et al. 2006; Silva et al. 2002). Published research on drug penetration of vanilloid ligands does not bring about clear strategies for influencing penetration kinetics of capsaicin or RTX through the bladder (Lazar et al. 2006; Magnussen and Koskinen 2000). The complex cellular localization of the TRPV1 receptor and the complex regulation of its function have not made this receptor a therapeutically viable target for enhanced potency or bioavailability of TRPV1 ligands for the pharmaceutical industry so far (Lazar et al. 2006). Botulinum toxin is a useful drug for treatment of severe refractory OAB by transurethral injection at up to 30 sites within the bladder (Rapp et al. 2004). Although improvements may be seen for at least 6 months after treatment, it has limited applicability due to its cumbersome way of administration.

2.5 *Other Ways to Manipulate Drug Distribution*

2.5.1 Binding to Alpha Acid Glycoprotein as a Mechanism to Achieve Enrichment in Target Tissues

Once absorbed, drug penetration to various bodily parts can be a passive process through diffusion of drug molecules, whereby molecular size, ionic charge, and lipophylic–hydrophilic balance of the drug determine where the drug can partition into the body. When the drug has affinity for transport systems in the body such as P-glycoproteins, organic-anion transporting polypeptides, multidrug resistance associates proteins, and others, this has an impact on limitation of drug distribution (and as such this may offer the opportunity for concentration in a target tissue). The use of transporter functions therefore offers the possibility of delivering a drug to the target organ, avoiding distribution to other organs (thereby reducing the chance of toxic side effects), controlling the elimination process, and/or improving oral bioavailability (Lewis 2000). For drugs under consideration in this chapter, this seems to be a theoretical potential only, since no published evidence seem to exist for any of these drugs.

An apparently less sophisticated way for compartmentalization of drugs is by selective drug binding, and the most common example is binding of drugs in plasma to proteins such as albumin, or alpha acid glycoprotein (AGP), or to low density lipoprotein (LDL). In this respect, it is relevant that drug binding to albumin and LDL is low-affinity binding, while drug binding to AGP is usually high-affinity binding. High-affinity drug binding suggests specificity that goes further than rough chemical characteristics such as basic or acidic groups at physiological pH or a certain degree of lipophilicity and an example is the α_1-adrenoceptor blocker and antihypertensive agent prazosin, where slight changes in oligosaccharide side chains of AGP were found to have a dramatic effect on the affinity constant for prazosin (Quin and Øie 1994). For the OAB drug oxybutynin, enantio-specific binding to AGP was demonstrated, with the (*S*)-enantiomer showing higher affinity than the (*R*)-enantiomer (Shibukawa et al. 2002). Since affinity constants of drugs binding to AGP can be in the range of the affinity constants of these drugs for their biological targets (receptor, enzyme, or ion channel), it is worthwhile to consider whether AGP binding is a potential factor to create differences in tissue distribution for certain drugs, and more importantly, whether this can have an impact clinically. Quite a number of drugs used for LUT diseases have a relatively strong plasma AGP binding (see Tables 1 and 2) and one may speculate whether and how this can be exploited as a mechanism to modify distribution or retention of drugs in target tissues. AGP is a so-called "acute phase protein" and AGP levels in plasma are known to increase in morbid states such as infections, inflammation, cancer, myocardial infarction, or trauma and in patients with renal impairment (Gabay and Kushner 1999; Kremer et al. 1988). For several antiviral and anticancer drugs, AGP was found to limit the distribution of the drug, thereby modulating its therapeutic profile (Bilello et al. 1995; Fuse et al. 2000; Sommadossi et al. 1995).

Table 2 Selected pharmacokinetic properties of drugs used in BPH

Drug	F^{a} (%)	Food effect	Vd[b]	Plasma binding	Metabolism	Active metabolites	References
Doxazosin (GITS™ formulation)	54–59	Yes	0.79–1.69 L/kg	98–99%	ND	No	Chung et al. (1999), Vashi et al. (1998)
Alfuzosin (as XL tablet)	49	Yes	3.2 L/kg	82–90%	Cyp3A4	No	Micomedex Healthcare series (2008c)
Tamsulosin (OCAS™ formulation)	~100	No	0.2 L/kg	AGP; 99%	Cyp3A4	No	Michel et al. (2005), Micomedex Healthcare series (2009)
Finasteride	~63	No		~90%	Cyp3A4	No	Steiner (1996)
Dutasteride	60	No	300–500 L	99%; AGP and albumin	Cyp3A4	Yes: 6-beta-hydroxydutasteride	Micomedex Healthcare series (2008a)

[a]*F* Bioavailability
[b]Distribution volume

In rat and human prostate, AGP was found to differ from plasma values and varied with morbid states (Andersen et al. 1996; Dubé et al. 1989), which makes that differences in AGP may be a factor of interest in LUTS-BPH. For OAB drugs, no plausible reasons were found to consider a therapeutically relevant application for AGP binding.

For the α_1-adrenoceptor blockers given in LUTS-BPH, an important issue is the relative effect of the drug at vascular α_1-adrenoceptors (having an impact on blood pressure regulation, with orthostatic hypotension as an inconvenient side effect) and the effect at prostatic α_1-adrenoceptors to induce relaxation of the prostatic capsule as the therapeutically desired effect. For tamsulosin, it was found that the cardiovascular effects were relatively more pronounced in rats and dogs than in humans. Plasma binding for tamsulosin is different in rats (±80%), dogs (±90%), and man (±99%), and the binding protein is predominantly AGP (Matsushima et al. 1998), which makes that the free fraction of tamsulosin in these species is higher than in man. This made it worthwhile to investigate potential AGP binding differences for tamsulosin in plasma and prostate in humans to explain the cardiovascular safety of the drug in humans. Data on this topic are available, but unfortunately were published in congress abstracts only (Korstanje et al. 2002; Romics et al. 2003; Wolters et al. 2004) and for that reason will be discussed in more detail here than usual for a reference paper.

In in vitro experiments with isolated tissue, tamsulosin was shown to have higher antagonist potency at α_1-adrenoceptor-induced contractile response in prostate tissue than at vascular tissue in both rats and humans (Yamada et al. 1994, 1999). However, in vivo studies in rats and dogs and clinical pharmacology studies in man show species differences in the selectivity of tamsulosin for urodynamic and cardiovascular parameters, and the selectivity of the drug for urodynamic effects decreases in the order man > dog > rat (Korstanje et al. 2002), while there is similarity in $\alpha_{1A\&D}$-adrenoceptor subtype dominance in prostate and bladder in rat, dog, and man. So, this implies that important other factors should be in place to account for the more "uroselective" clinical profile of the drug in humans. In dogs, the "uroselectivity" was attributed to higher tissue retention of tamsulosin in prostate and urethra than in plasma, but this was not studied over multiple time-points (Sato et al. 2001). Tissue distribution studies with i.v. injection of tritiated tamsulosin in rats showed no particular differences in drug binding to prostate as compared to other tissues, and under steady state plasma pharmacokinetics in rats the drug concentration in prostate was only twice that in plasma. In a clinical study with patients waitlisted for open prostatectomy, 0.4 mg tamsulosin was given for 6–21 days prior to open prostatectomy surgery via the suprapubic operation method. Prostate surgery was performed 4, 8, 24, or 48 h after last drug intake and plasma and prostate tissue samples were collected and analyzed for total tamsulosin, while ex vivo drug–tissue binding was analyzed using ^{14}C-labeled tamsulosin. Plasma and tissue concentration data were subjected to population pharmacokinetic analysis.

The $AUC_{0–24\ h}$ in prostate tissue amounted to 43% of the plasma $AUC_{0–24\ h}$ and consequently, the average concentration of tamsulosin HCl in prostate tissue over the day amounts to 43% of the average concentration in plasma. However, protein binding studies for tamsulosin in pooled plasma samples revealed the protein binding to be 99.6 ± 0.5%, thus having a free concentration, $f_u = 0.4\%$, while protein binding to prostate tissue was much lower, with f_u of 59%. Taking these differences into account between plasma and prostate drug binding and the total drug concentration in the samples, the $AUC_{0–24\ h}$ of unbound tamsulosin in prostate tissue was estimated to be 63-fold higher than the $AUC_{0–24\ h}$ in plasma. This is an unexpected finding, since it is assumed that under equilibrium conditions diffusion of unbound drug will lead to equal drug concentrations in all compartments in the body (Rowland and Tozer 1995), but since the data are obtained under presumed steady state pharmacokinetics, and since technical procedures have been vigorously checked and discussed by the authors and with peers from other institutes (Swart, personal communication), it looks that the findings have to be considered as real. For alfuzosin, slight differences are reported between plasma and prostate levels of the drug in man (Mottet et al. 2003), and since this drug does not have a very high plasma protein binding, it is not likely that its free concentration will differ greatly between plasma and prostate. In the context of "uroselectivity," a heavily debated topic for BPH-LUTS drugs at various discussion forums (Andersson 1998; McGrath et al. 1996), it appears that the study with tamsulosin is the only study with an acceptable level of scientific evidence to qualify for "specific PK aspects" relevant for clinical practise.

2.5.2 Irreversible Binding to Target Enzymes

Finasteride is used in LUTS-BPH and is a competitive and specific inhibitor of type II 5α-reductase. Finasteride slowly forms a stable enzyme complex and turnover from this complex is extremely slow (Moss et al. 1996). Dutasteride is a competitive inhibitor of both type I and type II 5α-reductase, is used in LUTS-BPH as well, and shows apparent irreversible inhibition of type II, like finasteride, and reversible inhibition of type I 5α-reductase (Stuart et al. 2001), the other type of 5α-reductase that is active in the prostate to produce dihydrotestosterone, the hormone that fuels benign prostate growth. The principle of irreversible inhibition of type II 5α-reductase as a tool to develop drugs to reduce prostatic levels of dihydrotestosterone has been adopted by all drug companies that have tried to develop 5α-reductase inhibitors for LUTS-BPH (McNulty et al. 2000). Since there are no clinically available type II 5α-reductase inhibitors with reversible enzyme inhibition kinetics, the value of irreversible inhibition of type II 5α-reductase versus reversible inhibition of type II 5α-reductase, unfortunately, cannot be assessed using publicly available documentation.

3 Conclusions

In both OAB and LUTS-BPH, extended release formulations to modulate drug plasma concentrations for alpha-blockers and anticholinergic drugs with short elimination half-lives have shown to be successful in reducing drug peak exposures and creating lower through – peak difference. There is evidence to show that these strategies generally have led to an efficacy similar to immediate release formulations, but with a reduction in side effects or increased tolerability. For drugs where active metabolites are formed in combination with metabolism of the parent drug, or prodrug via a metabolic route that gives rise to high interindividual differences in bioavailability (like the CYP2D6 enzyme), the merit is in creating a more predictable exposure (e.g., fesoterodine vs. tolterodine), which translates into a more reliable drug effect. For oxybutynin, strategies to reduce peak plasma exposure and reduce production of first-pass metabolites have not led to a further improvement of efficacy/tolerability ratio as those achieved with drugs such as darifenacin, solifenacin, and fesoterodine. Efficacy of anticholinergics via local application can be obtained via the intravesical route, but it is very inconvenient and gives no objectively measured higher efficacy. For two anticholinergics, solifenacin and fesoterodine, relatively high levels are excreted unchanged, and theoretically high enough concentrations in the bladder would be obtained to contribute to clinical efficacy after oral dosing. For fesoterodine this has not been verified. For solifenacin, data are suggestive that excreted urine from humans can have local efficacy in rat bladders, but how this translates to human practice is unclear. In LUTS-BPH, the 5α-reductase inhibitors are drugs where the irreversible kinetics of these drugs at the type II 5α-reductase enzyme is relevant for a stable pharmacodynamic effect, but clinical evidence for this is lacking. For α_1-adrenoceptor blockers in LUTS-BPH, extended release technologies have shown to be successful to reduce effects on blood pressure and orthostatic hypotension for those α_1-adrenoceptor blockers that show effects on the cardiovascular system like alfuzosin and doxazosin, whereas for tamsulosin (which does not show effect on blood pressure in patients in the capsule formulation), a further reduction of orthostatic symptoms was achieved with the OCAS™ formulation. The way differential binding of drugs to AGP, as proposed for tamsulosin, contributes to its low cardiovascular side effect profile, is plausible, but needs more solid evidence.

So, in short, it can be concluded that in OAB and LUTS-BPH modulation of pharmacokinetics of drugs by extended release, technologies plays a role in ameliorating tolerability issues some of the (mainly older) drugs may have and to increase dosing convenience. Although these are important assets on face value, this is not something unique to drugs used in OAB or LUTS-BPH, but a generally applicable principle in drug development. The irreversible enzyme kinetics of finasteride and dutasteride for type II 5α-reductase enzyme is clearly a consequence of clever chemical chemistry design and it is likely that reversible enzyme blockers could have a worse side effect profile. For α_1-adrenoceptor blockers used in LUTS-BPH, AGP may be a factor that contributes to a certain degree of

prostate selectivity of tamsulosin. Mentioned mechanisms for the 5α-reductase blockers and tamsulosin in this respect can be considered relatively specific for its urological indication.

References

Andersen UO, Bøg-Hansen TC, Kirkeby S (1996) Lectin-like receptor for α1-acid glycoprotein in the epithelium of the rat prostate gland and seminal vesicles. Prostate 29:356–361

Anderson RU, Mobley D, Blank B et al (1999) Once daily controlled versus immediate release oxybutynin choloride for urge urinary incontinence. J Urol 161:1809–1812

Andersson K-E (1998) The concept of uroselectivity. Eur Urol 33(suppl 2):7–11

Apodaca G (2004) The uroepithelium: not just a passive barrier. Traffic 5:117–128

Apostolidis A, Gonzales GE, Fowler CJ (2006) Effect of intravesical resiniferatoxin on lower urinary tract symptoms, urodynamic parameters, and quality of life of patients with urodynamic increased bladder sensation. Eur Urol 50:1299–1305

Appell RA, Chancellor MB, Zobrist RH et al (2003) Pharmacokinetics, metabolism and saliva output during transdermal and extended-release oral oxybutynin administration in healthy subjects. Mayo Clin Proc 78:696–702

Baldwin CM, Keating GM (2009) Transdermal oxybutynin. Drugs 69:327–337

Bilello JA, Bilello PA, Prichard M et al (1995) Reduction of the in vitro activity of A77003, an inhibitor of human immunodeficiency virus protease, by human serum 1 acid glycoprotein. J Infect Dis 171:559–565

Caramelli KE, Staskin DR, Volinn W (2008) Steady-state pharmacokinetics of an investigational oxybutynin gel in comparison with oxybutynin transdermal system. J Urol 179(suppl):1508

Chapple CR, Khullar V, Gabriel Z et al (2008) The effects of antimuscarinic treatments in overactive bladder: an update of a systematic review and meta-analysis. Eur Urol 54:543–562

Chuang YC, Thomas CA, Tyagi T et al (2008) Human urine with solifenacin intake but not tolterodine or darifenacin blocks detrusor overactivity. Int Urogynecol J 19:1353–1357

Chung M, Vashi V, Puente J et al (1999) Clinical pharmacokinetics of doxazosin in a controlled-release gastrointestinal therapeutic system (GITS) formulation. Br J Clin Pharmacol 48: 678–687

Cole P (2004) Fesoterodine, an advanced antimuscarinic for the treatment of overactive bladder: a safety update. Drugs Future 29:715–720

DeWachter S, Wyndaele J (2003) Intravesical oxybutynin: a local anesthetic effect on bladder C afferents. J Urol 169:1892–1895

Drugdex Evaluations (2008a) Fesoterodine. https://www.login.thomson-pharma.com/pcsso/sso

Drugdex Evaluations (2008b) Solifenacin. https://www.login.thomson-pharma.com/pcsso/sso

Drugdex Evaluations (2009a) Oxybutynin. https://www.login.thomson-pharma.com/pcsso/sso

Drugdex Evaluations (2009b) Tolterodine. https://www.login.thomson-pharma.com/pcsso/sso

Dubé JY, Paradis G, Tetu B, Tremblay RR (1989) Synthesis of α1-acid glycoprotein by the human prostate. Prostate 15:251–258

Fuse E, Hashimoto A, Sato N et al (2000) Physiological modeling of altered pharmacokinetics of a novel anticancer drug. UCN-01 (7-hydroxystaurosporine) caused by slow dissociation of UCN-01 from human 1-acid glycoprotein. Pharm Res 17:553–564

Gabay C, Kushner I (1999) Acute-phase proteins and other systemic responses to inflammation. N Engl J Med 340:448–454

Guay R (2003) Clinical pharmacokinetics of drugs used to treat urge incontinence. Clin Pharmacokinet 42:1243–1265

Gupta SK, Sathyan G (1999) Pharmacokinetics of an oral once-a-day controlled-release oxybutynin formulation compared with immediate-release oxybutynin. J Clin Pharmacol 39: 289–296

Hills CJ, Winter SA, Balfour JA (1998) Tolterodine. Drugs 55:813–820
Kaplinsky R (1996) Expanded follow-up of intravesical oxybutynin chloride use in children with neurogenic bladder. J Urol 156:753–756
Kim Y, Yoshimura N, Masida H et al (2005) Intravesical instillation of human urine after oral administration of trospium, tolterodine and oxybutynin in a rat model of detrusor overactivity. BJU Int 97:400–403
Korstanje C, de Wijn S, van den Berg S, Swart P (2002) Selective retention of tamsulosin in prostate and bladder & uroselectivity in rat as compared to dog. J Urol 167(suppl):218
Kremer JMH, Wilting J, HM JL (1988) Drug binding to human alpha-1-acid glycoprotein in health and disease. Pharmacol Rev 40:1–47
Lazar J, Braun DC, Toth A et al (2006) Kinetics of penetration influence the apparent potency of vanilloids on TRPV1. Mol Pharmacol 69:1166–1173
Lehtoranta K, Tainio H, Lukkari-Lax E et al (2002) Pharmacokinetics, efficacy, and safety of intravesical formulation of oxybutynin in patients with detrusor overactivity. Scand J Urol Nephrol 36:18–24
Lewis SA (2000) Everything you wanted to know about the bladder epithelium but were afraid to ask. Am J Renal Physiol 278:F867–F874
MacDiarmid SA (2009) The evolution of transdermal/topical overactive bladder therapy and its benefit over oral therapy. Rev Urol 11:1–6
Magnussen BM, Koskinen LD (2000) In vitro percutaneous penetration of topically applied capsaicin in relation to in vivo sensation responses. Int J Pharm 195:55–62
Malcowicz SB (2006) The role of intravescical chemotherapy in the treatment of bladder cancer. In: Lerner SP, Schoenberg M, Sternberg C (eds) Textbook of bladder cancer. Taylor and Francis, New York
Matsushima H, Kamimura H, Soeishi Y et al (1998) Pharmacokinetics and plasma protein binding of tamsulosin hydrochloride in rats, dogs, and humans. Drug Metab Disp 26:240–245
McGrath JC, Lepor H, Wyllie MG (1996) Report of a unique meeting between the alpha-blocker subcommittee and the pharmaceutical industry. Urology 48:665–667
McNulty AM, Audia JE, Bemis KG et al (2000) Kinetic analysis of LY320236: competitive inhibitor of type I and non-competitive inhibitor of type II human steroid 5α-reductase. J Steroid Biochem Mol Biol 72:13–21
Michel MC, Korstanje C, Krauwinkel W, Kuipers M (2005) The pharmacokinetic profile of tamsulosin oral controlled absorption system (OCAS(R)). Eur Urol 2(suppl 4):15–24
Micomedex Healthcare series (2008a) Dutasteride. https://www.login.thomson-pharma.com/pcsso/sso
Micomedex Healthcare series (2008b) Enablex. https://www.login.thomson-pharma.com/pcsso/sso
Micomedex Healthcare series (2008c) Uroxatral. https://www.login.thomson-pharma.com/pcsso/sso
Micromedex Healthcare series (2008d) Sanctura XR. https://www.login.thomson-pharma.com/pcsso/sso
Micomedex Healthcare series (2009) Tamsulosin. https://www.login.thomson-pharma.com/pcsso/sso
Mizuno N, Niwa T, Yotsumoto Y, Sugyama Y (2003) Impact of drug transporter studies on drug discovery and development. Pharmacol Rev 55:425–461
Moss ML, Kuzmic P, Stuart JD et al (1996) Inhibition of human 5alpha reductases type I and II by 6-aza-steroids: structural determinants of one-step vs. two-step mechanism. Biochemistry 35:3457–3464
Mottet N, Bressolle F, Delmas V et al (2003) Prostatic tissual distribution of alfuzosin in patients with benign prostatic hyperplasia following repeated oral administration. Eur Urol 44:101–105
Nilvebrant L (2000) The mechanism of action of tolterodine. Rev Contemp Pharmacother 11:13–27
Olsson B, Szamosi J (2001) Multiple dose pharmacokinetics of a new once daily extended release tolterodine formulation versus immediate release tolterodine. Clin Pharmacokinet 40:227–235

Quin M, Øie S (1994) Does α1-acid glycoprotein act as a non-functional receptor for α1-adrenergic antagonists? J Pharm Pharmacol 46:896–901

Rapp DE, Lucioni A, Katz EE et al (2004) Use of botulinum-A toxin for the treatment of refractory overactive bladder symptoms: an initial experience. Urology 63:1071–1075

Romics I, Kiss T, Kisbenedek L et al (2003) Tamsulosin drug ratio in prostate versus free fraction in plasma supports pharmacokinetic contribution to its uroselectivity. J Urol 169(suppl):288

Rowland M, Tozer TN (1995) Clinical pharmacokinetics. Williams & Wilkins, New York

Saito M, Watanabe T, Tabuchi M et al (2004) Urodynamic effects and safety of modified intravesical oxybutynin chloride in patients with neurogenic detrusor overactivity: 3 years experience. Int J Urol 11:592–596

Sathayan G, Chancellor MB, Gupta SK (2001) Effect or OROS controlled-release delivery on the pharmacokinetics and pharmacodynamics of oxybutynin chloride. Br J Clin Pharmacol 52:409–417

Sato S, Ohtake A, Matsushima H et al (2001) Pharmacological effect of tamsulosin in relation to dog plasma and tissue concentrations: prostatic and urethral retention possibly contributes to uroselectivity of tamsulosin. J Pharmacol Exp Ther 296:697–703

Shibukawa A, Ishizawa N, Kimura T et al (2002) Plasma protein binding study of oxybutynin by high-performance frontal analysis. J Chromatogr 768:177–188

Silva C, Rio M, Cruz F (2000) Desensitization of bladder sensory fibres by intravesical Resiniferatoxin, a capsaicin analogue: long-term results for the treatment of detrusor hyperreflexia. Eur Urol 38:444–452

Silva C, Ribeiro MJ, Cruz F (2002) The effect of intravesical resiniferatoxin in patients with idiopathic detrusor instability suggests that involuntary detrusor contractions are triggered by C-fiber input. J Urol 168:575–579

Simon HW, Malbotra B (2009) The pharmacokinetic profile of fesoterodine: similarities and differences to tolterodine. Swiss Med Wkly 139:146–151

Sommadossi J-P, Schinazi RF, McMillan A, et al. (1995) A human serum glycoprotein profoundly affects antiviral activity of the protease inhibitor SC-52151 by decreasing its cellular uptake. In: Program and abstracts for the second national conference of human retroviruses and related infections. Washington, DC

Steiner JF (1996) Clinical pharmacokinetics and pharmacodynamics of finasteride. Clin Pharmacokinet 30:16–27

Stuart JD, Lee FW, Simpson Noel D et al (2001) Pharmacokinetic parameters and mechanism of inhibition of rat type 1 and 2 steroid 5alpha-reductasees: determinants for different in vivo activities of GI198745 and finasteride in the rat. Biochem Pharmacol 62:933–942

Vashi V, Chung M, Puente J, Sweeney M (1998) Clinical pharmacokinetics of a controlled-release doxazosin gastrointestinal therapeutic system (GITS) developed for use in BPH. Eur Urol 33 (suppl 1):129

Versi E, Appell R, Mobley D et al (2000) Dry mouth with conventional and controlled-release oxybutynin in urinary incontinence. Obstet Gynecol 95:718–721

Waldeck K, Larsson B, Andersson KE (1997) Comparison of oxybutynin and its active metabolite N-desethyloxybutynin in the human detrusor and parotid gland. J Urol 157:1093–1097

Wolters FLC, Korstanje C, Swart PJ (2004) Uroselectivity of tamsulosin can be explained by higher free fraction in prostate tissue. Abstract Figon Dutch Medicines Days

Yamada S, Tanaka C, Kimura R, Kawabe K (1994) Alpha1-adrenoceptors in human prostate: characterization and binding characteristics of alpha1-antagonists. Life Sci 54:1845–1854

Yamada S, Ohkura T, Deguchi Y, Kimura R (1999) In vivo measurement by [3H] Tamsulosin of α1-adrenoceptors in rat tissues in relation to the pharmacokinetics. J Pharmacol Exp Ther 289:1575–1583

Zobrist RH, Quan D, Thomas HM et al (2003) Pharmacokinetics and metabolism of transdermal oxybutynin in vitro and in vivo performance of a novel delivery system. Pharm Res 20:103–109

α_1-Adrenoceptors in the Urinary Tract

Shizuo Yamada and Yoshihiko Ito

Contents

Abstract α_1-Adrenoceptors have been identified and characterized extensively by functional, radioligand-binding, and molecular biological techniques. Molecular clones have been isolated for three α_1-subtypes (α_{1a}, α_{1b}, and α_{1d}),

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and these subtypes are also functionally characterized. α_1-Adrenoceptors are present in the prostate, urethra, bladder (urothelium, smooth muscle, and afferent nerves), ureter, vas deferens, peripheral ganglia, nerve terminals, vascular tissues, and central nervous system (CNS), and they could all potentially influence overall urinary function and contribute to both the therapeutic and adverse effects of α_1-adrenoceptor antagonists in patients with benign prostatic hyperplasia (BPH) and lower urinary tract symptoms (LUTS). This review aimed to discuss the relevant physiological and pharmacological roles and molecular biology of α_1-adrenoceptor subtypes in the prostate, urethra, bladder, ureter, and CNS.

Keywords α1-Adrenoceptor antagonists · α1-Adrenoceptor subtypes · Lower urinary tract symptom · Urinary tract

1 Introduction

1.1 α_1-Adrenoceptor Subtypes

α_1-Adrenoceptors are activated by adrenaline and noradrenaline. Having bound a ligand, they mediate smooth muscle contraction and other functions through the activation of phospholipase C, generation of the second messengers inositol triphosphate and diacylglycerol, liberation of calcium from the endoplasmic reticulum, and activation of genes (Schwinn 2001). α_1-Adrenoceptors generally mediate their actions through members of the $G_{q/11}$ family of G proteins that stimulate the hydrolysis of inositol phosphate (Hawrylyshyn et al. 2004).

α_1-Adrenoceptors have been identified and characterized extensively by functional, radioligand-binding, and molecular biological techniques. Molecular clones have been isolated and characterized for three α_1-subtypes (α_{1a}, α_{1b}, and α_{1d}) (Schwinn and Michelotti 2000). The subtypes can be distinguished pharmacologically on the basis of their affinity for α_1-adrenoceptor antagonists (Bylund et al. 1994; Michel and Vrydag 2006). The α_{1A}-subtype generally regulates smooth muscle tone in the prostate and bladder neck, whereas the α_{1B}-subtype contributes to regulate blood pressure via contraction of the small resistance vessels (Guimaraes and Moura 2001). The α_{1D}-subtype may be involved in the bladder function and spinal cord innervations (Michel and Vrydag 2006).

Muramatsu et al. (1990) proposed classifying α_1-adrenoceptors with relatively low and high affinity for prazosin as α_{1L} and α_{1H}, respectively, from the antagonism of α-agonist-induced vascular smooth muscle responses, although a corresponding gene for the proposed α_{1L}-adrenoceptor has not been identified. Consequently, it is now generally assumed that the α_{1L}-adrenoceptor represents a phenotype of the cloned α_{1A}-adrenoceptor rather than a distinct receptor subtype.

1.2 Localization in the Urinary Tract

α_1-Adrenoceptors are present in the prostate, urethra, bladder, ureter, vas deferens, peripheral ganglia, nerve terminals, vascular tissues, and central nervous system (CNS), and they could all potentially influence overall urinary function and contribute to both the therapeutic and adverse effects of α_1-adreceptor antagonists.

Walden et al. (1997) have extensively investigated the localization of α_{1A}-adrenoceptor subtype in the prostate and urinary bladder of rats, monkeys, and humans, and they found that α_{1A}-adrenoceptor represented the major subtype in the smooth muscle of these species. The tissue distribution of the various subtypes is species-specific (Schwinn and Michelotti 2000). In humans, α_{1A}-adrenoceptors predominate in the prostate and urethra (Kobayashi et al. 1993; Walden et al. 1997; Kawabe 1998). A study of human prostate tissue found that about 70% of prostatic adrenoceptor mRNA was of the α_{1a}-subtype (Price et al. 1993), although the three α_1-subtypes are distributed differently in the prostatic epithelial and stromal components. Immunohistochemical analysis has revealed that α_{1A}-adrenoceptors are present solely in the stroma; α_{1B}-adrenoceptors are located predominantly in the epithelium, with only weak expression in the stroma; and α_{1D}-adrenoceptors are present in stromal elements, including blood vessels (Walden et al. 1999). However, the majority of α_{1D}-adrenoceptors are found in the detrusor muscle of the bladder, the bladder neck, and the sacral region of the spinal cord (Kawabe 1998; Smith et al 1999), and this subtype is also expressed in the prostate and C-fiber afferents (Price et al. 1993; Tseng-Crank et al. 1995; Nasu et al. 1996; Yokoyama et al. 2006). However, these immunohistochemical data should be interpreted with caution because most α_1-adrenoceptor antibodies lack the promised selectivity for their targets (Jensen et al. 2009; Pradidarcheep et al. 2009).

2 Pharmacology of α_1-Adrenoceptor Antagonists

2.1 Benign Prostatic Hyperplasia and Lower Urinary Tract Symptoms

Benign prostatic hyperplasia (BPH) is a highly prevalent disorder that affects more than 50% of men older than 50 years. Incidence rates increase incrementally with ages. (Boyle and Napalkov 1995; Fine and Ginsberg 2008). BPH is associated with voiding and storage lower urinary tract symptoms (LUTS), which may have a negative impact on the patient's quality of life. LUTS include urgency, frequency, nocturia, and a weak urine stream (Fine and Ginsberg 2008). More serious complications of BPH include acute urinary retention, renal insufficiency, urinary tract infection, gross hemaruria, renal insufficiency, urinary tract infection, gross hematuria, bladder stones, and renal failure.

Although the etiology of BPH has not been clearly defined, the disorder most likely involves age-related proliferation of stromal and glandular cells in the periurethral and transition zones of the prostate gland as well as long-term exposure of prostatic tissue to androgens (Oesterling 1996). The proliferative process that occurs in prostatic tissue may eventually result in an enlarged prostate, which may constrict the urethra and lead to bladder outlet obstruction (BOO). In addition, this process increases the smooth muscle tone of the prostate, which is also associated with urethral constriction and is mediated by α_1-adrenoceptors (Lepor and Lowe 2002). Thus, prostatic α_1-adrenoceptors have received considerable attention in the last decade due to the clinical success of α_1-adrenoceptor antagonists in the treatment of symptoms of BPH (Michel 2010).

2.2 *α_1-Adrenoceptor Antagonists*

Previous findings from pharmacological and molecular biological studies indicated that selective antagonists of α_{1A}-adrenoceptors could be effective in the treatment of urinary obstruction in symptomatic BPH with fewer cardiovascular side effects (Yamada et al. 1992, 1994a, b, c; Kawabe et al. 1994; Walden et al. 1997; Andersson 2002; Michel 2010). The currently prescribed α_1-adrenoceptor antagonists for the treatment of BPH and LUTS are terazosin, doxazosin, alfuzosin, tamsulosin, and silodosin (Schwinn and Roehrborn 2008). General characteristics of α_1-adrenoceptor antagonists (Forray et al. 1994; Kenny et al. 1996; Tatemichi et al. 2006) are summarized in Table 1. Alfuzosin, doxazosin, and terazosin are relatively long-acting and non-subtype selective in that they display equal affinity for all three α_1-adrenoceptor subtypes. In contrast, tamsulosin is selective, preferring the α_{1A}- and α_{1D}-adrenoceptors in vivo and in vitro, while it displays less affinity for the α_{1B}-subtype (Hatano et al. 1994; Ohkura et al. 1998; Yamada et al. 1992, 1994b, 1998, 1999). Silodosin ($\alpha_{1A} > \alpha_{1D} > \alpha_{1B}$) is highly subtype-selective antagonist. Yamada et al. (1994a) demonstrated that tamsulosin displayed about 12 times higher affinity for α_1-adrenoceptors in human prostate than aorta, while prazosin exerted a similar affinity in both tissues, indicating a relatively selective antagonist of human α_1-adrenoceptors. Furthermore, tamsulosin and silodosin administered intravenously and orally in rats exert a more selective and sustained binding of α_{1A}-adrenoceptors in the prostate and submaxillary gland (α_{1A}-subtype-rich tissues) than in the spleen and liver (α_{1B}-subtype-rich tissues) (Ohkura et al. 1998, 2002; Yamada et al. 1998, 1999, 2001). These results clearly indicate the α_{1A}-adrenoceptor selectivity of tamsulosin and silodosin under in vivo condition.

α_1-Adrenoceptor antagonists have comparable efficacy in improving symptoms and maximum urinary flow rate (Milani and Djavan 2005). The major difference may be their associated side effects. Terazosin can cause asthenia, dizziness, somnolence, hypotension, nasal congestion/rhinitis, and impotence (Wilde et al. 1993b); doxazosin can cause dizziness, fatigue, edema, dyspnea, and hypotension (Fulton et al. 1995); and

Table 1 Pharmacological characteristics of α_1-adrenoceptor antagonists used to treat lower urinary tract symptoms (LUTS)

α_1AR-subtype selectivity	Terazosin $\alpha_{1A} = \alpha_{1B} = \alpha_{1D}$	Doxazosin $\alpha_{1A} = \alpha_{1B} = \alpha_{1D}$	Alfuzosin $\alpha_{1A} = \alpha_{1B} = \alpha_{1D}$	Tamsulosin $\alpha_{1A} = \alpha_{1D} > \alpha_{1B}$	Silodosin $\alpha_{1A} > \alpha_{1D} > \alpha_{1B}$
Affinity for human α_1AR subtype (pKi)	8.16 8.71 8.46	8.56 8.98 8.78	8.20 8.53 8.40	9.70 8.90 9.80	10.41 8.19 8.66
α_{1A} α_{1B} α_{1D}					
Selectivity ratio (α_{1A}/α_{1B}) (α_{1D}/α_{1B})	0.3 0.6	0.4 0.6	0.5 0.7	6.3 7.9	166 3.0
Usual daily dose (mg)	1–10	1–8	7.5–10	0.4	4
Regimen (doses/day)	1	1	1–3	1	2
Side effects	Asthenia, dizziness, somnolence, hypotension, nasal congestion/rhinitis, and impotence	Dizziness, fatigue, edema, dyspnea, and hypotension	Dizziness, headache, nausea, dry mouth, diarrhea, and hypotension	Abnormal ejaculation, dizziness, infection, headache, and flu-like symptoms	Abnormal ejaculation, nasal congestion, and dizziness

(Modified from the references by Forray et al. (1994), Kenny et al. (1996), and Tatemichi et al. (2006))

alfuzosin can produce dizziness, headache, nausea, dry mouth, diarrhea, and hypotension (Wilde et al. 1993a). These agents were originally marketed as antihypertensive agents, so these mediations may cause the increased risk of hypotension or dizziness, which is likely related to their vasodilatory properties, when administered at therapeutic doses. Tamsulosin can cause abnormal ejaculation, dizziness, infection, headache, and flu-like symptoms, but this drug does not appear to affect blood pressure, particularly in elderly patients (Lepor 1998). This is probably due to the lack of α_{1B}-adrenoceptor binding. It has been hypothesized that age-relative changes in the distribution of vascular α_1-adrenoceptors may occur, with the greatest increase observed for the α_{1B}-subtype (Schwinn and Michelotti 2000). The most common adverse event of silodosin is retrograde ejaculation, followed by diarrhea and nasopharyngitis, and the incidence of orthostatic hypotension is low (Marks et al. 2009; Schilit and Benzeroual 2009).

In fact, to test the hypothesis that receptor selectivity allows fewer vasodilatory adverse events, a direct comparative study of terazosin (5 mg once daily) and tamsulosin (0.4 mg once daily) was conducted using nocturnal orthostatic stress testing in 50 elderly normotensive patients with LUTS. Symptomatic hypotensive or orthostatic stress occurred more frequently in the terazosin-treated group than in those who received tamsulosin (Harada et al. 1999). In addition, terazosin and doxazosin need to be titrated, and their full therapeutic doses are only achieved 2–4 weeks after the start of administration (de Mey et al. 1998). Alfuzosin and tamsulosin do not require titration. An 8-week, randomized, open-label comparative study of tamsulosin (0.4 mg/day) and terazosin (5 mg/day) demonstrated that subjects treated with tamsulosin had a statistically significant reduction of symptoms after 4 days treatment when compared with subjects treated with terazosin (Narayan et al. 2005). As many elderly patients with BPH may take multiple medications that, in combination, may also exacerbate age-related hypotension; appropriate drug selection is particularly important.

3 Prostate

3.1 mRNA Expression

A competitive study using RT-PCR showed the exclusive presence of α_{1a}-adrenoceptors in the rat prostate (Scofield et al. 1995). Using in situ hybridization, Walden et al. (1997) found that α_{1a}-adrenoceptors were predominantly distributed in the rat prostatic smooth muscle and in the monkey prostate. On the other hand, α_{1d}-adrenoceptors were found to be highly abundant in the rabbit prostate (Suzuki et al. 1997). In the human prostate, Hirasawa et al. (1993) detected only α_{1a}-adrenoceptor mRNA in RT-PCR assays, while α_{1d}-adrenoceptor mRNA has been detected by Northern blotting, RT-PCR, and RNase protection assays (Price et al. 1993; Tseng-Crank et al. 1995; Nasu et al. 1996). Thus, the approximate

relative ratio of α_{1a}-, α_{1b}-, and α_{1d}-adrenoceptor mRNA in the human prostate is 70:0:30%, respectively. Studies using in situ hybridization have also demonstrated the relative predominance of α_{1a}-adrenoceptors (Kawabe et al. 1994; Walden et al. 1997). At the cellular level, α_{1a}-adrenoceptor mRNA was detected in both stromal (smooth muscle) and epithelial (glandular) cells (Tseng-Crank et al. 1995).

3.2 Protein Expression

Prostatic α_1-adrenoceptor has been measured by the radioligand-binding method and quantitative autoradiography in vitro and in vivo (Yamada et al. 1987, 1991, 1992, 1994a, b, c, 1999, 2001; Kawabe et al. 1990, 1994). A study of the intraprostatic distribution of α_1-adrenoceptors found their density to be greater in the adenoma than in the submucosal tissue of the prostatic urethra in enucleated hyperplastic prostate (Kawabe et al. 1990). According to the studies using autoradiography, more α_1-adrenoceptors were located in the stroma (largely smooth muscle) than the glandular tissue of prostates of rats and humans (Chapple et al. 1989; Killam et al. 1995).

α_{1A}-Adrenoceptor in the prostate was characterized by an inhibition of radioligand binding by subtype-selective agents. Deng et al. (1996) obtained monophasic competition curves showing high affinity for 5-methylurapidil (α_{1A}-selective agent) and low affinity for α_{1D}-selective BMY-7378 in the rat prostate, suggesting the α_{1A}-subtype to be most abundant at the protein level. Studies with [^{3}H]tamsulosin have also shown a predominance of high-affinity sites for α_{1A}-adrenoceptors in the rat and human prostates (Yazawa and Honda 1993; Yamada et al. 1994c, 1998, 1999). In the human prostate, Testa et al. (1993) have shown that the ability of several α_1-adrenoceptor antagonists to compete in binding with [^{3}H]prazosin correlated well with that in binding to rabbit liver (α_{1A}-adrenoceptors), but not α_{1D}-adrenoceptor-rich tissue (chloroethylclonidine-treated rat hippocampus) or α_{1B}-adrenoceptor-rich tissue (rat liver). These results indicate the dominance of α_{1A}-adrenoceptors in the human prostate. In similar experiments with α_1-adrenoceptor antagonists, Michel et al. (1996) found the α_{1A}-adrenoceptor to be the most abundant subtype in the human prostate. A similar conclusion was obtained by Yamada et al. (1992, 1994a, b, c). Thus, the α_{1A}-subtype is considered the most abundant in the rat and human prostate at the protein level. The large amount of α_{1A}-adrenoceptor protein is consistent with the mRNA data.

The α_1-adrenoceptor mediating contraction of the rabbit prostate was shown to have characteristics of the α_{1L}-adrenoceptor with low affinity for prazosin (Michel and Vrydag 2006). Weak affinity for prazosin was also found in functional and radioligand-binding experiments with the canine prostate (Ohmura et al. 1993) and rat prostate (Killam et al. 1995). On the other hand, tamsulosin has similar affinity for the classical α_{1A}- and α_{1L}-adrenoceptor subtypes in rabbit prostate (Michel and Vrydag 2006) and this was confirmed in radioligand-binding experiments with [^{3}H]prazosin in bovine prostate, where tamsulosin had a similar high affinity for

prazosin's high- and low-affinity sites (Maruyama et al. 1998). Overall, the evidence suggests that the α_1-adrenoceptors mediating contraction of the rabbit prostate and canine prostate have relatively low affinity for prazosin and other quinazolines; in contrast, the receptors mediating contraction of the human prostate have only moderately lower affinity for prazosin and other quinazolines as would be expected based upon their affinity in binding experiments (Michel and Vrydag 2006).

Yamada et al. (1987, 1991) demonstrated that [^{3}H]prazosin and [^{3}H]bunazosin selectively label α_1-adrenoceptors in the hypertrophied human prostate. They have also showed that there is a greater density of prostatic [^{3}H]prazosin-binding sites in BPH patients than in normal subjects and that tamsulosin (YM-12617) binds α_1-adrenoceptors in the hypertrophied prostate with high affinity and in a stereoselective manner. These results have demonstrated the existence of pharmacologically relevant α_1-adrenoceptors and significant alterations in the human prostate with BPH.

3.3 Functions

3.3.1 In Vitro Effects

The contractile response of prostatic strips isolated from various species was measured with an organ bath technique, and the responsible α_1-subtype was identified using subtype-selective antagonists (Michel and Vrydag 2006). The contraction was predominantly mediated via α_1- rather than α_2-adrenoceptors as demonstrated by a potent and selective antagonism by prazosin of α-agonist- and field stimulation-induced contractions (Tsujii et al. 1992; Lepor et al. 1994; Guh et al. 1995). α_1-Adrenoceptor-mediated contractions in the rat prostate were effectively antagonized by α_{1A}-selective antagonists such as SNAP6383 (Chang et al. 2000) and A-61603 (Chang et al. 2000; Lagu et al. 2000), but not by chloroethylclonidine (α_{1B}-selective antagonist) (Yazawa and Honda 1993) or BMY-7378 (α_{1D}-selective antagonist) (Deng et al. 1996). Therefore, it appears that the contraction of the rat prostate is mediated predominantly, if not exclusively, by the α_{1A}-adrenoceptor. The contraction of the canine prostate also involves predominantly the α_{1A}-subtype (Chang et al. 2000; Lagu et al. 2000). Similarly, it has been reported that the contraction of the human prostate occurs predominantly via an α_{1A}-adrenoceptor, based on the marked potency of moderately α_{1A}-selective drugs, such as WB 4101 and tamsulosin (Marshall et al. 1995; Chueh et al. 1996; Noble et al. 1997), and on the correlation of the potency of antagonists with that for cloned receptor subtypes (Marshall et al. 1995; Kenny et al. 1996). Some studies in isolated human prostate have used field stimulation to release endogenous agonists (Yu et al. 1994; Chueh et al. 1996). Those studies showed α_{1A}-selective antagonists to be highly potent and hence demonstrate that contraction of the human prostate in response not only to exogenous but also to endogenous agonists occurs via this subtype.

The contraction of the isolated rabbit prostate occurs via an α_1-adrenoceptor with relatively low affinity for prazosin (Honda et al. 1985; Hiraoka et al. 1995). In addition, from the relative potency of α_{1A}- (tamsulosin, silodosin, and B8805-33) and α_{1D} (BMY-7378)-selective adrenoceptor antagonists (Delaflotte et al. 1996; Yamagishi et al. 1996; Eltze et al. 2001), it has been shown that the contraction of the rabbit prostate occurs via α_{1A} (α_{1L})-adrenoceptors with low affinity for prazosin. Taken together, the overall evidence demonstrates that contraction of the prostate of rats, dogs, and humans in response to agonists occurs largely via the α_{1A}-subtype.

3.3.2 In Vivo Effects

The alteration of intraurethral pressure (IUP) has been considered as a composite measure of the contractile force developed by the urethra and the surrounding prostate. In fact, the contribution of the prostate seems to dominate because phenylephrine-induced elevations of IUP were markedly reduced in prostate-ablated male rats as compared with prostate-intact rats (Akiyama et al. 1999). The systemic administration of α_1-agonists such as noradrenaline, adrenaline, and phenylephrine has been shown to increase IUP in anesthetized rats (Guilmard et al. 1996; Martin et al. 1997; Akiyama et al. 1999). Agonist-induced elevations of IUP were inhibited by α_1-adrenoceptor antagonists such as alfuzosin, doxazosin, tamsulosin, and terazosin (Kenny et al. 1994; Martin et al. 1997; Akiyama et al. 1999). A similar inhibition of the elevation of IUP induced by the stimulation of hypogastric nerves was reported (Lefevre-Borg et al. 1993; Sato et al. 2001).

On the basis of the studies that compared the effects of antagonists on IUP with those on blood pressure, it is likely that drugs having selectivity for α_{1A}-adrenoceptor subtypes exert greater inhibitory effects on IUP than on blood pressure (Kenny et al. 1996; Akiyama et al. 1999). These data, together with numerous studies in vitro on the isolated prostate, clearly indicate that the contraction is mainly mediated through the α_{1A}-adrenoceptor subtype. In fact, tamsulosin and silodosin are effective in lowering bladder outlet resistance in patients with BPH (Abrams et al. 1998; Schilit and Benzeroual 2009).

4 Urethra

4.1 mRNA and Protein Expression

The urethras of various species including humans seem to contain largely α_{1A}-adrenoceptors. The presence of α_1-adrenoceptors has been assessed at the mRNA and protein level. Using real-time PCR, Yono et al. (2004) showed the following rank order of expression: $\alpha_{1a} > \alpha_{1b} \geqq \alpha_{1d}$. RNase protection assays detected the

α_{1a}-adrenoceptor as the most abundant subtype in the male and female proximal urethra, while the α_{1d}-subtype mRNA was seen only in female tissues (Nasu et al. 1998). These authors also showed that the α_{1a}-signal predominated in the urethral smooth muscle using in situ hybridization.

Studies using radioligand binding and autoradiography have confirmed the presence of α_1 (possibly α_{1A}) adrenoceptors at the protein level in smooth and longitudinal muscle of urethras from monkeys and rabbits (Andersson et al. 1984; Larsson et al. 1986; Lefevre-Borg et al. 1993; Testa et al. 1993; Walden et al. 1997). The proximal and distal parts of the rabbit urethra expressed similar densities of α_1-adrenoceptors (Larsson et al. 1986). The density of α_1-adrenoceptor in the urethra was greatest in rats, followed by humans, dogs, and rabbits, and these receptors largely represented the α_{1A}-subtype (chloroethylclonidine-insensitive sites) (Testa et al. 1993).

4.2 In Vitro and In Vivo Effects

According to field stimulation studies, the proximal urethras of rabbits appear largely under the control of the parasympathetic nervous system, whereas the medial and distal urethra are largely controlled by the sympathetic nervous system (Deplanne et al. 1998). α_1-Adrenoceptor-mediated urethral contraction was demonstrated in rats (Lluel et al. 2003), rabbits, dogs (Testa et al. 1993), and humans (Kunisawa et al. 1985). In fact, experiments with subtype-selective antagonists have shown that the contraction of the rabbit urethra is mediated by α_{1A}-adrenoceptors (Testa et al. 1993), but the contraction exerts rather low affinity for prazosin, indicative of the α_{1L}-phenotype of α_{1A}-adrenoceptors. Furthermore, Testa et al. (1993) showed that the α_{1A}-adrenoceptor-mediated contraction of the rabbit urethra is sensitive to inhibition by the Ca^{2+} channel antagonist nifedipine. The α_1-adrenoceptors mediating contraction of the human urethra displayed high affinity for prazosin, indicative of the α_{1A}-subtype.

Takeda et al. (2003) reported that α_1-adrenoceptor agonists increased urethral pressure in anesthetized rats. The hypogastric nerve-stimulated urethral contraction in anesthetized cats was identified as an α_1-adrenoceptor-mediated response (Lefevre-Borg et al. 1993; Springer et al. 1994). The results of functional studies with α_1-agonists and α_1-antagonists in vivo may be associated with the effects on prostatic rather than urethral α_1-adrenoceptors (Akiyama et al. 1999). Yokoyama et al. (2007) have demonstrated that prostaglandin E2 enhances the micturition reflex through C-fiber afferents, and the intravenous injection of tamsulosin significantly increased the interval between bladder contractions in rats not treated with resiniferatoxin that were receiving intraurethral but not intravesical prostaglandin E2. They concluded that tamsulosin exerts an inhibitory effect on the urethral C-fiber afferent nerves.

5 Bladder

5.1 mRNA Expression

A competitive RT-PCR study showed that α_{1a}-, α_{1b}-, and α_{1d}-adrenoceptors accounted for 95, 1, and 4%, respectively, of all α_1-adrenoceptor mRNA in the rat bladder (Scofield et al. 1995), whereas a latter study revealed the presence of the three subtypes at a ratio of 70:5:25% (Hampel et al. 2002). The mRNA expression was markedly greater in the rat bladder base than in the detrusor (Yono et al. 2004). The predominance of the α_{1A}-subtype was confirmed quantitatively using in situ hybridization, which demonstrated strong expression in the urothelium and moderate expression in smooth muscle (Walden et al. 1997). These authors have shown similar distribution in the rhesus monkey bladder. Walden et al. (1997) also showed a moderate expression of α_{1A}-adrenoceptors in the human bladder dome smooth muscle, but not in the bladder urothelium or connective tissue. Nomiya and Yamaguchi (2003) have shown a moderate mRNA expression of α_{1A}-adrenoceptors in the human bladder, involving the α_{1a} (33%)-, α_{1b} (53%)-, and α_{1d} (14%)-subtypes.

Experiments by Sigala et al. (2004) using semiquantitative RT-PCR showed a dominance of α_{1a}- and α_{1d}-adrenoceptor mRNA with less α_{1b}-subtype mRNA in the human bladder. RNase protection assays by Malloy et al. (1998) revealed the α_{1a}-, α_{1b}-, and α_{1d}-adrenoceptors to be in a ratio of 34, 0, and 66%, respectively. Taken together, the total amount of α_1-adrenoceptor mRNA expressed in the detrusor seems low, while the α_{1A}-adrenoceptor is the most abundant subtype in the rat bladder and the α_{1A}- and α_{1D}-adrenoceptors may be present in the human bladder.

5.2 Protein Expression

The radioligand-binding assay and autoradiography with [^{125}I]BE 2254 ([^{125}I]HEAT) and [^{3}H]prazosin showed a low density of α_1-adrenoceptors in the rat bladder, representing a homogeneous population comprising the α_{1A}-subtype (Monneron et al. 2000; Hampel et al. 2002). Autoradiography with [^{3}H]prazosin showed very few α_1-adrenoceptors in the urothelial or smooth muscle of the rhesus monkey detrusor (Walden et al. 1997). It also showed a greater distribution of α_1-adrenoceptors in the monkey bladder base than detrusor, based on competitive inhibition by the α_{1A}-selective antagonist SNP 5272. (Walden et al. 1997).

α_1-Adrenoceptor was also found in the human bladder base (Levin et al. 1988). Studies in the human detrusor using [^{125}I]BE 2254 revealed low α_1-adrencoeptor density, and 60% of these receptors were described as α_{1D}-adrenoceptors (Malloy et al. 1998). Western blotting with subtype-selective antibodies by

Sigala et al. (2004) showed the presence of all three subtypes at the protein level in the human detrusor. Thus, the density of α_1-adrencoeptors in the detrusor of various species including humans is relatively low. Therefore, consistent with mRNA measurements, radioligand-binding and receptor autoradiography-based studies have shown low densities of α_1-adrenoceptors in the detrusor of several species including humans. α_1-Adrenoceptors seemed to be present in the trigone/bladder base/bladder neck region. The α_{1A}-adrenoceptor appears to be the most abundant subtype in rats, while the α_{1D}-adrenoceptor seems to be the most abundant subtype in humans.

5.3 Functions

5.3.1 In Vitro Effects

In most species including humans, the stimulation of α_1-adrenoceptors produces a relatively weak contraction of the detrusor, while a stronger contraction is observed for the trigone, bladder base, and bladder neck. The physiological relevance of these effects remains unclear, since the bladder neck appears largely under the control of the parasympathetic rather than sympathetic nervous system (Deplanne et al. 1998). In analogy to rats and rabbits, studies in the human detrusor have demonstrated only weak contraction by phenylephrine (Nomiya and Yamaguchi 2003). A stronger contraction was observed in the human bladder base and bladder neck (Caine et al. 1975). The contraction caused by α_1-agonists in the human bladder was markedly inhibited by a α_{1A}-adrenoceptor-selective antagonist, suggesting α_{1A}-subtype mediation (Chang et al. 2000).

α_1-Adrenoceptors in the bladder may affect the contractile response by modulating the release of neurotransmitters. In fact, α_1-adrenoceptor agonists enhanced the field stimulation-induced release of both acetylcholine and noradrenaline in the isolated rat bladder (Somogyi et al. 1995). Thus, it was concluded that cholinergic nerve terminals in rat bladder express α_1-adrenocoeptors that facilitate acetylcholine release. Szell et al. (2000) have shown that the enhancement of acetylcholine release was largely mediated by α_{1A}-adrenoceptors.

Trevisani et al. (2007) examined the influence of α_1-adrenoceptor agonists and antagonists on the release of substance P from primary sensory neurons of the lower urinary tract in rats. They concluded that α_1-adrenoceptors are functionally expressed by the capsaicin-sensitive, nociceptive, primary sensory neurons of the rat lower urinary tract, and their activation might contribute to the signaling of irritative and nociceptive responses arising from this region. Thus, part of the beneficial effects of α_1-adrenoceptor antagonists in the amelioration of storage symptoms in the lower urinary tract could be derived from their inhibitory effect on neurogenic inflammatory responses.

5.3.2 In Vivo Effects

The bladder receives innervation by autonomic nerves. Sacral parasympathetic nerves induce micturition, whereas lumbar sympathetic nerves modulate micturition and promote urine storage (de Groat et al. 1993). α_1-Adrenoceptors are present in the neural pathways to the bladder. Various factors are thought to be affected by actions mediated via the α_1-adrenoceptors, including the detrusor smooth muscle, the detrusor vasculature, afferent and efferent nerve terminals, intramural ganglia, the urothelium, and interstitial cells (Andersson and Gratzke 2007).

α_1-Adrenoceptors are also involved in the peripheral control of sympathetic supply to the bladder and thus storage. In this respect, stimulation of the hypogastric nerve has been shown to facilitate cholinergic transmission at the level of the pelvic ganglia by the actions of α_1-adrenoceptors (Keast et al. 1990) and thus also enhancing bladder contractions. The intravenous injection of α_1-adrenoceptor antagonists inhibited the sympathetic control of the bladder by reducing hypogastric nerve activity (Danuser and Thor 1995; Ramage and Wyllie 1995) and somatic activity to the urethra (Danuser and Thor 1995). In addition, α_1-adrenoceptors are assumed to suppress micturition via a peripheral mechanism (Jeong and Lee 2000).

Very recently, Okutsu et al. (2010) evaluated the effects of tamsulosin on bladder blood flow (BBF) by the fluoromicrosphere method and bladder function in rats with BOO. They demonstrated that subcutaneous treatment with tamsulosin significantly increased BBF in BOO rats with an improvement of the decrease in mean voided volume. The α_1-adrenoceptors expressed in the vesical artery were α_{1A}- > α_{1D}-adrenoceptors, with almost no expression of the α_{1B}-subtype detected. Thus, it was concluded that tamsulosin increased BBF in BOO rats via an antagonistic effect, presumably on the α_{1A}- and/or α_{1D}-adrenoceptors in the vesical artery, and improved the decrease in mean voided volume.

The effects of tamsulosin on bladder function and premicturition contraction in conscious rats with BOO were examined (Ohtake et al. 2006). Tamsulosin was shown to be effective against both premicturition contraction and IUP response in the same dose range in rats. These results partly support the idea that tamsulosin improved the storage symptoms as well as voiding symptoms in patients with LUTS associated with BOO by blocking α_1-adrenoceptors. Experimental findings have indicated involvement of the α_{1D}-adrenoceptor subtype in storage symptoms (Michel and Vrydag 2006). Thus, the α_1-adrenoceptor is considered responsible for the dynamic component of voiding and storage symptoms.

Birder and de Groat (2007) indicated the possible involvement of the urothelium in bladder dysfunction. Expression of α-adrenoceptors in the urothelium has been well documented. The upregulation can trigger the release of a number of mediators including ATP and nitric oxide. Ishihama et al. (2006) found the presence of α_{1D}-adrenoceptors in the rat urothelium and suggested that activation of these adrenoceptors facilitates the micturition reflex. They suggested that endogenous catecholamines act on α_{1D}-receptors in the urothelium to facilitate mechanosensitive bladder afferent nerve activity and reflex voiding. In addition,

Birder and de Groat (2007) assumed that catecholamines could be released from nerves adjacent to the urothelium or from nerves innervating nearly blood vessels. Whether or not such effects contribute to the clinical efficacy of α_1-adrenoceptor antagonists remains to be established.

6 Ureter

6.1 mRNA and Protein Expression

α_1-Adrenoceptors have been demonstrated in the ureters of humans, pigs, dogs, and horses, and indeed are more abundant than other adrenoceptors in the ureteral smooth muscle (Weiss et al. 1978; Morita et al. 1994). The relative mRNA expression levels of α_{1a}-, α_{1b}-, and α_{1d}-adrenoceptors were 74.5, 14.3, and 11.2, respectively, in the mouse ureter (Kobayashi et al. 2009a), while they were 24.1, 3.2, and 72.7%, respectively, in the dog ureter (Kobayashi et al. 2009b). Thus, the predominant subtype of ureteral α_1-adrenoceptors was α_{1a} in mice and α_{1d} in dogs. Tomiyama et al. (2007) determined mRNA levels and receptor protein expression by real-time quantitative RT-PCR and immunohistochemical analysis in hamster ureteral smooth muscle. The relative mRNA expression levels of α_{1a}-, α_{1b}-, and α_{1d}-adrenoceptors were 10.7, 1.2, and 88.1%, respectively, and protein expression was identified for α_{1A}- and α_{1D}-adrenoceptors immunohistochemically. The mRNA of three subtypes of α_1-adrenoceptor also reportedly expresses in the human ureteral smooth muscle (Sigala et al. 2005; Itoh et al. 2007).

6.2 In Vitro and In Vivo Effects

Adrenaline, noradrenaline, and phenylephrine produced a concentration-dependent contraction in isolated ureteral preparations of hamsters, mice, and dogs (Wanajo et al. 2005; Tomiyama et al. 2007; Kobayashi et al. 2009a, b). These agonist-induced contractions were competitively antagonized by silodosin, prazosin, and BMY-7378, with much higher potency of silodosin than BMY-7378. These findings indicate that the α_{1A}-adrenoceptor plays a major role in the α_1-agonist-induced contraction of the mouse and dog ureter. Tamsulosin has been found to inhibit ureteral contractions in humans (Küpeli et al 2004). Therefore, there seems to be no species differences among mice, hamsters, and humans in terms of the α_{1A}-adrenoceptor subtype participating in urethral contractions (Tomiyama et al. 2007; Sasaki et al. 2008).

The autonomic nervous system is known to play an important role in modulating ureteral motility (Schulman 1974). In an acute-ureteral-obstruction model using anesthetized dogs, phenylephrine elevated basal ureteral pressure

without enhancing peristalsis (Tomiyama et al. 2002). In another study, the intravenous administration of noradrenaline or phenylephrine reduced both bolus volume and the fluid flow of urine in anasthetized dogs (Morita et al. 1987). Thus, the stimulation of α_{1A}-adrenceptors is likely involved in the maintenance of ureteral tonus and resistance.

Urolithiasis, a common urologic condition, has been detected in 12% of the world's population, although the number of patients is increasing, particularly in Western countries (Menon et al. 1998; Tiselius 2003). α_1-Adrenoceptor antagonists facilitate the passage of ureteral stones in patients with urolithiasis (Yilmaz et al. 2005; Hollingsworth et al. 2006). In such patients, tamsulosin facilitates the spontaneous passage of stones and reduces the time taken to expel the stones (Beach and Mauro 2006; Parsons et al. 2007; Michel and de la Rosette 2006), indicating the close relation of α_{1A}- and/or α_{1D}-adreceptor stimulation to ureteral contraction. Currently, Sasaki et al. (2008) also found that the α_{1A}-adrenoceptor was mainly involved in phenylephrine-induced ureteral contractions in the isolated human ureter. The findings may indicate that α_{1A}-adrenoceptor antagonists could become useful medications for stone passage in urolithiasis patients. In fact, Tzortzis et al. (2009) noted in a review that "medical expulsive therapy," using α_1-adrenoceptor antagonists such as tamsulosin, augments the stone expulsion rate compared to standard therapy for moderately sized distal ureteral stones.

7 Central Nervous System

Many studies have shown that α_1-adrenoceptor mRNA is widely distributed in the brain and spinal cord, including within structures known to be involved in the control of micturition (Day et al. 1997; Domyancic and Morilak 1997; Smith et al. 1999; Thor and Donatucci 2004). Bladder and urethral activation through these pathways might involve excitatory α_1-adrenoceptors, which seem to be tonically active in both the sympathetic and somatic neural control of the lower urinary tract (Thor and Donatucci 2004).

The overall findings made in vivo suggest that spinal α_1-adrenoceptors are probably involved in mediating bladder contractions and decreasing the frequency of micturition. Thus, systemically administered α_1-adrenoceptor antagonists that penetrate the CNS are considered to inhibit bladder contractions. In anesthetized rats, intrathecal injections of prazosin inhibited the bladder contraction evoked from the locus caeruleus (Yoshimura et al. 1988). In addition, the intrathecal administration of α_1-adrenoceptor antagonists increased bladder capacity and decreased maximal vesical pressure during voiding (Ishizuka et al. 1997; Jeong and Lee 2000). Ishizuka et al. (1996) also showed that the intrathecal injection of doxazosin decreased micturition pressure and frequency in the continuous cystometry in conscious rats with and without BOO. The isovolumetric bladder contraction in rats was transiently abolished by the intrathecal injection of tamsulosin or naftopidil (Sugaya et al. 2002). The α_1-adrenoceptor selectivity of naftopidil is

$\alpha_{1D} \geqq \alpha_{1B} > \alpha_{1A}$. Therefore, in addition to the antagonistic effect of these agents on the α_{1A}-adrenoceptors of prostatic smooth muscles, it is speculated that both agents, especially naftopidil, also target the α_1(α_{1A} and/or α_{1D})-adrenoceptors in the lumbosacral cord. Yokoyama et al (2006) investigated the effect of naftopidil on cerebral infarction-induced overactivity of the detrusor in rats, with or without pretreatment with resiniferatoxin to functionally eliminate C-fibers. The intrathecal or intravenous injection of naftopidil enlarged bladder capacity without decreasing bladder contraction pressure or increasing residual volume in C-fiber-intact rats. By contrast, naftopidil did not increase the bladder capacity in C-fiber-desensitized rats with cerebral infarction, an observation that supports the hypothesis that naftopidil improves detrusor overactivity by inhibiting C-fiber afferents in the spinal cord. This concept is of particular interest considering the finding that α_{1D}-adrenocepoter mRNA is the predominant subtype in the human spinal cord (Smith et al. 1999).

α_{1D}-Adrenoceptors in the detrusor and their levels of expression might not always be relevant to the functional importance of this receptor subtype. The α_{1D}-adrenoceptors in bladder structures such as the urothelium involved in afferent signaling or the extravesical α_{1D}-subtype in afferent pathways, ganglia, and the CNS could be more important for the control of bladder function. The importance of α_{1D}-adrenoceptors for controlling micturition was further investigated in knockout mice. Nakamura et al. (2004) and Chen et al. (2005) showed that α_{1d}-knockout mice had greater bladder capacity and larger voided volumes than wild-type controls, supporting an important role for the α_{1D}-adrenoceptors in the control of voiding.

The permeability though the blood–brain barrier (BBB) is critical for the appearance of CNS effect of α_1-adrenoceptor antagonists in vivo. Alfuzosin and tamsulosin exhibit almost no penetration into the CNS. In fact, we found the absence of in vivo α_1-adrenoceptor binding of [^{3}H]prazosin, [^{3}H]tamsulosin, and [^{3}H]silodosin in the rat brain after the intravenous injection, possibly being due to the limited brain distribution by poor permeability through the BBB (Yamada et al. 1999, 2001). This idea is supported by the ex vivo observation that there is little alteration in α_1-adrenoceptor-binding parameters in brain tissues of rats after oral administration of prazosin and tamsulosin at pharmacological doses (Ohkura et al. 1998, 2002). Thus, it seems unlikely that these agents have some clinical effects on CNS.

8 Conclusion

α_1-Adrenoceptors in the urinary tract and CNS play a critical role in urinary function and also mediate many pharmacological effects of α_1-adrenoceptor antagonists. The downstream effectors beyond the receptors are increasingly being elucidated. This increased understanding may promote the development of new therapeutic agents in the near future. A knowledge of the pharmacology of α_1-adrenoceptor subtypes in the urinary tract should facilitate not only the rational use of α_1-adrenoceptor antagonists in the treatment of BPH and LUTS, but also the discovery of therapeutic agents for new target molecules. Finally, understanding the

molecular mechanism transducing the functional effects of α_1-adrenoceptors may yield further targets for future drug development.

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References

Abrams P, Tammela TL, Hellström P, Marberger M, Djavan BR, Lunglmayr G, Panos P, Labal KU (1998) European pressure-flow investigation of tamsulosin in men with lower urinary tract symptoms (LUTS) suggestive of benign prostatic obstruction (BPO) – ESPRIT study. J Urol 159:256

Akiyama K, Hora M, Tatemichi S, Masuda N, Nakamura S, Yamagishi R, Kitazawa M (1999) KMD-3213, a uroselective and long-acting α_{1a}-adrenoceptor antagonist, tested in a novel rat model. J Pharmacol Exp Ther 291:81–91

Andersson KE (2002) Alpha-adrenoceptors and benign prostatic hyperplasia: basic principles for treatment with alpha-adrenoceptor antagonists. World J Urol 19:390–396

Andersson KE, Gratzke C (2007) Pharmacology of α_1-adrenoceptor antagonists in the lower urinary tract and central nervous system. Nat Clin Pract Urol 4:368–378

Andersson KE, Larsson B, Sjogren C (1984) Characterization of the α-adrenoceptors in the female rabbit urethra. Br J Pharmacol 81:293–300

Beach MA, Mauro LS (2006) Pharmacologic expulsive treatment of ureteral calculi. Ann Pharmacother 40:1361–1368

Birder LA, de Groat WC (2007) Mechanisms of disease: involvement of the urothelium in bladder dysfunction. Nat Clin Pract Urol 4:46–54

Boyle P, Napalkov P (1995) The epidemiology of benign prostatic hyperplasia and observations on concomitant hypertension. Scand J Urol Nephrol Suppl 168:7–12

Bylund DB, Eikenberg DC, Hieble JP, Langer SZ, Lefkowitz RJ, Minneman KP, Molinoff PB, Ruffolo RR Jr, Trendelenburg U (1994) International Union of Pharmacology nomenclature of adrenoceptors. Pharmacol Rev 46:121–136

Caine M, Raz S, Zeigler M (1975) Adrenergic and cholinergic receptors in the human prostate, prostatic capsule and bladder neck. Br J Urol 47:193–202

Chang RS, Chen TB, O'Malley SS, Pettibone DJ, DiSalvo J, Francis B, Bock MG, Freidinger R, Nagarathnam D, Miao SW, Shen Q, Lagu B, Murali Dhar TG, Tyagarajan S, Marzabadi MR, Wong WC, Gluchowski C, Forray C (2000) In vitro studies on L-771, 688 (SNAP 6383), a new potent and selective α1a-adrenoceptor antagonist. Eur J Pharmacol 409:301–312

Chapple CR, Aubry ML, James S, Greengrass PM, Burnstock G, Turner-Warwick RT, Milroy EJ, Davey MJ (1989) Characterization of human prostatic adrenoceptors using pharmacology receptor binding and localization. Br J Urol 63:487–496

Chen Q, Takahashi S, Zhong S, Hosoda C, Zheng HY, Ogushi T, Fujimura T, Ohta N, Tanoue A, Tsujimoto G, Kitamura T (2005) Function of the lower urinary tract in mice lacking α_{1d}-adrenoceptor. J Urol 174:370–374

Chueh SC, Guh JH, Chen J, Lai MK, Ko FN, Teng CM (1996) Inhibition by tamsulosin of tension responses of human hyperplastic prostate to electrical field stimulation. Eur J Pharmacol 305:177–180

Danuser H, Thor KB (1995) Inhibition of central sympathetic and somatic outflow to the lower urinary tract of the cat by the α_1 adrenergic receptor antagonist prazosin. J Urol 153:1308–1312

Day HE, Campeau S, Watson SJ Jr, Akil H (1997) Distribution of α_{1a}-, α_{1b}- and α_{1d}-adrenergic receptor mRNA in the rat brain and spinal cord. J Chem Neuroanat 13:115–139

de Groat WC, Booth AM, Yoshimura N (1993) Neurophysiology of micturition and its modification in animal models of human disease. In: Maggi CA (ed) The autonomic nervous system nervous control of the urogenital system, vol 6. Harwood Academic Publishers, London, p 227

de Mey C, Michel MC, McEwen J, Moreland T (1998) A double-blind comparison of terazosin and tamsulosin on their differential effects on ambulatory blood pressure and nocturnal orthostatic stress testing. Eur Urol 33:481–488

Delaflotte S, Auguet M, Chabrier PE (1996) Pharmacological evidence that different α_1 adrenoceptor subtypes mediate contraction in rabbit prostate and hypogastric artery. Acta Physiol Scand 158:241–251

Deng XF, Chemtob S, Varma DR (1996) Characterization of α_{1d}- adrenoceptor subtype in rat myocardium, aorta and other tissues. Br J Pharmacol 119:269–276

Deplanne V, Palea S, Angel I (1998) The adrenergic, cholinergic and NANC nerve-mediated contractions of the female rabbit bladder neck and proximal, medial and distal urethra. Br J Pharmacol 123:1517–1524

Domyancic AV, Morilak DA (1997) Distribution of α_{1A} adrenergic receptor mRNA in the rat brain visualized by *in situ* hybridization. J Comp Neurol 386:358–378

Eltze M, Boer R, Michel MC, Hein P, Testa R, Ulrich WR, Kolassa N, Sanders KH (2001) In vitro and in vivo uroselectivity of B8805-033, an antagonist with high affinity at prostatic α_{1A}- vs. α_{1B}- and α_{1D}-adrenoceptors. Naunyn Schmiedebergs Arch Pharmacol 363:649–662

Fine SR, Ginsberg P (2008) Alpha-adrenergic receptor antagonists in older patients with benign prostatic hyperplasia: issues and potential complications. J Am Osteopath Assoc 108:333–337

Forray C, Bard JA, Wetzel JM, Chiu G, Shapiro E, Tang R, Lepor H, Hartig PR, Weinshank RL, Branchek TA, Gluchowski C (1994) The α_1-adrenergic receptor that mediates smooth muscle contraction in human prostate has the pharmacological properties of the cloned human α_{1c} subtype. Mol Pharmacol 45:703–708

Fulton B, Wagstaff AJ, Sorkin EM (1995) Doxazosin. An update of its clinical pharmacology and therapeutic applications in hypertension and benign prostatic hyperplasia. Drugs 49:295–320

Guh JH, Chueh SC, Ko FN, Teng CM (1995) Characterization of α_1-adrenoceptor subtypes in tension response of human prostate to electrical field stimulation. Br J Pharmacol 115:142–146

Guilmard C, Auguet M, Chabrier PE (1996) Pharmacological characterization of alpha$_1$-adrenoceptor subtype mediating regulation of arterial pressure and urethral perfusion pressure in the anaesthetized rat. J Auton Pharmacol 16:197–203

Guimaraes S, Moura D (2001) Vascular adrenoceptors: an update. Pharmacol Rev 53:319–356

Hampel C, Dolber PC, Smith MP, Savic SL, JW Throff, Thor KB, Schwinn DA (2002) Modulation of bladder α1-adrenergic receptor subtype expression by bladder outlet obstruction. J Urol 167:1513–1521

Harada K, Ohmori M, Kitoh Y, Sugimoto K, Fujimura A (1999) A comparison of the antagonistic activities of tamsulosin and terazosin against human vascular α_1-adrenoceptors. Jpn J Pharmacol 80:209–215

Hatano A, Takahashi H, Tamaki M, Komeyama T, Koizumi T, Takeda M (1994) Pharmacological evidence of distinct α_1-adrenoceptor subtypes mediating the contraction of human prostatic urethra and peripheral artery. Br J Pharmacol 113:723–728

Hawrylyshyn KA, Michelotti GA, Coge F, Guenin SP, Schwinn DA (2004) Update on human α_1-adrenoceptor subtype signaling and genomic organization. Trends Pharmacol Sci 25:449–455

Hiraoka Y, Ohmura T, Sakamoto S, Hayashi H, Muramatsu I (1995) Identification of α_1-adrenoceptor subtypes in the rabbit prostate. J Auton Pharmacol 15:271–278

Hirasawa A, Horie K, Tanaka T, Takagaki K, Murai M, Yano J, Tsujimoto G (1993) Cloning, functional expression and tissue distribution of human cDNA for the α_{1C}- adrenergic receptor. Biochem Biophys Res Commun 195:902–909

Hollingsworth JM, Rogers MA, Kaufman SR, Bradford TJ, Saint S, Wei JT, Hollenbeck BK (2006) Medical therapy to facilitate urinary stone passage: a meta-analysis. Lancet 368: 1171–1179

Honda K, Miyata-Osawa A, Takenaka T (1985) α_1-Adrenoceptor subtype mediating contraction of the smooth muscle in the lower urinary tract and prostate of rabbits. Naunyn Schmiedebergs Arch Pharmacol 330:16–21

Ishihama H, Momota Y, Yanase H, Wang X, de Groat WC, Kawatani M (2006) Activation of α_{1D} adrenergic receptors in the rat urothelium facilitates the micturition reflex. J Urol 175:358–364

Ishizuka O, Persson K, Mattiasson A, Naylor A, Wyllie M, Andersson KE (1996) Micturition in conscious rats with and without bladder outlet obstruction: role of spinal α_1-drenoceptors. Br J Pharmacol 117:962–966

Ishizuka O, Pandita RK, Mattiasson A, Steers WD, Andersson KE (1997) Stimulation of bladder activity by volume, L-dopa and capsaicin in normal conscious rats -effects of spinal α_1-adrenoceptor blockade. Naunyn Schmiedebergs Arch Pharmacol 355:787–793

Itoh Y, Kojima Y, Yasui T, Tozawa K, Sasaki S, Kohri K (2007) Examination of alpha 1 adrenoceptor subtypes in the human ureter. Int J Urol 14:749–753

Jensen BC, Swigart PM, Simpson PC (2009) Ten commercial antibodies for alpha-1-adrenergic receptor subtypes are nonspecific. Naunyn-Schmiedebergs Arch Pharmacol 379:409–412

Jeong MS, Lee JG (2000) The role of spinal and peripheral α_1- and α_2- adrenoceptors on bladder activity induced by bladder distension in anaesthetized rat. BJU Int 85:925–931

Kawabe K (1998) Current status of research on prostate-selective alpha 1-antagonists. Br J Urol 81 (Suppl 1):48–50

Kawabe K, Moriyama N, Hamada K, Ishima T (1990) Density and localization of alpha$_1$-adrenoceptors in hypertrophied prostate. J Urol 143:592–595

Kawabe K, Moriyama N, Yamada S, Taniguchi N (1994) Rationale for the use of α-blockers in the treatment of benign prostatic hyperplasia (BPH). Int J Urol 1:203–211

Keast JR, Kawatani M, De Groat WC (1990) Sympathetic modulation of cholinergic transmission in cat vesical ganglia is mediated by α_1- and α_2-adrenoceptors. Am J Physiol 258:R44–50

Kenny BA, Naylor AM, Carter AJ, Read AM, Greengrass PM, Wyllie MG (1994) Effect of alpha$_1$ adrenoceptor antagonists on prostatic pressure and blood pressure in the anesthetized dog. Urology 44:52–57

Kenny BA, Miller AM, Williamson IJ, O'Connell J, Chalmers DH, Naylor AM (1996) Evaluation of the pharmacological selectivity profile of α_1- adrenoceptor antagonists at prostatic α_1- adrenoceptors: binding, functional and in vivo studies. Br J Pharmacol 118:871–878

Killam AL, Watts SW, Cohen ML (1995) Role of α_1- adrenoceptors and 5-HT$_2$ receptors in serotonin-induced contraction of rat prostate: autoradiographical and functional studies. Eur J Pharmacol 273:7–14

Kobayashi S, Tang R, Shapiro E, Lepor H (1993) Characterization and localization of prostatic alpha 1 adrenoceptors using radioligand receptor binding on slide-mounted tissue section. J Urol 150:2002–2006

Kobayashi S, Tomiyama Y, Hoyano Y, Yamazaki Y, Hiroshi KUsama, Itoh Y, Kubota Y, Kohri K (2009a) Gene expressions and mechanical functions of α_1-adrenoceptor subtypes in mouse ureter. World J Urol. doi:10.1007/s00345-009-0396-y

Kobayashi S, Tomiyama Y, Hoyano Y, Yamazaki Y, Kusama H, Kubota Y, Sasaki S, Kohri K (2009b) Mechanical function and gene expression of α_1-adrenoceptor subtypes in dog intravesical ureter. Urology 74:458–462

Kunisawa Y, Kawabe K, Niijima T, Honda K, Takenaka T (1985) A pharmacological study of alpha adrenergic receptor subtypes in smooth muscle of human urinary bladder base and prostatic urethra. J Urol 134:396–398

Küpeli B, Irkilata L, Gürocak S, Tunç L, Kiraç M, Karaoğlan U, Bozkirli I (2004) Does tamsulosin enhance lower ureteral stone clearance with or without shock wave lithotripsy? Urology 64:1111–1115

Lagu B, Tian D, Jeon Y, Li C, Wetzel JM, Nagarathnam D, Shen Q, Forray C, Chang RS, Broten TP, Ransom RW, Chan TB, O'Malley SS, Schorn TW, Rodrigues AD, Kassahun K, Pettibone DJ, Freidinger RO, Gluchowski C (2000) *De novo* design of a novel oxazolidinone analogue as

a potent and selective α_{1A} adrenergic receptor antagonist with high oral bioavailability. J Med Chem 43:2775–2778

Larsson B, Sjogren C, Andersson KE (1986) Regional distribution of α-adrenoceptor subtypes in the female rabbit urethra. Acta Physiol Scand 126:39–43

Lefevre-Borg F, O'Connor SE, Schoemaker H, Hicks PE, Lechaire J, Gautier E, Pierre F, Pimoule C, Manoury P, Langer SZ (1993) Alfuzosin, a selective α_1-adrenoceptor antagonist in the lower urinary tract. Br J Pharmacol 109:1282–1289

Lepor H (1998) Phase III multicenter placebo-controlled study of tamsulosin in benign prostatic hyperplasia. Tamsulosin Investigator Group. Urology 51:892–900

Lepor H, Lowe FC (2002) Evaluation and nonsurgical management of benign prostatic hyperplasia. In: Walch PC, Tetik AB, Vaughan ED (eds) Campbell's urology, 8th edn. WB Saunders CO, Philadelphia, pp 1337–1378

Lepor H, Zhang W, Kobayashi S, Tang R, Wang B, Shapiro E (1994) A comparison of the binding and functional properties of *Alpha*-1 adrenoceptors and area density of smooth muscle in the human, canine and rat prostates. J Pharmacol Exp Ther 270:722–727

Levin RM, Ruggieri MR, Wein AJ (1988) Identification of receptor subtypes in the rabbit and human urinary bladder by selective radio-ligand binding. J Urol 139:844–848

Lluel P, Deplanne V, Heudes D, Bruneval P, Palea S (2003) Age-related changes in urethrovesical coordination in male rats: relationship with bladder instability? Am J Physiol Regul Integr Comp Physiol 284:R1287–1295

Malloy BJ, Price DT, Price RR, Bienstock AM, Dole MK, Funk BL, Rudner XL, Richardson CD, Donatucci CF, Schwinn DA (1998) α1-adrenergic receptor subtypes in human detrusor. J Urol 160:937–943

Marks LS, Gittelman MC, Hill LA, Volinn W, Hoel G (2009) Silodosin in the treatment of the signs and symptoms of benign prostatic hyperplasia: a 9-month, open-label extension study. Urol 74:1318–1322

Marshall I, Burt RP, Chapple CR (1995) Noradrenaline contractions of human prostate mediated by α_{1A}-(α_{1C}-) adrenoceptor subtype. Br J Pharmacol 115:781–786

Martin DJ, Lluel P, Guillot E, Coste A, Jammes D, Angel I (1997) Comparative *Alpha*-1 adrenoceptor subtype selectivity and functional uroselectivity of *Alpha*-1 adrenoceptor antagonists. J Pharmacol Exp Ther 282:228–235

Maruyama K, Nakamura T, Yoshihara T, Fukutomi J, Sugiyama K, Hattorim K, Ohnuki T, Watanabe K, Nagatomo T (1998) Tamsulosin: assessment of affinity of ^{3}H-prazosin bindings to two α_1-adrenoceptor subtypes (α_{1H} and α_{1L}) in bovine prostate and rat heart and brain. Gen Pharmacol 31:597–600

Menon M, Parulkar BG, Drach DW (1998) Urinary lithiasis: etiology, diagnosis and medical management. In: Walsh PC, Retik AB, Vaughan ED, Wein AJ (eds) Campbell's urology, vol 3, 7th edn. WB Saunders, Philadelphia, p 2702

Michel MC (2010) The foreront for novel therapeutic agents based on the pathophysiology of lower urinary tract dysfunction: α-blocker in the treatment of male voiding dysfunction – how do they work and why do they differ in tolerability? J Pharmacl Sci 112:151–157

Michel MC, de la Rosette JJ (2006) α-blocker treatment of urolithiasis. Eur Urol 50:213–214

Michel MC, Vrydag WV (2006) α_1-, α_2- and β-adrenoceptors in the urinary bladder, urethra and prostate. Br J Pharmacol 147:S88–S119

Michel MC, Grubbel B, Taguchi K, Verfurth F, Otto T, Kropfl D (1996) Drugs for treatment of benign prostatic hyperplasia: affinity comparison at cloned α_1-adrenoceptor subtypes and in human prostate. J Auton Pharmacol 16:21–28

Milani S, Djavan B (2005) Lower urinary tract symptoms suggestive of benign prostatic hyperplasia: latest update on α_1-adrenoceptor antagonists. BJU International 95(Suppl 4): 29–36

Monneron MC, Gillberg PG, Ohman B, Alberts P (2000) In vitro α-adrenoceptor autoradiography of the urethra and urinary bladder of the female pig, cat, guinea-pig and rat. Scand J Urol Nephrol 34:233–238

Morita T, Wada I, Suzuki T, Tsuchida S (1987) Characterization of α-adrenoceptor subtypes involved in regulation of ureteral fluid transport. Tohoku J Exp Med 152:111–118

Morita T, Ando M, Kihara K, Oshima H (1994) Function and distribution of autonomic receptors in canine ureteral smooth muscle. Neurourol Urodyn 13:315–321

Muramatsu I, Ohmura T, Kigoshi S, Hashimoto S, Oshita M (1990) Pharmacological subclassification of α_1-adrenoceptors in vascular smooth muscle. Br J Pharmacol 99:197–201

Nakamura Y, Tsujimoto G, Tanoue A, Ikegaki I, Shinozaki S, Nimura T, Matsuda Y, Kawatani M (2004) Is alpha-1D adrenergic receptor responsible for storage symptoms in mice?: effects of acetic acid on bladder function in mice lacking alpha-1D adrenergic receptor. Neurourol Urodyn 23:508

Narayan P, O'Leary M, Davidai G (2005) Early efficacy of tamsulosin vs. terazosin in the treatment of men with benign prostatic hyperplasia: a randomized, open-label trial. J Appl Res 5:237–245

Nasu K, Moriyama N, Kawabe K, Tsujimoto G, Murai M, Tanaka T, Yano J (1996) Quantification and distribution of α_1-adrenoceptor subtype mRNAs in human prostate: comparison of benign hypertrophied tissue and non-hypertrophied tissue. Br J Pharmacol 119:797–803

Nasu K, Moriyama N, Fukasawa R, Tsujimoto G, Tanaka T, Yano J, Kawabe K (1998) Quantification and distribution of α_1-adrenoceptor subtype mRNAs in human proximal urethra. Br J Pharmacol 123:1289–1293

Noble AJ, Chess-Williams R, Couldwell C, Furukawa K, Uchyiuma T, Korstanje C, Chapple CR (1997) The effects of tamsulosin, a high affinity antagonist at functional α_{1A}- and α_{1B}-adrenoceptor subtypes. Br J Pharmacol 120:231–238

Nomiya M, Yamaguchi O (2003) A quantitative analysis of mRNA expression of α1 and β-adrenoceptor subtypes and their functional roles in human normal and obstructed bladders. J Urol 170:649–653

Oesterling JE (1996) Benign prostatic hyperplasia: a review of its histogenesis and natural history. Prostate Suppl 6:67–73

Ohkura T, Yamada S, Deguchi Y, Kimura R, Matsushima H, Higuchi S, Inagaki O, Honda K, Takenaka T (1998) Ex vivo occupancy by tamsulosin of α_1-adrenoceptors in rat tissues in relation to the plasma concentration. Life Sci 63:2147–2155

Ohkura T, Yamada S, Abe Y, Kimura R (2002) Selective and sustained occupancy of prostatic α_1-adrenoceptors by oral administration of KMD-3213 and its plasma concentration in rats. J Pharm Pharmacol 54:975–982

Ohmura T, Sakamoto S, Hayashi H, Kigoshi S, Muramatsu I (1993) Identification of α_1-adrenoceptor subtypes in the dog prostate. Urol Res 21:211–215

Ohtake A, Ukai M, Saitoh C, Sonoda R, Noguchi Y, Okutsu H, Yuyama H, Sato S, Sasamata M, Miyata K (2006) Effect of tamsulosin on spontaneous bladder contraction in conscious rats with bladder outlet obstruction: comparison with effect on intraurethral pressure. Eur J Pharmacol 545:185–191

Okutsu H, Matsumoto S, Hanai T, Noguchi Y, Fujiyasu N, Ohtake A, Suzuki M, Sato S, Sasamata M, Uemura H, Kurita T (2010) Effects of tamsulosin on bladder blood flow and bladder function in rats with bladder outlet obstruction. Urol 75:235–240

Parsons JK, Hergan LA, Sakamoto K, Lakin C (2007) Efficacy of α-blockers for the treatment of urethral stones. J Urol 177:983–987

Pradidarcheep W, Stallen J, Labruyere WT, Dabhoiwala NF, Michel MC, Lamers WH (2009) Lack of specificity of commercially available antisera against muscarinergic and adrenergic receptors. Naunyn-Schmiedebergs Arch Pharmacol 379:397–402

Price DT, Schwinn DA, Lomasney JW, Allen LF, Caron MG, Lefkowitz RJ (1993) Identification, quantification, and localization of mRNA for three distinct alpha$_1$ adrenergic receptor subtypes in human prostate. J Urol 150:546–551

Ramage AG, Wyllie MG (1995) A comparison of the effects of doxazosin and terazosin on the spontaneous sympathetic drive to the bladder and related organs in anaesthetized cats. Eur J Pharmacol 294:645–650

Sasaki S, Itoh Y, Kojima Y, Kubota Y, Hayase M, Kobayashi M, Tomiyama Y, Kobayashi S, Kohri K (2008) Expression level and role in ureteral contraction of alpha1-adrenoceptor subtypes in human ureter. Neurourol Urodyn 179(suppl):244

Sato S, Ohtake A, Matsushima H, Saitoh C, Usuda S, Miyata K (2001) Pharmacological effect of tamsulosin in relation to dog plasma and tissue concentrations: prostatic and urethral retention possibly contributes to uroselectivity of tamsulosin. J Pharmacol Exp Ther 296:697–703

Schilit S, Benzeroual KE (2009) Silodosin: a selective alpha$_{1A}$-adrenergic receptor antagonist for the treatment of benign prostatic hyperplasia. Clin Ther 31:2489–2502

Schulman CC (1974) Electron microscopy of the human ureteric innervation. Br J Urol 46:609–623

Schwinn DA (2001) The role of α_1-adrenergic receptor subtypes in lower urinary tract symptoms. BJU Int 88(Suppl 2):27–34, discussion 49–50

Schwinn DA, Michelotti GA (2000) α_1-Adrenergic receptors in the lower urinary tract and vascular bed: potential role for the alpha1d subtype in filling symptoms and effects of ageing on vascular expression. BJU Int 85(Suppl 2):6–11

Schwinn DA, Roehrborn CG (2008) α_1-adrenoceptor subtypes and lower urinary tract symptoms. Int J Urol 15:193–199

Scofield MA, Liu F, Abel PW, Jeffries WB (1995) Quantification of steady state expression of mRNA for *Alpha*-1 adrenergic receptor subtypes using reverse transcription and a competitive polymerase chain reaction. J Pharmacol Exp Ther 275:1035–1042

Sigala S, Peroni A, Mirabella G, Fornari S, Palazzolo F, Pezzotti G, Simeone C, Cunico SC, Spano P (2004) Alpha$_1$ adrenoceptor subtypes in human urinary bladder: sex and regional comparison. Life Sci 76:417–427

Sigala S, Dellabella M, Milanese G, Fornari S, Faccoli S, Palazzolo F, Peroni A, Mirabella G, Cunico SC, Spano P, Muzzonigro G (2005) Evidence for the presence of α_1-adrenoceptor subtypes in the human ureter. Neurourol Urodyn 24:142–148

Smith MS, Schambra UB, Wilson KH, Page SO, Schwinn DA (1999) Alpha1-adrenergic receptors in human spinal cord: specific localized expression of mRNA encoding α_1-adrenergic receptor subtypes at four distinct levels. Brain Res Mol Brain Res 63:254–261

Somogyi GT, Tanowitz M, de Groat WC (1995) Prejunctional facilitatory α_1-adrenoceptors in the rat urinary bladder. Br J Pharmacol 114:1710–1716

Springer JP, Kropp BP, Thor KB (1994) Facilitatory and inhibitory effects of selective norepinephrine reuptake inhibitors on hypogastric nerve-evoked urethral contractions in the cat: a prominent role of urethral β-adrenergic receptors. J Urol 152:515–519

Sugaya K, Nishijima S, Miyazato M, Ashitomi K, Hatano T, Ogawa Y (2002) Effects of intrathecal injection of tamsulosin and naftopidil, alpha-1A and -1D adrenergic receptor antagonists, on bladder activity in rats. Neurosci Lett 328:74–76

Suzuki F, Miyamoto S, Takita M, Oshita M, Watanabe Y, Kakizuka A, Narumiya S, Taniguchi T, Muramatsu I (1997) Cloning, functional expression and tissue distribution of rabbit α_{1d}-adrenoceptor. Biochim Biophys Acta 1323:6–11

Szell EA, Yamamoto T, de Groat WC, Somogyi GT (2000) Smooth muscle and parasympathetic nerve terminals in the rat urinary bladder have different subtypes of α_1 adrenoceptors. Br J Pharmacol 130:1685–1691

Takeda H, Matsuzawa A, Igawa Y, Yamazaki Y, Kaidoh K, Akahane S, Kojima M, Miyata H, Akahane M, Nishizawa O (2003) Functional characterization of β-adrenoceptor subtypes in the canine and rat lower urinary tract. J Urol 170:654–658

Tatemichi S, Kobayashi K, Maezawa A, Kobayashi M, Yamazaki Y, Shibata N (2006) α_1-Adrenoceptor subtype selectivity and organ specificity of silodosin (KMD-3213). Yakugaku Zasshi 126:209–216

Testa R, Guarneri L, Ibba M, Strada G, Poggesi E, Taddei C, Simonazzi I, Leonardi A (1993) Characterization of α_1-adrenoceptor subtypes in prostate and prostatic urethra of rat, rabbit, dog and man. Eur J Pharmacol 249:307–315

Thor KB, Donatucci C (2004) Central nervous system control of the lower urinary tract: new pharmacological approaches to stress urinary incontinence in women. J Urol 172:27–33

Tiselius HG (2003) Epidemiology and medical management of stone disease. BJU Int 91:758–767

Tomiyama Y, Murakami M, Akiyama K, Kojima M, Akahane M, Park YC, Kurita T (2002) Modification of ureteral motility and promotion of urine flow around an intraureteral obstruction by CL-316243, phenylephrine, and furosemide in dogs. Neurourol Urodyn 21:251–257

Tomiyama Y, Kobayashi K, Tadachi M, Kobayashi S, Inada Y, Kobayashi M, Yamazaki Y (2007) Expressions and mechanical functions of α_1-adrenoceptor subtypes in hamster ureter. Eur J Pharmacol 573:201–205

Trevisani M, Campi B, Gatti R, Andre E, Materazzi S, Nicoletti P, Gazzieri D, Geppetti P (2007) The influence of $Alpha_1$-adrenoreceptors on neuropeptide release from primary sensory neurons of the lower urinary tract. Eur Urol 52:901–908

Tseng-Crank J, Kost T, Goetz A, Hazum S, Roberson KM, Haizlip J, Godinot N, Robertson CN, Saussy D (1995) The α_{1C}-adrenoceptor in human prostate: cloning, functional expression, and localization to specific prostatic cell types. Br J Pharmacol 115:1475–1485

Tsujii T, Azuma H, Yamaguchi T, Oshima H (1992) A possible role of decreased relaxation mediated by β-adrenoceptors in bladder outlet obstruction by benign prostatic hyperplasia. Br J Pharmacol 107:803–807

Tzortzis V, Mamoulakis C, Rioja J, Gravas S, Michel MC, De la Rosette JJMCH (2009) Medical expulsive therapy for distal ureteral stones. Drugs 69:677–692

Walden PD, Durkin MM, Lepor H, Wetzel JM, Gluchowski C, Gustafson EL (1997) Localization of mRNA and receptor binding sites for the α_{1a}-adrenoceptor subtype in the rat, monkey and human urinary bladder and prostate. J Urol 157:1032–1038

Walden PD, Gerardi C, Lepor H (1999) Localization and expression of the $alpha_{1A\text{-}1}$, $alpha_{1B}$ and $alpha_{1D}$-adrenoceptors in hyperplastic and non-hyperplastic human prostate. J Urol 161:635–640

Wanajo I, Tomiyama Y, Tadachi M, Kobayashi M, Yamazaki Y, Kojima M, Shibata N (2005) The potency of KUL-7211, a selective ureteral relaxant, in isolated canine ureter: comparison with various spasmolytics. Urol Res 33:409–414

Weiss RM, Bassett AL, Hoffman BF (1978) Adrenergic innervation of the ureter. Invest Urol 16:123–127

Wilde MI, Fitton A, McTavish D (1993a) Alfuzosin. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in benign prostatic hyperplasia. Drugs 45:410–429

Wilde MI, Fitton A, Sorkin EM (1993b) Terazosin. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in benign prostatic hyperplasia. Drugs Aging 3:258–277

Yamada S, Ashizawa N, Ushijima H, Nakayama K, Hayashi E, Honda K (1987) *Alpha*-1 adrenoceptors in human prostate: characterization and alteration in benign prostatic hypertrophy. J Pharmacol Exp Ther 242:326–330

Yamada S, Suzuki M, Matsuoka Y, Kato Y, Kimura R, Maruyama M, Kawabe K (1991) [^{3}H] bunazosin, a novel selective radioligand of alpha 1 adrenoceptors in human prostates. J Urol 146:877–880

Yamada S, Suzuki M, Kato Y, Kimura R, Mori R, Matsumoto K, Maruyama M, Kawabe K (1992) Binding characteristics of naftopidil and alpha 1-adrenoceptor antagonists to prostatic alpha-adrenoceptors in benign prostatic hypertrophy. Life Sci 50:127–135

Yamada S, Suzuki M, Tanaka C, Mori R, Kimura R, Inagaki O, Honda K, Asano M, Takenaka T, Kawabe K (1994a) Comparative study on α_1-adrenoceptor antagonist binding in human prostate and aorta. Clin Exp Pharmacol Physiol 21:405–411

Yamada S, Tanaka C, Kimura R, Kawabe K (1994b) Alpha 1-adrenoceptors in human prostate: characterization and binding characteristics of alpha 1-antagonists. Life Sci 54:1845–1854

Yamada S, Tanaka C, Ohkura T, Mori R, Kimura R, Inagaki O, Honda K, Kawabe K (1994c) High-affinity specific [^{3}H]tamsulosin binding to alpha 1-adrenoceptors in human prostates with benign prostatic hypertrophy. Urol Res 22:273–278

Yamada S, Ohkura T, Kimura R, Kawabe K (1998) In vivo receptor binding of novel alpha1-adrenoceptor antagonists for treatment of benign prostatic hyperplasia. Life Sci 62:1585–1589

Yamada S, Ohkura T, Deguchi Y, Kimura R (1999) In vivo measurement by [^{3}H]tamsulosin of alpha1 adrenoceptors in rat tissues in relation to the pharmacokinetics. J Pharmacol Exp Ther 289:1575–1583

Yamada S, Okura T, Kimura R (2001) In vivo demonstration of α_1-adrenoceptor subtype selectivity of KMD-3213 in rat tissues. J Pharmacol Exp Ther 296:160–167

Yamagishi R, Akiyama K, Nakamura S, Hora M, Masuda N, Matsuzawa A, Murata S, Ujiie A, Kurashina Y, Iizuka K, Kitazawa M (1996) Effect of KMD-3213, an α_{1a}-adrenoceptor-selective antagonist, on the contractions of rabbit prostate and rabbit and rat aorta. Eur J Pharmacol 315:73–79

Yazawa H, Honda K (1993) α_1-adrenoceptor subtype in the rat prostate is preferentially the alpha 1A type. Jpn J Pharmacol 62:297–304

Yilmaz E, Batislam E, Basar MM, Tuglu D, Ferhat M, Basar H (2005) The comparison and efficacy of 3 difference α_1-adrenergic blockers for distal ureteral stones. J Urol 173:2010–2012

Yokoyama O, Yusup A, Oyama N, Aoki Y, Tanase K, Matsuta Y, Miwa Y, Akino H (2006) Improvement of bladder storage function by α_1-blocker depends on the suppression of C-fiber afferent activity in rats. Neurourol Urodyn 25:461–467

Yokoyama O, Yusup A, Oyama N, Aoki Y, Miwa Y, Akino H (2007) Improvement in bladder storage function by tamsulosin depends on suppression of C-fiber urethral afferent activity in rats. J Urol 177:771–775

Yono M, Foster HE Jr, Shin D, Takahashi W, Pouresmail M, Latifpour J (2004) Doxazosin-induced up-regulation of α_{1A}-adrenoceptor mRNA in the rat lower urinary tract. Can J Physiol Pharmacol 82:872–878

Yoshimura N, Sasa M, Ohno Y, Yoshida O, Takaori S (1988) Contraction of urinary bladder by central norepinephrine originating in the locus coeruleus. J Urol 139:423–427

Yu SM, Ko FN, Chueh SC, Chen J, Chen SC, Chen CC, Teng CM (1994) Effects of dicentrine, a novel α_1-adrenoceptor antagonist, on human hyperplastic prostates. Eur J Pharmacol 252:29–34

β-Adrenergic Receptor Subtypes in the Urinary Tract

Martin C. Michel

Contents

Abstract Within the urinary tract, β-adrenergic receptors (AR) are found largely on smooth muscle cells but are also present, at least in the bladder, in the urothelium and on afferent nerves. Our understanding of β-AR subtype expression and function is hampered by a lack of well-validated tools, particularly with regard to β_3-AR. Moreover, the β-AR subtypes involved in a specific function may differ between species. In the ureter, β-AR can modulate pacemaker activity and smooth muscle tone involving multiple subtypes. In the human bladder, β-AR promote urine storage. Bladder smooth muscle relaxation primarily involves β_3-AR, and the agonists selective for this subtype are in clinical development to treat bladder dysfunction. While prostate and urethra also express β-AR, the overall physiological role in these tissues remains unclear.

Keywords β-Adrenergic receptor · Bladder · Prostate · Ureter · Urethra

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1 Introduction

β-Adrenergic receptors (ARs) mediate many of the effects of norepinephrine released from the sympathetic nervous system and epinephrine released from the adrenals. For a long time, these effects were attributed to two receptor subtypes, the β_1- and β_2-ARs. During the past two decades, it has become clear that a third subtype exists, the β_3-AR, but much less is known about this than the two other subtypes, particularly due to a lack of selective tools (see Sect. 15.2). It appears that in humans, the urinary tract is a key tissue in which β_3-ARs are important, particularly in the bladder. We have recently comprehensively reviewed the expression and function of β-AR subtypes in bladder, prostate, and urethra (Michel and Vrydag 2006). As limited novel data has emerged on prostatic or urethral β-AR, this chapter will only summarize the earlier data for these tissues and the older bladder data. More space will be given to novel data on β-AR and the bladder and to the role of β-AR in the ureter.

2 Methodological Aspects

Based upon the long-standing interest in β_1- and β_2-ARs, many well-validated tools are available to study these subtypes at the protein level, i.e., highly selective radioligands, agonists, and antagonists. In contrast, selective agonists and antagonists are only available to a limited extent for β_3-ARs (Vrydag and Michel 2007). For example, the often used antagonist SR 59,230A, contrary to common belief lacks selectivity for this subtype in humans, is a partial agonist and can also act on targets other than β-ARs. Many other compounds also have poor selectivity for β_3-ARs and/or ancillary properties (Mori et al. 2010). Moreover, no suitable radioligands are available to label β_3-ARs in tissues. Finally, recent data indicate that commercially available antibodies for all three β-AR subtypes lack the promised selectivity for their cognate receptors (Pradidarcheep et al. 2009; Hamdani and van der Velden 2009). These limitations need to be kept in mind in the interpretation of available data on β-AR in the urinary tract. Specifically, some studies may have underestimated a contribution from β_3-ARs or claimed their presence based on insufficient data.

3 Ureter

The ureter provides unidirectional transport of urine from the kidneys to the bladder by means of propulsive contractions originating from pacemaker cells in the renal pelvis. This peristalsis is primarily regulated by myogenic mechanisms, but several factors including neuronally released norepinephrine contribute to the regulation of ureter smooth muscle tone (Canda et al. 2007). Norepinephrine appears to mainly

act via α_1-ARs rather than β-ARs in the ureter as the net response across several species is contraction rather than relaxation both in vitro (Weiss et al. 1978; Morita et al. 1994; Labadia et al. 1987) and in vivo (Hernandez et al. 1992; McLeod et al. 1973). However, the balance between α_1- and β-AR may depend on the ureter segment, with the distal segments exhibiting a somewhat stronger β-AR component (Tindall 1972).

All three β-AR subtypes are expressed in human ureter smooth muscle at the mRNA level (Park et al. 2000), whereas only β_1- and β_2-AR mRNA has been reported in the rat (β_3-AR mRNA not investigated) (Meister et al. 1994). The density of β-AR protein as detected by radioligand binding in the ureter has been reported to be low, e.g., in dogs (Levin and Wein 1979) and humans (Park et al. 2000), but the techniques being used are unsuitable to detect possibly present β_3-AR.

β-AR agonists can have two major effects on the ureter activity. Firstly, they can alter the pacemaker activity in vitro (Morita and Suzuki 1984; Oostendorp et al. 2000) and in vivo (Peters et al. 1979). Although the receptor subtypes mediating such alterations have not been well characterized, some data in dogs indicate that β_1-AR agonists (dobutamine) may enhance whereas β_2-AR agonists (terbutaline) may inhibit the pacemaker (Morita and Suzuki 1984). In contrast, β_1-AR in mice, at least when stimulated via the noncatecholamine recognition site activated by CGP 12,177, can enhance ureteral pacemaker activity (Oostendorp et al. 2000).

Secondly, β-AR agonists can relax ureter smooth muscle, and related studies have been the main thrust of research in this field. This relaxation involves a reduction of both the frequency and the force of contraction in vitro (Morita et al. 1994) and in vivo (Mayo and Halbert 1981; Hernandez et al. 1992; Murakami et al. 2000; Danuser et al. 2001). The β-AR subtypes involved in the relaxation have been explored in several species. However, the findings in most species are not fully conclusive, either due to limited value of the tools, which have been used or due to somewhat controversial data. Nevertheless, it appears that the subtypes being involved in ureter relaxation may differ between species (for summary see Table 1).

Table 1 β-Adrenergic receptor subtypes involved in urinary tract smooth muscle relaxation in various species

Species	Ureter	Bladder detrusor	Prostate	Urethra
Rat	β_1	$\beta_3 \geq \beta_2$	β_2	$\beta_2 > \beta_3$
Mouse	n.d.	β_2	n.d.	n.d.
Rabbit	β_2 and/or β_3	β_2	n.d.	n.d.
Guinea pig	β_2 and/or β_3	β_1, β_2 and/or β_3	β_1, β_2	n.d.
Ferret	n.d.	β_3	n.d.	n.d.
Cat	n.d.	β_1	n.d.	n.d.
Dog	β_2 and/or β_3	β_3	n.d.	β_2
Pig	β_2 and/or β_3	$\beta_2 \geq \beta_3$	n.d.	β_3
Horse	β_2	n.d.	n.d.	β_2
Monkey	n.d.	β_3	n.d.	n.d.
Human	β_2 and/or β_3	β_3	β_2	n.d.

Of note, conclusions for several species are based on limited data. For references, see main text (ureter) and Michel and Vrydag (2006) (other tissues)
n.d. Not determined

Thus, β_1-AR may be involved in rats (Tomiyama et al. 1998). In contrast, β_2- and/or β_3-AR have been proposed for rabbits (Tomiyama et al. 1998, 2003a), guinea pigs (Yamamoto and Koike 2000), pigs (Wanajo et al. 2004), dogs (Morita et al. 1994; Tomiyama et al. 1998, 2003a, b), horses (Labadia et al. 1987), and humans (Park et al. 2000).

Reduction of ureter smooth muscle tone is clinically relevant in the context of stone disease, and currently available medical treatments to enhance stone expulsion have limited efficacy (Tzortzis et al. 2009). Therefore, several studies have addressed the potential of β-AR agonists to promote stone expulsion. β-AR agonists can cause ureter relaxation in a dog model of acute obstruction (Murakami et al. 2000) and have been reported to be effective in a rabbit in vitro model of stone disease (Miyatake et al. 2001). Two clinical pilot studies, one based on systemic administration of the partial agonist oxprenolol (Bajor 1980) and one based on topical application of isoproterenol during renal pelvic irrigation (Jung et al. 2008), have yielded promising results. Based on such data, the mixed $\beta_{2/3}$-AR agonist KUL-7211 has been proposed to be a potential therapeutic for the treatment of ureteral stones based upon animal data (Tomiyama et al. 2003a; Wanajo et al. 2005), but this remains to be confirmed in clinical studies.

4 Bladder

The bladder translates the continuous process of urine formation into the discontinuous event of micturition. The associated increase in intravesical volume of >10-fold requires marked smooth muscle relaxation lasting for several hours (Michel and Parra 2008). While the physiological role of the sympathetic system in this long-lasting relaxation remains unclear, β-AR stimulation is an effective mechanism to increase bladder capacity.

At the mRNA level, all three subtypes are detectable in the bladder. Whereas in the rat bladder, the abundance of the three subtypes appears similar (Barendrecht et al. 2009), in the human bladder, >95% of all β-AR mRNA belongs to the β_3-subtype (Nomiya and Yamaguchi 2003). At the protein level, β_1- and β_2-AR have been identified by radioligand binding in the bladder of humans (Goepel et al. 1997) and several animals species (Michel and Vrydag 2006), whereas a lack of β_3-AR detection is primarily attributable to a lack of suitable tools rather than a lack of presence of this subtype.

Bladder smooth muscle relaxation upon β-AR stimulation has been demonstrated in many species including rats, rabbits, guinea pigs, ferrets, cats, dogs, pigs, monkeys, and humans; the maximum relaxation appears similar across species, but agonist potency may differ between them (Michel and Vrydag 2006). Moreover, efficacy and potency of β-AR agonists depends markedly on the stimulus used to induce bladder contraction (Michel and Vrydag 2006; Michel and Sand 2009). Of note, bladder relaxation occurs mainly in the detrusor and not necessarily in the bladder neck. At the cellular level, bladder smooth muscle relaxation induced

by β-AR stimulation involves cAMP formation to only a minor, if any, extent, whereas activation of BK_{Ca} potassium channels and perhaps also inhibition of the L-type calcium channels appear to play an important role (Frazier et al. 2008; Takemoto et al. 2008; Brown et al. 2008; Hristov et al. 2008).

Many studies have investigated the β-AR subtypes involved in bladder smooth muscle relaxation, and the results differ between species (Table 1). These experiments largely rely on the use of subtype-selective agonists; while experiments with β_1- and β_2-AR antagonists have also been performed, truly β_3-selective antagonists such as L-748,337 have rarely been used. Based on these tools and their limitations, it appears that human bladder relaxation involves predominantly, if not exclusively, β_3-AR (Igawa et al. 1998, 2001; Takeda et al. 1999; Nomiya and Yamaguchi 2003; Biers et al. 2006; Yamanishi et al. 2006; Badawi et al. 2007; Takasu et al. 2007; Otsuka et al. 2008a; Wuest et al. 2009), whereas it involves additional and/or alternative subtypes in some animals species (Table 1).

Bladder smooth muscle relaxation by β-AR agonists apparently does not display major gender differences (Frazier et al. 2006). In contrast, ageing has repeatedly been reported to be associated with reductions in β-AR binding sites, reflecting only alterations of β_1- and β_2-AR for technical reasons, whereas the potency and/or efficacy of β-AR-mediated smooth muscle relaxation exhibited only inconsistent and mostly small reductions with age (Michel and Barendrecht 2008). Whether the observed impairment of relaxation reflects specific desensitization of β-AR responses or rather a general impairment of relaxation, e.g., due to structural changes of the bladder, remains unclear. Moreover, almost all of these studies were based on rats, and data on other species, specifically humans, are scarce. Alterations of bladder β-AR responsiveness under pathophysiological conditions such as bladder outlet obstruction (Barendrecht et al. 2009) appear absent or of limited magnitude (Michel and Barendrecht 2008), but some exceptions such as chronic NO deficiency may exist (Monica et al. 2008).

In vivo studies demonstrate that β-AR agonists reduce intravesical pressure and enhance functional bladder capacity (Michel and Vrydag 2006). Therapeutically, perhaps more importantly, β-AR agonists can also reduce nonvoiding bladder contraction, a frequently used proxy parameter for urgency (Michel and Chapple 2009), or increase bladder capacity in animal models of bladder dysfunction. These include bladder irritation by intravesical installation of prostaglandin E_2 (Takeda et al. 2002; Yamazaki et al. 2002). While such models may be more representative of disease states such as interstitial cystitis, β-AR agonists also improve bladder function, e.g., after cerebral infarction (Kaidoh et al. 2002). Ex vivo studies with bladder tissue from patients with low compliance, hyperreflexic bladders (Igawa et al. 2001), those with urgency incontinence (Hudman et al. 2001), or those with outlet obstruction (Nomiya and Yamaguchi 2003) have also shown maintained smooth muscle relaxation by β-AR agonists.

Of note, most previous studies into β-AR and bladder function have focused on their role as inductors of detrusor smooth muscle relaxation. Comparison between intraperitoneal and intrathecal administration of β-AR agonists confirm the idea that such drugs primarily act via a peripheral site of action (Durant et al. 1988), but

an additional central site of action is possible for agonists with good penetration into the brain and/or spinal cord. Such peripheral effects may not be limited to direct smooth muscle relaxation but may also involve modulation of the contractile effects mediated by muscarinic receptors (Ehlert et al. 2007; Klausner et al. 2009; Michel and Sand 2009; Witte et al. 2009). However, this does not necessarily imply bladder smooth muscle as their primary site of action, particularly not under pathophysiological conditions. Of note, recent studies demonstrate the existent of β-AR in the urothelium, where they apparently can modulate the release of factors altering smooth muscle function (Murakami et al. 2007; Otsuka et al. 2008b; Tyagi et al. 2009). Additionally, the possibility has been raised that β-AR agonists may also modulate bladder function by acting on afferent neurons (Aizawa et al. 2010). However, the relative role of smooth muscle, urothelium, and afferent neurons in the modulation of bladder function in response to β-AR stimulation has not been well explored (Leon et al. 2008).

All of these studies indicate that β-AR agonists may become useful therapeutic agents for bladder dysfunction. Indeed small pilot studies with terbutaline (Lindholm and Lose 1986) or clenbuterol (Gruneberger 1984) have reported promising results. As human detrusor relaxation occurs via β_3-AR, a subtype with limited function in the human cardiovascular system (Ursino et al. 2009), selective β_3-AR agonists are under active preclinical and clinical evaluation (see Sect. 15.7).

5 Prostate

The prostate contains glandular/epithelial as well as smooth muscle cells. At the mRNA level, the presence of both β_2- and β_3-AR has been reported in rats and humans, respectively, whereas at the protein level, mostly β_2-AR have been detected in the prostate of various species including humans (Michel and Vrydag 2006), but the radioligands, which have been used in those studies, are not suitable to detect a possible presence of β_3-AR (see Sect. 15.2).

At the cellular level, prostatic β-AR couple to stimulation of adenylyl cyclase. At the tissue level they have little effect on basal prostate tone but can inhibit α_1-AR-stimulated or receptor-independent prostate contraction in rats (Kalodimos and Ventura 2001), guinea pigs (Haynes and Hill 1997), dogs (Normandin and Lodge 1996), horses (Garcia-Sacristan et al. 1984), and humans (Tsujii et al. 1992; Drescher et al. 1994), and in most cases, this involved β_2-AR. While little is known about the expression and function of β-AR in prostatic epithelium, recent data with the epithelium-derived prostate cancer cell line PC-3 indicates that norepinephrine (in a propranolol-sensitive manner) stimulates cell growth in vitro and metastasis of PC-3 cells in nude mice in vivo (Palm et al. 2006).

Prostatic β-AR expression and/or function may be reduced in patients with benign prostatic hyperplasia (Tsujii et al. 1992) and in a rat model of type 1 diabetes (Gousse et al. 1991; Fukumoto et al. 1993; Carmena et al. 1997). Moreover, prostatic β-AR

are regulated by androgens (Poyet et al. 1986; Purvis et al. 1986; Collins et al. 1988; Guthrie et al. 1990).

While these findings would be compatible with a contribution of reduced β-AR function to symptoms of benign prostatic hyperplasia and of enhanced β-AR function in prostate cancer, none of these possibilities has been explored in a meaningful way in clinical studies.

6 Urethra

While the urethra contributes to bladder outlet resistance in females, its relative role is much smaller in males where the prostate is more important. The presence of β-AR protein has been reported by radioligand binding studies in the urethra of rabbits and humans (Michel and Vrydag 2006). Similar to the other urinary tract tissues, urethral β-AR couple to smooth muscle relaxation, but its effects in the urethra are considerably smaller than those in the bladder (Takeda et al. 2003). Depending upon species, this appears to involve mainly β_2- and β_3-AR (Table 1). Nevertheless, β-AR agonists can reduce urethral pressure in vivo in rats (Takeda et al. 2003), cats (Springer et al. 1994), and humans (Thind et al. 1993a, b). While such urethral relaxation and hence reduction of bladder outlet resistance may be undesirable in the treatment of overactive bladder and urgency incontinence, the smaller magnitude of effects in the urethra as compared to those in the bladder make it unlikely that direct effects on the urethra limit the usefulness of β-AR agonists, specifically β_3-selective drugs, in the treatment of bladder dysfunction.

7 Drugs in Clinical Development

Based upon the above, it is obvious that drugs targeting β-AR in the LUT are most likely agonists, specifically β_3-selective agonists. The most advanced candidate drug in this regard is mirabegron (also known as YM 178) intended for the treatment of overactive bladder (Takasu et al. 2007; Vrydag et al. 2008). Most importantly, positive results from a clinical proof of concept study (Chapple et al. 2008) as well as a dose-ranging study (Chapple et al. 2010) have recently been reported for this drug. Accordingly, Astellas has announced on June 18th 2010 that it had submitted mirabegron for marketing authorization in Japan, implying that positive phase III results have also been obtained. Other β_3-selective agonists currently in various states of preclinical or clinical development for the treatment of overactive bladder include AJ-9677 (Otsuka et al. 2008a), compound 138-3350020 (Yamanishi et al. 2006), FK 175 (Imanishi et al. 2008), FK 4664 (Imanishi et al. 2008; Vrydag et al. 2008), solabegron (Biers et al. 2006; Hicks et al. 2007), TRK-380 (also known as TAK-301) (Yoshikawa et al. 2009a, b), and ZD-7114 (Badawi et al. 2007). Ritobegron (also known as KUC 7483) is also in clinical development for OAB

treatment (Yamazaki et al. 2002), but on July 2nd, 2010, Kissei announced that ritobegron had failed to reach the primary efficacy endpoint in phase III studies. β-AR agonists acting on other subtypes such as KUL-7211 may be useful in the treatment of ureteral stones, but this concept remains to be explored clinically.

8 Conclusions

β-AR, mostly representing β_2- and β_3-AR, are expressed in all parts of the urinary tract where they mediate smooth muscle relaxation. However, specifically in the urinary bladder, additional sites of action including the urothelium and afferent nerves may also represent cellular targets of β-AR ligands. While β-AR may contribute to prostatic function, the overactive bladder syndrome and perhaps ureteral stone expulsion are more likely to represent valid indications for the use of β-AR agonists. Recently published clinical proof of concept data are encouraging in this regard (Chapple et al. 2008).

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References

Aizawa N, Igawa Y, Nishizawa O et al (2010) Effects of CL316, 243, a beta3-adrenoceptor agonist, and intravesical prostaglandin E2 on the primary bladder afferent activity of the rat. Neurourol Urodyn 29(5):771–776

Badawi JK, Seja T, Uecelehan H et al (2007) Relaxation of human detrusor muscle by selective beta-2 and beta-3 agonists and endogenous catecholamines. Urology 69:785–790

Bajor G (1980) Beta-blocking agent facilitating the spontaneous passage of ureteral stones. Int Urol Nephrol 22:33–36

Barendrecht MM, Frazier EP, Vrydag W et al (2009) The effect of bladder outlet obstruction on α_1- and β-adrenoceptor expression and function. Neurourol Urodyn 28:349–355

Biers SM, Reynard JM, Brading AF (2006) The effects of a new selective β_3-adrenoceptor agonist (GW427353) on spontaneous activity and detrusor relaxation in human bladder. BJU Int 98:1310–1314

Brown SM, Bentcheva-Petkova LM, Liu L et al (2008) β-Adrenergic relaxation of mouse urinary bladder smooth muscle in the absence of large-conductance Ca^{2+}-activated K^+-channel. Am J Physiol 295:F1149–F1157

Canda AE, Turna B, Cinar GM et al (2007) Physiology and pharmacology of the human ureter: basis for current and future treatments. Urol Int 78:289–298

Carmena MJ, Clemente C, Carrero I et al (1997) G-Proteins and β-adrenergic stimulation of adenylate cyclase activity in the diabetic rat prostate. Prostate 33:46–54

Chapple CR, Yamaguchi O, Ridder A et al (2008) Clinical proof of concept study (Blossom) shows novel β3 adrenoceptor agonist YM178 is effective and well tolerated in the treatment of symptoms of overactive bladder. Eur Urol Suppl 7:239–239

Chapple C, Wyndaele J-J, van Kerrebroeck P et al (2010) Dose-ranging study of once-daily mirabegron (YM178), a novel selective β3-adrenoceptor agonist, in patients with overactive bladder (OAB). Eur Urol Suppl 9:249–249

Collins S, Quarmby VE, French FS et al (1988) Regulation of the β_2-adrenergic receptor and its mRNA in the rat ventral prostate by testosterone. FEBS Lett 233:173–176

Danuser H, Weiss R, Abel D et al (2001) Systemic and topical drug administration in the pig ureter: effect of phosphodiesterase inhibitors α1, β and β2-adrenergic receptor agonists and antagonists on the frequency and amplitude of ureteral contractions. J Urol 166:714–720

Drescher P, Eckert RE, Madsen PO (1994) Smooth muscle contractility in prostatic hyperplasia: role of cyclic adenosine monophosphate. Prostate 25:76–80

Durant PA, Lucas PC, Yaksh TL (1988) Micturition in the unanaesthetized rat: spinal vs. peripheral pharmacology of the adrenergic system. J Pharmacol Exp Ther 245:426–435

Ehlert FJ, Ahn S, Pak KJ et al (2007) Neuronally release acetylcholine acts on the M_2 muscarinic receptor to oppose the relaxant effect of isoproterenol on cholinergic contractions in mouse urinary bladder. J Pharmacol Exp Ther 322:631–637

Frazier EP, Schneider T, Michel MC (2006) Effects of gender, age and hypertension on β-adrenergic receptor function in rat urinary bladder. Naunyn-Schmiedeberg's Arch Pharmacol 373:300–309

Frazier EP, Peters SLM, Braverman AS et al (2008) Signal transduction underlying control of urinary bladder smooth muscle tone by muscarinic receptors and β-adrenoceptors. Naunyn-Schmiedeberg's Arch Pharmacol 377:449–462

Fukumoto Y, Yoshida M, Dokita S et al (1993) The reversal effect of insulin on diabetes-induced alterations in beta adrenergic and muscarinic receptors in rat prostate. J Urol 149:1602–1607

Garcia-Sacristan A, Casanueva CR, Castilla C et al (1984) Adrenergic receptors in the urethra and prostate of the horse. Res Vet Sci 36:57–60

Goepel M, Wittmann A, Rübben H et al (1997) Comparison of adrenoceptor subtype expression in porcine and human bladder and prostate. Urol Res 25:199–206

Gousse A, Yoshida M, Weiss RM et al (1991) Beta adrenergic receptor alterations in diabetic rat prostate: effects of insulin and dietary myoinositol. Prostate 19:121–131

Gruneberger A (1984) Treatment of motor urge incontinence with clenbuterol and flavoxate hydrochloride. Br J Obst Gynaecol 91:275–278

Guthrie PD, Freeman MR, Liao ST et al (1990) Regulation of gene expression in rat prostate by androgen and β-adrenergic receptor pathways. Mol Endocrinol 4:1343–1353

Hamdani N, van der Velden J (2009) Lack of specificity of antibodies directed against human beta-adrenergic receptors. Naunyn-Schmiedeberg's Arch Pharmacol 379:403–407

Haynes JM, Hill SJ (1997) β-Adrenoceptor-mediated inhibition of α_1-adrenoceptor-mediated and field stimulation-induced contractile responses in the prostate of the guinea pig. Br J Pharmacol 122:1067–1074

Hernandez M, Prieto D, Simonsen U et al (1992) Noradrenaline modulates smooth muscle activity of the isolated intravesical ureter of the pig through different types of adrenoceptors. Br J Pharmacol 107:931

Hicks A, McCafferty GP, Riedel E et al (2007) GW427353 (solabegron), a novel, selective β_3-adrenergic receptor agonist, evokes bladder relaxation and increases micturition reflex threshold in the dog. J Pharmacol Exp Ther 323:202–209

Hristov KL, Cui X, Brown SM et al (2008) Stimulation of β3-adrenoceptor relaxes rat urinary bladder smooth muscle via activation of the large-conductance Ca^{2+}-activated K^+ channels. Am J Physiol 295:C1344–C1353

Hudman D, Elliott RA, Whitakker P et al (2001) Inhibition of the contractile responses of isolated human and rat bladders by clenbuterol. J Urol 166:1969–1973

Igawa Y, Yamazaki Y, Takeda H et al (1998) Possible β3-adrenoceptor-mediated relaxation of the human detrusor. Acta Physiol Scand 164:117–118

Igawa Y, Yamazaki Y, Takeda H et al (2001) Relaxant effects of isoproterenol and selective β3-adrenoceptor agonists on normal, low compliant and hyperreflexic human bladders. J Urol 165:240–244

Imanishi M, Tomishima Y, Itou S et al (2008) Discovery of a novel series of biphenyl benzoic acid derivatives as potent and selective human β_3-adrenergic receptor agonists with good oral bioavailability. Part I. J Med Chem 51:1925–1944

Jung H, Norby B, Frimodt-Moller PC et al (2008) Endoluminal isoproterenol irrigation decreases renal pelvic pressure during flexible ureteroscopy: a clinical, randomized, controlled study. Eur Urol 54:1404–1413

Kaidoh K, Igawa Y, Takeda H et al (2002) Effects of selective β_2 and β_3-adrenoceptor agonists in detrusor hyperreflexia in conscious cerebral infarcted rats. J Urol 168:1247–1252

Kalodimos PJ, Ventura S (2001) β_2-Adrenoceptor-mediated inhibition of field stimulation induced contractile responses of the smooth muscle of the rat prostate gland. Eur J Pharmacol 431:81–89

Klausner AP, Rourke KF, Miner AS et al (2009) Potentiation of carbachol-induced detrusor smooth muscle contractions by β-adrenoceptor activation. Eur J Pharmacol 606:191–198

Labadia A, Rivera L, Costa G et al (1987) Alpha- and beta-adrenergic receptors in the horse ureter. Rev Esp Fisiol 43:421–425

Leon LA, Hoffman BE, Gardner SD et al (2008) Effects of the β3-adrenergic receptor agonist disodium 5[(2R)-2-[[(2R)-2-(3-chlorophenyl)-2-hydroxyethyl]amino]propyl]-1, 3-benzodioxole-2, 2, dicarboxylate (L-316243) on bladder micturition reflex in spontaneously hypertensive rats. J Pharmacol Exp Ther 326:178–185

Levin RM, Wein AJ (1979) Quantitative analysis of alpha and beta adrenergic receptor densities in the lower urinary tract of the dog and the rabbit. Invest Urol 17:75–77

Lindholm P, Lose G (1986) Terbutaline (Bricanyl) in the treatment of female urge incontinence. Urol Int 41:158–160

Mayo ME, Halbert SA (1981) The effect of autonomic drugs on ureteric peristalsis: a canine in vivo study. Urol Res 9:209–216

McLeod DG, Reynolds DG, Swan KG (1973) Adrenergic mechanisms in the canine ureter. Am J Physiol 224:1054–1058

Meister B, Dagerlind A, Nicholas AP et al (1994) Patterns of messenger RNA expression for adrenergic receptor subtypes in rat kidney. J Pharmacol Exp Ther 268:1605–1611

Michel MC, Barendrecht MM (2008) Physiological and pathological regulation of the autonomic control of urinary bladder contractility. Pharmacol Ther 117:297–312

Michel MC, Chapple CR (2009) Basic mechanisms of urgency: basic and clinical evidence. Eur Urol 56:298–308

Michel MC, Parra S (2008) Similarities and differences in the autonomic control of airway and urinary bladder smooth muscle. Naunyn-Schmiedeberg's Arch Pharmacol 378:217–224

Michel MC, Sand C (2009) Effect of pre-contraction on β-adrenoceptor-mediated relaxation of rat urinary bladder. World J Urol 27:711–715

Michel MC, Vrydag W (2006) α_1-, α_2- and β-adrenoceptors in the urinary bladder, urethra and prostate. Br J Pharmacol 147:S88–S119

Miyatake R, Tomiyama Y, Murakami M et al (2001) Effects of isoproterenol and butylscopolamine on the friction between an artificial stone and the intraureteral wall in anaesthetized rabbits. J Urol 166:1083–1087

Monica FZT, Bricola AAO, Bau FR et al (2008) Long-term nitric oxide deficiency causes muscarinic supersensitivity and reduces β_3-adrenoceptor-mediated relaxation, causing rat detrusor overactivity. Br J Pharmacol 153:1659–1668

Mori A, Miwa T, Sakamoto K et al (2010) Pharmacological evidence for the presence of functional β_3-adrenoceptors in rat retinal blood vessels. Naunyn-Schmiedeberg's Arch Pharmacol 382 (2):119–126

Morita T, Suzuki T (1984) Effects of beta-adrenergic agents on the pacemaker of ureteral peristalsis. Urol Int 39:154–158

Morita T, Ando M, Kihara K et al (1994) Function and distribution of autonomic receptors in canine ureteral smooth muscle. Neurourol Urodyn 13:315–321

Murakami M, Tomiyama Y, Hayakawa K et al (2000) Effects of β-adrenergic stimulation on acutely obstructed ureter in dogs. J Pharmacol Exp Ther 292:67–75

Murakami S, Chapple CR, Akino H et al (2007) The role of the urothelium in mediating bladder responses to isoprenaline. BJU Int 99:669–673

Nomiya M, Yamaguchi O (2003) A quantitative analysis of mRNA expression of α1 and β-adrenoceptor subtypes and their functional roles in human normal and obstructed bladders. J Urol 170:649–653

Normandin DE, Lodge NJ (1996) Pharmacoalogical characterization of the isolated canine prostate. J Urol 155:1758–1761

Oostendorp J, Preitner F, Moffatt J et al (2000) Contribution of β-adrenoceptor subtypes to relaxation of colon and oesophagus and pacemaker activity of ureter in wild-type and β_3-adrenoceptor knockout mice. Br J Pharmacol 130:747–758

Otsuka A, Shinbo H, Hasebe K et al (2008a) Effects of a novel β_3-adrenoceptor agonist, AJ-9677, on relaxation of the detrusor muscle: an in vitro study. Int J Urol 15:1072–1076

Otsuka A, Shinbo H, Matsumoto R et al (2008b) Expression and functional role of β-adrenoceptors in the human urinary bladder. Naunyn-Schmiedeberg's Arch Pharmacol 377:473–481

Palm D, Lang K, Niggemann B et al (2006) The norepinephrine-driven metastasis development of PC-3 human prostate cancer cells in BALB/c nude mice is inhibited by β-blockers. Int J Cancer 118:2744–2749

Park Y-C, Tomiyama Y, Hayakawa K et al (2000) Existence of a β3-adrenoceptor and its functional role in the human ureter. J Urol 164:1364–1370

Peters HJ, Parekh N, Popa G (1979) Effects of adrenergic and cholinergic agents on ureteral functions in dogs. Urol Int 34:137–146

Poyet P, Gagne B, Labrie F (1986) Characteristics of the β-adrenergic stimulation of adenylate cyclase activity in rat ventral prostate and its modulation by androgens. Prostate 9:237–245

Pradidarcheep W, Stallen J, Labruyere WT et al (2009) Lack of specificity of commercially available antisera against muscarinic and adrenergic receptors. Naunyn-Schmiedeberg's Arch Pharmacol 379:397–402

Purvis K, Rui H, Gordeladze JO et al (1986) Hormonal activation of the adenylyl cyclases of the rat and human prostate gland. Prostate 8:11–24

Springer JP, Kropp BP, Thor KB (1994) Facilitatory and inhibitory effects of selective norepinephrine reuptake inhibitors on hypogastric nerve-evoked urethral contractions in the cat: a prominent role of urethral β-adrenergic receptors. J Urol 152:515–519

Takasu T, Ukai M, Sato S et al (2007) Effect of YM178, a novel selective β_3-adrenoceptor agonist, on bladder function. J Pharmacol Exp Ther 321:642–647

Takeda M, Obara K, Mizusawa T et al (1999) Evidence for β_3-adrenoceptor subtypes in relaxation of the human urinary bladder detrusor: analysis by molecular biological and pharmacologocial methods. J Pharmacol Exp Ther 288:1367–1373

Takeda H, Yamazaki Y, Igawa Y et al (2002) Effects of β_3-adrenoceptor stimulation on prostaglandin E_2-induced bladder hyperreactivity and on the cardiovascular system in conscious rats. Neurourol Urodyn 21:558–565

Takeda H, Matsuzawa A, Igawa Y et al (2003) Functional characterization of β-adrenoceptor subtypes in the canine and rat lower urinary tract. J Urol 170:654–658

Takemoto J, Masumiya H, Nunoki K et al (2008) Potentiation of potassium currents by beta-adrenoceptor agonists in human urinary bladder smooth muscle cells: a possible electrical mechanism of relaxation. Pharmacology 81:251–258

Thind P, Lose G, Colstrup H et al (1993a) The effect of pharmacological stimulation and blockade of autonomic receptors on the urethral pressure and power generation during coughing and squeezing of the pelvic floor in healthy females. Scand J Urol Nephrol 27:519–525

Thind P, Lose G, Colstrup H et al (1993b) The influence of β-adrenoceptor and muscarinic receptor agonists and antagonists on the static urethral closure function in healthy females. Scand J Urol Nephrol 27:31–38

Tindall AR (1972) Preliminary observations on the mechanical and electrical activity of the rat ureter. J Physiol (London) 223:633–647

Tomiyama Y, Hayakawa K, Shinagawa K et al (1998) β-Adrenoceptor subtypes in the ureteral smooth muscle of rats, rabbits and dogs. Eur J Pharmacol 352:269–278

Tomiyama Y, Murakami M, Hayakawa K et al (2003a) Pharmacological profile of KUL-7211, a selective β-adrenoceptor agonist, in isolated ureteral smooth muscle. J Pharmacol Sci 92:411–419

Tomiyama Y, Murakami M, Yamazaki Y et al (2003b) Comparison between CL-316243- and CGP-12177A-induced relaxations in isolated canine ureter. Pharmacology 68:140–146

Tsujii T, Azuma H, Yamaguchi T et al (1992) A possible role of decreased relaxation mediated by β-adrenoceptors in bladder outlet obstruction by benign prostatic hypertrophy. Br J Pharmacol 107:803–807

Tyagi P, Thomas CA, Yoshimura N et al (2009) Investigations into the presence of functional β1, β2 and β3-adrenoceptors in urothelium and detrusor of human bladder. Int Braz J Urol 35:76–83

Tzortzis V, Mamoulakis C, Rioja J et al (2009) Medical expulsive therapy for distal ureteral stones. Drugs 69:677–692

Ursino MG, Vasina V, Raschi E et al (2009) The β_3-adrenoceptor as a therapeutic target: current perspectives. Pharmacol Res 59:221–234

Vrydag W, Michel MC (2007) Tools to study β_3-adrenoceptors. Naunyn-Schmiedeberg's Arch Pharmacol 374:385–398

Vrydag W, Alewijnse AE, Michel MC (2008) Do gene polymorphisms alone or in combination affect the function of human β_3-adrenoceptors? Br J Pharmacol 156:127–134

Wanajo I, Tomiyama Y, Yamazaki Y et al (2004) Pharmacological characterization of β-adrenoceptor subtypes mediating relaxation in porcine isolated utereral smooth muscle. J Urol 172:1155–1159

Wanajo I, Tomiyama T, Tadachi M et al (2005) The potency of KUL-7211, a selective ureteral relaxant, in isolated canine ureter: comparison with various spasmolytics. Urol Res 33:409–414

Weiss RM, Basset AL, Hoffman BF (1978) Adrenergic innervation of the ureter. Invest Urol 16:123–127

Witte LPW, de Haas N, Mammen M et al (2009) Roles of M_2 and M_3 muscarinic receptors in the modulation of β-adrenoceptor-mediated relaxation of the rat urinary bladder. pA2 online 6: http://www.pA2online.org/abstracts/Vol6Issue4abst053.pdf

Wuest M, Eichhorn B, Grimm MO et al (2009) Catecholamines relax detrusor through β_2-adrenoceptors in mouse and β_3-adrenoceptors in man. J Pharmacol Exp Ther 328:213–222

Yamamoto Y, Koike K (2000) The effects of β-adrenoceptor agonists on KCl-induced rhythmic contraction in the ureter of guinea pig. J Smooth Muscle Res 36:13–19

Yamanishi T, Yasuda K, Kitahara S et al (2006) Effects of 138-355, a β_3-adrenoceptor selective agonist, on relaxation of the human detrusor muscle in vitro. Neurourol Urodyn 25:815–819

Yamazaki Y, Tatemichi S, Maruyama I et al (2002) Characterization of KUC-7483 and its active metabolite, KUC-7322, a selective beta3-adrenoceptor agonist, on bladder function in rats. https://www.icsoffice.org/publications/2002/pdf/095.pdf

Yoshikawa S, Kanie S, Kobayashi R et al (2009) TRK-380, a novel B3-adrenoceptor (AR) agonist, decreases voiding frequency in rats with formalin-induced pollakiuria and suppresses the non-voiding contractions (NVCS) in bladder outlet obstruction (BOO). https://www.icsoffice.org/ASPNET_Membership/Membership/Abstracts/Publish/47/000505.pdf

Yoshikawa S, Morimoto T, Okazaki S et al (2009) TRK-380, a novel B3-adrenoceptor (AR) agonist with potent agonistic activity, functionally relaxes detrusor strips in some species including humans. https://www.icsoffice.org/ASPNET_Membership/Membership/Abstracts/Publish/47/000502.pdf

Muscarinic Acetylcholine Receptors in the Urinary Tract

K.-E. Andersson

Contents

Abstract Muscarinic receptors comprise five cloned subtypes, encoded by five distinct genes, which correspond to pharmacologically defined receptors (M_1–M_5). They belong to the family of G-protein-coupled receptors and couple differentially

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to the G-proteins. Preferentially, the inhibitory muscarinic M_2 and M_4 receptors couple to $G_{i/o}$, whereas the excitatory muscarinic M_1, M_3, and M_5 receptors preferentially couple to $G_{q/11}$. In general, muscarinic M_1, M_3, and M_5 receptors increase intracellular calcium by mobilizing phosphoinositides that generate inositol 1,4,5-trisphosphate (InsP3) and 1,2-diacylglycerol (DAG), whereas M_2 and M_4 receptors are negatively coupled to adenylyl cyclase. Muscarinic receptors are distributed to all parts of the lower urinary tract. The clinical use of antimuscarinic drugs in the treatment of detrusor overactivity and the overactive bladder syndrome has focused interest on the muscarinic receptors not only of the detrusor, but also of other components of the bladder wall, and these have been widely studied. However, the muscarinic receptors in the urethra, prostate, and ureter, and the effects they mediate in the normal state and in different urinary tract pathologies, have so far not been well characterized. In this review, the expression of and the functional effects mediated by muscarinic receptors in the bladder, urethra, prostate, and ureters, under normal conditions and in different pathologies, are discussed.

Keywords Bladder · Cholinergic nerves · Muscarinic receptor agonist · Muscarinic receptor antagonist · Prostate · Ureter · Urethra

1 Introduction

The parasympathetic part of the autonomic nervous system is composed of neurons arising from the brainstem and sacral spinal cord. The main transmitter is acetylcholine (ACh), which is released at both ganglionic synapses and at postganglionic neuroeffector junctions. Nerves containing ACh are called cholinergic, a term introduced by Dale to *describe* neurons that liberate ACh. It should be remembered, however, that such nerves may contain other transmitters and that they can also be found postjunctionally in the sympathetic part of the autonomic nervous system (sweat glands and prostate). In all ganglia, released ACh stimulates nicotinic receptors. However, the postjunctional effects of ACh released from cholinergic nerves, mediating important functional actions in smooth muscle and other structures of the urogenital region, are mediated via muscarinic receptors (Andersson 1993; Andersson and Wein 2004). ACh may be released not only from cholinergic nerves; in isolated human bladder tissue, a basal ACh release of nonneuronal origin has also been demonstrated (Yoshida et al. 2004, 2006, 2008). The released ACh was at least partly generated by the urothelium.

1.1 Muscarinic Acetylcholine Receptors

Muscarinic receptors comprise five subtypes, encoded by five distinct genes (Caulfield and Birdsall 1998). The five gene products correspond to pharmacologically defined

receptors, and M_1–M_5 are used to describe both the molecular and pharmacological subtypes. The muscarinic receptors belong to the family of G-protein-coupled receptors. The G-proteins, consisting of α-, β-, and γ-subunits, are subdivided into G_s, $G_{i/o}$, Gq, and G_{12} depending on the primary sequence homology of their α-subunits (Gudermann et al. 1996). The muscarinic receptor subtypes couple differentially to the G-proteins, and the subunits of G-proteins activate distinct cellular pathways. Preferentially, the inhibitory muscarinic M_2 and M_4 receptors couple to $G_{i/o}$, whereas the excitatory muscarinic M_1, M_3, and M_5 receptors preferentially couple to $G_{q/11}$ (Wu et al. 2000). Muscarinic M_1, M_3, and M_5 receptors increase intracellular calcium by mobilizing phosphoinositides that generate inositol 1,4,5-trisphosphate (InsP3) and 1,2-diacylglycerol (DAG), whereas M_2 and M_4 receptors are negatively coupled to adenylyl cyclase (van Koppen and Kaiser 2003).

2 Bladder

2.1 Cholinergic Nerves

Although histochemical methods that stain for acetylcholine-esterase (AChE) and choline acetyl transferase (ChAT) are not specific for acetylcholine-containing nerves (Lincoln and Burnstock 1993), they have been widely used as an indirect indicator of cholinergic nerves. The vesicular acetylcholine transporter (VAChT) is considered a specific marker for cholinergic nerve terminals (Arvidsson et al. 1997) and should more reliably detect cholinergic nerves. In rat bladder, smooth muscle bundles were supplied with a very rich number of VAChT-positive terminals also containing neuropeptide Y, nitric oxide synthase (NOS), and vasoactive intestinal polypeptide (VIP) (Persson et al. 1997). Similar findings have been reported in human bladders of neonates and children (Dixon et al. 2000). The muscle coat of the bladder was richly innervated by a plexus of branching VAChT-immunoreactive neurons scattered throughout the detrusor muscle. VAChT-positive clusters of ganglion cells could be observed in the muscle coat, but VAChT-immunoreactive nerves, and occasionally, ganglion cells were also observed in the lamina propria. The function of these nerves is unclear, but an afferent function or a neurotrophic role with respect to the urothelium cannot be excluded (Dixon et al. 2000).

2.2 Muscarinic Acetylcholine Receptors

Muscarinic receptors are the physiologically most important mechanisms to elicit contraction of the urinary bladder (Andersson 1993). In the human bladder, the mRNAs and proteins of all muscarinic receptor subtypes have been demonstrated (Sigala et al. 2002; Bschleipfer et al. 2007), with a predominance of M_2 and M_3

receptors (Yamaguchi et al. 1996; Sigala et al. 2002; Giglio and Tobin 2009). These receptors are functionally coupled to G-proteins, but the signal transduction systems vary (Eglen et al. 1996; Hegde and Eglen 1999; Chess-Williams 2002; Giglio and Tobin 2009). Carbachol-induced contraction was associated with phosphoinositide hydrolysis in human detrusor (Andersson et al. 1991; Harriss et al. 1995). In cat detrusor muscle, contraction induced by acetylcholine was found to be mediated via M_3 receptor-dependent activation of $G_{q/11}$ and PLC-β_1 and IP_3-dependent Ca^{2+} release (An et al. 2002). Jezior et al. (2001) found that bethanechol-induced contractions in the rabbit detrusor were practically abolished by inhibitors of Rho-kinase (Y-27632, HA 1077) in combination with a nonselective cation channel inhibitor (LOE-908). They suggested that muscarinic receptor activation of detrusor muscle includes both nonselective cation channels and activation of Rho-kinase. Supporting a role of Rho-kinase in the regulation of rat detrusor contraction and tone, Wibberley et al. (2003) found that Rho-kinase inhibitors (Y-27632 and HA 1077) inhibited contractions evoked by carbachol without affecting the contraction response to KCl. They also demonstrated high levels of Rho-kinase isoforms (I and II) in the bladder. In the human detrusor, Schneider et al. (2004a), demonstrated that the phospholipase C inhibitor U 73122 did not significantly affect carbachol-stimulated bladder contraction. This inhibitor was shown to block IP_3 generation in rat bladder (Schneider et al. 2004b). They concluded that carbachol-induced contraction of human urinary bladder is mediated via M_3 receptors and largely depends on Ca^{2+} entry through nifedipine-sensitive channels and activation of the Rho-kinase pathway. Thus, it may be that the main pathways for muscarinic receptor activation of the human detrusor via M_3 receptors are calcium influx via L-type calcium channels, and increased sensitivity to calcium of the contractile machinery via inhibition of myosin light chain phosphatase through activation of Rho-kinase (Schneider et al. 2004a, b; Andersson 2004a, 2004b; Frazier et al. 2007).

The inhibitory muscarinic M_2 and M_4 receptors may affect adenylyl cyclase activity (Hegde et al. 1997) and inhibit potassium channels (K_{ATP}: Bonev and Nelson 1993; Nakamura et al. 2002; BK: Werner et al. 2007), nonselective cation channels (Kotlikoff et al. 1999; Yamamoto et al. 2008), and transient receptor potential channels (Yoshimura et al. 2008).

2.3 *Receptor Distribution and Function*

Detrusor smooth muscle contains muscarinic receptors of mainly the M_2 and M_3 subtypes (Eglen et al. 1996; Hegde and Eglen 1999; Chess-Williams 2002; Schneider et al. 2004a, b; Giglio and Tobin 2009). In the human detrusor, Goepel et al. (1998), using immunoprecipitation, found a ratio of M_2:M_3 receptors of 3:1. In agreement with this, Mansfield et al. (2005) reported that of the total muscarinic receptor population, 70% were of the M_2 subtype, 20% of the M_3 subtype, and 10% of the M_1 subtype. Also in most animal species, detrusor smooth muscle contains

muscarinic receptors of the M_2 and M_3 subtypes (Eglen et al. 1996; Hegde and Eglen 1999; Chess-Williams 2002). The dominance of muscarinic M_2 receptors is consistently reported. The ratio between muscarinic M_2 and M_3 receptors in binding studies has been estimated as 9:1 and 3:1 in rats and humans, respectively (Wang et al. 1995; Yamanishi et al. 2000).

Muscarinic receptors have also been demonstrated on the *urothelium* and in *suburothelium* (*lamina propria*). The porcine urothelium was found to express a high density of muscarinic receptors, even higher than the bladder smooth muscle (Hawthorn et al. 2000), and, in the rat and human urothelium, the receptor proteins and mRNAs, respectively, for all muscarinic receptor subtypes (M_1–M_5) were demonstrated (Tyagi et al. 2006). In these studies, not only the urothelium but also part of the lamina propria was included in the tissues investigated. However, the expression pattern of the different subtypes in the human urothelium was reported to differ: the M_1 receptors on basal cells, M_2 receptors on umbrella cells, M_3 and M_4 receptors homogenously, and M_5 receptors with a decreasing gradient from luminal to basal cells (Bschleipfer et al. 2007). Mansfield et al. (2005) found, using Real Time-Polymerase Chain Reaction (RT-PCR) analysis, an abundant expression of muscarinic M_2 receptors in the human bladder mucosa (urothelium + lamina propria). Some of these receptors may occur at other locations than the urothelium, e.g., on suburothelial interstitial cells of Cajal (ICC; Mukerji et al. 2006; Grol et al. 2009).

It should be emphasized that there have been concerns about the selectivity of G-protein receptor antibodies (Michel et al. 2009), including antibodies for muscarinic receptor subtypes (Pradidarcheep et al. 2008, 2009; Jositsch et al. 2009). This raises the question of the accuracy of some of the receptor subtype distribution data. Using a well-characterized antibody, Grol et al. (2009) showed that the muscarinic receptors in the lamina propria were located specifically on the ICC. The physiological role of these cells is unknown and consequently the significance of what appears to be a cholinergic signaling system is unclear. It has been suggested that they may be involved in the release of an unknown inhibitory factor (Hawthorn et al. 2000; Chess-Williams 2002).

Muscarinic receptors may also be located on the *presynaptic nerve terminals* and participate in the regulation of transmitter release. The inhibitory prejunctional muscarinic receptors have been classified as M_2 in the rabbit (Tobin and Sjögren 1995; Inadome et al. 1998) and rat (Somogyi and de Groat 1992), and M_4 in the guinea pig (Alberts 1995), rat (D'Agostino et al. 1997) and human (D'Agostino et al. 2000) bladder. Prejunctional facilitatory muscarinic receptors appear to be of the M_1 subtype in the rat and rabbit urinary bladder (Somogyi and de Groat 1992; Tobin and Sjögren 1995; Inadome et al. 1998), but have also been detected in human bladders (Somogyi and de Groat 1999). The muscarinic facilitatory mechanism seems to be upregulated in overactive bladders from chronic spinal cord transected rats. The facilitation in these preparations was primarily mediated by M_3 muscarinic receptors (Somogyi and de Groat 1999; Somogyi et al. 2003). Due to the limitations (selectivity) of available pharmacological tools, it has been difficult to establish whether the prejunctional inhibitory autoreceptors in the human bladder

belong to the M_2 or M_4 receptor subtypes. Provided that results obtained in mice are also representative for human bladder, this ambiguity may be clarified by the use of M_2 and M_4 receptor knockout (KO) animals. In electrically stimulated bladder segments, preincubated with [^{3}H]choline and superfused with oxotremorine, ipratropium stimulated tritium outflow in wild-type and M_2 receptor KO bladders, but had no effect in M_4 receptor KO bladders (Zhou et al. 2002). These studies clearly implicate a key role of M_4 receptors in regulating ACh release from postganglionic cholinergic nerves via a negative feedback mechanism. The existence of facilitatory M_1 receptors, whose presence has been demonstrated pharmacologically, has yet to be confirmed by gene KO studies.

The use of mutant mice deficient in one or more muscarinic receptor subtype has given important information on the contribution of the five muscarinic receptor subtypes to bladder function. In M_3 receptor KO mice, bladder contractile responses to carbachol were found to be attenuated by 95%, consistent with findings from pharmacological studies that have ascribed a major role of M_3 receptors in mediating direct contraction of the bladder (Matsui et al. 2002; Ehlert et al. 2005). Interestingly, cystometric studies (Igawa et al. 2004) showed that, although M_3 KO animals have longer voiding intervals and larger micturition volumes and bladder capacity than wild-type controls, the bladder residual volume in these animals was unchanged, indicating no major functional impairment of bladder emptying (at least up to a certain age). A possible explanation for this finding is that chronic absence of the M_3 receptor is compensated by either another muscarinic receptor or non-cholinergic mechanisms (ATP). The residual (5%) direct contractile responses that persist in M_3 KO mice were completely lost in mice lacking both M_2 and M_3 receptors, implying that the M_2 receptor is capable of mediating small direct contractile responses (Matsui et al. 2002).

The functional role for the M_2 receptors has not been clarified, but cystometric studies have demonstrated small increases in voiding intervals and micturition volumes in M_2 receptor KO mice (Igawa et al. 2004). It has been suggested that M_2 receptors may oppose sympathetically (β_3-AR) mediated smooth muscle relaxation by inhibition of adenylyl cyclase (Hegde et al. 1997). The indirect cAMP-related contractile effects of M_2 receptor activation have also been confirmed using M_2 and M_3 KO animals (Ehlert et al. 2005). In bladders from M_3 KO mice that were precontracted with $PGF_{2\alpha}$ and relaxed with isoprenaline or forskolin, oxotremorine produced concentration-dependent contractile responses that exhibited an M_2 receptor profile in competitive antagonism studies and were completely abolished in M_2/M_3 receptor KO mice. However, the biochemical mechanisms underlying the potential crosstalk between M_2 and M_3 receptors have not been definitely established. Ehlert et al. (2005) suggested that the M_2 receptor mediates contractions indirectly in the bladder by enhancing M_3 receptor-mediated contractions and inhibiting relaxation. In certain disease states, M_2 receptors may contribute to contraction of the bladder (see below).

In the trigone, two distinct layers of smooth muscle can be distinguished. The deeper one is believed to be a continuation of the detrusor and therefore predominantly innervated by cholinergic nerves. The superficial trigone, which develops

from an outgrowth of the mesonephric duct, is predominantly innervated by adrenergic nerves, but it has also a cholinergic innervation, contains muscarinic receptors, and can be contracted by muscarinic receptor agonists (Speakman et al. 1988; Roosen et al. 2008). Sigala et al. (2002) studied the mRNA expression of muscarinic receptor subtypes in human bladder, including the trigone. No separation between deep and superficial layers was done. All muscarinic receptor subtypes were expressed, but M_2 and M_5 subtypes seemed to be selectively expressed in the male samples.

2.4 Receptor Changes in Disease

Not unexpectedly, the density and function of muscarinic receptors can be changed in different urinary tract pathologies (see, e.g., Ruggieri and Braverman 2006). Thus, muscarinic receptor functions may be changed in, e.g., bladder outlet obstruction, neurogenic, and idiopathic DO, and diabetes. However, it is not always clear what the changes mean in terms of changes in detrusor function.

There is good evidence that *bladder outlet obstruction* may change the cholinergic functions of the bladder. Thus, detrusor denervation as a consequence of outflow obstruction has been demonstrated in several species including humans (Gosling et al. 1986; Sibley 1987; Speakman et al. 1987; Pandita et al. 2000). In pigs with experimental outflow obstruction, detrusor response to intramural nerve stimulation was decreased, but there was a postjunctional supersensitivity to acetylcholine (Sibley 1984). Similar changes were found in bladders of obstructed patients with DO (Harrison et al. 1987). It was suggested that the supersensitivity was due to partial denervation of the bladder, postjunctional muscarinic receptor changes, and that one consequence of this may be DO (Sibley 1987). On the other hand, Yokoyama et al. (1991) found that the responses to acetylcholine of detrusor strips from obstructed patients with DO were not significantly different from those without. However, they suggested that bladder overdistension caused by infravesical obstruction may lead to supersensitivity of the detrusor muscle secondary to denervation. The obstructed human bladder often shows an increased (up to ≈50%) atropine-resistant contractile component (Sjögren et al. 1982; Sibley 1984; Bayliss et al. 1999). This may be taken as indirect evidence of changes in the cholinergic functions of the bladder, since normally, the atropine-resistant component is almost negligible (Andersson 1993; Tagliani et al. 1997; Bayliss et al. 1999).

In the obstructed rat bladder, M_3 receptors were found to play a predominant role in mediating detrusor contraction (Krichevsky et al. 1999). However, Braverman and Ruggieri (2003) found that in obstructed, hypertrophied rat bladders, there was an increase in total and M_2 receptor density, whereas there was a reduction in M_3 receptor density. The functional significance of such a change for voiding function has not been established.

Turner and Brading (1997) suggested that in *DO* alterations of the smooth muscle (supersensitivity), as mentioned previously, may be seen as a consequence

of "patchy denervation" of the detrusor. Immunohistological investigations of the obstructed mouse bladder (exhibiting DO) revealed that the nerve distribution patterns were markedly changed. In large parts of the detrusor, the smooth muscle bundles were completely devoid of accompanying VAChT-immunoreactive varicose terminals ("patchy denervation"). In other parts, the densities of nerve structures were nearly normal (Pandita et al. 2000). The importance of patchy denervation was supported by studies on human overactive bladders (Charlton et al. 1999; Mills et al. 2000).

An increased sensitivity to muscarinic receptor stimulation has been demonstrated in smooth muscle preparations from patients with *idiopathic and neurogenic DO* (Stevens et al. 2007). German et al. (1995) found that isolated detrusor strips from patients with neurogenic DO were supersensitive to both carbachol and KCl, but responded like normal controls to intramural nerve stimulation. The results were interpreted to suggest a state of postjunctional supersensitivity of the detrusor secondary to a partial parasympathetic denervation. However, other investigators have arrived at other conclusions. Kinder and Mundy (1987) compared detrusor muscle from human normal bladders to that from patients with idiopathic or neurogenic DO. They found no significant differences in the degree of inhibition of electrically induced contractions produced by tetrodotoxin or atropine in detrusor strips from any of these bladders, and no significant differences in the concentration–response curves for acetylcholine. In neurogenic DO, a decreased number of muscarinic receptors were demonstrated (Restorick and Mundy 1989), but its relation to overactivity was unclear. In patients with myelomeningocele and detrusor dysfunction, Gup et al. (1989a) found no supersensitivity to carbachol and no changes in the binding properties of the muscarinic receptors.

In the denervated (decentralized) rat bladder, M_2 receptors, or a combination of M_2 and M_3, mediated contractile responses and the two types of receptor seemed to act in a facilitatory manner to mediate contraction (Braverman et al. 1998, 1999, 2002). Again, the functional significance of this change for voiding function has not been established.

Mansfield et al. (2007) found that M_3 receptor mRNA expression was significantly less in mucosa from patients with idiopathic DO than from age-matched controls. M_2 receptor expression was not significantly different. This is in contrast to previous findings by Mukerji et al. (2006) who found an increase in M_2 and M_3 immunostaining in suburothelial ICCs in clinical bladder syndromes, which was correlated with clinical scores of urgency and frequency.

Patients with *diabetes mellitus* and voiding dysfunction show a variety of urodynamic abnormalities, including DO and impaired detrusor contractility (Kaplan et al. 1995). In bladders from diabetic animals, an increased density of muscarinic receptors accompanied by an enhanced muscarinic receptor-mediated phosphoinositide hydrolysis was found (Latifpour et al. 1989; Mimata et al. 1995). Supersensitivity of postjunctional muscarinic receptors may develop (Hashitani and Suzuki 1996). Stevens et al. (2006) demonstrated detrusor supersensitivity to carbachol already after 1 week of untreated diabetes in the rat. This enhanced sensitivity could partly be explained by an increase in receptor density, but there

appeared to be no change in receptor/G-protein coupling. Tong et al. (2006) found that in rats with streptozotocin-induced diabetes there was an increased mRNA and protein expression of the M_2 receptor both in the urothelium and in the muscle layer. Further study (Cheng et al. 2007) reported that in such animals, both mRNA and protein expression of the M_3 receptor also increased in these structures. However, it is unclear what these receptor changes mean for the functional bladder disorders seen in diabetic patients.

Modulations of the muscarinic receptor functions may also occur during *cystitis* (Giglio and Tobin 2009). For example, cyclophosphamide-induced cystitis caused an upregulation of muscarinic receptors (M_5 receptors) (Giglio et al. 2005). In bladders from cats with feline interstitial cystitis, there was an increase in urothelial ACh release and/or mucosal muscarinic receptor sensitivity in the mucosal layer (Ikeda et al. 2009).

3 Urethra

The female (Dass et al. 2001) and male (Koraitim 2008) human urethra have two distinct smooth muscle layers: an outer circular and an inner longitudinal. In other species, e.g., the pig, a third outer, longitudinal layer can sometimes be demonstrated. Innervation, receptor distribution, and function may differ between these layers.

3.1 Cholinergic Nerves

Urethral smooth muscle receives a rich cholinergic innervation. For example, in dogs, pigs, and humans, ChAT immunoreactivity and AChE staining, indicative of cholinergic innervation, were shown to be widely distributed all over the lower urinary tract (LUT) (Ek et al. 1977; Persson et al. 1995; Arrighi et al. 2008). Throughout the human urethra the distribution of AChE-positive nerve fibers was uniform, but the number was clearly less than in the urinary bladder (Ek et al. 1977). Most probably, the cholinergic nerves cause relaxation of the outflow region at the start of micturition by releasing NO and other relaxant transmitters (Persson et al. 1995), but otherwise their functional role is largely unknown.

3.2 Receptor Distribution and Function

The distribution and number of muscarinic receptors in different parts of the urethra seems to vary. Compared to the bladder, the number of muscarinic receptor binding sites in the rabbit urethra was lower (Johns 1983), and by autoradiography, it was

demonstrated that muscarinic receptors were abundant in the outer parts of the urethral wall and decreased in density in luminal direction (Mattiasson et al. 1990).

Muscarinic receptor agonists contract isolated urethral smooth muscle from several species, including humans, but these responses seem to be mediated mainly by the longitudinal muscle layer (Ek et al. 1977; Andersson 1993). Taki et al. (1999), investigating the whole length of the female human urethra, found that ACh contracted only the proximal part and the bladder neck. If this contractile activation is exerted in the longitudinal direction, it should be expected that the urethra is shortened and that the urethral pressure decreases. Experimentally, in vitro resistance to flow in the urethra was increased only by high concentrations of ACh (Persson and Andersson 1976; Andersson et al. 1978).

Prejunctional muscarinic receptors may influence the release of both noradrenaline and ACh in the bladder neck/urethra. In urethral tissue from both rabbit and humans, carbachol decreased and scopolamine increased concentration dependently the release of [^{3}H]noradrenaline from adrenergic and of [^{3}H]choline from cholinergic nerve terminals (Mattiasson et al. 1984). At least theoretically, this would mean that released ACh could inhibit noradrenaline release, thereby decreasing urethral tone and intraurethral pressure. However, in humans, tolerable doses of the muscarinic receptor agonist, bethanechol (Ek et al. 1978), and the antagonist, emeprone (Ulmsten and Andersson 1977), had little effect on intraurethral pressure.

The muscarinic receptor subtypes involved in contractile effects on smooth muscle, or controlling transmitter release in the urethra, have not been established. It has been reported that M_1, M_2, and M_3 receptors all mediate contraction of the circular muscle of the rabbit urethra after stimulation with carbachol (Mutoh et al. 1997; Nagahama et al. 1998).

3.3 Changes in Disease

In cats with complete and partial sacral decentralization, urethral supersensitivity to bethanechol chloride was demonstrated (el-Salmy et al. 1985). After complete lesions, the urethra showed exaggerated constriction responses to the drug. Part of the response was indirect and adrenergically mediated, suggesting that muscarinic receptor stimulation caused release of noradrenaline, but part was a direct effect mediated via muscarinic receptors. Also after partial decentralization the urethra responded to subthreshold doses of subcutaneous bethanechol. As mentioned previously, the muscarinic facilitatory mechanism observed on prejunctional nerve terminals (M_3 receptor mediated) seems to be upregulated in overactive bladders from chronic spinal cord transected rats (Somogyi and de Groat 1999; Somogyi et al. 2003). This may also be the case in the urethra. el-Salmy et al. (1985) suggested that urethral supersensitivity to bethanechol might be responsible for a nonvoiding outcome after bethanechol injection in patients with complete cauda equina lesions.

4 Prostate

4.1 Cholinergic Nerves

Chapple et al. (1991) reported that AChE-positive nerves can be found in various parts of the human prostate. The greatest density of nerves was found in the proximal central prostate, followed by the anterior capsule and distal central prostate, with the least density in the peripheral prostate. AChE-positive axons can be demonstrated near the base of the epithelium. Since muscarinic receptors are concentrated more or less exclusively to the secretory epithelium (Hedlund et al. 1985), these cholinergic nerves were suggested to be secretory. Supporting such a view, prostatic cholinergic nerves also contain NOS and VIP (Hedlund et al. 1997), which are known to stimulate secretion.

4.2 Muscarinic Receptors

Several studies have investigated the presence of muscarinic receptor protein in the human prostate by radioligand binding using either [^{3}H]-methylquinuclidinyl benzilate (QNB) or [^{3}H]N-methylscopolamine (NMS) as the radioligand (Witte et al. 2008). Quantitatively, the density seemed to be higher than that of α_1-ARs. Using [^{3}H]-NMS, Anisuzzaman et al. (2008) were able to demonstrate M_1 and M_2, but not M_3, receptors in the human prostate. The expression of mRNA encoding muscarinic receptors in a primary culture of stromal and epithelial cells from the human prostate (patients with BPE) was studied by Obara et al. (2000). mRNA for all five muscarinic receptor subtypes was detected, however, primary stromal cultures only showed M_2, M_3, and M_4 receptors with M_2 apparently being most abundant. In contrast, primary epithelial cultures exhibited M_1, M_2, and M_5 receptor mRNA.

4.3 Distribution and Function

Autoradiographic studies have been performed, allowing association of the receptors with specific anatomic structures. Most muscarinic receptors were found in the prostatic epithelium, whereas the stroma contained far fewer, if any, muscarinic receptors (Lepor and Kuhar 1984; Hedlund et al. 1985; James et al. 1989). Thus, muscarinic receptors in the human prostate are primarily found in the epithelial rather than the stromal cells.

Based on a rank order of potency of subtype-selective antagonists, it was proposed that stromal cells in the prostate mainly express M_2 receptors; however, it should be noted that the compounds used may allow a differentiation between M_1, M_2, and M_3 receptors, but not from M_4 and M_5 receptors (Witte et al. 2008).

However, a preponderance of M_2 receptors in prostatic stromal cells is in good agreement with the available mRNA data (Obara et al. 2000).

Ruggieri et al. (1995) assessed the muscarinic receptor subtypes in prostatic homogenates from humans and found the majority of receptors to be of the M_1 subtype. Immunoprecipitation of muscarinic receptors with subtype-selective antibodies for M_{1-4} receptors confirmed that M_1 receptors are more abundant than the other three subtypes combined. Using the M_1 antibody for immunohistochemistry staining of the prostatic epithelium, but not stroma, was observed. Taken together, these data suggest that the human prostate mainly expresses M_1 receptors on epithelial cells at the protein level, whereas the much smaller population of receptors on the stromal cells may belong to the M_2 subtype.

Various investigators have studied a possible contractile effect of muscarinic receptor agonists in human prostate. Some studies did not detect contractile responses (Caine et al. 1975; Hedlund and Andersson 1985), whereas others do report such effects (Gup et al. (1989a, b); Kester et al. 2003). Although contractions were sensitive to muscarinic antagonists such as atropine (Kester et al. 2003), their magnitude was small as compared to the contractile effects of α_1-AR agonists and were seen only with very high agonist concentrations. Therefore, it appears that muscarinic receptors contribute to human prostate contraction in a minor way only, which is in line with their primary expression on epithelial rather than stromal cells.

Considering the distribution of muscarinic receptors in the human prostate, a role for muscarinic receptors in the regulation of human prostatic secretion is likely, but has to be definitely established. Since the predominant muscarinic receptor expression is found in the prostatic epithelium, it could be expected that muscarinic receptors contribute to the regulation of prostatic growth. Indeed, primary cultures from patients with either enlarged prostates or prostate cancer exhibited growth stimulation on treatment with carbachol (Rayford et al. 1997).

4.4 Changes in Disease

Blanco and Robinson (2004) studied the relationship of muscarinic receptors to differentiation in prostate cancer. They found that the presence of M_3 receptors, as demonstrated by immunostaining, distinguished normal glandular epithelium and low-grade carcinomas from poorly differentiated tumors. They suggested that its demonstration could be a useful tool in distinguishing grade of tumor clinically. It could be expected that in, for example, benign prostatic hyperplasia (BPH) and lower urinary tract symptoms (LUTS), the densities of muscarinic receptors would change. However, comparisons of the densities of NMS binding sites in samples from asymptomatic patients with those from symptomatic patients with enlarged prostates obtained either by open prostatectomy or transurethral resection (Gup et al. 1990) showed no significant differences between the groups.

5 Ureter

5.1 Cholinergic Nerves

In the whole human ureter, two neuronal networks, one in the lamina propria and one in the wall smooth muscle, were demonstrated by confocal laser scanning microscopy (Nemeth et al. 2001). These nerves were in continuity with the neuronal network of the renal pelvis. Correspondingly, a rich supply of cholinergic nerve fibers was found in ureters from both humans and animals, forming dense neuromuscular, subepithelial, and perivascular plexuses, as demonstrated by staining for AChE and ChAT and by electron microscopy (Schulman 1975; Prieto et al. 1994; Rolle et al. 2002, 2008).

5.2 Muscarinic Receptors

In the human ureter, only M_2, M_3, and M_5 receptor subtypes were identified by reverse RT-PCR. However, the presence of all five muscarinic receptor subtypes (M_1–M_5) was immunohistochemically demonstrated (Sakamoto et al. 2006). An interesting finding was the identification of relatively high levels of M_5 receptors by both immunocytochemical and RT-PCR analysis. In a study of pig ureter, using [^{3}H]-QNB binding, the antagonist profile clearly indicated the existence of an M_2 receptor population. In addition, the presence of a minor non-M_2 population, possibly formed by a mixture of several muscarinic subtypes (i.e., M_1, M_3, and/or M_4), could not be excluded (Hernández et al. 1995).

5.3 Distribution and Function

It has generally been observed that muscarinic receptor agonists produce an excitatory effect by a direct stimulation of muscarinic receptors in ureteral smooth muscle from several animal species. However, in, for example, dogs, cholinergic stimulation may induce ureteral contractions via an indirect release of catecholamines (see, Tomiyama et al. 2004). Information on human ureters seems to be scarce – contractile responses to ACh have been demonstrated, but they were weak and inconsistent (Long and Nergårdh 1978).

Tomiyama et al. (2003) investigated the functional muscarinic receptor subtypes in the isolated canine ureter. They found that carbachol concentration dependently increased the frequency of the rhythmic contractions in isolated spiral preparations, whereas the drug induced relaxation of longitudinal preparations. They suggested that, based on their experiments with subtype selective drugs, that the carbachol-induced rhythmic contractions were mediated via the M_3 receptor, while the relaxation

in the longitudinal preparation probably was mediated mainly via the M_4 receptor. In functional experiments on the pig intravesical ureter, Hernández et al. (1993) found evidence for a tonic response mediated via M_1 receptors, whereas the phasic activity could involve either both M_2 and M_3 or an M_4 muscarinic receptor.

5.4 Receptor Changes in Disease

Generally, stimulation by cholinergic agonists induces contractions in isolated ureteral preparations and in anesthetized animals. However, Tomiyama et al. (2004), evaluating the effects of carbachol and atropine on completely (COU) and partially obstructed ureters (POU) in anesthetized dogs, found that intravenous carbachol dose dependently decreased elevated intraureteral pressure in COU and peristalsis in POU. They suggested that these effects were mediated via stimulation of M_4 receptors on the smooth muscle. Their conclusion was that when ureteral motility is increased by obstruction, muscarinic receptor stimulation may have a suppressive effect on the obstructed ureter (at least in the anesthetized dog). Whether or not this is the case in humans remains to be established.

6 Muscarinic Receptor Agonists

Bladder emptying requires a coordinated contraction of adequate magnitude of the detrusor muscle, a concomitant lowering of the outflow resistance at the level of the smooth and striated sphincter, and absence of anatomic obstruction. If these requirements are not fulfilled, urinary retention or residual urine will result (Andersson and Wein 2004). Detrusor underactivity (DU) is defined as a contraction of reduced strength and/or duration, resulting in prolonged bladder emptying and/or a failure to achieve complete bladder emptying within a normal time span (Taylor and Kuchel 2006). Theoretically, when the primary cause of emptying failure is insufficient strength of the detrusor, drugs stimulating bladder contraction via action on muscarinic receptors may be indicated. Carbachol and bethanechol are the two main agents that have been used clinically. Both are quaternary amines, not easily hydrolyzed by cholinesterases, fairly selective for muscarinic receptors with little (bethanechol) or some (carbachol) effect on nicotinic receptors, and they have no distinct receptor subtype selectivity (bethanechol possibly for M_3 receptors). For unclear reasons, both drugs have been considered to have some selectivity for the urinary bladder (Finkbeiner 1985). When bethanechol is given subcutaneously to neurologically normal individuals, the effect on the bladder is characterized by an increased stiffness of the bladder wall at rest. This is reflected in high pressures in the cystometrogram, decreased capacity, and an increased awareness of the distended bladder, effects that may not result in an increase in urine flow rate during voiding. De Wachter and Wyndaele (2001) determined the bladder electrical

threshold in healthy volunteers receiving 5 mg bethanechol subcutaneously and demonstrated a marked decrease in the volume at which various filling sensations occurred and that the electrical threshold decreased. De Wachter et al. (2003) treated 18 women with impaired detrusor contraction with subcutaneous bethanechol (5 mg four times daily) for 10 days. At the end of treatment 61% of the patients voided without a postvoid residual volume. It was also found that in these women the sensation of filling and electrical sensitivity were significantly increased compared to before treatment. It might be that bethanechol-induced voiding requires intact bladder reflex pathways, as was demonstrated in cats (Twiddy et al. 1980). De Wachter et al. (2003) suggested that patients likely to respond to bethanechol can be identified by determination of the bladder electrical perception threshold. Riedl et al. (2000) tested electromotive administration of intravesical bethanechol in patients with detrusor areflexia. A mean pressure increase of 34 cm H_2O during the electromotive administration was found in 24 of 26 patients with areflexia and neurological disease. Patients with BPH, receiving prazosin to relieve their outflow obstruction, had no effect on urodynamic variables, or improvement of bladder emptying when treated with oral carbachol in a dose that caused side effects (perspiration and gastrointestinal discomfort) (Hedlund and Andersson 1988).

A recent systematic review of controlled clinical studies on the use of direct and indirect parasympathetic agonists in patients with an underactive detrusor showed that these drugs do not exhibit consistent benefit and may even be harmful (Barendrecht et al. 2007).

7 Muscarinic Receptor Antagonists

Terminology. Since ACh acts on both muscarinic and nicotinic receptors, the term "anticholinergic drugs" should cover agents with effects on both types of receptor. However, most often "anticholinergic" is used synonymously with "antimuscarinic." Since the commonly used drugs for treatment of LUT disorders are selective for muscarinic receptors, "antimuscarinics" is the preferred name for these agents.

Mechanism of action. Antimuscarinics, by definition, block muscarinic receptors (Abrams and Andersson 2007), and this is the basis for their effects, including the beneficial action in the treatment of DO/OAB. However, where and when they act is still a matter of discussion. The traditional view is that antimuscarinics act by blocking the muscarinic receptors on the detrusor muscle, thereby inhibiting the voiding contraction. This may be the case in neurogenic DO, where the goal is to "paralyse" the bladder. However, in idiopathic DO/OAB, the treatment goal is to eliminate involuntary detrusor contractions and urgency and at the same time maintain the ability of the bladder to contract. At the doses of antimuscarinics used for treatment of idiopathic DO/OAB, the drugs act mainly during the storage phase, decreasing urgency, and increasing bladder capacity. Importantly, during this phase, there is normally no spinal parasympathetic input to the LUT (Andersson 2004a, b). Furthermore, commonly used antimuscarinics are competitive antagonists. During the voiding contraction, there is a massive release of ACh from parasympathetic nerve endings, overriding the blocking effects of the

antimuscarinics. Undeniably, high doses of antimuscarinics can produce urinary retention in humans, but in the dose range used for beneficial effects in OAB/DO, there is little evidence for a significant reduction of the voiding contraction (Finney et al. 2006). However, there is good experimental evidence that the drugs act during the filling phase by decreasing the activity in bladder afferent nerves (both C- and Aδ-fibers) (De Laet et al. 2006; Iijima et al. 2007).

As mentioned previously, muscarinic receptors are found on bladder mucosa (urothelium + lamina propria), where their density can be even higher than in detrusor muscle. The commonly used and clinically effective muscarinic receptor antagonists were found to bind to receptors located both on the bladder mucosa and the detrusor, suggesting that muscarinic receptors in the mucosa may represent an important site of action for these agents in OAB (Mansfield et al. 2009). However, whether the muscarinic receptors on urothelial cells can influence micturition in humans has not yet been established, even if there is experimental evidence showing that it is possible to initiate micturition via urothelial stimulation (Andersson et al. 2009). A basal ACh release has been demonstrated in isolated human bladder tissue (Yoshida et al. 2004, 2006, 2008). The released ACh was probably of nonneuronal origin and, at least partly, generated by the urothelium. Thus, during the storage phase, ACh may be released from both neuronal and nonneuronal sources and directly or indirectly (by ATP release?) excite afferent nerves in the suburothelium and within the detrusor. Thus, both these mechanisms may be important in the pathophysiology of overactive bladder and represent possible targets for antimuscarinic drugs.

Pharmacologic properties. Generally, antimuscarinics are tertiary or quaternary amines (Guay 2003; Abrams and Andersson 2007), differing with regard to lipophilicity, molecular charge, and even molecular size. Tertiary compounds generally have higher lipophilicity and molecular charge than quaternary agents. Among the drugs used for treatment of DO/OAB (Andersson et al. 2009), atropine, darifenacin, fesoterodine (and its active metabolite 5-hydroxymethyl tolterodine), oxybutynin, propiverine, solifenacin, and tolterodine are tertiary amines. They are generally well absorbed from the gastrointestinal tract and should theoretically be able to pass into the central nervous system (CNS), dependent on their individual physicochemical properties. High lipophilicity, small molecular size, and less charge will increase the possibilities to pass the blood–brain barrier, but for some of the drugs this is counteracted by active transport out of the CNS by P-glycoprotein. Quaternary ammonium compounds, such as propantheline and trospium, are not well absorbed, pass into the CNS to a limited extent, and have a low incidence of CNS side effects (Guay 2003; Abrams and Andersson 2007).

Many antimuscarinics are metabolized by the P450 enzyme system to active and/or inactive metabolites (Guay 2003; Michel and Hegde 2006). The most commonly involved P450 enzymes are CYP2D6 and CYP3A4. The metabolic conversion creates a risk for drug–drug interactions, resulting in either reduced (enzyme induction) or increased (enzyme inhibition and substrate competition) plasma concentration/effect of the antimuscarinic and/or interacting drug.

Antimuscarinics secreted by the renal tubules (e.g., trospium) may theoretically be able to interfere with the elimination of other drugs using this mechanism.

Antimuscarinics are still the most widely used treatment for urgency and urgency incontinence (Andersson 2004a, b; Andersson et al. 2009). However, currently used drugs lack selectivity for the bladder, and effects on other organ systems may result in side effects, which limit their usefulness. One way of avoiding many of the antimuscarinic side effects is to administer the drugs intravesically. However, this is practical only in a limited number of patients.

Clinical efficacy. The clinical relevance of the efficacy of antimuscarinic drugs relative to placebo has been questioned (Herbison et al. 2003). However, large meta-analyses of studies performed with the currently most widely used drugs (Chapple et al. 2005, 2008; Novara et al. 2008) clearly show that antimuscarinics are of significant clinical benefit.

It should be emphasized that none of the antimuscarinic drugs in common clinical use (darifenacin, fesoterodine, oxybutynin, propiverine, solifenacin, tolterodine, or trospium) is ideal as a first-line treatment for *all* DO/OAB patients. Optimal treatment should be individualized, implying that the patient's comorbidities and concomitant medications, and the pharmacological profiles of the different drugs, should be taken into consideration (Chapple et al. 2008).

Tolerability and safety. An extensive literature supports that antimuscarinics for the treatment of OAB symptoms are generally well tolerated. The adverse effect profiles of the different drugs are determined by their organ and muscarinic receptor subtype selectivities and pharmacokinetic parameters. The most commonly reported adverse effects are dry mouth, constipation, headache, and blurred vision (Chapple et al. 2008).

Among the more serious concerns related to antimuscarinic use is the risk of cardiac adverse effects, particularly QT prolongation and induction of polymorphic ventricular tachycardia (torsade de pointes), and increases in heart rate. It should be emphasized that QT prolongation and its consequences are not related to blockade of muscarinic receptors, but rather linked to inhibition of the hERG potassium channel in the heart (Roden 2004). Thus, QT prolongation is not a class effect of antimuscarinics. In general, the cardiovascular safety for antimuscarinic drugs seems to be acceptable. However, the potential of the different agents to increase heart rate or to prolong the QT time has not been extensively explored. Differences between the different drugs cannot be excluded, but risk assessments based on available evidence are not possible.

Another concern is that antimuscarinic drugs commonly used to treat overactive bladder can be associated with CNS side effects including cognitive dysfunction, memory impairment, dizziness, fatigue, and headache. With the exception for oxybutynin, CNS-related side effects are not commonly found when investigated. The potential to cause CNS-related adverse effects may differ between drugs, but in the absence of comparative trials relative risk assessments are not possible.

For detailed discussion of the clinical efficacy, tolerability, and safety of the individual antimuscarinics, see Andersson et al. (2009).

8 Summary and Perspectives

Our knowledge of muscarinic receptors in the urinary tract has increased greatly during the last decade. It has been established that in the urinary bladder, the M_3 receptor is the principal receptor for detrusor contraction. The functional role of the M_2 receptors is still unclear – they may enhance contractions mainly by inhibition of detrusor relaxation. However, alterations in the muscarinic receptor signaling systems may contribute to the pathogenesis of functional bladder disorders. Further studies of the muscarinic receptors of afferent nerves, urothelium, ICC in the suburothelium and detrusor muscle, and even on the detrusor muscle are required to establish their roles in bladder function when both normal and changed by disease. Our knowledge of the distribution of muscarinic receptor subtypes and their roles for the functions of the urethra, prostate, and ureters is incomplete and should be fruitful fields for further research. Even if the general mechanisms for muscarinic receptor-induced activation of the bladder seem established, we still do not know how to use muscarinic receptor agonists to stimulate bladder emptying. This is a field where the medical need is urgent. Treatment of DO/OAB with antimuscarinics is still the gold standard, but is far from ideal and can probably be improved. Whether or not this can be achieved by individually tailored treatment with existing drugs, or requires new drugs with different receptor subtype selectivities and pharmacokinetic properties, awaits further study.

References

Abrams P, Andersson KE (2007) Muscarinic receptor antagonists for overactive bladder. BJU Int 100(5):987–1006

Alberts P (1995) Classification of the presynaptic muscarinic receptor subtype that regulates 3H-acetylcholine secretion in the guinea pig urinary bladder in vitro. J Pharmacol Exp Ther 274(1):458–468

An JY, Yun HS, Lee YP, Yang SJ, Shim JO, Jeong JH, Shin CY, Kim JH, Kim DS, Sohn UD (2002) The intracellular pathway of the acetylcholine-induced contraction in cat detrusor muscle cells. Br J Pharmacol 137(7):1001–1010

Andersson KE (1993) Pharmacology of lower urinary tract smooth muscles and penile erectile tissues. Pharmacol Rev 45(3):253–308

Andersson KE (2004a) Antimuscarinics for treatment of overactive bladder. Lancet Neurol 3(1):46–53

Andersson K-E (2004b) Detrusor contraction – focus on muscarinic receptors. Scand J Urol Nephrol Suppl 215:54–57

Andersson KE, Wein AJ (2004) Pharmacology of the lower urinary tract: basis for current and future treatments of urinary incontinence. Pharmacol Rev 56(4):581–631

Andersson KE, Persson CG, Alm P, Kullander S, Ulmsten U (1978) Effects of acetylcholine, noradrenaline, and prostaglandins on the isolated, perfused human fetal urethra. Acta Physiol Scand 104(4):394–401

Andersson KE, Holmquist F, Fovaeus M, Hedlund H, Sundler R (1991) Muscarinic receptor stimulation of phosphoinositide hydrolysis in the human isolated urinary bladder. J Urol 146(4):1156–1159

Andersson K-E, Chapple CR, Cardozo L et al (2009) Pharmacological treatment of urinary incontinence. In: Abrams P, Cardozo L, Khoury S, Wein A (eds) Incontinence, 4th international consultation on incontinence. Plymouth. Plymbridge Distributors Ltd, Plymouth, UK, pp 631–699

Anisuzzaman AS, Morishima S, Suzuki F, Tanaka T, Yoshiki H, Sathi ZS, Akino H, Yokoyama O, Muramatsu I (2008) Assessment of muscarinic receptor subtypes in human and rat lower urinary tract by tissue segment binding assay. J Pharmacol Sci 106(2):271–279

Arrighi S, Bosi G, Cremonesi F, Domeneghini C (2008) Immunohistochemical study of the pre- and postnatal innervation of the dog lower urinary tract: morphological aspects at the basis of the consolidation of the micturition reflex. Vet Res Commun 32(4):291–304

Arvidsson U, Riedl M, Elde R, Meister B (1997) Vesicular acetylcholine transporter (VAChT) protein: a novel and unique marker for cholinergic neurons in the central and peripheral nervous systems. J Comp Neurol 378(4):454–467

Barendrecht MM, Oelke M, Laguna MP, Michel MC (2007) Is the use of parasympathomimetics for treating an underactive urinary bladder evidence-based? BJU Int 99(4):749–752

Bayliss M, Wu C, Newgreen D, Mundy AR, Fry CH (1999) A quantitative study of atropine-resistant contractile responses in human detrusor smooth muscle, from stable, unstable and obstructed bladders. J Urol 162(5):1833–1839

Blanco M, Robinson MJ (2004) Muscarinic M3 acetylcholine receptor immunostaining in paraffin-embedded normal and neoplastic prostatic gland tissue. Ann Diagn Pathol 8(6):333–336

Bonev AD, Nelson MT (1993) Muscarinic inhibition of ATP-sensitive K+ channels by protein kinase C in urinary bladder smooth muscle. Am J Physiol 265(6 Pt 1):C1623–C1628

Braverman AS, Ruggieri MR Sr (2003) Hypertrophy changes the muscarinic receptor subtype mediating bladder contraction from M3 toward M2. Am J Physiol Regul Integr Comp Physiol 285(3):R701–R708

Braverman AS, Luthin GR, Ruggieri MR (1998) M2 muscarinic receptor contributes to contraction of the denervated rat urinary bladder. Am J Physiol 275:R1654–R1660

Braverman A, Legos J, Young W, Luthin G, Ruggieri M (1999) M2 receptors in genito-urinary smooth muscle pathology. Life Sci 64:429–436

Braverman AS, Tallarida RJ, Ruggieri MR Sr (2002) Interaction between muscarinic receptor subtype signal transduction pathways mediating bladder contraction. Am J Physiol Regul Integr Comp Physiol 283(3):R663–R668

Bschleipfer T, Schukowski K, Weidner W, Grando SA, Schwantes U, Kummer W, Lips KS (2007) Expression and distribution of cholinergic receptors in the human urothelium. Life Sci 80(24–25):2303–2307

Caine M, Raz S, Zeigler M (1975) Adrenergic and cholinergic receptors in the human prostate, prostatic capsule and bladder neck. Br J Urol 47(2):193–202

Caulfield MP, Birdsall NJM (1998) International Union of Pharmacology: XVII. Classification of muscarinic acetylcholine receptors. Pharmacol Rev 50:279–290

Chapple CR, Crowe R, Gilpin SA, Gosling J, Burnstock G (1991) The innervation of the human prostate gland – the changes associated with benign enlargement. J Urol 146 (6):1637–1644

Chapple C, Khullar V, Gabriel Z, Dooley JA (2005) The effects of antimuscarinic treatments in overactive bladder: a systematic review and meta-analysis. Eur Urol 48(1):5–26

Chapple CR, Khullar V, Gabriel Z, Muston D, Bitoun CE, Weinstein D (2008) The effects of antimuscarinic treatments in overactive bladder: an update of a systematic review and meta-analysis. Eur Urol 54(3):543–562

Charlton RG, Morley AR, Chambers P, Gillespie JI (1999) Focal changes in nerve, muscle and connective tissue in normal and unstable human bladder. BJU Int 84(9):953–960

Cheng JT, Yu BC, Tong YC (2007) Changes of M3-muscarinic receptor protein and mRNA expressions in the bladder urothelium and muscle layer of streptozotocin-induced diabetic rats. Neurosci Lett 423(1):1–5

Chess-Williams R (2002) Muscarinic receptors of the urinary bladder: detrusor, urothelial and prejunctional. Auton Autacoid Pharmacol 22(3):133–145

D'Agostino G, Barbieri A, Chiossa E, Tonini M (1997) M4 muscarinic autoreceptor-mediated inhibition of -3H-acetylcholine release in the rat isolated urinary bladder. J Pharmacol Exp Ther 283(2):750–756

D'Agostino G, Bolognesi ML, Lucchelli A, Vicini D, Balestra B, Spelta V, Melchiorre C, Tonini M (2000) Prejunctional muscarinic inhibitory control of acetylcholine release in the human isolated detrusor: involvement of the M4 receptor subtype. Br J Pharmacol 129(3):493–500

Dass N, McMurray G, Greenland JE, Brading AF (2001) Morphological aspects of the female pig bladder neck and urethra: quantitative analysis using computer assisted 3-dimensional reconstructions. J Urol 165(4):1294–1299

De Laet K, De Wachter S, Wyndaele JJ (2006) Systemic oxybutynin decreases afferent activity of the pelvic nerve of the rat: new insights into the working mechanism of antimuscarinics. Neurourol Urodyn 25(2):156–161

De Wachter S, Wyndaele JJ (2001) Does bladder tone influence sensation of filling and electrosensation in the bladder? A blind controlled study in young healthy volunteers using bethanechol. J Urol 165(3):802–804

De Wachter S, Van Meel TD, Wyndaele JJ (2003) Study of the afferent nervous system and its evaluation in women with impaired detrusor contractility treated with bethanechol. Urology 62(1):54–58

Dixon JS, Jen PY, Gosling JA (2000) The distribution of vesicular acetylcholine transporter in the human male genitourinary organs and its co-localization with neuropeptide Y and nitric oxide synthase. Neurourol Urodyn 19(2):185–194

Eglen RM, Hegde SS, Watson N (1996) Muscarinic receptor subtypes and smooth muscle function. Pharmacol Rev 48:531–565

Ehlert FJ, Griffin MT, Abe DM, Vo TH, Taketo MM, Manabe T, Matsui M (2005) The M 2 muscarinic receptor mediates contraction through indirect mechanisms in mouse urinary bladder. J Pharmacol Exp Ther 313:368–378

Ek A, Alm P, Andersson KE, Persson CG (1977) Adrenergic and cholinergic nerves of the human urethra and urinary bladder. A histochemical study. Acta Physiol Scand 3:345–352

Ek A, Andersson KE, Ulmsten U (1978) The effects of norephedrine and bethanechol on the human urethral closure pressure profile. Scand J Urol Nephrol 12(2):97–104

el-Salmy S, Downie JW, Awad SA (1985) Bladder and urethral function and supersensitivity to subcutaneously administered bethanechol in cats with chronic cauda equina lesions. J Urol 134(5):1011–1018

Finkbeiner AE (1985) Is bethanechol chloride clinically effective in promoting bladder emptying? A literature review. J Urol 134(3):443–449

Finney SM, Andersson KE, Gillespie JI, Stewart LH (2006) Antimuscarinic drugs in detrusor overactivity and the overactive bladder syndrome: motor or sensory actions? BJU Int 98(3):503–507

Frazier EP, Braverman AS, Peters SLM, Michel MC, Ruggieri MR Sr (2007) Does phospholipase C mediate muscarinic receptor-induced rat urinary bladder contraction? J Pharmacol Exp Ther 322:998–1002

German K, Bedwani J, Davies J, Brading AF, Stephenson TP (1995) Physiological and morphometric studies into the pathophysiology of detrusor hyperreflexia in neuropathic patients. J Urol 153:1678–1683

Giglio D, Tobin G (2009) Muscarinic receptor subtypes in the lower urinary tract. Pharmacology 83(5):259–269

Giglio D, Ryberg AT, To K, Delbro DS, Tobin G (2005) Altered muscarinic receptor subtype expression and functional responses in cyclophosphamide induced cystitis in rats. Auton Neurosci 122(1–2):9–20

Goepel M, Gronewald A, Krege S, Michel MC (1998) Muscarinic receptor subtypes in porcine detrusor: comparison with humans and regulation by bladder augmentation. Urol Res 26:149–154

Gosling JA, Gilpin SA, Dixon JS, Gilpin CJ (1986) Decrease in the autonomic innervation of human detrusor muscle in outflow obstruction. J Urol 136:501–504
Grol S, Essers PB, van Koeveringe GA, Martinez-Martinez P, de Vente J, Gillespie JI (2009) M(3) muscarinic receptor expression on suburothelial interstitial cells. BJU Int 104(3):398–405
Guay DR (2003) Clinical pharmacokinetics of drugs used to treat urge incontinence. Clin Pharmacokinet 42(14):1243–1285
Gudermann T, Kalkbrenner F, Schultz G (1996) Diversity and selectivity of receptor-G protein interaction. Annu Rev Pharmacol Toxicol 36:429–459
Gup DI, Baumann M, Lepor H, Shapiro E (1989a) Muscarinic cholinergic receptors in normal pediatric and myelodysplastic bladders. J Urol 142(2 Pt 2):595–599, discussion 603–605
Gup DI, Shapiro E, Baumann M, Lepor H (1989b) Contractile properties of human prostate adenomas and the development of infravesical obstruction. Prostate 15(2):105–114
Gup DI, Shapiro E, Baumann M, Lepor H (1990) Autonomic receptors in human prostate adenomas. J Urol 143(1):179–185
Harrison SCV, Hunnam GR, Farman P, Doyle PT (1987) Bladder instability and denervation in patients with bladder outflow obstruction. Br J Urol 60:519–522
Harriss DR, Marsh KA, Birmingham AT, Hill SJ (1995) Expression of muscarinic M3-receptors coupled to inositol phospholipid hydrolysis in human detrusor cultured smooth muscle cells. J Urol 154(3):1241–1245
Hashitani H, Suzuki H (1996) Altered electrical properties of bladder smooth muscle in streptozotocin-induced diabetic rats. Br J Urol 77(6):798–804
Hawthorn MH, Chapple CR, Cock M, Chess-Williams R (2000) Urothelium-derived inhibitory factor(s) influences on detrusor muscle contractility in vitro. Br J Pharmacol 129(3):416–419
Hedlund H, Andersson KE (1988) Effects of prazosin and carbachol in patients with benign prostatic obstruction. Scand J Urol Nephrol 22(1):19–22
Hedlund H, Andersson KE, Larsson B (1985) α-adrenoceptors and muscarinic receptors in the isolated human prostate. J Urol 134(6):1291–1298
Hedlund P, Ekström P, Larsson B, Alm P, Andersson KE (1997) Heme oxygenase and NO-synthase in the human prostate – relation to adrenergic, cholinergic and peptide-containing nerves. J Auton Nerv Syst 63(3):115–126
Hegde SS, Eglen RM (1999) Muscarinic receptor subtypes modulating smooth muscle contractility in the urinary bladder. Life Sci 64(6–7):419–428
Hegde SS, Choppin A, Bonhaus D et al (1997) Functional role of M-2 and M-3 muscarinic receptors in the urinary bladder of rats in vitro and in vivo. Br J Pharmacol 120:1409–1418
Herbison P, Hay-Smith J, Ellis G, Moore K (2003) Effectiveness of anticholinergic drugs compared with placebo in the treatment of overactive bladder: systematic review. Br Med J 326:841–844
Hernández M, Símonsen U, Prieto D, Rivera L, García P, Ordaz E, García-Sacristán A (1993) Different muscarinic receptor subtypes mediating the phasic activity and basal tone of pig isolated intravesical ureter. Br J Pharmacol 110(4):1413–1420
Hernández M, García-Sacristán A, Orensanz LM (1995) Muscarinic binding sites of the pig intravesical ureter. J Auton Pharmacol 15(5):351–359
Igawa Y, Zhang X, Nishizawa O, Umeda M, Iwata A, Taketo MM, Manabe T, Matsui M, Andersson KE (2004) Cystometric findings in mice lacking muscarinic M 2 or M 3 receptors. J Urol 172:2460–2464
Iijima K, De Wachter S, Wyndaele JJ (2007) Effects of the M3 receptor selective muscarinic antagonist darifenacin on bladder afferent activity of the rat pelvic nerve. Eur Urol 52(3):842–847
Ikeda Y, Birder L, Buffington C, Roppolo J, Kanai A (2009) Mucosal muscarinic receptors enhance bladder activity in cats with feline interstitial cystitis. J Urol 181(3):1415–1422
Inadome A, Yoshida M, Takahashi W, Yono M, Seshita H, Miyamoto Y, Kawano T, Ueda S (1998) Prejunctional muscarinic receptors modulating acetylcholine release in rabbit detrusor smooth muscles. Urol Int 61(3):135–141

James S, Chapple CR, Phillips MI, Greengrass PM, Davey MJ, Turner-Warwick RT, Milroy EJ, Burnstock G (1989) Autoradiographic analysis of alpha-adrenoceptors and muscarinic cholinergic receptors in the hyperplastic human prostate. J Urol 142(2 Pt 1):438–444

Jezior JR, Brady JD, Rosenstein DI, McCammon KA, Miner AS, Ratz PH (2001) Dependency of detrusor contractions on calcium sensitization and calcium entry through LOE-908-sensitive channels. Br J Pharmacol 134(1):78–87

Johns A (1983) Alpha- and beta-adrenergic and muscarinic cholinergic binding sites in the bladder and urethra of the rabbit. Can J Physiol Pharmacol 61(1):61–66

Jositsch G, Papadakis T, Haberberger RV, Wolff M, Wess J, Kummer W (2009) Suitability of muscarinic acetylcholine receptor antibodies for immunohistochemistry evaluated on tissue sections of receptor gene-deficient mice. Naunyn Schmiedebergs Arch Pharmacol 379(4):389–395

Kaplan SA, Te AE, Blaivas JG (1995) Urodynamic findings in patients with diabetic cystopathy. J Urol 153:342–344

Kester RR, Mooppan UM, Gousse AE, Alver JE, Gintautas J, Gulmi FA, Abadir AR, Kim H (2003) Pharmacological characterization of isolated human prostate. J Urol 170(3):1032–1038

Kinder RB, Mundy AR (1987) Pathophysiology of idiopathic detrusor instability and detrusor hyper-reflexia. An in vitro study of human detrusor muscle. Br J Urol 60(6):509–515

Koraitim MM (2008) The male urethral sphincter complex revisited: an anatomical concept and its physiological correlate. J Urol 179(5):1683–1689

Kotlikoff MI, Dhulipala P, Wang YX (1999) M2 signaling in smooth muscle cells. Life Sci 64(6–7):437–442

Krichevsky VP, Pagala MK, Vaydovsky I, Damer V, Wise GJ (1999) Function of M3 muscarinic receptors in the rat urinary bladder following partial outlet obstruction. J Urol 161(5): 1644–1650

Latifpour J, Gousse A, Kondo S, Morita T, Weiss RM (1989) Effects of experimental diabetes on biochemical and functional characteristics of bladder muscarinic receptors. J Pharmacol Exp Ther 248:81–88

Lepor H, Kuhar MJ (1984) Characterization and localization of the muscarinic cholinergic receptor in human prostatic tissue. J Urol 132(2):397–402

Lincoln J, Burnstock G (1993) Autonomic innervation of the urinary bladder and urethra. In: Maggi CA (ed) The autonomic nervous system, vol 6, Nervous control of the urogenital system, Chapter 2. Harwood Academic, London, pp 33–68

Long S, Nergårdh A (1978) Autonomic receptor functions of the human ureter: an in vitro study. Scand J Urol Nephrol 12(1):23–26

Mansfield KJ, Liu L, Mitchelson FJ, Moore KH, Millard RJ, Burcher E (2005) Muscarinic receptor subtypes in human bladder detrusor and mucosa, studied by radioligand binding and quantitative competitive RT-PCR: changes in ageing. Br J Pharmacol 144(8):1089–1099

Mansfield KJ, Liu L, Moore KH, Vaux KJ, Millard RJ, Burcher E (2007) Molecular characterization of M2 and M3 muscarinic receptor expression in bladder from women with refractory idiopathic detrusor overactivity. BJU Int 99(6):1433–1438

Mansfield KJ, Chandran JJ, Vaux KJ, Millard RJ, Christopoulos A, Mitchelson FJ, Burcher E (2009) Comparison of receptor binding characteristics of commonly used muscarinic antagonists in human bladder detrusor and mucosa. J Pharmacol Exp Ther 328(3):893–899

Matsui M, Motomura D, Fujikawa T, Jiang J, Takahashi S, Manabe T, Taketo MM (2002) Mice lacking M2 and M3 muscarinic acetylcholine receptors are devoid of cholinergic smooth muscle contractions but still viable. J Neurosci 22(24):10627–10632

Mattiasson A, Andersson KE, Sjögren C (1984) Adrenoceptors and cholinoceptors controlling noradrenaline release from adrenergic nerves in the urethra of rabbit and man. J Urol 131(6): 1190–1195

Mattiasson A, Andersson KE, Andersson PO, Larsson B, Sjögren C, Uvelius B (1990) Nerve-mediated functions in the circular and longitudinal muscle layers of the proximal female rabbit urethra. J Urol 143(1):155–160

Michel MC, Hegde SS (2006) Treatment of the overactive bladder syndrome with muscarinic receptor antagonists: a matter of metabolites? Naunyn Schmiedebergs Arch Pharmacol 374(2): 79–85

Michel MC, Wieland T, Tsujimoto G (2009) How reliable are G-protein-coupled receptor antibodies? Naunyn Schmiedebergs Arch Pharmacol 379(4):385–388

Mills IW, Greenland JE, McMurray G, McCoy R, Ho KHT, Noble JG, Brading AF (2000) Studies of the pathophysiology of idiopathic detrusor instability: the physiological properties of the detrusor smooth muscle and its pattern of innervation. J Urol 163:646–651

Mimata H, Wheeler MA, Fukomoto Y, Takigawa H, Nishimoto T, Weiss RM, Latifpour J (1995) Enhancement of muscarinic receptor-coupled phosphatidyl inositol hydrolysis in diabetic bladder. Mol Cell Biochem 152:71–76

Mukerji G, Yiangou Y, Grogono J, Underwood J, Agarwal SK, Khullar V, Anand P (2006) Localization of M2 and M3 muscarinic receptors in human bladder disorders and their clinical correlations. J Urol 176(1):367–373

Mutoh S, Latifpour J, Saito M, Weiss RM (1997) Evidence for the presence of regional differences in the subtype specificity of muscarinic receptors in rabbit lower urinary tract. J Urol 157(2): 717–721

Nagahama K, Tsujii T, Morita T, Azuma H, Oshima H (1998) Differences between proximal and distal portions of the male rabbit posterior urethra in the physiological role of muscarinic cholinergic receptors. Br J Pharmacol 124(6):1175–1180

Nakamura T, Kimura J, Yamaguchi O (2002) Muscarinic M2 receptors inhibit Ca2+-activated K+ channels in rat bladder smooth muscle. Int J Urol 9(12):689–696

Nemeth L, O'Briain DS, Puri P (2001) Demonstration of neuronal networks in the human upper urinary tract using confocal laser scanning microscopy. J Urol 166(1):255–258

Novara G, Galfano A, Secco S, D'Elia C, Cavalleri S, Ficarra V, Artibani W (2008) A systematic review and meta-analysis of randomized controlled trials with antimuscarinic drugs for overactive bladder. Eur Urol 54(4):740–763

Obara K, Arai K, Miyajima N, Hatano A, Tomita Y, Takahashi K (2000) Expression of m2 muscarinic acetylcholine receptor mRNA in primary culture of human prostate stromal cells. Urol Res 28(3):196–200

Pandita RK, Fujiwara M, Alm P, Andersson KE (2000) Cystometric evaluation of bladder function in non-anesthetized mice with and without bladder outlet obstruction. J Urol 164(4): 1385–1389

Persson CG, Andersson KE (1976) Adrenoceptor and cholinoceptor mediated effects in the isolated urethra of cat and guinea-pig. Clin Exp Pharmacol Physiol 3(5):415–426

Persson K, Alm P, Johansson K, Larsson B, Andersson KE (1995) Co-existence of nitrergic, peptidergic and acetylcholine esterase-positive nerves in the pig lower urinary tract. J Auton Nerv Syst 52(2–3):225–236

Persson K, Johansson K, Alm P, Larsson B, Andersson KE (1997) Morphological and functional evidence against a sensory and sympathetic origin of nitric oxide synthase-containing nerves in the rat lower urinary tract. Neuroscience 77(1):271–281

Pradidarcheep W, Labruyere WT, Dabhoiwala NF, Lamers WH (2008) Lack of specificity of commercially available antisera: better specifications needed. J Histochem Cytochem 56: 1099–1111

Pradidarcheep W, Stallen J, Labruyere WT, Dabhoiwala NF, Michel MC, Lamers WH (2009) Lack of specificity of commercially available antisera against muscarinic and adrenergic receptors. Naunyn Schmiedebergs Arch Pharmacol 379:397–402

Prieto D, Simonsen U, Martín J, Hernández M, Rivera L, Lema L, García P, García-Sacristán A (1994) Histochemical and functional evidence for a cholinergic innervation of the equine ureter. J Auton Nerv Syst 47(3):159–170

Rayford W, Noble MJ, Austenfeld MA, Weigel J, Mebust WK, Shah GV (1997) Muscarinic cholinergic receptors promote growth of human prostate cancer cells. Prostate 30(3):160–166

Restorick JM, Mundy AR (1989) The density of cholinergic and alpha and beta adrenergic receptors in the normal and hyper-reflexic human detrusor. Br J Urol 63(1):32–35
Riedl CR, Stephen RL, Daha LK, Knoll M, Plas E, Pflüger H (2000) Electromotive administration of intravesical bethanechol and the clinical impact on acontractile detrusor management: introduction of a new test. J Urol 164(6):2108–2111
Roden DM (2004) Drug-induced prolongation of the QT interval. N Engl J Med 350 (10):1013–1022
Rolle U, Chertin B, Nemeth L, Puri P (2002) Demonstration of nitrergic and cholinergic innervation in whole-mount preparations of rabbit, pig, and human upper urinary tract. Pediatr Surg Int 18(5–6):315–318
Rolle U, Brylla E, Tillig B, Chertin B, Cascio S, Puri P (2008) Demonstration of intrinsic innervation of the guinea pig upper urinary tract using whole-mount preparation. Neurourol Urodyn 27(4):341–347
Roosen A, Wu C, Sui GP, Fry CH (2008) Synergistic effects in neuromuscular activation and calcium-sensitization in the bladder trigone. BJU Int 101(5):610–614
Ruggieri MR Sr, Braverman AS (2006) Regulation of bladder muscarinic receptor subtypes by experimental pathologies. Auton Autacoid Pharmacol 26(3):311–325
Ruggieri MR, Colton MD, Wang P, Wang J, Smyth RJ, Pontari MA, Luthin GR (1995) Human prostate muscarinic receptor subtypes. J Pharmacol Exp Ther 274(2):976–982
Sakamoto K, Suri D, Rajasekaran M (2006) Characterization of muscarinic receptor subtypes in human ureter. J Endourol 20(11):939–942
Schneider T, Fetscher C, Krege S, Michel MC (2004a) Signal transduction underlying carbachol-induced contraction of human urinary bladder. J Pharmacol Exp Ther 309(3):1148–1153
Schneider T, Hein P, Michel MC (2004b) Signal transduction underlying carbachol-induced contraction of rat urinary bladder. I. Phospholipases and Ca2+ sources. J Pharmacol Exp Ther 308(1):47–53
Schulman CC (1975) Ultrastructural evidence for adrenergic and cholinergic innervation of the human ureter. J Urol 113(6):765–771
Sibley GN (1984) A comparison of spontaneous and nerve-mediated activity in bladder muscle from man, pig and rabbit. J Physiol 354:431–443
Sibley GNA (1987) The physiological response of the detrusor muscle to experimental bladder outflow obstruction in the pig. Br J Urol 60:332–336
Sigala S, Mirabella G, Peroni A, Pezzotti G, Simeone C, Spano P, Cunico SC (2002) Differential gene expression of cholinergic muscarinic receptor subtypes in male and female normal human urinary bladder. Urology 60(4):719–725
Sjögren C, Andersson KE, Husted S, Mattiasson A, Moller-Madsen B (1982) Atropine resistance of transmurally stimulated isolated human bladder muscle. J Urol 128(6):1368–1371
Somogyi GT, de Groat WC (1992) Evidence for inhibitory nicotinic and facilitatory muscarinic receptors in cholinergic nerve terminals of the rat urinary bladder. J Auton Nerv Syst 37 (2):89–97
Somogyi GT, de Groat WC (1999) Function, signal transduction mechanisms and plasticity of presynaptic muscarinic receptors in the urinary bladder. Life Sci 64(6–7):411–418
Somogyi GT, Zernova GV, Yoshiyama M, Rocha JN, Smith CP, de Groat WC (2003) Change in muscarinic modulation of transmitter release in the rat urinary bladder after spinal cord injury. Neurochem Int 43(1):73–77
Speakman MJ, Brading AF, Gilpin CJ, DixonJS GSA, Gosling J (1987) Bladder outflow obstruction – a cause of denervation supersensitivity. J Urol 138:1461–1466
Speakman MJ, Walmsley D, Brading AF (1988) An in vitro pharmacological study of the human trigone – a site of non-adrenergic, non-cholinergic neurotransmission. Br J Urol 61(4): 304–309
Stevens LA, Sellers DJ, McKay NG, Chapple CR, Chess-Williams R (2006) Muscarinic receptor function, density and G-protein coupling in the overactive diabetic rat bladder. Auton Autacoid Pharmacol 26(3):303–309

Stevens LA, Chapple CR, Chess-Williams R (2007) Human idiopathic and neurogenic overactive bladders and the role of M2 muscarinic receptors in contraction. Eur Urol 52(2):531–538

Tagliani M, Candura SM, Di Nucci A, Franceschetti GP, D'Agostino G, Ricotti P, Fiori E, Tonini M (1997) A re-appraisal of the nature of the atropine-resistant contraction to electrical field stimulation in the human isolated detrusor muscle. Naunyn Schmiedebergs Arch Pharmacol 356(6):750–755

Taki N, Taniguchi T, Okada K, Moriyama N, Muramatsu I (1999) Evidence for predominant mediation of alpha1-adrenoceptor in the tonus of entire urethra of women. J Urol 162(5): 1829–1832

Taylor JA III, Kuchel GA (2006) Detrusor underactivity: clinical features and pathogenesis of an underdiagnosed geriatric condition. J Am Geriatr Soc 54(12):1920–1932

Tobin G, Sjögren C (1995) In vivo and in vitro effects of muscarinic receptor antagonists on contractions and release of [3 H]acetylcholine in the rabbit urinary bladder. Eur J Pharmacol 281:1–8

Tomiyama Y, Wanajo I, Yamazaki Y, Murakami M, Kojima M, Shibata N (2003) Functional muscarinic cholinoceptors in the isolated canine ureter. Naunyn Schmiedebergs Arch Pharmacol 367(4):348–352

Tomiyama Y, Wanajo I, Yamazaki Y, Kojima M, Shibata N (2004) Effects of cholinergic drugs on ureteral function in anesthetized dogs. J Urol 172(4 Pt 1):1520–1523

Tong YC, Cheng JT, Hsu CT (2006) Alterations of M(2)-muscarinic receptor protein and mRNA expression in the urothelium and muscle layer of the streptozotocin-induced diabetic rat urinary bladder. Neurosci Lett 406(3):216–221

Turner WH, Brading AF (1997) Smooth muscle of the bladder in the normal and the diseased state: pathophysiology, diagnosis and treatment. Pharmacol Ther 75(2):77–110

Twiddy DA, Downie JW, Awad SA (1980) Response of the bladder to bethanechol after acute spinal cord transection in cats. J Pharmacol Exp Ther 215(2):500–506

Tyagi S, Tyagi P, Van-le S, Yoshimura N, Chancellor MB, de Miguel F (2006) Qualitative and quantitative expression profile of muscarinic receptors in human urothelium and detrusor. J Urol 176(4 Pt 1):1673–1678

Ulmsten U, Andersson KE (1977) The effects of emeprone on intravesical and intra-urethral pressure in women with urgency incontinence. Scand J Urol Nephrol 11(2):103–109

van Koppen CJ, Kaiser B (2003) Regulation of muscarinic acetylcholine receptor signaling. Pharmacol Ther 98(2):197–220

Wang P, Luthin GR, Ruggieri MR (1995) Muscarinic acetylcholine receptor subtypes mediating urinary bladder contractility and coupling to GTP binding proteins. J Pharmacol Exp Ther 273:959–966

Werner ME, Knorn AM, Meredith AL, Aldrich RW, Nelson MT (2007) Frequency encoding of cholinergic- and purinergic-mediated signaling to mouse urinary bladder smooth muscle: modulation by BK channels. Am J Physiol Regul Integr Comp Physiol 292(1): R616–R624

Wibberley A, Chen Z, Hu E, Hieble JP, Westfall TD (2003) Expression and functional role of Rho-kinase in rat urinary bladder smooth muscle. Br J Pharmacol 138(5):757–766

Witte LP, Chapple CR, de la Rosette JJ, Michel MC (2008) Cholinergic innervation and muscarinic receptors in the human prostate. Eur Urol 54(2):326–334

Wu G, Bogatkevich GS, Mukhin YV, Benovic JL, Hildebrandt JD, Lanier SM (2000) Identification of Gβγ binding sites in the third intracellular loop of the M 3 -muscarinic receptor and their role in receptor regulation. J Biol Chem 275:9026–9034

Yamaguchi O, Shishido K, Tamura K et al (1996) Evaluation of mRNAs encoding muscarinic receptor subtypes in human detrusor muscle. J Urol 156:1208–1213

Yamamoto M, Unno T, Matsuyama H, Kohda M, Masuda N, Nishimura M, Ishii T, Komori S (2008) Two types of cation channel activated by stimulation of muscarinic receptors in guinea-pig urinary bladder smooth muscle. J Pharmacol Sci 108(3):248–257

Yamanishi T, Chapple CR, Yasuda K, Chess-Williams R (2000) The role of M2 -muscarinic receptors in mediating contraction of the pig urinary bladder in vitro. Br J Pharmacol 131:1482–1488

Yokoyama O, Nagano K, Kawaguchi K, Hisazumi H (1991) The response of the detrusor muscle to acetylcholine in patients with infravesical obstruction. Urol Res 19:117–121

Yoshida M, Miyamae K, Iwashita H, Otani M, Inadome A (2004) Management of detrusor dysfunction in the elderly: changes in acetylcholine and adenosine triphosphate release during aging. Urology 63(3 Suppl 1):17–23

Yoshida M, Inadome A, Maeda Y, Satoji Y, Masunaga K, Sugiyama Y, Murakami S (2006) Non-neuronal cholinergic system in human bladder urothelium. Urology 67(2):425–430

Yoshida M, Masunaga K, Satoji Y, Maeda Y, Nagata T, Inadome A (2008) Basic and clinical aspects of non-neuronal acetylcholine: expression of non-neuronal acetylcholine in urothelium and its clinical significance. J Pharmacol Sci 106(2):193–198

Yoshimura N, Kaiho Y, Miyazato M, Yunoki T, Tai C, Chancellor MB, Tyagi P (2008) Therapeutic receptor targets for lower urinary tract dysfunction. Naunyn Schmiedebergs Arch Pharmacol 377(4–6):437–448

Zhou H, Meyer A, Starke K, Gomeza J, Wess J, Trendelenburg AU (2002) Heterogeneity of release-inhibiting muscarinic autoreceptors in heart atria and urinary bladder: a study with M (2)- and M(4)-receptor-deficient mice. Naunyn Schmiedebergs Arch Pharmacol 365(2): 112–122

Modulation of Urinary Bladder Innervation: TRPV1 and Botulinum Toxin A

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Contents

Abstract The persisting interest around neurotoxins such as vanilloids and botulinum toxin (BoNT) derives from their marked effect on detrusor overactivity refractory to conventional antimuscarinic treatments. In addition, both are administered by intravesical route. This offers three potential advantages. First, intravesical therapy is an easy way to provide high concentrations of pharmacological agents in the

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bladder tissue without causing unsuitable levels in other organs. Second, drugs effective on the bladder, but inappropriate for systemic administration, can be safely used as it is the case of vanilloids and BoNT. Third, the effects of one single treatment might be extremely longlasting, contributing to render these therapies highly attractive to patients despite the fact that the reasons to the prolonged effect are still incompletely understood. Attractive as it may be, intravesical pharmacological therapy should still be considered as a second-line treatment in patients refractory to conventional oral antimuscarinic therapy or who do not tolerate its systemic side effects. However, the increasing off-label use of these neurotoxins justifies a reappraisal of their pharmacological properties.

Keywords Bladder · BoNT/A · Pain · Prostate · TRPV1b · TRPV1

1 General Characteristics of the TRPV1 Receptor

The TRPV1 receptor is a protein with six transmembrane domains that forms a pore between the fifth and the sixth domain (Caterina et al. 1997). This protein allows the passage of cations, especially calcium, when activated by vanilloids, noxious heat, and low pH (Caterina et al. 1997). Although the TRPV1 heat threshold was established around 43°C, it is known that in acidosis, the receptor is activated at body temperature (Tominaga et al. 1998; Caterina et al. 2000). Several studies have shown that TRPV1 agonists act on different domains of the protein (Welch et al. 2000a). For instance, vanilloids and endovanilloids, such as anandamide, bind to the intracellular region of the third domain (Jordt and Julius 2002), while protons and other ions act on the extracellular loop that forms the pore (Jordt et al. 2000; Ahern 2005).

1.1 Localization of TRPV1 Protein in Lower Urinary Tract

The TRPV1 receptor was first described in the perikarya and in the central and peripheral processes of a subset of small-diameter sensory neurons (Caterina et al. 1997). Later, the presence of the receptor was described in other neuronal structures, such as the trigeminal ganglia (Guo et al. 1999; Ichikawa and Sugimoto 2001) and the vagus nerves (Holzer 1991), among others (Ichikawa and Sugimoto 2001; Michael and Priestley 1999; Kadowaki et al. 2004; Koike et al. 2004). The receptor was also observed in other areas of the nervous system. Several studies reported the presence of TRPV1 in some areas of the brain (Mezey et al. 2000; Tóth et al. 2005). TRPV1 receptor expression has also been shown in cells outside the nervous system, a subject that has generated some controversy. The expression of the TRPV1 receptor in smooth muscle cells is still a matter of debate

(Ost et al. 2002; Yang et al. 2008; Kark et al. 2008; Wang et al. 2008a; Ito et al. 2008). Nevertheless, the expression of this receptor in mast cells and keratinocytes is now widely accepted (Birder et al. 2007; Turner et al. 2003; Ständer et al. 2004; Bodó et al. 2005). It has been shown that TRPV1 activation in those cells promotes the release of proinflammatory molecules (Birder et al. 2007; Southall et al. 2003).

In the urinary bladder and ureter, TRPV1 is expressed in nerve fibers (Avelino et al. 2002) and in urothelial cells (Birder et al. 2001; Lazzeri et al. 2004a; Charrua et al. 2009a). Dense varicose nerve fibers immunoreactive for TRPV1 occur among detrusor smooth muscle fibers and underneath the epithelium (Avelino et al. 2002). In the prostate, TRPV1-expressing fibers were found in the prostatic urethral mucosa, verumontanum, ejaculatory ducts, and periurethral prostatic acini (Dinis et al. 2005).

A recent work published by Everaerts et al. (2009) revealed that antibodies against TRPV1 may have a positive reaction in TRPV1 KO mice, indicating an absolute lack of specificity. The consequence of these observations is the need to confirm any positive TRPV1 immunostainning with a negative control using TRPV1 KO tissues. In addition, the combination of TRPV1 immunoreaction with other methodologies to detect the presence of the receptor, such as reverse transcriptase polymerase chain reaction, blotting analysis, in situ hybridization, among others, is highly recommended.

1.2 The Role of TRPV1 on Lower Urinary Tract Function and Dysfunction

The role of TRPV1 in normal urinary bladder is still controversial. In cystometries performed in awake TRPV1 knockout (KO) mice, these animals have more small volume voids than their wild-type (WT) controls (Birder et al. 2002). However, in anesthetized animals, the findings are conflicting, some groups reporting that the animals have nonvoiding contractions (Birder et al. 2002; Wang et al. 2008b), whereas others found totally normal cystometric traces (Charrua et al. 2007).

TRPV1 activation outside lower urinary tract (LUT) is essential for the development of somatic thermal hyperalgesia during inflammation (Caterina et al. 2000; Davis et al. 2000). TRPV1 receptor activation elicits the release of proinflammatory molecules, which consequently leads to the development of neurogenic inflammation in visceral organs (Veronesi et al. 2000; McVey and Vigna 2001). In LUT, Charrua et al. (2007) have shown that TRPV1 is essential for the development of hyperreflexia and pain associated with cystitis. While inflamed WT mice exhibit bladder hyperactivity and intense spinal Fos expression, TRPV1 KO mice do not (Charrua et al. 2007). In agreement, TRPV1 antagonists were able to reduce the bladder hyperactivity and noxious input associated with cystitis (Charrua et al. 2009b).

1.3 TRPV1 Regulatory Mechanisms During LUT Inflammation

1.3.1 TRPV1 Expression During Inflammatory Conditions

Most of the information about TRPV1 expression in inflammation comes from somatic models. During somatic inflammation, there is an increased number of dorsal root ganglia (DRG) neurons expressing TRPV1 (Carlton and Coggeshall 2001; Amaya et al. 2003). Moreover, the quantification of TRPV1 protein in DRG cells by Western blotting analysis was consistent with these findings (Ji et al. 2002). The same was observed in visceral inflammation. Similarly, Avelino and Cruz (2010) observed an increase in the number of DRG neurons expressing TRPV1 during cystitis. In agreement with these experimental findings, patients with bladder pain syndrome exhibit a higher number of TRPV1-IR fibers in the bladder mucosa (Mukerji et al. 2006).

Interestingly, the increase in the protein levels in DRG cells does not seem to be accompanied by an increase in TRPV1 mRNA, indicating that the process is regulated at a posttranslation level. This observation was made in both somatic (Ji et al. 2002; Tohda et al. 2001; Voilley et al. 2001) and bladder inflammation models (Charrua et al. 2008). However, the receptor translation does increase in urothelial cells upon inflammation (Charrua et al. 2009b). These observations may indicate diverse forms of regulation in different cells.

1.3.2 Endovanilloid Release During Inflammation

TRPV1 can be directly activated by proinflammatory molecules such as *N*-arachidonoyl-ethanolamine, also known as anandamide (Zygmunt et al. 1999), *N*-arachidonoyl-dopamine (Huang et al. 2002), *N*-oleoyl-dopamine (Chu et al. 2003), eicosanoid acids, and leucotrienes (Hwang et al. 2000; Shin et al. 2002). In the urinary bladder, anandamide levels increase during cystitis (Dinis et al. 2004a) and contribute to a TRPV1-dependent bladder overactivity and increased noxious input (Dinis et al. 2004a). Nevertheless, endovanilloids are low-potency TRPV1 activators in the urinary bladder when compared to capsaicin and resiniferatoxin (Dinis et al. 2004a). Therefore, one might speculate that TRPV1 sensitization might be a prerequisite for a robust TRPV1 activation by endovanilloids (Lee et al. 2005).

Anandamide is also a cannabinoid receptor 1 (CB1) agonist (de Haro et al. 2003). This is curious, since CB1 activation promotes analgesia (Stein et al. 1996) and decreases detrusor contractility (Tyagi et al. 2009). However, it is now well demonstrated that CB1 can either enhance or diminish TRPV1 response to agonists, depending on whether or not cyclic adenosine monophosphate (cAMP)-mediated signaling pathway has been concomitantly activated (Hermann et al. 2003). Therefore, anandamide can activate TRPV1 receptor also through CB1 activation. This receptor was shown to induce TRPV1 phosphorylation, through a phosphoinositide

phospholipase C (PLC) pathway, and eliminate phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P2)-mediated TRPV1 inhibition (Hermann et al. 2003). This can explain why the blockade of CB1 receptor in the urinary bladder significantly enhanced anandamide-induced bladder reflex activity in naive but not in cyclophosphamide-injected animals (Dinis et al. 2004a).

1.3.3 TRPV1 Sensitization

TRPV1 phosphorylation can lead to the receptor sensitization (Fig. 1). It is known that TRPV1 needs to be phosphorylated by Ca^{2+}/calmodulin-dependent protein kinase to be activated by vanilloids (Hermann et al. 2003). Phosphorylation of all putative sites by protein kinase A (PKA) or protein kinase C (PKC) also leads to TRPV1 sensitization (Jung et al. 2004). Protease-activated receptor 2 (Amadesi et al. 2006) or 5-hydroxytryptamine 7 receptor (Ohta et al. 2006) is known to be involved in PKA-induced TRPV1 sensitization. Conversely, activation of group II metabotropic glutamate receptors (Carlton et al. 2009) or μ-opioid receptor (Vetter et al. 2006, 2008) inhibits TRPV1 activation by modulation of cAMP/PKA pathway. Receptors, such as bradykinin receptor (Premkumar and Ahern 2000; Vellani et al. 2001; Sugiura et al. 2002; Carr et al. 2003; Tang et al. 2004; Mizumura et al. 2005), purinergic receptors (Tominaga et al. 2001, 2003; Moriyama et al. 2003; Lakshmi and Joshi 2005), tyrosine kinase receptor A (trk A) (Bonnington and McNaughton 2003; Zhuang et al. 2004; Zhu and Oxford 2007), among others, when activated, are known to sensitize TRPV1 receptor through a PKC-dependent mechanism. Furthermore, activation of bradykinin receptor can induce PLC and phospholipase A_2 activation, which leads to the production of arachidonic acid

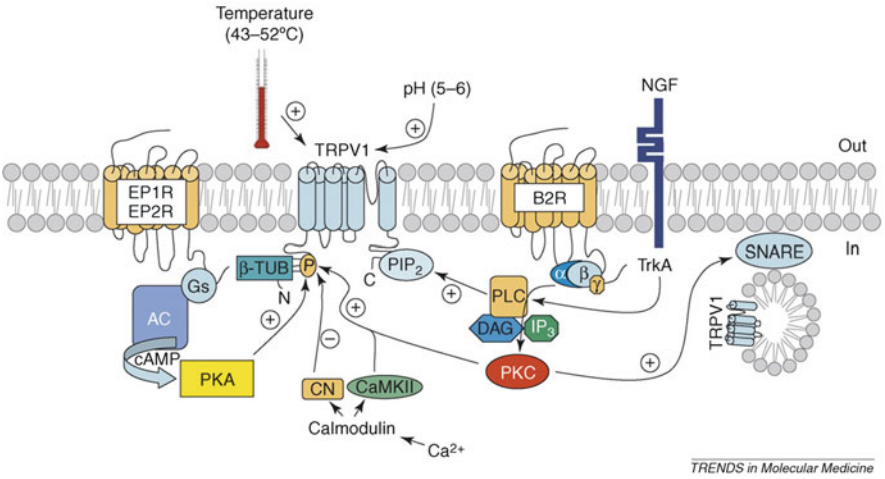

Fig. 1 Schematic model of TRPV1 activation and sensitization [in Szallasi et al. (2006); with Elsevier permission]

metabolites that can activate the TRPV1 channel (Shin et al. 2002; Tang et al. 2004; Ferreira et al. 2004).

Another mechanism of TRPV1 sensitization results from the receptor interaction with phosphoinositides. It has been shown that PtdIns(4,5)P2 sensitizes TRPV1 in the presence of high concentration of some agonists (Prescott and Julius 2003; Liu et al. 2005), but inhibits the receptor at low concentration of agonists (Chuang et al. 2001; Rohacs et al. 2008). Although functional analysis performed in patches from acutely dissociated dorsal root neurons provided evidence that PtdIns(4,5)P2 is in fact a TRPV1 sensitizer (Stein et al. 2006), the mechanisms that lead to such an antagonic effect are still unveiled (Voets and Nilius 2007). TRPV1's direct interaction with ATP molecules is another mechanism of the receptor sensitization. ATP has a binding domain within the ankyrin domain present in the N-terminal of TRPV1 molecule (Lishko et al. 2007). When ATP binds to the receptor, it increases the response of the receptor to further stimulus (Lishko et al. 2007).

1.3.4 TRPV1 Trafficking to the Membrane

Another mechanism that might increase TRPV1-mediated neuronal activity is related with an increase in TRPV1 trafficking to the plasma membrane. In fact, it is known that PKC activation promotes a rapid vesicular TRPV1 transport to the membrane in a soluble NSF attachment protein receptor (SNARE)-dependent mechanism (Morenilla-Palao et al. 2004). Furthermore, TRPV1 phosphorylation by PKA seems to be crucial for TRPV1 cytoplasmic membrane expression, since functional analysis associated with point mutation of putative sites of PKA phosphorylation almost abolished TRPV1 cytoplasmic membrane expression (Bhave et al. 2002). Besides promoting TRPV1 phosphorylation, receptor tyrosine kinase, such as trk A and insulin receptor, promotes TRPV1 trafficking to the membrane through a phosphoinositide 3-kinase (PI3K)-dependent pathway (Stein et al. 2006; Van Buren et al. 2005).

1.3.5 Expression of TRPV1 Splice Variants

The most recently discovered mechanism of TRPV1 modulation results from the receptor interaction with its own splice variants (Wang et al. 2004; Vos et al. 2006; Eilers et al. 2007). The splice variant TRPV1b differs from TRPV1 protein by completely missing exon 7 (Lu et al. 2005). This splice variant, the only one found to be expressed in humans, is known to have a dominant-negative effect over the TRPV1 receptor (Vos et al. 2006). That is, whenever TRPV1 is coexpressed with TRPV1b, the receptor became unresponsive to vanilloids, protons, and heat (Vos et al. 2006). Recently, it was shown that, during urinary bladder inflammation, there is a decrease in the expression of TRPV1b mRNA (Charrua et al. 2008). One possible consequence is the reduction in the amount of TRPV1b available to form heterotetrameric, inactive TRPV1 channels (Wang et al. 2004;

Eilers et al. 2007; Lu et al. 2005). This effect was restricted to neurons that innervate the inflamed urinary bladder, since, during LUT inflammation, TRPV1 and TRPV1b levels were maintained in the perikarya of neurons present in DRG that do not receive bladder afferent fibers (Charrua et al. 2008). It might be interesting to recall at this point that cystitis did not increase the expression of TRPV1 (Charrua et al. 2008).

1.4 Mechanisms of TRPV1 Desensitization

Vanilloid-induced desensitization of urinary bladder type C sensory afferents has been used as a therapeutic approach to ameliorate pain and hyperreflexia associated with some urinary bladder diseases (Fowler et al. 1992, 1994; Geirsson et al. 1995; Chandiramani et al. 1996; Das et al. 1996; Lazzeri et al. 1996, 1997, 1998, 2004b; Cruz et al. 1997a, b; de Ridder et al. 1997; de Séze et al. 1998, 1999, 2004, 2006; Wiart et al. 1998; Chancellor and de Groat 1999; Fagerli et al. 1999; Silva et al. 2000; Giannantoni et al. 2002; Igawa et al. 2003; Kim et al. 2003). However, the mechanisms that lead to TRPV1 desensitization are complex and not fully understood. It is known that desensitization depends on the agonist or agonists that are acting on the receptor (Novakova-Tousova et al. 2007; Vyklický et al. 2008), the phosphorylation state of the receptor (Dinis et al. 2004a; Hermann et al. 2003), the membrane potential (Piper et al. 1999), and the interaction between the receptor and molecules such as ATP, calmodulin, and PtdIns(4,5)P2 (Lishko et al. 2007; Koplas et al. 1997; Mohapatra and Nau 2005). TRPV1 desensitization might be achieved either by applications of high doses of vanilloids onto the bladder, which induces a transient burning sensation followed by a prolonged decrease in pain sensation and urinary bladder frequency (Das et al. 1996; Cruz et al. 1997a, b; de Ridder et al. 1997; Giannantoni et al. 2002; Igawa et al. 2003), or by successive applications of low doses of vanilloids, which improved pain and bladder micturition frequency in each consecutive application (Lazzeri et al. 1996). TRPV1 desensitization by vanilloids occurs after the opening of the phosphorylated channel and it is intrinsically connected to the influx of calcium ions (de Sèze et al. 1999; Docherty et al. 1996). The increase in $[Ca^{2+}]i$ leads to a series of events, which include activation of protein phosphatase 2B (PP2B, calcineurin), which dephosphorylates TRPV1 receptor (Mohapatra and Nau 2005; Docherty et al. 1996) and changes the allosteric coupling between TRPV1 and PtdIns(4,5)P2 (Vyklický et al. 2008). Through the activation of PLC, PtdIns(4,5)P2 is cleaved into diacyl glycerol and inositol 1,4,5-triphosphate, concomitantly with ATP displacement, allowing Ca^{2+}–calmodulin binding to both ankyrin repeat domain and C terminus, promoting the channel desensitization (Lishko et al. 2007; Vyklický et al. 2008). Nevertheless, the use of vanilloids as intravesical therapeutic agents has been hampered by the pungency of capsaicin solutions (Cruz et al. 1997a) and the lack of stability of the resiniferatoxin solutions (Brady et al. 2004b).

1.5 TRPV1 Antagonists

The importance of finding a reliable molecule that blocks bladder TRPV1 is based on the observations that TRPV1 KO mice do not develop bladder hyperreflexia and noxious input during cystitis (Charrua et al. 2007). Therefore, TRPV1 antagonists could offer bladder analgesia, which is difficult to obtain with current analgesics and, in addition, to contribute to control bladder reflex activity.

The first molecule to be used as a competitive TRPV1 antagonist was capsazepine (Urban and Dray 1991; Dickenson and Dray 1991; Perkins and Campbell 1992; Bevan et al. 1992; Szallasi et al. 1993; Walpole et al. 1994). Although in some studies this molecule proved to be extremely useful for TRPV1 investigation, others demonstrated that it is not specific enough and effective as a TRPV1 antagonist (Dinis et al. 2004a; Kuenzi and Dale 1996; Liu et al. 2008; Docherty et al. 1997; Oh et al. 2001; Mahmmoud 2008). Furthermore, capsazepine is not able to counteract all TRPV1 modes of activation (McIntyre et al. 2001; Walker et al. 2003). Although several other TRPV1 antagonists have been produced and commercialized, there is still a lack of information concerning specificity and efficacy in most of them (Szallasi et al. 2007; Wong and Gavva 2008). The most recently synthesized TRPV1 antagonist molecules, which already reached clinical trials, are delivered via oral route (Gunthorpe and Chizh 2009). Japan Tobacco is testing, in Japan, one of those molecules, JTS-653, to treat urinary bladder overactivity, although there is still no data available from that study (Gunthorpe and Chizh 2009; Khairatkar-Joshi and Szallasi 2009). Using an animal model, Charrua et al. (2009b) have shown that oral administration of GRC-6211, a specific TRPV1 antagonist that is already in phase II for neuropathic pain treatment, abolishes both urinary bladder hyperreflexia and hyperalgesia associated with LUT inflammation in rodents and bladder hyperreflexia in animal models of spinal cord transection (Silva et al. 2008a). One undesirable side effect of TRPV1 antagonists is that, by acting on visceral TRPV1 (Jones et al. 2005), they can cause hyperthermia (Steiner et al. 2007; Swanson et al. 2005) after promoting vasoconstriction and increasing thermogenesis (Jones et al. 2005; Gavva et al. 2007). However, recent data have shown that hyperthermia is attenuated after repeated application of TRPV1 antagonist, which renews the interest in already synthesized molecules (Wong and Gavva 2008). Moreover, some compounds, like GRC-6211, did not show this side effect (Charrua et al. 2009b; Silva et al. 2008a).

One curious aspect of TRPV1 antagonist on bladder function is a capacity to decrease bladder reflex activity (Charrua et al. 2009b). In high doses, GRC-6211 was able to transiently block bladder contractions in rats and WT mice. However, the same dose did not produce any effect on bladder activity in TRPV1 KO mice, suggesting that TRPV1 may have mechanoreceptor proprieties, either directly or indirectly. It is tempting to speculate that the formation of TRPV1/TRPV4 dimers may contribute to this unexpected function of TRPV1 receptor in the urinary bladder (Charrua et al. 2009b) and other viscera (Gavva et al. 2008).

1.6 Clinical Perspective of TRPV1 Targeting

1.6.1 TRPV1 Desensitization

Pathologies such as spinal cord injury (De Groat 1997), chronic bladder outlet obstruction (Chai et al. 1998), and in patients with idiopathic detrusor overactivity (IDO) (Silva et al. 2002) have in common the overactivity of a spinal C-fiber initiated micturition reflex. This reflex is ultimately the origin of detrusor overactivity as indirectly shown by the suppression of nonvoluntary detrusor contractions after C-fiber desensitization induced by intravesical application of vanilloids such as capsaicin or resiniferatoxin (Fowler et al. 1994; Lazzeri et al. 1997, 1998; Cruz et al. 1997a, b; de Ridder et al. 1997; de Séze et al. 1998; Silva et al. 2002; Liu and Kuo 2007b).

The effect of vanilloids on bladder C-fibers was clearly demonstrated in patients with neurogenic detrusor overactivity (NDO) of spinal origin. Intravesical resiniferatoxin resulted in a decreased expression of TRPV1 in bladder sensory fibers (Brady et al. 2004b; Silva et al. 2002, 2005) and in urothelial cells (Apostolidis et al. 2005a), which correlated with an increase in bladder capacity and a decrease in urgency (Silva et al. 2007). Intravesical resiniferatoxin was administered either as a single high-dose instillation or as multiple low-dose instillations, in both cases with very positive results (Lazzeri et al. 2000, 2004a; Silva et al. 2007; Dinis et al. 2004b; Kuo 2003, 2005a; Kuo et al. 2006; Chen et al. 2005; Apostolidis et al. 2006a; Peng et al. 2007; Payne et al. 2005). Placebo control trials showed a clear benefit on the resiniferatoxin arm (Silva et al. 2005; Kuo et al. 2006). However, resiniferatoxin instillation did not gain worldwide popularity despite its low pungency (Cruz et al. 1997b) due to technical difficulties in obtaining stable solutions.

1.6.2 TRPV1 Antagonists

Initial experiments with TRPV1 antagonists on rodent models of bladder inflammation or detrusor overactivity induced by spinal cord injury suggest that this new class of drugs will have a relevant future for the treatment of bladder pain, urinary frequency, and urinary incontinence (Charrua et al. 2009b; Silva et al. 2008a). From a theoretical point of view, TRPV1 antagonists will be of special interest in bladder disorders such as interstitial cystitis, a chronic inflammatory condition of the bladder of unknown etiology where TRPV1 expression is increased (Mukerji et al. 2006).

For the same reasons, TRPV1 antagonists might be relevant in detrusor overactivity. In the particular case of NDO, the capacity of TRPV1 antagonists to cause detrusor paralysis makes this class of drugs very appealing (Brady et al. 2004a).

2 General Characteristics of Botulinum Toxin

Botulinum toxin (BoNT) is a neurotoxic protein produced by a great variety of Gram-positive spore-forming bacteria that form *Clostridium botulinum* (Mohanty et al. 2001). Among these bacteria, some produce only one serotype and others produce more than one of the seven known BoNT serotypes A, B, C, D, E, F, and G (Fujinaga et al. 1995; Santos-Buelga et al. 1998; Hill et al. 2007). *Clostridium butyricum* and *Clostridium baratii* also produce type E (Meng et al. 1997) and type F of BoNT (McCroskey et al. 1991), respectively. All BoNT serotypes genes differ in nucleotide sequence and are preceded by the nontoxic nonhemagglutinin gene and other genes that encode toxin-associated proteins (Hill et al. 2007; Popoff and Marvaud 1999). BoNT/A presents four subtypes: BoNT/A1, BoNT/A2, BoNT/A3, and BoNT/A4 that can have up to 15% amino acid differences (Hill et al. 2007). Nevertheless, all BoNT/A genes are translated into a single polypeptide chain (≈150 kDa), afterward being cleaved into a heavy subunit (≈100 kDa) and a light subunit (≈50 kDa) (Simpson 1981; Lacy and Stevens 1999; Binz et al. 2002). The resulting subunits stay connected by disulfide bridges and by noncovalent interactions (Binz et al. 2002; Strauss and Keller 2008). After being produced, the toxin forms a complex with other proteins, becoming more resistant to proteolysis and denaturation (Wagman 1954). The light chain (LC) of BoNT has an amino acid sequence with a motif His-Glu-x-x-His in their intermedial portion (Schiavo et al. 1994; Humeau et al. 2000). This motif is characteristic of the catalytic domain of all Zn-dependent endopeptidases (Binz et al. 2002; Humeau et al. 2000; Li et al. 2000; Rigoni et al. 2001). The heavy chain (HC) of BoNT is subdivided into three portions: a α-helical domain of 50 kDa (H_N), responsible for the LC translocation into the neuronal cytoplasm, and another 50 kDa fragment subdivided into two domains of 25 kDa (H_{CN} and H_{CC}) (Binz et al. 2002; Lalli et al. 1999). The H_N fragment is integrated in the membrane, due to the acidic compartment of the endosome, and forms a selective cationic channel (Blaustein et al. 1987; Kalandakanond and Coffield 2001). It is through this channel that the LC can pass, after conformation rearangement, possibly helped by the belt region, which has chaperone characteristics (Koriazova and Montal 2003; Fischer and Montal 2007a, b; Galloux et al. 2008; Montal 2009). The function of H_{CN}, the structure of which resembles a lectin, is still unveiled (Binz et al. 2002). The H_{CC} function is associated with both the recognition of neuronal specific areas (nonmyelinated, with complex polysialogangliosides, especially the terminal NAcGalβ3-1Galβ of GT1b and GD1a and glicosfingolipids), and the toxin internalization (Rummel et al. 2004).

2.1 BoNT Mechanism of Action

The BoNT mechanisms of action comprise three steps: the connection of the toxin to the neuronal membrane, its internalization, and finally its intracellular effects (Fig. 2).

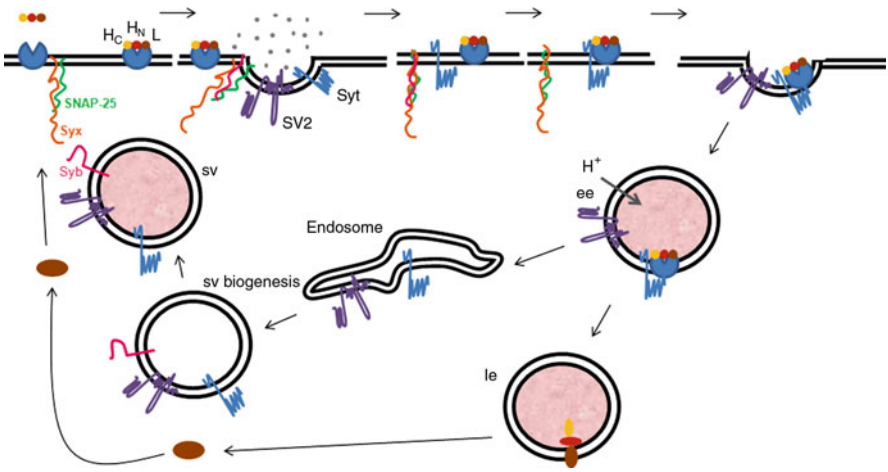

Fig. 2 Schematic model of BoNT toxin mechanism of action [adapted from Binz and Rummel (2009); with Wiley InterScience permission]

The H_{CC} fraction of BoNT/A connects to the terminal portion of the abundant low-affinity receptor NAcGalβ3-1Galβof polysialogangliosides G_{T1b}, G_{Q1b}, G_{D1a}, and G_{D1b}, maintaining the BoNT/A attached to the neuronal membrane (Rummel et al. 2004; Simpson 1984; Yowler and Schengrund 2004; Binz and Rummel 2009; Brunger and Rummel 2009). During exocytosis, the H_{CC} fraction of BoNT/A will bind to the scarce, but now available, high-affinity receptor synaptic vesicle protein 2 – SV2 (Rummel et al. 2004; Simpson 1984; Yowler and Schengrund 2004; Binz and Rummel 2009; Brunger and Rummel 2009; Dong et al. 2006; Mahrhold et al. 2006). The SV2 has three isoforms: A, B, and C, the latter having the highest affinity to the BoNT (Dong et al. 2006; Mahrhold et al. 2006). Once connected to the SV2 receptor, the toxin is internalized inside synaptic vesicles during the process of recycling (Schiavo et al. 1994).

Only the LC of the toxin is translocated from the synaptic vesicle to the neuronal cytosol, after being pulled apart from the rest of the chain following the reduction of the disulfide bridge (Galloux et al. 2008; Fischer et al. 2008). LC translocation is processed through a pore formed by H_N fragment, once this part of the BoNT/A molecule is incorporated into the synaptic vesicle membrane (Blaustein et al. 1987; Kalandakanond and Coffield 2001; Fischer et al. 2008). Although the LC dimension seems to be far superior to the pore diameter, it is believed that it passes through the pore partially unfolded (Koriazova and Montal 2003; Fischer and Montal 2007a, b; Galloux et al. 2008; Montal 2009; Cai et al. 2006). This conformation change is dependent on a low pH inside the synaptic vesicle (Koriazova and Montal 2003; Fischer and Montal 2007a, b; Galloux et al. 2008; Montal 2009; Cai et al. 2006). Before translocation, LC portion is depleted from Zn, which is regained once LC reaches the neuron cytosol (Simpson et al. 2001). Once in the cytosol, the higher pH of this cellular compartment prompts LC to regain its neutral conformation (Schiavo et al. 1994). It is known that LC can be phosphorylated by Src family of tyrosine kinases in the tyrosine-71, which will increase the thermal stability of the endopeptidase (Ferrer-Montiel et al. 1996; Ibañez et al. 2004).

2.1.1 Effect of BoNT in Neurotransmitter Release

BoNT/A prevents neurotransmitter release from intoxicated cells, much of which neurons, in a calcium-dependent manner (Schiavo et al. 1994; Sakaba et al. 2005). The basic fusion machinery that leads to synaptic vesicle and plasma membrane fusion is formed by several molecules, such as the vesicle proteins VAMP2 (synaptobrevin 2; v-SNARE; associated with synaptophysin and V-ATPase) and synaptotagmin, the soluble NSF (SNAP/NSF complex), and the membrane proteins syntaxin 1 and SNAP-25 (Schiavo et al. 1994). SNAP-25 is associated with the internal leaf of neuronal cytoplasmic membrane by palmitoylation (Schiavo et al. 1994; Prescott et al. 2009). Syntaxin and SNAP-25 form the t-SNAREs (Schiavo et al. 1994). t-SNAREs together with v-SNARE form *trans*-SNARE or *SNARE* core complex (Schiavo et al. 1994; McNew et al. 2000). In order for neurotransmission to occur, the C-terminal of syntaxin 1 binds to the C- and N-terminal of SNAP-25 and to the major part of the cytoplasmic domain of VAMP2 forming a *trans*-SNARE complex (Jahn and Scheller 2003; Jahn et al. 2003). The *trans*-SNARE complex is in association with other factors, such as α-SNAP/NSF complex (ATPase that uncouple *trans*-SNARE complex), Rab proteins, and SM protein (such as Munc18 or Munc 13, which organizes spatial and temporal *trans*-SNARE complex) and complexin (Brunger et al. 2008; Vassilieva and Nusrat 2008; Wickner and Schekman 2008). The free energy released during the formation of this complex (35 k_BT, ≈20 kcal/mol) together with calcium-induced synaptotagmin activation can allow the fusion of the two membranes (Chen et al. 1999; Südhof and Rothman 2009). In fact, it is necessary 50–100 k_BT for the fusion to occur, which is obtained with the formation of three or more *trans*-SNARE complexes (Südhof and Rothman 2009).

In spite of SNARE complex cleavage by BoNT/A, synaptic vesicles are still able to bind the cytoplasmic membrane (Humeau et al. 2000; Muller et al. 1987; Marsal et al. 1989). However, this binding might occur far from voltage-sensitive calcium channels (Ibañez et al. 2004; Atlas et al. 2001). This will prevent the calcium-dependent fusion between synaptic vesicles and the cytoplasmatic membrane (Ibañez et al. 2004; Wonnacots et al. 1978; López-Alonso et al. 1995). This observation is confirmed in experiments in which an increase in calcium influx allows this ion to be available to the fusion process even in vesicles primed away from the places of calcium entry (Ibañez et al. 2004; de Haro et al. 2003).

This same mechanism might hold true for afferent neurons. It was recently showed that in rat trigeminal ganglia, CGRP is coexpressed with SNAP-25, syntaxin 1, VAMP, and synaptotagmin, VAMP being extremely important for CGRP release (McVary et al. 1998). Vanilloid-induced or bradykinin-induced CGRP release was only partially abolished by BoNT/A (McVary et al. 1998). This ineffectiveness of BoNT/A could be explained by the massive calcium entry to the cell caused by capsaicin stimulation (McVary et al. 1998).

A less well discussed mechanism of action of BoNT/A deals with its effect on the energetic machinery of the cells. Neuronal ATP and creatine phosphate levels decrease in cholinergic neurons after BoNT/A administration (Dunant et al. 1987,

1988, 1990). In consequence, one might expect that the nerve terminal affected by the toxin will be impaired.

2.2 *BoNT/A and the Urinary Bladder*

BoNT/A injections in the bladder wall may induce detrusor paralysis, provided that appropriate doses of neurotoxin are administered. In humans, detrusor paralysis and urinary retention were observed in patients with NDO and IDO (Apostolidis et al. 2005b, 2006b; da Silva and Cruz 2009). In animal experiments, normal animals will also display detrusor paralysis, provided that adequate doses of BoNT/A are injected in the bladder.

BoNT/A was first thought to induce bladder paralysis by preventing acetylcholine release in the neuronal–muscular junction (Edmunds and Long 1923; Dickson and Shevky 1923; Ambache 1949; Burgen et al. 1949). In the skeletal muscle, BoNT/A causes muscular paralysis that recovers within 2–4 months time after BoNT/A injection. During this period, a decrease of extrajunctional acetylcholinesterase can be detected and axons develop lateral sprouts that eventually disappear once synaptic transmission recovers (Thesleff et al. 1990; de Paiva et al. 1999; Meunier et al. 2002). Curiously, neuronal sprouting during detrusor paralysis was never documented in the urinary bladder (Haferkampa et al. 2004). In addition, no changes could be detected in the detrusor smooth muscle cells or nerve fibers coursing the urinary bladder (Haferkampa et al. 2004). On the other end, fragments of cleaved SNAP-25 seem to last much longer in the human bladder than in striated muscle (Schulte-Baukloh et al. 2007) and the average duration of BoNT/A in the bladder, around 9 months, largely exceeds that of skeletal muscle. Altogether, these facts indicate that additional mechanisms of action are probably operative in the bladder.

Among possible mechanisms, interruption of sensory input from the bladder, has received great attention. About 50% of the human bladder afferents express SV2 and SNAP-25, indicating their sensitivity to the neurotoxin (Coelho 2009). BoNT/A decreases the release of both substance P and CGRP from bladder afferent peripheral terminals in the bladder wall (Apostolidis et al. 2006b; Welch et al. 2000b; Chuang et al. 2004; Rapp et al. 2006; Lucioni et al. 2008), which is expected to limit neurogenic inflammation in the bladder. In addition, BoNT/A also inhibits the spinal cord release of glutamate, substance P, and CGRP by sensory nerves (Meng et al. 1997; Purkiss et al. 2000; Duggan et al. 2002; Aoki 2005), decreasing the excitation of second-order spinal cord sensory neurons.

BoNT/A was shown to prevent TRPV1 trafficking to the membrane during bladder inflammation (Morenilla-Palao et al. 2004). This process is expected to not only affect the noxious transmission in the bladder, but also prevent the activation of micturition reflex by bladder filling. In accordance with these experimental findings, TRPV1 and P2X3 immunoreactivity in the urinary bladder of patients with detrusor overactivity was shown to decrease after BoNT/A administration (Apostolidis et al. 2005b).

Another mechanism by which BoNT/A might interfere with the sensory arm of micturion reflex deals with the decrease of ATP release after BoNT/A injection in the bladder wall in animal models of spinal cord injury (Khera et al. 2004; Smith et al. 2008). ATP activates P2X3 receptors in the suburothelium, which was shown to be essential for bladder contractions (Cockayne et al. 2000). The decrease of ATP release was initially suggested to be dependent on a direct effect of BoNT/A on urothelial cells (Khera et al. 2004; Smith et al. 2008). However, rodents and human urothelial cells do not express SV2 or SNAP-25 (Coelho 2009). The decrease of ATP release should therefore be interpreted as either a direct effect of BoNT/A on ATP producing machinery (Dunant et al. 1987, 1988, 1990), a possible effect on other urothelial proteins involved in ATP release, such as connexins, nucleoside transporters, or the ABC cassette system (Grossman et al. 1994; Wang et al. 2005), or an indirect consequence of the intravesical pressure decrease following detrusor paralysis (Vlaskovska et al. 2001).

A marked decrease in urine concentration of nerve growth factor (NGF) was observed in NDO and IDO patients after BoNT/A injections (Giannantoni et al. 2006a; Liu et al. 2009). The mechanism of NGF release impaired by BoNT/A is unknown at this moment. Although an effect on urothelial cells could be suggested on the basis of NGF released from those cells (Birder et al. 2007), a recent observation from the same group seems to exclude such hypothesis. In fact, in contrast with urine levels (Kim et al. 2005; Yokoyama et al. 2008; Kuo et al. 2009), NGF concentration in mucosal samples of patients with detrusor overactivity is not superior to those of normal subjects (Birder et al. 2007). Whatever the mechanisms of NGF decrease in urine, the lack of this neurotrophin is expected to reduce excitability of bladder sensory fibers (Dmitrieva and McMahon 1996). Quite recently a decrease of brain-derived nerve factor concentration was also found after BoNT/A administration (Pinto et al. 2010).

The impairment of bladder nociceptive input following BoNT/A application in the bladder (Smith et al. 2004) opened the opportunity of BoNT/A injection in patients with bladder pain syndrome/interstitial cystitis (BPS/IC) (Giannantoni et al. 2006a; Liu and Kuo 2007a). Due to the concentration of bladder nociceptive fibers in the trigonal region, BoNT/A injections restricted to the trigone were recently evaluated in BPS/IC (Pinto et al. 2010). Pain alleviation proved to be highly effective in spite of the limited area of injection (Pinto et al. 2010). In addition, the risk of micturition dysfunction was minimized when compared with whole bladder injection (Pinto et al. 2010).

2.3 BoNT/A and the Prostate

The effect of BoNT/A was recently investigated in the prostate. Injections in the rat, dog, and human prostate were shown to decrease prostate volume (Chuang et al. 2005; Silva et al. 2008b, c). In a study carried out in rats, the prostate volume

reduction was correlated with an activation of apoptotic mechanisms (Silva et al. 2008b). Interestingly, prostate apoptosis was shown to be dependent on sympathetic nerve impairment and consequent decrease of the adrenergic stimulation of gland (Silva et al. 2008b). In addition to prostate volume changes, BoNT/A was shown to induce a dose-dependent decrease on the contractile function of the dog prostate (Schaible et al. 2005), further reinforcing the importance of sympathetic nerve signaling.

These findings suggest for intraprostatic BoNT/A, a totally different mechanism than proposed for intradetrusor BoNT/A (see Sect. 2.2), where parasympathetic impairment seems to play the key role. However, sympathetic fibers are the most numerous nerves in the prostate, whereas parasympathetic nerves predominate in the bladder (McVary et al. 1998; Schaible et al. 2005). Indirectly the differences between the bladder and the prostate indicate that BoNT/A effect in a particular organ depends on the impairment of the most important/numerous nerve fibers coursing through it.

2.4 Clinical Implications in the Usage of BoNT/A

The use of BoNT/A has become the treatment of excellence for patients with NDO (Cruz and Silva 2006), since it is simple and reproducible. It consists of BoNT/A injection (200–300 U of Botox® or 500–1,000 of Dysport®) distributed by 30 detrusor sites of injections under cystoscopic control. The treatment resulted in a reduction of incontinence episodes in neurogenic detrusor overactive patients (Reitz et al. 2004; Kalsi et al. 2007; Schurch et al. 2005; Ehren et al. 2007), with a mean duration between 6 and 9 months. More complete studies comparing BoNT/A with a placebo will prompt the definitive approval of this drug for NDO treatment.

Several studies, although also limited in size, have demonstrated clinical and urodynamics improvement in patients with refractory IDO after BoNT/A administration (Schmid et al. 2006; Sahai et al. 2007; Schulte-Baukloh et al. 2005; Brubaker et al. 2008). Furthermore, patients with benign prostate hyperplasia (Maria et al. 2003; Kuo 2005b; Chuang et al. 2005; Silva et al. 2008c; Doggweiler et al. 1998) and patients with BPS/IC (Giannantoni et al. 2006b, 2008; Pinto et al. 2009) presented an improvement in their symptoms after treatment with BoNT/A.

3 Future Directions

The key role of motor and sensory nerve fibers of somatic and autonomic origin for the LUT function makes the nervous system a foreseeable target for the control of micturition dysfunction. TRPV1 receptors are the first example of these targets that

are expected to be more and more relevant as new antagonist will come into therapeutic use. BoNT/A represents a nonspecific, albeit extremely potent, neurotoxin. However, the capacity to impair LUT innervation for long periods of time makes this toxin extremely attractive for the treatment of LUT dysfunction refractive to standard treatment. Future directions in BoNT/A will inevitably pass through the definition of doses and choice of injection places according to pathologies to treat, taking into consideration the different distribution of somatic, autonomic, and sensory fibers throughout the LUT.

References

Ahern GP, Brooks IM, Miyares RL, Wang XB (2005) Extracellular cations sensitize and gate capsaicin receptor TRPV1 modulating pain signalling. J Neurosci 25:5109–5116

Amadesi S, Cottrell GS, Divino L, Chapman K, Grady EF, Bautista F, Karanjia R, Barajas-Lopez C, Vanner S, Vergnolle N, Bunnett NW (2006) Protease-activated receptor 2 sensitizes TRPV1 by protein kinase Cepsilon- and A-dependent mechanisms in rats and mice. J Physiol 575:555–571

Amaya F, Oh-hashi K, Naruse Y, Iijima N, Ueda M, Shimosato G, Tominaga M, Tanaka Y, Tanaka M (2003) Local inflammation increases vanilloid receptor 1 expression within distinct subgroups of DRG neurons. Brain Res 963:190–196

Ambache N (1949) The peripheral action of Cl. Botulinum toxin. J Physiol 108:127–141

Aoki KR (2005) Review of a proposed mechanism for the antinociceptive action of botulinum toxin type A. Neurotoxicology 26:785–793

Apostolidis A, Brady CM, Yiangou Y, Davis J, Fowler CJ, Anand P (2005a) Capsaicin receptor TRPV1 in urothelium of neurogenic human bladders and effect of intravesical resiniferatoxin. Urology 65:400–405

Apostolidis A, Popat R, Yiangou Y, Cockayne D, Ford AP, Davis JB, Dasgupta P, Fowler CJ, Anand P (2005b) Decreased sensory receptors P2X3 and TRPV1 in suburothelial nerve fibers following intradetrusor injections of botulinum toxin for human detrusor overactivity. J Urol 174:977–982

Apostolidis A, Gonzales GE, Fowler CJ (2006a) Effect of intravesical resiniferatoxin (RTX) on lower urinary tract symptoms, urodynamic parameters, and quality of life of patients with urodynamic increased bladder sensation. Eur Urol 50:1299–1305

Apostolidis A, Dasgupta P, Fowler CJ (2006b) Proposed mechanism for the efficacy of injected botulinum toxin in the treatment of human detrusor overactivity. Eur Urol 49:644–650

Atlas D, Wiser O, Trus M (2001) The voltage-gated Ca^{2+} channel is the Ca^{2+} sensor of fast neurotransmitter release. Cell Mol Neurobiol 21:717–731

Avelino A, Cruz F (2010) TRPV1 in visceral pain and other visceral disorders. In: Gomtsyan A, Faltynek CR (eds) Vanilloid receptor TRPV1 in drug discovery. Wiley Ed, New York, pp 206–238, Chap 8

Avelino A, Cruz C, Nagy I, Cruz F (2002) Vanilloid receptor 1 expression in the rat urinary tract. Neuroscience 109:787–798

Bevan S, Hothi S, Hughes G, James IF, Rang HP, Shah K, Walpole CSJ, Yeats JC (1992) Capsazepine: a competitive antagonist of the sensory neurone excitant capsaicin. Br J Pharmacol 107:544–552

Bhave G, Zhu W, Wang H, Brasier DJ, Oxford GS, Gereau RW 4th (2002) cAMP-dependent protein kinase regulates desensitization of the capsaicin receptor (VR1) by direct phosphorylation. Neuron 35:721–731

Binz T, Rummel A (2009) Cell entry strategy of clostridial neurotoxins. J Neurochem 109:1584–1595

Binz T, Bade S, Rummel A, Kollewe A, Alves J (2002) Arg(362) and Tyr(365) of the botulinum neurotoxin type a light chain are involved in transition state stabilization. Biochemistry 41:1717–1723

Birder LA, Kanai AJ, de Groat WC, Kiss S, Nealen ML, Burke NE et al (2001) Vanilloid receptor expression suggests a sensory role for urinary bladder epithelial cells. Proc Natl Acad Sci 98:13396–13401

Birder LA, Nakamura Y, Kiss S, Nealen ML, Barrick S, Kanai AJ, Wang F, Ruiz G, de Groat WC, Apadoca G, Watkins S, Caterina MJ (2002) Altered urinary bladder function in mice lacking the vanilloid receptor TRPV1. Nat Neurosci 5:856–860

Birder LA, Wolf-Johnston A, Griffiths D, Resnick NM (2007) Role of urothelial nerve growth factor in human bladder function. Neurourol Urodyn 26:405–409

Blaustein RO, Germann WJ, Finkelstein A, DasGupta BR (1987) The N-terminal half of the heavy chain of botulinum type A neurotoxin forms channels in planar phospholipid bilayers. FEBS Lett 226:115–120

Bodó E, Bíró T, Telek A, Czifra G, Griger Z, Tóth BI, Mescalchin A, Ito T, Bettermann A, Kovács L, Paus R (2005) A hot new twist to hair biology: involvement of vanilloid receptor-1 (VR1/TRPV1) signaling in human hair growth control. Am J Pathol 166:985–998

Bonnington JK, McNaughton PA (2003) Signalling pathways involved in the sensitisation of mouse nociceptive neurons by nerve growth factor. J Physiol 551:433–446

Brady CM, Apostolidis AN, Harper M, Yiangou Y, Beckett A, Jacques TS, Freeman A, Scaravilli F, Fowler CJ, Anand P (2004a) Parallel changes in bladder suburothelial vanilloid receptor TRPV1 and pan-neuronal marker PGP9.5 immunoreactivity in patients with neurogenic detrusor overactivity after intravesical resiniferatoxin treatment. BJU Int 93:770–776

Brady CM, Apostolidis A, Yiangou Y, Baecker PA, Ford AP, Freeman A, Jacques TS, Fowler CJ, Anand P (2004b) P2X3-immunoreactive nerve fibres in neurogenic detrusor overactivity and the effect of intravesical resiniferatoxin. Eur Urol 46:247–253

Brubaker L, Richter HE, Visco A, Mahajan S, Nygaard I, Braun TM, Barber MD, Menefee S, Schaffer J, Weber AM, Wei J, Pelvic Floor Disorders Network (2008) Refractory idiopathic urge urinary incontinence and botulinum A injection. J Urol 180(1):217–222

Brunger AT, Rummel A (2009) Receptor and substrate interactions of clostridial neurotoxins. Toxicon 54:550–560

Brunger AT, Jin R, Breidenbach MA (2008) Highly specific interactions between botulinum neurotoxins and synaptic vesicle proteins. Cell Mol Life Sci 65:2296–2306

Burgen ASV, Dickens F, Zatman LJ (1949) The action of botulinum toxin on the neuro-muscular junction. J Physiol 109:10–24

Cai S, Kukreja R, Shoesmith S, Chang TW, Singh BR (2006) Botulinum neurotoxin light chain refolds at endosomal pH for its translocation. Protein J 25:455–462

Carlton SM, Coggeshall RE (2001) Peripheral capsaicin receptors increase in the inflamed rat hindpaw: a possible mechanism for peripheral sensitization. Neurosci Lett 310:53–56

Carlton SM, Du J, Zhou S (2009) Group II metabotropic glutamate receptor activation on peripheral nociceptors modulates TRPV1 function. Brain Res 1248:86–95

Carr MJ, Lollarik M, Meeker SN, Undem BJ (2003) A role for TRPV1 in bradykinin-induced excitation of vagal airway afferent nerve terminals. J Pharmacol Exp Ther 304:1275–1279

Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D (1997) The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 389:816–824

Caterina MJ, Lefber A, Malmberg AB, Martin WJ, Trafton J, Petersen KR, Koltzenburg M, Basbaum AI, Julius D (2000) Impaired nociception and pain sensation in mice lacking the capsaicin receptor. Science 288:306–313

Chai TC, Gray ML, Steers W (1998) The incidence of a positive ice water test in bladder outlet obstructed patients: evidence for bladder neural plasticity. J Urol 160:34–38

Chancellor MB, de Groat WC (1999) Intravesical capsaicin and resiniferatoxin therapy: spicing up the ways to treat the overactive bladder. J Urol 162:3–11

Chandiramani VA, Peterson T, Duthie GS, Fowler CJ (1996) Urodynamic changes during therapeutic intravesical instillations of capsaicin. Br J Urol 77:792–797

Charrua A, Cruz CD, Cruz F, Avelino A (2007) Transient receptor potential vanilloid subfamily 1 is essential for the generation of noxious bladder input and bladder overactivity in cystitis. J Urol 177:1537–1541

Charrua A, Reguenga C, Paule CC, Nagy I, Cruz F, Avelino A (2008) Cystitis is associated with TRPV1b-downregulation in rat dorsal root ganglia. NeuroReport 19:1469–1472

Charrua A, Reguenga C, Cordeiro JM, Correiade-Sá P, Paule C, Nagy I, Cruz F, Avelino A (2009a) Functional transient receptor potential vanilloid 1 is expressed in human urothelial cells. J Urol 182:2944–2950

Charrua A, Cruz CD, Narayanan S, Gharat L, Gullapalli S, Cruz F, Avelino A (2009b) GRC-6211, a new oral specific TRPV1 antagonist, decreases bladder overactivity and noxious bladder input in cystitis animal models. J Urol 181:379–386

Chen YA, Scales SJ, Patel SM, Doung YC, Scheller RH (1999) SNARE complex formation is triggered by Ca^{2+} and drives membrane fusion. Cell 97:165–174

Chen TY, Corcos J, Camel M, Ponsot Y, Tu le M (2005) Prospective, randomized, double-blind study of safety and tolerability of intravesical resiniferatoxin (RTX) in interstitial cystitis (IC). Int Urogynecol J Pelvic Floor Dysfunct 16:293–297

Chu CJ, Huang SM, De Petrocellis L, Bisogno T, Ewing SA, Miller JD, Zipkin RE, Daddario N, Appendino G, Di Marzo V, Walker JM (2003) N-oleoyldopamine, a novel endogenous capsaicin-like lipid that produces hyperalgesia. J Biol Chem 278:13633–13639

Chuang HH, Prescott ED, Kong H, Shields S, Jordt SE, Basbaum AI, Chao MV, Julius D (2001) Bradykinin and nerve growth factor release the capsaicin receptor from PtdIns(4, 5)P2-mediated inhibition. Nature 411:957–962

Chuang YC, Yoshimura N, Huang CC, Chiang PH, Chancellor MB (2004) Intravesical botulinum toxin a administration produces analgesia against acetic acid induced bladder pain responses in rats. J Urol 172:1529–1532

Chuang YC, Chiang PH, Huang CC, Yoshimura N, Chancellor MB (2005) Botulinum toxin type A improves benign prostatic hyperplasia symptoms in patients with small prostates. Urology 66:775–779

Cockayne DA, Hamilton SG, Zhu QM, Dunn PM, Zhong Y, Novakovic S, Malmberg AB, Cain G, Berson A, Kassotakis L, Hedley L, Lachnit WG, Burnstock G, McMahon SB, Ford AP (2000) Urinary bladder hyporeflexia and reduced pain-related behaviour in P2X3-deficient mice. Nature 407:1011–1015

Coelho A, Dinis P, Pinto R, Gorgal T, Silva C, Silva A, Silva J, Cruz CD, Cruz F, Avelino A (2009) Mechanisms of action of botulinum toxin type A: distribution of its high affinity binding site and intracellular target in the human bladder. Eur Urol 56:134–140

Cruz F, Silva C (2006) Refractory neurogenic detrusor overactivity. Int J Clin Pract 60:22–26

Cruz F, Guimarães M, Silva C, Rio ME, Coimbra A, Reis M (1997a) Desensitization of bladder sensory fibres by intravesical capsaicin has long lasting clinical and urodynamic effects in patients with hyperactive or hypersensitive bladder dysfunction. J Urol 157: 585–589

Cruz F, Guimaräes M, Silva C, Reis M (1997b) Suppression of bladder hyperreflexia by intravesical resiniferatoxin. Lancet 350:640–641

da Silva CM, Cruz F (2009) Has botulinum toxin therapy come of age: what do we know, what do we need to know, and should we use it? Curr Opin Urol 19:347–352

Das A, Chancellor MB, Watanabe T, Sedor J, Rivas DA (1996) Intravesical capsaicin in neurologic impaired patients with detrusor hyperreflexia. J Spinal Cord Med 19:190–193

Davis JB, Gray J, Gunthorpe MJ, Hatcher JP, Davey PT, Overend P, Harries MH, Latcham J, Clapham C, Atkinson K, Hughes SA, Rance K, Grau E, Harper AJ, Pugh PL, Rogers DC,

Bingham S, Randall A, Sheardown SA (2000) Vanilloid receptor-1 is essential for inflammatory thermal hyperalgesia. Nature 405:183–187

de Groat WC (1997) A neurologic basis for the overactive bladder. Urology 50:36–52

de Haro L, Quetglas S, Iborra C, Lévêque C, Seagar M (2003) Calmodulin-dependent regulation of a lipid binding domain in the v-SNARE synaptobrevin and its role in vesicular fusion. Biol Cell 95:459–464

de Paiva A, Meunier FA, Molgó J, Aoki KR, Dolly JO (1999) Functional repair of motor endplates after botulinum neurotoxin type A poisoning: biphasic switch of synaptic activity between nerve sprouts and their parent terminals. Proc Natl Acad Sci U S A 96:3200–3205

de Ridder D, Chandiramani V, Dasgupta P, Van Poppel H, Baert L, Fowler CJ (1997) Intravesical capsaicin as a treatment for refractory detrusor hyperreflexia: a dual center study with long-term followup. J Urol 158:2087–2092

de Séze M, Wiart L, Joseph PA, Dosque JP, Mazaux JM, Barat M (1998) Capsaicin and neurogenic detrusor hyperreflexia. A double blind placebo controlled study in 20 patients with spinal cord lesions. Neurourol Urodyn 17:513–523

de Sèze M, Wiart L, Ferrière J, de Sèze MP, Joseph P, Barat M (1999) Intravesical instillation of capsaicin in urology: a review of the literature. Eur Urol 36:267–277

de Sèze M, Wiart L, de Sèze MP, Soyeur L, Dosque JP, Blajezewski S, Moore N, Brochet B, Mazaux JM, Barat M, Joseph PA (2004) Intravesical capsaicin versus resiniferatoxin for the treatment of detrusor hyperreflexia in spinal cord injured patients: a double-blind, randomized, controlled study. J Urol 171:251–255

de Sèze M, Gallien P, Denys P, Labat JJ, Serment G, Grise P, Salle JY, Blazejewski S, Hazane C, Moore N, Joseph PA (2006) Intravesical glucidic capsaicin versus glucidic solvent in neurogenic detrusor overactivity: a double blind controlled randomized study. Neurourol Urodyn 25:752–757

Dickenson AH, Dray A (1991) Selective antagonism of capsaicin by capsazepine: evidence for a spinal receptor site in capsaicin-induced antinociception. Br J Pharmacol 104:1045–1049

Dickson EC, Shevky R (1923) Botulism. Studies on the manner in which the toxin of *clostridium botulinum* acts upon the body: I. The effect upon the autonomic nervous system. J Exp Med 37:711–731

Dinis P, Charrua A, Avelino A, Yaqoob M, Bevan S, Nagy I, Cruz F (2004a) Anandamide-evoked activation of vanilloid receptor 1 contributes to the development of bladder hyperreflexia and nociceptive transmission to spinal dorsal horn neurons in cystitis. J Neurosci 24:11253–11263

Dinis P, Silva J, Ribeiro MJ, Avelino A, Reis M, Cruz F (2004b) Bladder C-fiber desensitization induces a long-lasting improvement of BPH-associated storage LUTS: a pilot study. Eur Urol 46:88–93

Dinis P, Charrua A, Avelino A, Nagy I, Quintas J, Ribau U, Cruz F (2005) The distribution of sensory fibers immunoreactive for the TRPV1 (capsaicin) receptor in the human prostate. Eur Urol 48:162–167

Dmitrieva N, McMahon SB (1996) Sensitisation of visceral afferents by nerve growth factor in the adult rat. Pain 66:87–97

Docherty RJ, Yeats JC, Bevan S, Boddeke HW (1996) Inhibition of calcineurin inhibits the desensitization of capsaicin-evoked currents in cultured dorsal root ganglion neurones from adult rats. Pflugers Arch 431:828–837

Docherty RJ, Yeats JC, Piper AS (1997) Capsazepine block of voltage-activated calcium channels in adult rat dorsal root ganglion neurons in culture. Br J Pharmacol 121:1461–1467

Doggweiler R, Zermann DH, Ishigooka M, Schmidt RA (1998) Botox-induced prostatic involution. Prostate 37:44–50

Dong M, Yeh F, Tepp WH, Dean C, Johnson EA, Janz R, Chapman ER (2006) SV2 is the protein receptor for botulinum neurotoxin A. Science 312:592–596

Duggan MJ, Quinn CP, Chaddock JA, Purkiss JR, Alexander FC, Doward S, Fooks SJ, Friis LM, Hall YH, Kirby ER, Leeds N, Moulsdale HJ, Dickenson A, Green GM, Rahman W, Suzuki R,

Shone CC, Foster KA (2002) Inhibition of release of neurotransmitters from rat dorsal root ganglia by a novel conjugate of a *Clostridium botulinum* toxin A endopeptidase fragment and Erythrina cristagalli lectin. J Biol Chem 277:34846–34852

Dunant Y, Esquerda JE, Loctin F, Marsal J, Muller D (1987) Botulinum toxin inhibits quantal acetylcholine release and energy metabolism in the Torpedo electric organ. J Physiol 385:677–692

Dunant Y, Loctin F, Marsal J, Muller D, Parducz A, Rabasseda X (1988) Energy metabolism and quantal acetylcholine release: effects of botulinum toxin, 1-fluoro-2, 4-dinitrobenzene, and diamide in the Torpedo electric organ. J Neurochem 50(2):431–439

Dunant Y, Esquerda JE, Loctin F, Marsal J, Muller D (1990) Type A botulinum toxin disorganizes quantal acetylcholine release and inhibits energy metabolism. J Physiol Paris 84(3):211–219

Edmunds CW, Long PH (1923) Contribution to the pathologic physiology of botulism J. Am Med Ass 81:542–547

Ehren I, Volz D, Farrelly E, Berglund L, Brundin L, Hultling C, Lafolie P (2007) Efficacy and impact of botulinum toxin A on quality of life in patients with neurogenic detrusor overactivity: a randomised, placebo-controlled, double-blind study. Scand J Urol Nephrol 41(4): 335–340

Eilers H, Lee SY, Hau CW, Logvinova A, Schumacher MA (2007) The rat vanilloid receptor splice variant VR.5'sv blocks TRPV1 activation. NeuroReport 18:969–973

Everaerts W, Sepulveda MR, Gevaert T, Roskams T, Nilius B, de Ridder D (2009) Where is TRPV1 expressed in the bladder, do we see the real channel? Naunyn Schmiedebergs Arch Pharmacol 379:421–425

Fagerli J, Fraser MO, deGroat WC, Chancellor MB, Flood HD, Smith D, Jordan ML (1999) Intravesical capsaicin for the treatment of interstitial cystitis: a pilot study. Can J Urol 6:737–744

Ferreira J, da Silva GL, Calixto JB (2004) Contribution of vanilloid receptors to the overt nociception induced by B2 kinin receptor activation in mice. Br J Pharmacol 141:787–794

Ferrer-Montiel AV, Canaves JM, DasGupta BR, Wilson MC, Montal M (1996) Tyrosine phosphorylation modulates the activity of clostridial neurotoxins. J Biol Chem 271:18322–18325

Fischer A, Montal M (2007a) Crucial role of the disulfide bridge between botulinum neurotoxin light and heavy chains in protease translocation across membranes. J Biol Chem 282:29604–29611

Fischer A, Montal M (2007b) Single molecule detection of intermediates during botulinum neurotoxin translocation across membranes. Proc Natl Acad Sci USA 104:10447–10452

Fischer A, Mushrush DJ, Lacy DB, Montal M (2008) Botulinum neurotoxin devoid of receptor binding domain translocates active protease. PLoS Pathog 4:e1000245

Fowler CJ, Jewkes D, McDonald WI, Lynn B, de Groat WC (1992) Intravesical capsaicin for neurogenic bladder dysfunction. Lancet 339:1239

Fowler CJ, Beck RO, Gerrard S, Betts CD, Fowler CG (1994) Intravesical capsaicin for treatment of detrusor hyperreflexia. J Neurol Neurosurg Psychiatry 57:169–173

Fujinaga Y, Takeshi K, Inoue K, Fujita R, Ohyama T, Moriishi K, Oguma K (1995) Type A and B neurotoxin genes in a *Clostridium botulinum* type AB strain. Biochem Biophys Res Commun 213:737–745

Galloux M, Vitrac H, Montagner C, Raffestin S, Popoff MR, Chenal A, Forge V, Gillet D (2008) Membrane interaction of botulinum neurotoxin A translocation (T) domain. The belt region is a regulatory loop for membrane interaction. J Biol Chem 283:27668–27676

Gavva NR, Bannon AW, Surapaneni S, Hovland DN Jr, Lehto SG, Gore A, Juan T, Deng H, Han B, Klionsky L, Kuang R, Le A, Tamir R, Wang J, Youngblood B, Zhu D, Norman MH, Magal E, Treanor JJ, Louis JC (2007) The vanilloid receptor TRPV1 is tonically activated in vivo and involved in body temperature regulation. J Neurosci 27:3366–3374

Gavva NR, Treanor JJ, Garami A, Fang L, Surapaneni S, Akrami A, Alvarez F, Bak A, Darling M, Gore A, Jang GR, Kesslak JP, Ni L, Norman MH, Palluconi G, Rose MJ, Salfi M, Tan E,

Romanovsky AA, Banfield C, Davar G (2008) Pharmacological blockade of the vanilloid receptor TRPV1 elicits marked hyperthermia in humans. Pain 136:202–210

Geirsson G, Fall M, Sullivan L (1995) Clinical and urodynamic effects of intravesical capsaicin treatment in patients with chronic traumatic spinal detrusor hyperreflexia. J Urol 154: 1825–1829

Giannantoni A, Di Stasi SM, Stephen RL, Navarra P, Scivoletto G, Mearini E, Porena M (2002) Intravesical capsaicin versus resiniferatoxin in patients with detrusor hyperreflexia: a prospective randomized study. J Urol 167:1710–1714

Giannantoni A, Di Stasi SM, Nardicchi V, Zucchi A, Macchioni L, Bini V, Goracci G, Porena M (2006a) Botulinum-A toxin injections into the detrusor muscle decrease nerve growth factor bladder tissue levels in patients with neurogenic detrusor overactivity. J Urol 175:2341–2344

Giannantoni A, Costantini E, Di Stasi SM, Tascini MC, Bini V, Porena M (2006b) Botulinum A toxin intravesical injections in the treatment of painful bladder syndrome: a pilot study. Eur Urol 49(4):704–709

Giannantoni A, Porena M, Costantini E, Zucchi A, Mearini L, Mearini E (2008) Botulinum A toxin injection in patients with painful bladder syndrome: 1-year follow-up. J Urol 179: 1031–1034

Grossman HB, Liebert M, Lee IW, Lee SW (1994) Decreased connexin expression and intercellular communication in human bladder cancer cells. Cancer Res 54:3062–3065

Gunthorpe MJ, Chizh BA (2009) Clinical development of TRPV1 antagonists: targeting a pivotal point in the pain pathway. Drug Discov Today 14:56–67

Guo A, Vulchanova L, Wang J, Li X, Elde R (1999) Immunocytochemical localization of the vanilloid receptor 1 (VR1): relationship to neuropeptides, the P2X3 purinoceptor and IB4 binding sites. Eur J Neurosci 11:946–958

Haferkampa A, Schurch B, Reitzb A, Krengela U, Grosse J, Kramer G, Schumachera S, Bastiana PJ, Buttnere R, Mullera SC, Stohrer M (2004) Lack of ultrastructural detrusor changes following endoscopic injection of botulinumtoxintype A in overactive neurogenic bladder. Eur Urol 46:784–791

Hermann H, De Petrocellis L, Bisogno T, Bisogno T, Schiano Moriello A, Lutz B, Di Marzo V (2003) Dual effect of cannabinoid CB1 receptor stimulation on a vanilloid VR1 receptor-mediated response. Cell Mol Life Sci 60:607–616

Hill KK, Smith TJ, Helma CH, Ticknor LO, Foley BT, Svensson RT, Brown JL, Johnson EA, Smith LA, Okinaka RT, Jackson PJ, Marks JD (2007) Genetic diversity among Botulinum Neurotoxin-producing clostridial strains. J Bacteriol 189:818–832

Holzer P (1991) Capsaicin: cellular target, mechanism of action and selectivity for thin sensory neurons. Pharmacol Rev 43:143–201

Huang SM, Bisogno T, Trevisani M, Al-Hayani A, De Petrocellis L, Fezza F, Tognetto M, Petros TJ, Krey JF, Chu CJ, Miller JD, Davies SN, Geppetti P, Walker JM, Di Marzo V (2002) An endogenous capsaicin-like substance with high potency at recombinant and native vanilloid VR1 receptors. Proc Natl Acad Sci USA 99:8400–8405

Humeau Y, Doussau F, Grant NJ, Poulain B (2000) How botulinum and tetanus neurotoxins block neurotransmitter release. Biochimie 82:427–446

Hwang SW, Cho H, Kwak J, Lee SY, Kang CJ, Jung J, Cho S, Min KH, Suh YG, Kim D, Oh U (2000) Direct activation of capsaicin receptors by products of lipoxygenases: endogenous capsaicin-like substances. Proc Natl Acad Sci USA 97:6155–6160

Ibañez C, Blanes-Mira C, Fernández-Ballester G, Planells-Cases R, Ferrer-Montiel A (2004) Modulation of botulinum neurotoxin A catalytic domain stability by tyrosine phosphorylation. FEBS Lett 578:121–127

Ichikawa H, Sugimoto T (2001) VR1-immunoreactive primary sensory neurons in the rat trigeminal ganglion. Brain Res 890:184–188

Igawa Y, Satoh T, Mizusawa H, Seki S, Kato H, Ishizuka O, Nishizawa O (2003) The role of capsaicin-sensitive afferents in autonomic dysreflexia in patients with spinal cord injury. BJU Int 91:637–641

Ito S, Kume H, Naruse K, Kondo M, Takeda N, Iwata S, Hasegawa Y, Sokabe M (2008) A novel Ca^{2+} influx pathway activated by mechanical stretch in human airway smooth muscle cells. Am J Respir Cell Mol Biol 38:407–413

Jahn R, Scheller RH (2003) SNAREs-engines for membrane fusion. Nat Rev Mol Cell Biol 7:631–643

Jahn R, Lang T, Sudhof TC (2003) Membrane fusion. Cell 112:519–533

Ji RR, Samad TA, Jin SX, Schmoll R, Woolf CJ (2002) p38 MAPK activation by NGF in primary sensory neurons after inflammation increases TRPV1 levels and maintains heat hyperalgesia. Neuron 36:57–68

Jones RC, Xu L, Gebhart GF (2005) The mechanosensitivity of mouse colon afferent fibers and their sensitization by inflammatory mediators require transient receptor potential vanilloid 1 and acid-sensing ion channel 3. J Neurosci 25:10981–10989

Jordt SE, Julius D (2002) Molecular basis for species-specific sensitivity to "hot" chili peppers. Cell 108:421–430

Jordt SE, Tominaga M, Julius D (2000) Acid potentiation of the capsaicin receptor determined by a key extracellular site. Proc Natl Acad Sci U S A 97:8134–8139

Jung J, Shin JS, Lee SY, Hwang SW, Koo J, Cho H, Oh U (2004) Phosphorylation of vanilloid receptor 1 by Ca^{2+}/calmodulin-dependent kinase II regulates its vanilloid binding. J Biol Chem 279:7048–7054

Kadowaki M, Kuramoto H, Takaki M (2004) Combined determination with functional and morphological studies of origin of nerve fibers expressing transient receptor potential vanilloid 1 in the myenteric plexus of rat jejunum. Auton Neurosci 116:11–18

Kalandakanond S, Coffield JA (2001) Cleavage of SNAP-25 by botulinum toxin type A requires receptor-mediated endocytosis, pH-dependent translocation, and zinc. J Pharmacol Exp Ther 296:980–986

Kalsi V, Gonzales G, Popat R, Apostolidis A, Elneil S, Dasgupta P, Fowler CJ (2007) Botulinum injections for the treatment of bladder symptoms of multiple sclerosis. Ann Neurol 62(5): 452–457

Kark T, Bagi Z, Lizanecz E, Pásztor ET, Erdei N, Czikora A, Papp Z, Edes I, Pórszász R, Tóth A (2008) Tissue-specific regulation of microvascular diameter: opposite functional roles of neuronal and smooth muscle located vanilloid receptor-1. Mol Pharmacol 73:1405–1412

Khairatkar-Joshi N, Szallasi A (2009) TRPV1 antagonists: the challenges for therapeutic targeting. Trends Mol Med 15:14–22

Khera M, Somogyi GT, Kiss S, Boone TB, Smith CP (2004) Botulinum toxin A inhibits ATP release from bladder urothelium after chronic spinal cord injury. Neurochem Int 45:987–993

Kim JH, Rivas DA, Shenot PJ, Green B, Kennelly M, Erickson JR, O'Leary M, Yoshimura N, Chancellor MB (2003) Intravesical resiniferatoxin for refractory detrusor hyperreflexia: a multicenter, blinded, randomized, placebo-controlled trial. J Spinal Cord Med 26:358–363

Kim JC, Park EY, Hong SH, Seo SI, Park YH, Hwang TK (2005) Changes of urinary nerve growth factor and prostaglandins in male patients with overactive bladder symptom. Int J Urol 12:875–878

Koike S, Uno T, Bamba H, Shibata T, Okano H, Hisa Y (2004) Distribution of vanilloid receptors in the rat laryngeal innervation. Acta Otolaryngol 124:515–519

Koplas PA, Rosenberg RL, Oxford GS (1997) The role of calcium in the desensitization of capsaicin responses in rat dorsal root ganglion neurons. J Neurosci 17:3525–3537

Koriazova LK, Montal M (2003) Translocation of botulinum neurotoxin light chain protease through the heavy chain channel. Nat Struct Biol 10:13–18

Kuenzi FM, Dale N (1996) Effect of capsaicin and analogues on potassium and calcium currents and vanilloid receptors in Xenopus embryo spinal neurones. Br J Pharmacol 119:81–90

Kuo HC (2003) Effectiveness of intravesical resiniferatoxin for anticholinergic treatment refractory detrusor overactivity due to nonspinal cord lesions. J Urol 170:835–839

Kuo HC (2005a) Multiple intravesical instillation of low-dose resiniferatoxin is effective in the treatment of detrusor overactivity refractory to anticholinergics. BJU Int 95:1023–1027

Kuo HC (2005b) Prostate botulinum A toxin injection: an alternative treatment for benign prostatic obstruction in poor surgical candidates. Urology 65:670–674

Kuo HC, Liu HT, Yang WC (2006) Therapeutic effect of multiple resiniferatoxin intravesical instillations in patients with refractory detrusor overactivity: a randomized, double-blind, placebo controlled study. J Urol 176:641–645

Kuo HC, Liu HT, Chancellor MB (2009) Urinary nerve growth factor is a better biomarker than detrusor wall thickness for the assessment of overactive bladder with incontinence. Neurourol Urodyn 29:482–487

Lacy BD, Stevens RC (1999) Sequence homology and structural analysis of the clostridial neurotoxins. J Mol Biol 291:1091–1104

Lakshmi S, Joshi PG (2005) Co-activation of P2Y2 receptor and TRPV channel by ATP: implications for ATP induced pain. Cell Mol Neurobiol 25:819–832

Lalli G, Herreros J, Osborne SL, Montecucco C, Rossetto O, Schiavo G (1999) Functional characterisation of tetanus and botulinum neurotoxins binding domains. J Cell Sci 112:2715–2724

Lazzeri M, Beneforti P, Benaim G, Maggi CA, Lecci A, Turini D (1996) Intravesical capsaicin for treatment of severe bladder pain: a randomized placebo controlled study. J Urol 156: 947–952

Lazzeri M, Beneforti P, Turini D (1997) Urodynamic effects of intravesical resiniferatoxin in humans: preliminary results in stable and unstable detrusor. J Urol 158:2093–2096

Lazzeri M, Spinelli M, Beneforti P, Zanollo A, Turini D (1998) Intravesical resiniferatoxin for the treatment of detrusor hyperreflexia refractory to capsaicin in patients with chronic spinal cord diseases. Scand J Urol Nephrol 32:331–334

Lazzeri M, Beneforti M, Spinelli A, Zanollo A, Barbagli G, Turini D (2000) Intravesical resiniferatoxin for the treatment of hypersensitive disorder: a randomized placebo controlled study. J Urol 164:676–679

Lazzeri M, Vannucchi MG, Zardo C, Spinelli M, Beneforti P, Turini D, Faussone-Pellegrini MS (2004a) Immunohistochemical evidence of vanilloid receptor 1 in normal human urinary bladder. Eur Urol 46:792–798

Lazzeri M, Spinelli M, Zanollo A, Turini D (2004b) Intravesical vanilloids and neurogenic incontinence: ten years experience. Urol Int 72:145–149

Lee SY, Lee JH, Kang KK, Hwang SY, Choi KD, Oh U (2005) Sensitization of vanilloid receptor involves an increase in the phosphorylated form of the channel. Arch Pharm Res 28:405–412

Li L, Binz T, Niemann H, Singh BR (2000) Probing the mechanistic role of glutamate residue in the zinc-binding motif of type A botulinum neurotoxin light chain. Biochemistry 39: 2399–2405

Lishko PV, Procko E, Jin X, Phels CB, Gaudet R (2007) The ankyrin repeats of TRPV1 bind multiple ligands and modulate channel sensitivity. Neuron 54:905–918

Liu HT, Kuo HC (2007a) Intravesical botulinum toxin A injections plus hydrodistension can reduce nerve growth factor production and control bladder pain in interstitial cystitis. Urology 70:463–468

Liu HT, Kuo HC (2007b) Increased expression of transient receptor potential vanilloid subfamily 1 in the bladder predicts the response to intravesical instillations of resiniferatoxin in patients with refractory idiopathic detrusor overactivity. BJU Int 100:1086–1090

Liu B, Zhang C, Qin F (2005) Functional recovery from desensitization of vanilloid receptor TRPV1 requires resynthesis of phosphatidylinositol 4, 5-biphosphate. J Neurosci 25:4835–4843

Liu HT, Chancellor MB, Kuo HC (2008) Urinary nerve growth factor levels are elevated in patients with detrusor overactivity and decreased in responders to detrusor botulinum toxin-A injection. Eur Urol 56(4):700–707

Liu HT, Chancellor MB, Kuo HC (2009) Urinary nerve growth factor levels are elevated in patients with detrusor overactivity and decreased in responders to detrusor botulinum toxin-A injection. Eur Urol 56:700–706

López-Alonso E, Canaves J, Arribas M, Casanova A, Marsal J, González-Ros JM, Solsona C (1995) Botulinum toxin type A inhibits $Ca(^{2+})$-dependent transport of acetylcholine in

reconstituted giant liposomes made from presynaptic membranes from cholinergic nerve terminals. Neurosci Lett 196:37–40
Lu G, Henderson D, Liu L, Reinhart PH, Simon SA (2005) TRPV1b, a functional human vanilloid receptor splice variant. Mol Pharmacol 67:1119–1127
Lucioni A, Bales GT, Lotan TL, McGehee DS, Cook SP, Rapp DE (2008) Botulinum toxin type A inhibits sensory neuropeptide release in rat bladder models of acute injury and chronic inflammation. BJU Int 101:366–370
Mahmmoud YA (2008) Capsazepine, a synthetic vanilloid that converts the Na, K-ATPase to Na-ATPase. Proc Natl Acad Sci USA 105:1757–1761
Mahrhold S, Rummel A, Bigalke H, Davletov B, Binz T (2006) The synaptic vesicle protein 2C mediates the uptake of botulinum neurotoxin A into phrenic nerves. FEBS Lett 580:2011–2014
Maria G, Brisinda G, Civello IM, Bentivoglio AR, Sganga G, Albanese A (2003) Relief by botulinum toxin of voiding dysfunction due to benign prostatic hyperplasia: results of a randomized, placebo-controlled study. Urology 62:259–264
Marsal J, Egea G, Solsona C, Rabasseda X, Blasi J (1989) Botulinum toxin type A blocks the morphological changes induced by chemical stimulation on the presynaptic membrane of Torpedo synaptosomes. Proc Natl Acad Sci USA 86:372–376
McCroskey LM, Hatheway CL, Woodruff BA, Greenberg JA, Jurgenson P (1991) Type F botulism due to neurotoxigenic *Clostridium baratii* from an unknown source in an adult. J Clin Microbiol 29(11):2618–2620
McIntyre P, McLatchie LM, Chambers A, Phillips E, Clarke M, Savidge J, Toms C, Peacock M, Shah K, Winter J, Weerasakera N, Webb M, Rang HP, Bevan S, James IF (2001) Pharmacological differences between the human and rat vanilloid receptor 1 (VR1). Br J Pharmacol 132:1084–1094
McNew JA, Parlati F, Fukuda R, Johnston RJ, Paz K, Paumet F, Söllner TH, Rothman JE (2000) Compartmental specificity of cellular membrane fusion encoded in SNARE proteins. Nature 407(6801):153–159
McVary KT, McKenna KE, Lee C (1998) Prostate innervation. Prostate Suppl 8:2–13
McVey DC, Vigna SR (2001) The capsaicin VR1 receptor mediates substance P release in toxin A-induced enteritis in rats. Peptides 22:1439–1446
Meng X, Karasawa T, Zou K, Kuang X, Wang X, Lu C, Wang C, Yamakawa K, Nakamura S (1997) Characterization of a neurotoxigenic *Clostridium butyricum* strain isolated from the food implicated in an outbreak of food-borne type E botulism. J Clin Microbiol 35(8):2160–2162
Meunier FA, Schiavo G, Molgó J (2002) Botulinum neurotoxins: from paralysis to recovery of functional neuromuscular transmission. J Physiol Paris 96:105–113
Mezey E, Tóth ZE, Cortright DN, Arzubi MK, Krause JE, Elde R, Guo A, Blumberg PM, Szallasi A (2000) Distribution of mRNA for vanilloid receptor subtype 1 (VR1), and VR1-like immunoreactivity, in the central nervous system of the rat and human. Proc Natl Acad Sci USA 97:3655–3660
Michael GJ, Priestley JV (1999) Differential expression of the mRNA for the vanilloid receptor subtype 1 in cells of the adult rat dorsal root and nodose ganglia and its downregulation by axotomy. J Neurosci 19:1844–1854
Mizumura K, Sugiur T, Koda H, Katanosaka K, Kumar BR, Giron R, Tominaga M (2005) Pain and bradykinin receptors – sensory transduction mechanism in the nociceptor terminals and expression change of bradykinin receptors in inflamed condition. Nihon Shinkei Seishin Yakurigaku Zasshi 25:33–38
Mohanty S, Dhawan B, Chaudhry R (2001) Botulism: an update. Indian J Med Microbiol 19: 35–43
Mohapatra DP, Nau C (2005) Regulation of Ca^{2+}-dependent desensitization in the vanilloids receptor TRPV1 by calcineurin and cAMP-dependent protein kinase. J Biol Chem 280: 13424–13432
Montal M (2009) Translocation of botulinum neurotoxin light chain protease by the heavy chain protein-conducting channel. Toxicon 54:565–569

Morenilla-Palao C, Planells-Cases R, García-Sanz N, Ferrer-Montiel A (2004) Regulated exocytosis contributes to protein kinase C potentiation of vanilloid receptor activity. J Biol Chem 279:25665–25672

Moriyama T, Iida T, Kobayashi K, Higashi T, Fukuoka T, Tsumura H, Leon C, Suzuki N, Inoue K, Gachet C, Noguchi K, Tominaga M (2003) Possible involvement of P2Y2 metabotropic receptors in ATP-induced transient receptor potential vanilloid receptor 1-mediated thermal hypersensitivity. J Neurosci 23:6058–6062

Mukerji G, Yiangou Y, Agarwal SK, Anand P (2006) Transient receptor potential vanilloid receptor subtype 1 in painful bladder syndrome and its correlation with pain. J Urol 176:797–801

Muller D, Garcia-Segura LM, Parducz A, Dunant Y (1987) Brief occurrence of a population of presynaptic intramembrane particles coincides with transmission of a nerve impulse. Proc Natl Acad Sci USA 84:590–594

Novakova-Tousova K, Vyklicky L, Susankova K, Benedikt J, Samad A, Teisinger J, Vlachova V (2007) Functional changes in the vanilloids receptor subtype 1 channel during and after acute desensitization. Neuroscience 149:144–154

Oh GS, Pae HO, Seo WG, Kim NY, Pyun KH, Kim IK, Shin M, Chung HT (2001) Capsazepine, a vanilloid receptor antagonist, inhibits the expression of inducible nitric oxide synthase gene in lipopolysaccharide-stimulated RAW264.7 macrophages through the inactivation of nuclear transcription factor-kappa B. Int Immunopharmacol 1:777–784

Ohta T, Ikemi Y, Murakami M, Imagawa T, Otsuguro K, Ito S (2006) Potentiation of transient receptor potential V1 functions by the activation of metabotropic 5-HT receptors in rat primary sensory neurons. J Physiol 576:809–822

Ost D, Roskams T, Van Der Aa F, De Ridder D (2002) Topography of the vanilloid receptor in the human bladder: more than just the nerve fibers. J Urol 168:293–297

Payne CK, Mosbaugh PG, Forrest JB, Evans RJ, Whitmore KE, Antoci JP, Perez-Marrero R, Jacoby K, Diokno AC, O'Reilly KJ, Griebling TL, Vasavada SP, Yu AS, Frumkin LR (2005) Intravesical resiniferatoxin for the treatment of interstitial cystitis: a randomized, double-blind, placebo controlled trial. J Urol 173:1590–1594

Peng CH, Kuo HC (2007) Multiple intravesical instillations of low-dose resiniferatoxin in the treatment of refractory interstitial cystitis. Urol Int 78:78–81

Perkins MN, Campbell EA (1992) Capsazepine reversal of the antinociceptive action of capsaicin *in vivo*. Br J Pharmacol 107:329–333

Pinto R, Silva A, Lopes T, Silva J, Silva C, Cruz F, Dinis P (2009) Intra-trigonal injection of botulinum toxin A in patients with bladder pain syndrome. Results at 9-months of follow-up. J Urol 181:20

Pinto R, Lopes T, Frias B, Silva A, Silva JA, Silva CM, Cruz C, Cruz F, Dinis P (2010) Trigonal injection of botulinum toxin A in patients with refractory bladder pain syndrome/interstitial cystitis. Eur Urol 58:360–365

Piper AS, Yeats JC, Bevan S, Docherty RJ (1999) A study of the voltage dependence of capsaicin-activated membrane currents in rat sensory neurones before and after acute desensitization. J Physiol 518:721–733

Popoff M, Marvaud JC (1999) Structural and genomic features of clostridial neurotoxins. In: Alouf JE, Freer JH (eds) The comprehensive sourcebook of bacterial protein toxins. Academic, London, pp 202–228

Premkumar LS, Ahern GP (2000) Induction of vanilloid receptor channel activity by protein kinase C. Nature 408:985–990

Prescott ED, Julius D (2003) A modular PIP2 binding site as a determinant of capsaicin receptor sensitivity. Science 300:1284–1288

Prescott GR, Gorleku OA, Greaves J, Chamberlain LH (2009) Palmitoylation of the synaptic vesicle fusion machinery. J Neurochem 110:1135–1149

Purkiss J, Welch M, Doward S, Foster K (2000) Capsaicin-stimulated release of substance P from cultured dorsal root ganglion neurons: involvement of two distinct mechanisms. Biochem Pharmacol 59:1403–1406

Rapp DE, Turk KW, Bales GT, Cook SP (2006) Botulinum toxin type A inhibits calcitonin gene-related peptide release from isolated rat bladder. J Urol 175:1138–1142

Reitz A, Stöhrer M, Kramer G, Del Popolo G, Chartier-Kastler E, Pannek J, Burgdörfer H, Göcking K, Madersbacher H, Schumacher S, Richter R, von Tobel J, Schurch B (2004) European experience of 200 cases treated with botulinum-A toxin injections into the detrusor muscle for urinary incontinence due to neurogenic detrusor overactivity. Eur Urol 45:510–515

Rigoni M, Caccin P, Johnson EA, Montecucco C, Rossetto O (2001) Site-directed mutagenesis identifies active-site residues of the light chain of botulinum neurotoxin type A. Biochem Biophys Res Commun 288:1231–1237

Rohacs T, Thyagarajan B, Lukacs V (2008) Phospholipase C mediated modulation of TRPV1 channels. Mol Neurobiol 37:153–163

Rummel A, Mahrhold S, Bigalke H, Binz T (2004) The HCC-domain of botulinum neurotoxins A and B exhibits a singular ganglioside binding site displaying serotype specific carbohydrate interaction. Mol Microbiol 51:631–643

Sahai A, Khan MS, Dasgupta P (2007) Efficacy of botulinum toxin-A for treating idiopathic detrusor overactivity: results from a single center, randomized, double-blind, placebo-controlled trial. J Urol 177:2231–2236

Sakaba T, Stein A, Jahn R, Neher E (2005) Distinct kinetic changes in neurotransmitter release after SNARE protein cleavage. Science 309:491–494

Santos-Buelga JA, Collins MD, East AK (1998) Characterization of the genes encoding the botulinum neurotoxin complex in a strain of *Clostridium botulinum* producing type B and F neurotoxins. Curr Microbiol 37:312–318

Schaible HG, Del Rosso A, Matucci-Cerinic M (2005) Neurogenic aspects of inflammation. Rheum Dis Clin North Am 31:77–101

Schiavo G, Rossetto O, Benfenati F, Poulain B, Montecucco C (1994) Tetanus and botulinum neurotoxins are zinc proteases specific for components of the neuroexocytosis apparatus. Ann NY Acad Sci 710:65–75

Schmid DM, Sauermann P, Werner M, Schuessler B, Blick N, Muentener M, Strebel RT, Perucchini D, Scheiner D, Schaer G, John H, Reitz A, Hauri D, Schurch B (2006) Experience with 100 cases treated with botulinum-A toxin injections in the detrusor muscle for idiopathic overactive bladder syndrome refractory to anticholinergics. J Urol 176:177–185

Schulte-Baukloh H, Weiss C, Stolze T, Herholz J, Stürzebecher B, Miller K, Knispel HH (2005) Botulinum-A toxin detrusor and sphincter injection in treatment of overactive bladder syndrome: objective outcome and patient satisfaction. Eur Urol 48:984–990

Schulte-Baukloh H, Zurawski TH, Knispel HH, Miller K, Haferkamp A, Dolly JO (2007) Persistence of the synaptosomal-associated protein-25 cleavage product after intradetrusor botulinum toxin A injections in patients with myelomeningocele showing an inadequate response to treatment. BJU Int 100:1075–1080

Schurch B, de Sèze M, Denys P, Chartier-Kastler E, Haab F, Everaert K, Plante P, Perrouin-Verbe B, Kumar C, Fraczek S, Brin MF, Botox Detrusor Hyperreflexia Study Team (2005) Botulinum toxin type A is a safe and effective treatment for neurogenic urinary incontinence: results of a single treatment, randomized, placebo controlled 6-month study. J Urol 174(1): 196–200

Shin J, Cho H, Hwang SW, Jung J, Shin CY, Lee SY, Kim SH, Lee MG, Choi YH, Kim J, Haber NA, Reichling DB, Khasar S, Levine JD, Oh U (2002) Bradykinin-12-lipoxygenase-VR1 signaling pathway for inflammatory hyperalgesia. Proc Natl Acad Sci USA 99: 10150–10155

Silva C, Rio ME, Cruz F (2000) Desensitization of bladder sensory fibers by intravesical resiniferatoxin, a capsaicin analog: long-term results for the treatment of detrusor hyperreflexia. Eur Urol 38:444–452

Silva C, Ribeiro MJ, Cruz F (2002) The effect of intravesical resiniferatoxin in patients with idiopathic detrusor instability suggests that involuntary detrusor contractions are triggered by C-fiber input. J Urol 168:575–579

Silva C, Silva J, Ribeiro MJ, Avelino A, Cruz F (2005) Urodynamic effect of intravesical resiniferatoxin in patients with neurogenic detrusor overactivity of spinal origin: results of a double-blind randomized placebo-controlled trial. Eur Urol 48:650–655

Silva C, Silva J, Castro H, Reis F, Dinis P, Avelino A, Cruz F (2007) Bladder sensory desensitization decreases urinary urgency. BMC Urol 11:7–9

Silva A, Cruz CD, Charrua A, Pinto R, Avelino A, Silva C, Dinis P, Cruz F (2008a) GRC 6211, a new oral TRPV1 antagonist, decreases neurogenic detrusor overactivity in a rat model of spinal cord transection. Neurourol Urodyn 27(7):597–598

Silva J, Pinto R, Carvallho T, Coelho A, Avelino A, Dinis P, Cruz F (2008b) Mechanisms of prostate atrophy after glandular botulinum neurotoxin type A injection: an experimental study in the rat. Eur Urol 56:134–141

Silva J, Silva C, Saraiva L, Silva A, Pinto R, Dinis P, Cruz F (2008c) Intraprostatic botulinum toxin type A injection in patients unfit for surgery presenting with refractory urinary retention and benign prostatic enlargement. Effect on prostate volume and micturition resumption. Eur Urol 53:153–159

Simpson LL (1981) The origin, structure, and pharmacological activity of botulinum toxin. Pharmacol Rev 33:155–188

Simpson LL (1984) Botulinum toxin and tetanus toxin recognize similar membrane determinants. Brain Res 305(1):177–180

Simpson LL, Maksymowych AB, Hao S (2001) The role of zinc binding in the biological activity of botulinum toxin. J Biol Chem 276:27034–27041

Smith CP, Radziszewski P, Borkowski A, Somogyi GT, Boone TB, Chancellor MB (2004) Botulinum toxin a has antinociceptive effects in treating interstitial cystitis. Urology 64:871–875

Smith CP, Gangitano DA, Munoz A, Salas NA, Boone TB, Aoki KR, Francis J, Somogyi GT (2008) Botulinum toxin type A normalizes alterations in urothelial ATP and NO release induced by chronic spinal cord injury. Neurochem Int 52:1068–1075

Southall MD, Li T, Gharibova LS, Pei Y, Nicol GD, Travers JB (2003) Activation of epidermal vanilloid receptor-1 induces release of proinflammatory mediators in human keratinocytes. J Pharmacol Exp Ther 304:217–222

Ständer S, Moormann C, Schumacher M, Buddenkotte J, Artuc M, Shpacovitch V, Brzoska T, Lippert U, Henz BM, Luger TA, Metze D, Steinhoff M (2004) Expression of vanilloid receptor subtype 1 in cutaneous sensory nerve fibers, mast cells, and epithelial cells of appendage structures. Exp Dermatol 13:129–139

Stein EA, Fuller SA, Edgemond WS, Campbell WB (1996) Physiological and behavioural effects of the endogenous cannabinoid, arachidonylethanolamide (anandamide), in the rat. Br J Pharmacol 119:107–114

Stein AT, Ufret-Vincenty CA, Hua L, Santana LF, Gordon SE (2006) Phosphoinositide 3-kinase binds to TRPV1 and mediates NGF-stimulated TRPV1 trafficking to the plasma membrane. J Gen Physiol 128:509–522

Steiner AA, Turek VF, Almeida MC, Burmeister JJ, Oliveira DL, Roberts JL, Bannon AW, Norman MH, Louis JC, Treanor JJS, Gavva NR, Romanovsky AA (2007) Nonthermal activation of transient receptor potential vanilloid-1 channels in abdominal viscera tonically inhibits autonomic cold-defense effectors. J Neurosci 27:7459–7468

Strauss HM, Keller S (2008) Pharmacological interference with protein-protein interactions mediated by coiled-coil motifs. Handb Exp Pharmacol 186:461–482

Südhof TC, Rothman JE (2009) Membrane fusion: grappling with SNARE and SM proteins. Science 323:474–477

Sugiura T, Tominaga M, Katsuya H, Mizumura K (2002) Bradykinin lowers the threshold temperature for heat activation of vanilloid receptor 1. J Neurophysiol 88:544–548

Swanson DM, Dubin AE, Shah C, Nasser N, Chang L, Dax SL, Jetter M, Breitenbucher JG, Liu C, Mazur C, Lord B, Gonzales L, Hoey K, Rizzolio M, Bogenstaetter M, Codd EE, Lee DH, Zhang SP, Chaplan SR, Carruthers NI (2005) Identification and biological evaluation of

4-(3-trifluoromethylpyridin-2-yl)piperazine-1-carboxylic acid (5-trifluoromethylpyridin-2-yl) amide, a high affinity TRPV1 (VR1) vanilloid receptor antagonist. J Med Chem 48:1857–1872

Szallasi A, Goso C, Blumberg PM, Manzini S (1993) Competitive inhibition by capsazepine of [^{3}H]Resiniferatoxin binding to central (spinal cord and dorsal root ganglia) and peripheral (urinary bladder and airways) vanilloid (capsaicin) receptors in the rat. J Pharmcol Exp Ther 267:728–733

Szallasi A, Cruz F, Geppetti P (2006) TRPV1: a therapeutic target for novel analgesic drugs? Trends Mol Med 12(11):545–554

Szallasi A, Cortright DN, Blum CA, Eid SR (2007) The vanilloid receptor TRPV1: 10 years from channel cloning to antagonist proof-of concept. Nat Rev Drug Discov 6:357–372

Tang HB, Inoue A, Oshita K, Nakata Y (2004) Sensitization of vanilloid receptor 1 induced by bradykinin via the activation of second messenger signaling cascades in rat primary afferent neurons. Eur J Pharmacol 498:37–43

Thesleff S, Molgó J, Tågerud S (1990) Trophic interrelations at the neuromuscular junction as revealed by the use of botulinal neurotoxins. J Physiol Paris 84:167–173

Tohda C, Sasaki M, Konemura T, Sasamura T, Itoh M, Kuraishi Y (2001) Axonal transport of VR1 capsaicin receptor mRNA in primary afferents and its participation in inflammation-induced increase in capsaicin sensitivity. J Neurochem 76:1628–1635

Tominaga M, Caterina MJ, Malmberg AB, Rosen TA, Gilbert H, Skinner K, Raumann BE, Basbaum AI, Julius D (1998) The cloned capsaicin receptor integrates multiple pain-producing stimuli. Neuron 21:531–543

Tominaga M, Wada M, Masu M (2001) Potentiation of capsaicin receptor activity by metabotropic ATP receptors as a possible mechanism for ATP-evoked pain and hyperalgesia. Proc Natl Acad Sci USA 98:6951–6956

Tominaga M, Numazaki M, Iida T, Tominaga T (2003) Molecular mechanisms of nociception. Nihon Shinkei Seishin Yakurigaku Zasshi 23:139–147

Tóth A, Boczán J, Kedei N, Lizanecz E, Bagi Z, Papp Z, Edes I, Csiba L, Blumberg PM (2005) Expression and distribution of vanilloid receptor 1 (TRPV1) in the adult rat brain. Brain Res Mol Brain Res 135:162–168

Turner H, Fleig A, Stokes A, Kinet JP, Penner R (2003) Discrimination of intracellular calcium store subcompartments using TRPV1 (transient receptor potential channel, vanilloid subfamily member 1) release channel activity. Biochem J 371:341–350

Tyagi V, Philips BJ, Su R, Smaldone MC, Erickson VL, Chancellor MB, Yoshimura N, Tyagi P (2009) Differential expression of functional cannabinoid receptors in human bladder detrusor and urothelium. J Urol 181(4):1932–1938

Urban L, Dray A (1991) Capsazepine, a novel capsaicin antagonist, selectively antagonises the effects of capsaicin in the mouse spinal cord in vitro. Neurosci Lett 134:9–11

Van Buren JJ, Bhat S, Rotello R, Pauza ME, Premkumar LS (2005) Sensitization and translocation of TRPV1 by insulin and IGF-I. Mol Pain 1:17–28

Vassilieva EV, Nusrat A (2008) Vesicular trafficking: molecular tools and targets. Methods Mol Biol 440:3–14

Vellani V, Mapplebeck S, Moriondo A, Davis JB, McNaughton PA (2001) Protein kinase C activation potentiates gating of the vanilloid receptor VR1 by capsaicin, protons, heat and anandamide. J Physiol 534:813–825

Veronesi B, Oortgiesen M, Roy J, Carter JD, Simon SA, Gavett SH (2000) Vanilloid (capsaicin) receptors influence inflammatory sensitivity in response to particulate matter. Toxicol Appl Pharmacol 169:66–76

Vetter I, Wyse BD, Monteith GR, Roberts-Thomson SJ, Cabot PJ (2006) The mu opioid agonist morphine modulates potentiation of capsaicin-evoked TRPV1 responses through a cyclic AMP-dependent protein kinase A pathway. Mol Pain 2:22–37

Vetter I, Cheng W, Peiris M, Wyse BD, Roberts-Thomson SJ, Zheng J, Monteith GR, Cabot PJ (2008) Rapid, opioid-sensitive mechanisms involved in transient receptor potential vanilloid 1 sensitization. J Biol Chem 283:19540–19550

Vlaskovska M, Kasakov L, Rong W, Bodin P, Bardini M, Cockayne DA, Ford AP, Burnstock G (2001) P2X3 knock-out mice reveal a major sensory role for urothelially released ATP. J Neurosci 21:5670–5677

Voets T, Nilius B (2007) Modulation of TRPs by PIPs. J Physiol 582:939–944

Voilley N, de Weille J, Mamet J, Lazdunski M (2001) Nonsteroid anti-inflammatory drugs inhibit both the activity and the inflammation-induced expression of acid-sensing ion channels in nociceptors. J Neurosci 21:8026–8033

Vos MH, Neelands TR, McDonald HA, Choi W, Kroeger PE, Puttfarcken PS, Faltynek CR, Moreland RB, Han P (2006) TRPV1b overexpression negatively regulates TRPV1 responsiveness to capsaicin, heat and low pH in HEK293 cells. J Neurochem 99:1088–1102

Vyklický L, Nováková-Tousová K, Benedikt J, Samad A, Touska F, Vlachová V (2008) Calcium-dependent desensitization of vanilloid receptor TRPV1: a mechanism possibly involved in analgesia induced by topical application of capsaicin. Physiol Res 57:S59–S68

Wagman J (1954) Isolation and sedimentation study of low molecular weight forms of type A botulinus toxin. Arch Biochem Biophys 50:104–112

Walker KM, Urban L, Medhurst SJ, Patel S, Panesar M, Fox AJ, McIntyre P (2003) The VR1 antagonist capsazepine reverses mechanical hyperalgesia in models of inflammatory and neuropathic pain. J Pharmacol Exp Ther 304:56–62

Walpole CS, Bevan S, Bovermann G, Boelsterli JJ, Breckenridge R, Davies JW, Hughes GA, James I, Oberer L, Winter J, Wrigglesworth R (1994) The discovery of capsazepine, the first competitive antagonist of the sensory neuron excitants capsaicin and resiniferatoxin. J Med Chem 37:1942–1954

Wang C, Hu HZ, Colton CK, Wood JD, Zhu MX (2004) An alternative splicing product of the murine trpv1 gene dominant negatively modulates the activity of TRPV1 channels. J Biol Chem 279:37423–37430

Wang EC, Lee JM, Ruiz WG, Balestreire EM, von Bodungen M, Barrick S, Cockayne DA, Birder LA, Apodaca G (2005) ATP and purinergic receptor-dependent membrane traffic in bladder umbrella cells. J Clin Invest 115:2412–2422

Wang YX, Wang J, Wang C, Liu J, Shi LP, Xu M, Wang C (2008a) Functional expression of transient receptor potential vanilloid-related channels in chronically hypoxic human pulmonary arterial smooth muscle cells. J Membr Biol 223:151–159

Wang ZY, Wang P, Merriam FV, Bjorling DE (2008b) Lack of TRPV1 inhibits cystitis-induced increased mechanical sensitivity in mice. Pain 139:158–167

Welch JM, Simon SA, Reinhart PH (2000a) The activation mechanism of rat vanilloid receptor 1 by capsaicin involves the pore domain and differs from the activation by either acid or heat. Proc Natl Acad Sci USA 97:13889–13894

Welch MJ, Purkiss JR, Foster KA (2000b) Sensitivity of embryonic rat dorsal root ganglia neurons to *Clostridium botulinum* neurotoxins. Toxicon 38:245–258

Wiart L, Joseph PA, Petit H, Dosque JP, de Sèze M, Brochet B, Deminière C, Ferrière JM, Mazaux JM, N'Guyen P, Barat M (1998) The effects of capsaicin on the neurogenic hyperreflexic detrusor. A double blind placebo controlled study in patients with spinal cord disease. Preliminary results. Spinal Cord 36:95–99

Wickner W, Schekman R (2008) Membrane fusion. Nat Struct Mol Biol 15:658–664

Wong GY, Gavva NR (2008) Therapeutic potential of vanilloid receptor TRPV1 agonists and antagonists as analgesics: recent advances and setbacks. Brain Res Rev 60:267–277

Wonnacots S, Marchbank M, Fiol C (1978) Ca^{2+} uptake by synaptosomes and its effect on the inhibition of acetylcholine release by botulinum toxin. J Neurochem 30:1127–1134

Yang X, Han JQ, Liu R (2008) Effects of experimental colitis on the expressions of calcitonin gene-related peptide and vanilloid receptor 1 in rat spinal cord sensory neurons. Acta Physiol Sinica 60:143–148

Yokoyama T, Kumon H, Nagai A (2008) Correlation of urinary nerve growth factor level with pathogenesis of overactive bladder. Neurourol Urodyn 27:417–420

Yowler BC, Schengrund CL (2004) Glycosphingolipids: sweets for botulinum neurotoxin. Glycoconj J 21:287–293

Zhu W, Oxford GS (2007) Phosphoinositide-3-kinase and mitogen activated protein kinase signaling pathways mediate acute NGF sensitization of TRPV1. Mol Cell Neurosci 34:689–700

Zhuang ZY, Xu H, Clapham DE, Ji RR (2004) Phosphatidylinositol 3-kinase activates ERK in primary sensory neurons and mediates inflammatory heat hyperalgesia through TRPV1 sensitization. J Neurosci 24:8300–8309

Zygmunt PM, Petersson J, Andersson DA, Chuang H, Sorgard M, Di Marzo V, Julius D, Högestatt ED (1999) Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. Nature 400:452–457

Ion Channel Modulators and Urinary Tract Function

A.F. Brading and K.L. Brain

Contents

Abstract The membrane potential fulfils an important role in initiating smooth muscle contraction, through its depolarization and the subsequent influx of Ca^{2+} through voltage-gated Ca^{2+} channels. Changes in membrane potential can also co-ordinate contraction across great distances, utilizing the speed of electrical current flow through gap junctions. Hence, regulating membrane potential can greatly influence smooth muscle function. In this chapter, we will consider the influence of ion channels, as dynamic gatekeepers of membrane permeability, on urogenital

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function. Through their ability to act as key regulators of both the resting membrane potential and its dynamic changes, they provide important pharmacological targets for influencing urogenital function.

Urogenital smooth muscle and urothelia contain a diverse range of molecularly and functionally distinct K^+ channels, which are key to regulating the resting membrane and for re-establishing the normal membrane potential following both active and passive changes. The voltage-gated Ca^{2+} channels are key to initiating contraction and causing rapid depolarization, supplemented in some smooth muscles by rapid Na^+ conductances. The Cl^- channels, often assumed to be passive, can actively change the membrane potential, and hence, cellular function, because Cl^- is not usually at its equilibrium potential. The useful ways in which these ion channels can be targeted therapeutically in the ureter, bladder and urethra are discussed, focussing particularly on treatments for ureteric obstruction and detrusor overactivity. Current treatments for many urinary tract disorders, particularly the overactive bladder, are complicated by side effects. While ion channels have traditionally been considered as poor therapeutic targets by the pharmaceutical industry, our increasing knowledge of the molecular diversity of K^+ and Cl^- channels gives new hope for more narrowly focused drug targeting, while the exciting discoveries of active currents in interstitial cells give us a new set of cellular targets for drugs.

Keywords Calcium channels · Chloride channels · Detrusor overactivity · Interstitial cells of cajal · Potassium channels · TRP channels · Ureter · Urethra · Urinary bladder

1 Introduction

Animals adapt to changes in their environment, and this has consequences when one is considering drug treatment, since continuous use of a drug can trigger changes. This occurs mainly at the level of cell membranes that contain a variety of chemically receptive sites, including ion channels.

G-protein-coupled receptors are designed specifically to respond to signalling molecules and to change their sensitivity to ensure that the functional response is maintained even if the strength of the signal varies. Thus, a drug designed to alter the function of a receptor may automatically trigger adaptive changes that may reduce the therapeutic benefits. A good example here is the use of antimuscarinic drugs in the treatment of bladder overactivity. In this case, in normal use, 80% of patients have stopped taking them within 12 months, because they no longer provide effective relief (Hegde 2006). The bladder has adapted to their presence.

Other receptors are themselves ion channels, such as the P2X receptor family, which respond to ATP and are important in some parts of the urogenital system. For example, P2X1 receptors are present on smooth muscle cells of the urinary bladder, where they are activated by ATP co-released from parasympathetic nerves to initiate action potentials and hence contraction (Hashitani et al. 2000b; Young

et al. 2008). In humans, there is particular interest in this ATP-mediated or purinergic transmission, as its importance increases with age (Yoshida et al. 2004). The pharmacology of this family of receptors has been reviewed by Jarvis and Khakh (2009).

Simpler ion channels, however, may also be therapeutic targets. Possibly their main advantage is that they have not evolved with the specific ability to alter their properties in the complex ways that the G-protein-coupled receptors have, and thus if a drug can be found that modulates their activity, they are less likely to adapt to its presence. Drugs interacting with ion channels may do so in quite subtle ways, not just activating or blocking, but also altering their properties to decrease or increase the likelihood of channel opening. Thus, changing the numbers or relative proportions of the various open channels gives considerable potential for manipulating tissue behaviour – it should for instance be possible to increase or decrease spontaneous activity and correct many disorders. The main disadvantage is that the effects of modulating channel activity are likely to be very widespread, and tissue selectivity is thus of overriding importance.

The most important ion channel targets relevant for controlling the behaviour of the urinary tract will be K^+ and Ca^{2+} channels in the autonomic nervous system and its effectors, but there is also scope for drugs acting on other channels and in regions of the central nervous system (CNS) that control autonomic outflow.

In this chapter, we first consider in more detail the properties of the various channels, and the drugs known to interact with them, before giving some brief examples of the responses of specific urinary tract smooth muscles. It should, however, be emphasized that the outcome of such manipulations is not always straightforward to predict. For instance, one might expect that opening K^+ channels would hyperpolarize membranes, and opening Ca^{2+} channels would depolarize, since the reversal potential for K^+ is more negative than the resting potential, and for Ca^{2+} far more positive. However, this is far from true. For example, inwardly rectifying K^+ channels exist, which over a certain range of $[K^+]_o$ will depolarize the membrane when opened, and Ca^{2+} entry through Ca^{2+} channels will hyperpolarize if it reaches Ca^{2+}-activated K^+ channels. Ca^{2+}-activated Cl^- channels also exist, but the reversal potential for Cl^- channels in smooth muscles is negative to the resting potential and thus their activation will depolarize the membrane. Table 1 shows typical values estimated for the various ionic concentrations and their reversal potentials.

Table 1 Typical ion concentrations and Nernst potentials in mammalian cells

Ion	Intracellular conc. (mM)	Extracellular conc. (mM)	Reversal potential at 38°C
K^+	150	4.5	−92.9
Na^+	9	140	+72.7
Cl^-	(3–50) 30	100	−31.9
HCO_3^-	12	24	−18.4
Ca^{2+}	0.0003	2.4	+119
Mg^{2+}	0.4	1.5	+17.3
X^- net charge on macromolecules	118	28	

2 Potassium Channels

K^+ channels are normally composed of four subunits: complex, membrane-spanning proteins that share a common amino acid sequence on one of the pore-forming loops, which gives the channel its selectivity. It now appears that there are three main classes of K^+ channel, based on the number of transmembrane segments of each receptor subunit (see Fig. 1). They are the two, four, and six transmembrane groups.

The 4TM proteins may be the ones that confer the basic K^+ leak conductance. The 2TM group includes the inward rectifier channels, the ATP-selective and the G-protein-coupled channels, whereas the 6TM group includes the Ca^{2+}-activated and delayed rectifier channels. The even number of membrane-spanning loops and their arrangement means that the two terminals are intracellular. Frequently there are regulatory subunits, additional proteins that can interact with the channel to modify channel behaviour.

2.1 4TM Channels

These probably are responsible for the inherent potassium selectivity of cell membranes and the net negative membrane potential. Each subunit has two pore-forming

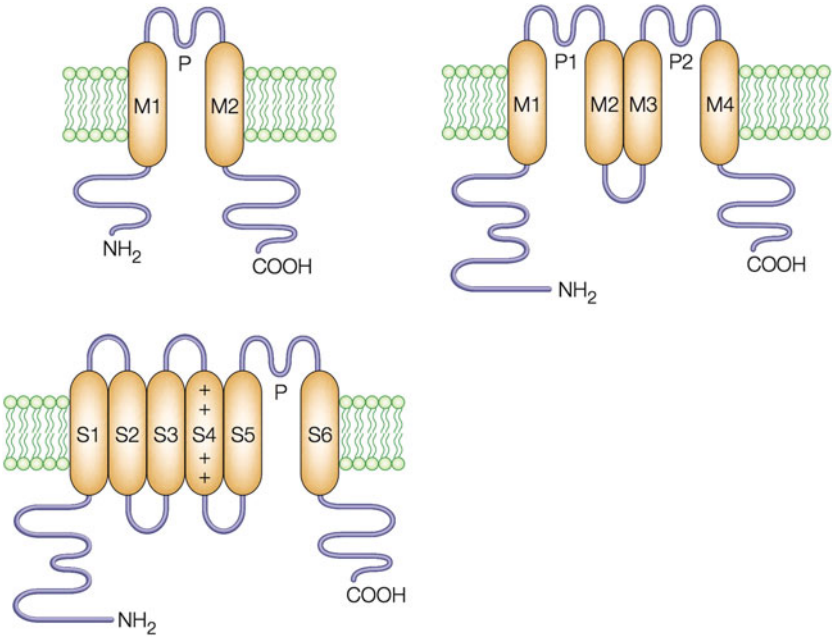

Fig. 1 Proposed structures of potassium channel subunits. Four subunits combine and may also interact with additional regulatory subunits to form the functional potassium channel. Reprinted by permission from Macmillan Publishers Ltd: Nature Neuroscience (Choe 2002), copyright 2002

loops (P domains). The channel homologue in the human genome is called TWIK-1. When these channels are expressed in *Xenopus* oocytes, the current through them is time-independent and has a nearly linear I–V relationship, which, however, saturates at large depolarizations (positive to 0 mV) as long as internal Mg^{2+} ions are present. TWIK-1 channel activity is blocked by Ba^{2+}, quinine and quinidine. This channel is of particular interest because its mRNA is widely distributed in human tissues and is particularly abundant in brain and heart. The channel activity is indirectly upregulated by activation of protein kinase C and downregulated by internal acidification (Lesage et al. 1996).

2.2 2TM Channels

The 2TM channels include the inward rectifier channels, i.e. the ones that allow current to pass into the cell more easily than out of it. They also include the ATP-sensitive and G-protein-coupled channels. Such channels can have very specific functions in the body, and drugs that can activate or inhibit them will have rather precise effects.

2.2.1 Inward Rectifiers

These are found in many types of excitable cells, and are so called because ions flow through the open channels more easily into the cell than out. This rectification does not seem to be due to any intrinsic voltage sensitivity of the channel proteins, but is the result of large intracellular ions that cannot pass through the channel; so when the cell membrane is positive to the potassium equilibrium potential, thus providing a driving force for current flow out of the cell, these ions will block the channel. Examples of blocking molecules are divalent cations such as Mg^{2+}, or organic molecules such as spermine, spermidine, putrescine and cadaverine (Lopatin et al. 1994). These channels have been studied in arterial smooth muscles (for Nelson and Quayle (1995)), and unlike other K^+ channels, have activity that is sensitive to extracellular $[K^+]$. Changing the potassium equilibrium potential changes the driving force on the ions, and in these channels outward current remains small but current flows inward when $[K^+]_o$ and the driving force are changed appropriately. The channels in arterial smooth muscle are not blocked by charybdotoxin and iberiotoxin, or 10 μM glibenclamide, or 1 mM tetraethylammonium (TEA), but are sensitive to Ba^{2+} (Nelson and Quayle 1995).

2.2.2 ATP-Sensitive $K_{IR}6$ Channels

These control insulin release from pancreatic β cells (for references, see Ashcroft and Gribble (1998)). The regulatory subunits of these channels bind to, and are

inhibited by, an increasing intracellular ATP, so that when glucose levels are high, the cell takes up glucose and metabolizes it to increase intracellular ATP. This increase in ATP switches off the channels and causes depolarization, Ca^{2+} entry and insulin secretion. If this system does not work, it causes a neonatal form of type I diabetes mellitus (Gloyn et al. 2004). Sulfonylureas, including oral hypoglycaemic drugs such as tolbutamide, can be useful: they act by binding to the regulatory subunit of the K_{ATP} channel and blocking its activation. Thus, they can depolarize and cause the secretion of insulin from any surviving pancreatic β cells. Channels in arterial smooth muscle can be blocked by Ba^{2+} (half block at around 100 μM), 4-aminopyridine (4AP; half block at around 0.2 mM) and TEA (half block at 7 mM), but are not sensitive to charybdotoxin or iberiotoxin.

2.2.3 G-Protein-Coupled Channels

It has been known for some time that in the heart, the βγ subunits of GTP-binding proteins can directly activate muscarinic K^+ channels (Logothetis et al. 1987). More recently, Lopes et al. (2007) have provided evidence that G-protein-coupled 2TM channels exist in myometrial smooth muscle. However, they suggest that this form of interaction may occur in all K^+ channel classes (Fig. 2).

For instance, Toro et al. (1990) examined a channel in the myometrium that possesses β-adrenergic receptors coupled to a GTP-dependent protein that could directly gate calcium-activated K^+ channels of a conductance of about 260 pS, which would now be classified as $K_{Ca}1.1$ channel (see below). Furthermore, the K^+ channels, β-adrenergic receptors and G-proteins could be reconstituted in lipid bilayers as a stable, functionally coupled, molecular complex. More recently, Bhattacharyya et al. (2002) have looked at a similar channel in the ciliary epithelium that was blocked by iberiotoxin from the outside and barium from the inside and is a $K_{Ca}1.1$ (maxi K) channel in which β-receptor stimulation increased the open probability of the channel through a direct G-protein-gated pathway.

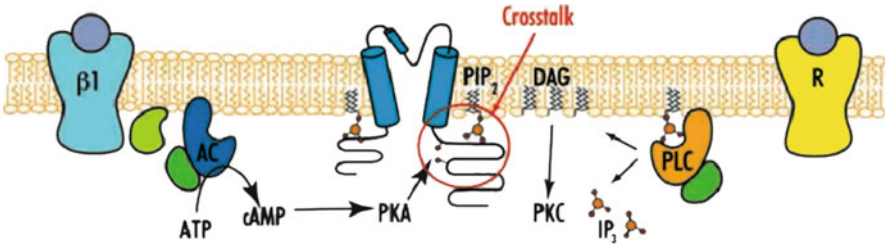

Fig. 2 A scheme showing a model where PKA phosphorylation sites in the channel directly regulate channel interactions with PIP_2, thus regulating the G_q-coupled modulation of the channel; from Lopes et al. (2007)

2.3 6TM Channels

2.3.1 Delayed Rectifier Channels

These are those thought classically to be responsible for repolarization of action potentials. It is interesting that these channels were first described in cold-blooded animals, such as in the squid giant axon. In these animals, the rate of spontaneous repolarization after inactivation of the voltage-sensitive Na^+ channels would be sufficiently slow to restrict the frequency of firing action potentials, and activating the K^+ channel allows more rapid repolarization. In warm-blooded animals everything is much faster, and it is not always necessary to involve K^+ channels to allow repetitive firing of action potentials, although they do have a role in ventricular cardiac muscle. Thus, in mammalian myelinated nerves, voltage-sensitive K^+ channels are not present in the nodes, although some are still present in the internodal membranes and play a role in the pathology of demyelinating diseases.

2.3.2 Ca^{2+}-Activated Channels

These are particularly important in those tissues such as cardiac and smooth muscles where Ca^{2+} ions carry a portion of the inward current in the action potential. Here, when active, these channels will generate a hyperpolarizing after potential and play an important role in determining the frequency of action potential firing. There are basically three types: small, medium and large conductance channels.

Large conductance ($K_{Ca}1.1$; previously or otherwise known as maxi K or BK) channels are voltage- and calcium-sensitive potassium channels that are fundamental to the control of smooth muscle tone and neuronal excitability. They are formed by two subunits: the pore-forming α subunit and the modulatory β subunit, which may partially inactivate or slightly decrease the activation time of the channels. Charybdotoxin and apamine are often cited as selective blockers of these channels, of which the first selective inhibitor reported was charybdotoxin, found by Miller et al. (1985) to be a high-affinity inhibitor of the channels from mammalian skeletal muscle that they studied. Externally applied TEA, which is known to block many different K^+ channels, could be displaced by charybdotoxin, and the results were interpreted to suggest that charybdotoxin may be binding to a site near to the TEA-binding site in the external mouth of the channel.

The $K_{Ca}1.1$ channels are of particular importance in regulating the effects of β-adrenoceptor agonists, such as circulating adrenaline. Activation of β-adrenoceptors in the bladder is known to increase cAMP, and hence, protein kinase A activity, but it also activates $K_{Ca}1.1$ channels by a direct action of the G-proteins (Uchida et al. 2005). However, the relative importance of this pathway has been disputed (Frazier et al. 2005; Yamaguchi and Chapple 2007).

Small and intermediate conductance channels are less well understood than the large conductance channels. Their unit conductances are 2–20 and 20–85 pS, respectively. They are more sensitive to $[Ca^{2+}]_i$ than the large conductance channels and are gated solely by internal Ca^{2+} ions. The intermediate channels are more common in smooth muscles than the small channels, the latter being more highly expressed in the CNS. However, the protein-coding regions of these two channels do not contain consensus Ca^{2+}-binding motifs. Fanger et al. (1999) studying in particular the intermediate conductance channel have shown that this Ca^{2+} sensitivity is mediated by calmodulin, which is prebound to the cytoplasmic C-tail.

3 TRP Channels

These are non-selective cation channels, which although having a similar topography to the 6TM K^+ channels, are only distantly related to them. TRP channels are found in all eukaryotic cells and are probably involved in several different functions such as mediation of the responses to painful stimuli, receptor-mediated excitation and modulation of the cell cycle. A common theme that has been proposed to link the TRP channels is their activation or modulation by phosphatidylinositol signal transduction pathways (Clapham 2004).

There are three main subfamilies: the TRPC (canonical), TRPV (vanilloid receptor, osm9-like) and TRPM (melastatin) subfamilies. In the TRPC family, TPRC3, TRPC6 and TRPC7 are highly expressed in smooth and cardiac muscle cells and are candidates for the receptor-activated non-selective cation channels known to exist in these sites. In the TRPV family, TRPV1 is a Ca^{2+}-permeant channel that is potentiated by heat and decreased pH and inhibited by cellular phosphatidylinositol-4,5-bisphosphate (PIP_2). Stretch reportedly increases TRPV2 translocation; increased temperature also activates TRPV3 and TRPV4. TRPV4 current is potentiated by hypotonicity (cell swelling). TRPV5 and TRPV6 comprise a distinct subfamily of homomeric and heteromeric channels found in transporting epithelia of the kidney and intestine.

In the TRPM family, TRPM1 is widely expressed in normal tissues, but its function and electrophysiological properties have not been described. TRPM2 forms a Ca^{2+}-permeant channel that is gated by binding of ADP ribose and nicotinamide adenine dinucleotide. TRPM3 forms a Ca^{2+}-permeant non-selective channel that is constitutively active when heterologously expressed. Its activity is increased by hypotonicity, but there is little homology to TRPV4 that might suggest a common mechanism of activation. TRPM4 is expressed primarily in kidney distal-collecting-duct epithelium and in the CNS. TRPM4 and TRPM5 are widely distributed and are the only monovalent-selective ion channels of the TRP family. They are activated through GPCRs coupled to PLC-dependent endoplasmic reticulum Ca^{2+} release, perhaps by direct Ca^{2+} binding to the channel (Clapham 2003).

The molecular diversity of this channel family makes it an excellent target for specific pharmacological intervention, but the pharmacology of TRP receptors is

currently so poor (except for TRPV1) that therapies await the development of subtype-specific drugs.

4 Ca^{2+} Channels

Since Ca^{2+} is the main trigger for activating numerous pathways, channels allowing Ca^{2+} entry are clearly important targets for drugs. This chapter considers some pathways allowing entry from the extracellular space, but will not cover the channels that mediate release from the endoplasmic reticulum.

Two important channels allowing Ca^{2+} entry are the L-type and T-type Ca^{2+} channels. L-type channels have long single channel open times and are large conductance channels that are activated by relatively large depolarizations. T-type channels in contrast are transient, have a tiny single channel conductance and are activated by small depolarizations. Classical L-type channel blockers are the dihydropyridines, such as nifedipine and nicardipine, and the non-dihydropyridines, such as verapamil (a phenylalkylamine) and diltiazem (a benzothiazepine). It is also interesting to note that the 'antimuscarinic' drug propiverine has effects in vivo mediated by its metabolites, one of which directly inhibits L-type Ca^{2+} channels (Zhu et al. 2008).

In contrast, development of selective T-type blockers has not been so successful. Mibefradil was the first to be used, but was shown to have deleterious side effects and was withdrawn from use in 1998.

Later discovery of other Ca^{2+} channels activated by large depolarizations led to the proposal of N-type channels (neural, neither L nor T), and from there came an array of further channels, such as P/Q and R channels. These have been identified by their ability to be blocked by specific toxins. N-type channels are blocked by ω-conotoxin GVIA; P/Q-type channels are blocked by ω-agatoxins; and R-type channels are resistant to the toxins.

The L-type Ca^{2+} channel antagonists, such as nifedipine, are very effective at inhibiting the contraction of urogenital smooth muscle in response to the stimulation of the cholinergic nerves and the action of acetylcholine at muscarinic receptors. At first glance this is surprising, because the activation of the G-protein-coupled muscarinic receptors rarely cause a significant junction potential (a change in the membrane potential) in urogenital smooth muscle, and so why a voltage-sensitive Ca^{2+} channel might be so important is not at all clear. For example, in the rabbit or rodent urinary bladder, muscarinic stimulation causes either a slow, or no, membrane potential change (Creed et al. 1983; Fujii 1988; Hashitani and Suzuki 1995). It does, however, increase the frequency of 'spontaneous' smooth muscle action potentials for a few seconds following the end of the field stimulus train (Hashitani et al. 2000, 2004a); it is this increased firing rate of smooth muscle action potentials, in the absence of an obvious junction potential, that causes the nifedipine-sensitive contraction; the mechanism by which these action potentials are initiated is far from clear. It is also the case that muscarinic activation causes an

IP_3-mediated release of Ca^{2+} from intracellular stores (Andersson et al. 1991), so it is possible that the store-filling state chronically depends on the influx of Ca^{2+} through L-type Ca^{2+} channels, so indirectly affecting the response to cholinergic transmission.

4.1 Store-Operated Ca^{2+} Channels

Another interesting mechanism that occurs in cells is the store-operated system. When release of intracellular Ca^{2+} stores is activated, much of the Ca^{2+} will escape from the cell, and in order to maintain homeostasis, the stores need to be refilled from the extracellular space (for references, see Parekh (2006)). It has been found that anything that empties the stores does indeed trigger Ca^{2+} entry. The channels involved are often called CRAC (calcium release-activated channels). I_{CRAC} is non-voltage sensitive, inwardly rectifying and remarkably selective for Ca^{2+}. The conductance of the individual channels is tiny – probably less than 1 pS. Unlike several other divalent ion-permeant channels, the selectivity does not depend on the presence of divalent cations (Ca^{2+} and Mg^{2+}) in the channel, and in their absence, single channel conductance is still very low. The channels are selectively blocked by the trivalent ion Gd^{3+}, and also by the Synta compound BTP2 manufactured by GlaxoSmithKline, UK (Ng et al. 2008).

5 Cl^- Channels

Early studies on striated muscle suggested that chloride might be passively distributed in this tissue, since E_{Cl} was close to the membrane potential, and evidence suggested that there was a high permeability to Cl^- (see e.g., Adrian (1961)). High Cl^- permeability would tend to stabilize the resting potential and damp down the responses to activation or inhibition of other channels. However, after the introduction of ion-sensitive microelectrodes (Neild and Thomas 1973), measurements of $[Cl^-_i]$ in heart (Vaughan-Jones 1982) and smooth muscle (Aickin and Brading 1983) showed that E_{Cl} is considerably positive to the membrane potential, that the passive Cl^- permeability is quite low in these tissues (Vaughan-Jones 1982; Aickin and Brading 1983), and that active transport is required to maintain the equilibrium. Anything that increases Cl^- permeability will now depolarize the tissue, and this provides a useful strategy for cells to respond to external stimuli. Chloride movement across the plasma membrane is involved in key cellular events including cell volume regulation, transepithelial fluid transport, muscle contraction and neuro-excitation (Verkman and Galietta 2009). Genetic damage to these channels can result in serious disease.

5.1 Types of Cl^- Channels and Their Functions

Mammalian chloride channels broadly fall into five classes based on their regulation: cystic fibrosis transmembrane conductance regulator (CFTR), which is activated by cyclic AMP-dependent phosphorylation; calcium-activated chloride channels (CaCCs); voltage-gated chloride channels (ClCs); ligand-gated chloride channels (GABA and lysine-activated); and volume-regulated chloride channels (see Nilius and Droogmans (2003), Verkman and Galietta (2009)).

Classical Cl^- channel blockers include the disulphonic stilbenes (DIDS and SITS) that block most Cl^- channels. Other interesting blockers are the sulfonylureas (e.g. glibenclamide and tolbutamide) that block the CFTR channels, tamoxifen that blocks ClCs and niflumic acid that blocks CaCCs. A useful list of blockers can be found in Suzuki et al. (2006).

6 Na^+ Channels

Voltage-dependent Na^+ channels have probably been studied more than any other type of channel. Early on, the squid giant axon provided a wonderful model, because simple wire electrodes could be inserted and the membrane could be current or voltage clamped sufficiently rapidly to allowing detailed studies of rapidly activating and inactivating channels (Curtis and Cole 1940; Hodgkin and Huxley 1939); for review see Hodgkin and Huxley (1990). Tetrodotoxin (TTX) is one of the most specific channel blockers known, and TTX sensitivity is used to classify these channels. In tissues where rapid activation is less important, Ca^{2+} ions are frequently the main carriers of the inward current, and this appears to be the case in most smooth muscles (see for example Brading et al. (1969)). Nevertheless, more recent evidence suggests that genuine Na^+ channels may be present in some smooth muscles, and there may be both TTX-sensitive and TTX-insensitive Na^+ channels, although there remain questions about whether or not they function in normal tissues, or are only induced under pathological conditions; for example, see Platoshyn et al. (2005), Berra-Romani et al. (2005), Nakajima et al. (2008). Most of the urogenital effects of TTX, however, are attributed to the nerves.

7 Ion Channels in Urinary Tract Smooth Muscles

7.1 Distribution of Ion Channels

The important ion channels available for modulation are not just on the smooth muscle myocytes, but also on the interstitial cells that occur within the walls of the organs. The importance and roles of interstitial cells are reviewed in Brading and

McCloskey (2005). These cells appear to be involved in the initiation of spontaneous activity and characteristically express kit-like immunoreactivity. They have also been called myofibroblasts, for example by Drake et al. (2003), and there is now some consensus for using the term ICC (McCloskey 2010), originating from 'Interstitial Cell of Cajal'.

7.2 Potential Benefit of Modulating Ion Channels

The pathophysiological changes that need treatment include renal colic due to ureteric obstruction (usually caused by ureteric spasm induced by passage of kidney stones), overactive bladders, stress and urge incontinence due to urethral damage. Drugs acting on ion channels provide therapeutic benefit, and although there is relatively little specific detail of the molecular composition of urinary tract ion channels, it is worth examining some of the experimental studies on the effects of various channel modulators in the ureter, bladder and urethra. Useful techniques include simple studies of the effects of drugs on the contractile activity of smooth muscle strips, or more sophisticated studies of the responses of the membrane potentials of myocytes and interstitial cells to drugs using surface recordings (e.g. sucrose gap methods) or microelectrodes.

7.2.1 Ureter

The ureter is a particularly interesting organ. It is periodically activated by spontaneous depolarizations that arise in the renal pelvis and sweep the length of the ureter triggering contraction that pushes urine down into the bladder. Detailed examination of the cell structure in the renal pelvis and upper ureter reveals two cell types other than myocytes: 'atypical' smooth muscle cells that are the pacemakers and 'ICC-like cells' [also called intermediate cells; (Lang 1989)].

In human upper tracts, 'atypical' pacemaker cells form the muscle coat of the minor calyces in the kidney pelvis and extend distally to create an inner layer within the wall of the major calyces and renal pelvis (Dixon and Gosling 1973, 1982). The number of myocytes increases with distance from the papilla base and they become tightly packed into bundles as they progress down the tracts (Dixon and Gosling 1982; Klemm et al. 1999). The stellate 'ICC-like' cells are the targets of the autonomic nerves (Lang et al. 1995), and are involved both in linking the myocytes to the pacemaker cells and in determining the extent of coupling between them, and thus how often a pacemaker potential will generate a full-blown ureteric peristaltic contraction.

The depolarizations of the myocytes are often called action potentials, but consist of a fast initial transient depolarization followed by a prolonged plateau phase that lasts the length of each contraction (Lang 1989). The ion channels

underlying this activity and their modulation by various drugs and ions have been the subjects of considerable study.

The effects on the action potential and the transmembrane ionic currents in the myocytes produced by changing external [Na^+] and [Ca^{2+}] suggest that the initial fast component of the action potential is due to Ca^{2+} influx, whereas the plateau is due to Na^+ influx (Kuriyama and Tomita 1970; Shuba 1977). Seki and Suzuki (1990) also found that in the rabbit renal pelvis increasing extracellular K^+, decreasing Na^+ or inhibition of the Na^+/K^+ pump with ouabain induced a depolarization and an increase in the frequency of the slow waves. Hyperpolarization with applied current decreased their frequency. On the other hand, decreasing extracellular Ca^{2+} decreased the amplitude of slow waves with little change in their frequency. References to more recent work can be found in Burdyga and Wray (2005); see also Maggi and Meli (1984) and Maggi et al. (1996).

7.2.2 Bladder

Spontaneous activity in the bladder is necessary for the bladder to keep its shape during filling and thus allow rapid emptying when activated. However, synchronous activity in the myocytes needs to be avoided to prevent a build-up of intravesical pressure, which would compromise filling. The myocytes are arranged in small bundles and are less well coupled electrically than in most other smooth muscles.

Microelectrodes have been used to study the effects of various K^+ channel blocking and activating drugs on the bladder. For instance, Fujii et al. (1990) investigated the effect of various K^+ channel blockers in guinea-pig bladder myocytes and their interaction with the K^+ channel opener cromakalim. Their results suggested that there were at least two types of K^+ channel present, one of which was blocked by apamin and responsible for the after-hyperpolarizations of the spikes. This channel was also blocked by procaine and partially blocked by quinidine and was not involved in the effects of cromakalim. The other channel, responsible for the falling phase of the spike was resistant to both apamin and 4-aminopyridine but blocked by procaine, TEA and quinidine, and activated by cromakalim.

Hashitani and colleagues, using a variety of blockers, propose that spike repolarization is determined by inactivation of L-type Ca channels as well as the delayed rectifier K^+ channels and that other Ca^{2+}-activated and voltage-dependent channels are involved in modulation of the resting potential and action potential frequency (Hashitani et al. 2000, 2001, 2004a; Hashitani and Brading 2003a, b).

ICC-like cells occur at the edges of the myocyte bundles, as well as below the urothelium and in the lamina propria (Davidson and McCloskey 2005). The electrophysiological properties of the ICC-like cells have been investigated by Hashitani et al. (2004b), Kubota et al. (2008) and by Davidson and McCloskey (2005), McCloskey (2005, 2006), McCloskey et al. (2009). The ICC-like cells are not thought to be responsible for initiating the spontaneous activity, but to modulate the activity and potentially modulate neural input.

7.2.3 Urethra

Extensive patch clamp experiments examining K^+ channel properties in isolated pig urethral myocytes have been undertaken (e.g. Brading and Turner 1996; Teramoto and Brading 1996). The use of various K^+ channel blockers leads to the conclusion that the myocytes have predominantly three types of K^+ channel: small and large Ca^{2+}-activated K^+ channels and K_{ATP} channels. Isolated strips generate continuous spontaneous tone, which requires Ca^{2+} entry and is reduced by L-type Ca^{2+} channel blockers, NO donors and K_{ATP} openers.

The rabbit isolated urethral strips do not develop spontaneous tone, but microelectrode studies (Hashitani et al. 1996) show that the tissue develops regular slow waves and presumably under the right conditions; these slow waves may give rise to action potentials and phasic contractions. Similar slow waves have been recorded in guinea-pig urethra (Hashitani and Edwards 1999). In 2000, the Belfast group (Sergeant et al. 2000) published striking evidence that the rabbit urethra possessed interstitial cells with structural and morphological properties similar to those found in the interstitial cells of Cajal in the gut. They isolated and recorded from these cells and showed that they generated slow waves very similar to the slow waves recorded in the urethral smooth muscles with microelectrodes (Hashitani et al. 1996). Ca^{2+} imaging demonstrated that most of these interstitial cells show spontaneous oscillations in $[Ca^{2+}]_i$. Evidence now strongly suggests that these slow waves are in fact generated by interstitial cells through a combination of Ca^{2+} release from the internal stores and the opening of Ca^{2+}-activated Cl^- channels (Hollywood et al. 2003). Depolarizing current is then injected into the neighbouring smooth muscle cells to produce slow waves. The interstitial cells respond to transmitters, and the complex excitatory and inhibitory innervation that is found in the urethra may have input onto both cell types.

8 Conclusion

8.1 A Speculative Approach

In order to benefit from targeting ion channels, we need to know details of the channel types present in the smooth muscles we hope to modulate and have specific drugs available to interact with them without causing undesirable side effects. The latter is the main problem. One approach that to our knowledge that has not been exploited might come from the fact that we are dealing with organs that transport or store urine. Urine is elaborated in the kidney by filtration and then secretion or reabsorption along the kidney tubule. Transport mechanisms are present for organic anions and cations that have fairly nonspecific requirements, and it is possible to synthesize potential drug molecules with attached groups that allow them to be actively secreted into the urine. One could thus administer a drug systemically at

a dose that would be concentrated into the effective range only in the urine. This pharmacokinetic targeting of the urinary bladder, particularly through actions on the urothelium, could solve the core, general problem of urinary tract pharmacology: effective local action without systemic side effects.

Furthermore, potentiating ligands, analogous to the allosteric potentiating ligands targeting the nicotinic receptors, might provide a key future direction for targeting urogenital ion channels. The idea is not to attempt to either block or activate a channel, but rather to modify its function so that it becomes more, or less, sensitive to endogenous regulation or membrane potential signals. Work on identifying allosteric binding site for P2X ligand-gated ion channels has already commenced (Evans 2009), and perhaps a similar approach will work for other ion channels once more structural information is available.

Most urogenital smooth muscle functions as a syncytium, and perhaps by targeting emergent network properties of such syncytia, we can effectively target organ function. For example, the problem of detrusor activity occurs not so much because individual smooth muscle cells are more active, but rather because the smooth muscle activity is more coordinated. Identifying which ion channels can influence such macroscopic properties is difficult, but several groups are now applying computational approaches, usually reserved for the study of central networks, to understand bladder function.

Finally, an explosion of interest in ICCs now provides new cellular targets for drug therapies. These cells modulate both urothelial to smooth muscle communication and signalling from nerves. If ICCs prove to have a unique or characteristic distribution of ion channels, it may be possible to selectively target their predominant channel subtypes pharmacologically and hence modify urogenital tract function.

So, driven by unmet clinical needs and bolstered by new molecular and cellular data, the pharmacological targeting of urogenital ion channels may become a key area of therapeutics over coming decades.

References

Adrian RH (1961) Internal chloride concentration and chloride efflux of frog muscle. J Physiol 156:623–632

Aickin CC, Brading AF (1983) Towards an estimate of chloride permeability in the smooth muscle of guinea-pig vas deferens. J Physiol 336:179–197

Andersson KE, Holmquist F, Fovaeus M, Hedlund H, Sundler R (1991) Muscarinic receptor stimulation of phosphoinositide hydrolysis in the human isolated urinary bladder. J Urol 146 (4):1156–1159

Ashcroft FM, Gribble FM (1998) Correlating structure and function in ATP-sensitive K^+ channels. Trends Neurosci 21(7):288–294

Berra-Romani R, Blaustein MP, Matteson DR (2005) TTX-sensitive voltage-gated Na^+ channels are expressed in mesenteric artery smooth muscle cells. Am J Physiol Heart Circ Physiol 289 (1):H137–H145. doi:10.1152/ajpheart.01156.2004

Bhattacharyya BJ, Lee E, Krupin D, Hockberger P, Krupin T (2002) (-)-Isoproterenol modulation of maxi-K^+ channel in nonpigmented ciliary epithelial cells through a G-protein gated pathway. Curr Eye Res 24(3):173–181

Brading AF, McCloskey KD (2005) Mechanisms of disease: specialized interstitial cells of the urinary tract – an assessment of current knowledge. Nat Clin Prac 2(11):546–554

Brading AF, Turner WH (1996) Potassium channels and their modulation in the urogenital tract smooth muscles. In: Evans JM, Hamilton TC, Longham SD, Stemp G (eds) Potassium channels and their modulators: from synthesis to clinical experience. Taylor and Francis, London, pp 335–359

Brading A, Bulbring E, Tomita T (1969) The effect of sodium and calcium on the action potential of the smooth muscle of the guinea-pig taenia coli. J Physiol 200(3):637–654

Burdyga T, Wray S (2005) Action potential refractory period in ureter smooth muscle is set by Ca sparks and BK channels. Nature 436(7050):559–562

Choe S (2002) Potassium channel structures. Nat Rev Neurosci 3(2):115–121. doi:10.1038/nrn727

Clapham DE (2003) TRP channels as cellular sensors. Nature 426(6966):517–524

Clapham DE (2004) Mammalian TRP channels as molecular targets. Novartis Foundation Symposium, vol 258. Wiley, Chichester

Creed KE, Ishikawa S, Ito Y (1983) Electrical and mechanical activity recorded from rabbit urinary bladder in response to nerve stimulation. J Physiol 338:149–164

Curtis HJ, Cole KS (1940) Membrane action potentials from the squid giant axon. J Cell Comp Physiol 15:147–157

Davidson RA, McCloskey KD (2005) Morphology and localization of interstitial cells in the guinea pig bladder: structural relationships with smooth muscle and neurons. J Urol 173 (4):1385–1390

Dixon JS, Gosling JA (1973) The fine structure of pacemaker cells in the pig renal calices. Anat Rec 175(2):139–153. doi:10.1002/ar.1091750203

Dixon JS, Gosling JA (1982) The musculature of the human renal calices, pelvis and upper ureter. J Anat 135(1):129–137

Drake MJ, Hedlund P, Andersson KE, Brading AF, Hussain I, Fowler C, Landon DN (2003) Morphology, phenotype and ultrastructure of fibroblastic cells from normal and neuropathic human detrusor: absence of myofibroblast characteristics. J Urol 169(4):1573–1576. doi:10.1097/01.ju.0000054928.34777.37

Evans RJ (2009) Orthosteric and allosteric binding sites of P2X receptors. Eur Biophys J 38 (3):319–327. doi:10.1007/s00249-008-0275-2

Fanger CM, Ghanshani S, Logsdon NJ, Rauer H, Kalman K, Zhou J, Beckingham K, Chandy KG, Cahalan MD, Aiyar J (1999) Calmodulin mediates calcium-dependent activation of the intermediate conductance K_{Ca} channel, IKCa1. J Biol Chem 274(9):5746–5754

Frazier EP, Mathy MJ, Peters SL, Michel MC (2005) Does cyclic AMP mediate rat urinary bladder relaxation by isoproterenol? J Pharmacol Exp Ther 313(1):260–267. doi:jpet.104.077768 [pii] 10.1124/jpet.104.077768

Fujii K (1988) Evidence for adenosine triphosphate as an excitatory transmitter in guinea-pig, rabbit and pig urinary bladder. J Physiol 404:39–52

Fujii K, Foster CD, Brading AF, Parekh AB (1990) Potassium channel blockers and the effects of cromakalim on the smooth muscle of the guinea-pig bladder. Br J Pharmacol 99(4): 779–785

Gloyn AL, Pearson ER, Antcliff JF, Proks P, Bruining GJ, Slingerland AS, Howard N, Srinivasan S, Silva JM, Molnes J, Edghill EL, Frayling TM, Temple IK, Mackay D, Shield JP, Sumnik Z, van Rhijn A, Wales JK, Clark P, Gorman S, Aisenberg J, Ellard S, Njolstad PR, Ashcroft FM, Hattersley AT (2004) Activating mutations in the gene encoding the ATP-sensitive potassium-channel subunit Kir6.2 and permanent neonatal diabetes. N Engl J Med 350(18):1838–1849. doi:10.1056/NEJMoa032922

Hashitani H, Brading AF (2003a) Electrical properties of detrusor smooth muscles from the pig and human urinary bladder. Br J Pharmacol 140(1):146–158. doi:10.1038/sj.bjp. 0705319

Hashitani H, Brading AF (2003b) Ionic basis for the regulation of spontaneous excitation in detrusor smooth muscle cells of the guinea-pig urinary bladder. Br J Pharmacol 140(1): 159–169. doi:10.1038/sj.bjp. 0705320

Hashitani H, Edwards FR (1999) Spontaneous and neurally activated depolarizations in smooth muscle cells of the guinea-pig urethra. J Physiol 514(2):459–470
Hashitani H, Suzuki H (1995) Electrical and mechanical responses produced by nerve stimulation in detrusor smooth muscle of the guinea-pig. Eur J Pharmacol 284(1–2):177–183
Hashitani H, Van Helden DF, Suzuki H (1996) Properties of spontaneous depolarizations in circular smooth muscle cells of rabbit urethra. Br J Pharmacol 118(7):1627–1632
Hashitani H, Bramich NJ, Hirst GD (2000) Mechanisms of excitatory neuromuscular transmission in the guinea-pig urinary bladder. J Physiol 524:565–579
Hashitani H, Fukuta H, Takano H, Klemm MF, Suzuki H (2001) Origin and propagation of spontaneous excitation in smooth muscle of the guinea-pig urinary bladder. J Physiol 530:273–286
Hashitani H, Brading AF, Suzuki H (2004a) Correlation between spontaneous electrical, calcium and mechanical activity in detrusor smooth muscle of the guinea-pig bladder. Br J Pharmacol 141(1):183–193. doi:10.1038/sj.bjp. 0705602
Hashitani H, Yanai Y, Suzuki H (2004b) Role of interstitial cells and gap junctions in the transmission of spontaneous Ca2+ signals in detrusor smooth muscles of the guinea-pig urinary bladder. J Physiol 559(Pt 2):567–581. doi:10.1113/jphysiol.2004.065136
Hegde SS (2006) Muscarinic receptors in the bladder: from basic research to therapeutics. Br J Pharmacol 147(S2):S80–S87. doi:10.1038/sj.bjp.0706560
Hodgkin AL, Huxley AF (1939) Action potentials recorded from inside a nerve fibre. Nature 144(3651):710–711
Hodgkin AL, Huxley AF (1990) A quantitative description of membrane current and its application to conduction and excitation in nerve. 1952. Bull Math Biol 52(1-2):25–71, discussion 25–23
Hollywood MA, Sergeant GP, McHale NG, Thornbury KD (2003) Activation of Ca^{2+}-activated Cl^- current by depolarizing steps in rabbit urethral interstitial cells. Am J Physiol Cell Physiol 285(2):C327–C333. doi:10.1152/ajpcell.00413.2002
Jarvis MF, Khakh BS (2009) ATP-gated P2X cation-channels. Neuropharmacology 56(1): 208–215. doi:10.1016/j.neuropharm.2008.06.067
Klemm MF, Exintaris B, Lang RJ (1999) Identification of the cells underlying pacemaker activity in the guinea-pig upper urinary tract. J Physiol 519(3):867–884
Kubota Y, Hashitani H, Shirasawa N, Kojima Y, Sasaki S, Mabuchi Y, Soji T, Suzuki H, Kohri K (2008) Altered distribution of interstitial cells in the guinea pig bladder following bladder outlet obstruction. Neurourol Urodyn 27(4):330–340. doi:10.1002/nau.20502
Kuriyama H, Tomita T (1970) The action potential in the smooth muscle of the guinea pig taenia coli and ureter studied by the double sucrose-gap method. J Gen Physiol 55(2):147–162
Lang RJ (1989) Identification of the major membrane currents in freshly dispersed single smooth muscle cells of guinea-pig ureter. J Physiol 412:375–395
Lang RJ, Zhang Y, Exintaris B, Vogalis F (1995) Effects of nerve stimulation on the spontaneous action potentials recorded in the proximal renal pelvis of the guinea-pig. Urol Res 23(5): 343–350
Lesage F, Guillemare E, Fink M, Duprat F, Lazdunski M, Romey G, Barhanin J (1996) TWIK-1, a ubiquitous human weakly inward rectifying K^+ channel with a novel structure. EMBO J 15(5): 1004–1011
Logothetis DE, Kurachi Y, Galper J, Neer EJ, Clapham DE (1987) The beta gamma subunits of GTP-binding proteins activate the muscarinic K^+ channel in heart. Nature 325(6102):321–326. doi:10.1038/325321a0
Lopatin AN, Makhina EN, Nichols CG (1994) Potassium channel block by cytoplasmic polyamines as the mechanism of intrinsic rectification. Nature 372(6504):366–369. doi:10.1038/372366a0
Lopes CM, Remon JI, Matavel A, Sui JL, Keselman I, Medei E, Shen Y, Rosenhouse-Dantsker A, Rohacs T, Logothetis DE (2007) Protein kinase A modulates PLC-dependent regulation and PIP_2-sensitivity of K^+ channels. Channels (Austin) 1(2):124–134. doi:4322 [pii]

Maggi CA, Meli A (1984) Eserine-induced hypertone of guinea pig distal colon in vivo: a new pharmacological procedure for testing smooth muscle relaxants. J Pharmacol Methods 12(2): 91–96

Maggi CA, Giuliani S, Santicioli P, Brading AF (1996) Role of intracellular Ca^{2+} in the K channel opener action of CGRP in the guinea-pig ureter. Br J Pharmacol 118(6):1493–1503

McCloskey KD (2005) Characterization of outward currents in interstitial cells from the guinea pig bladder. J Urol 173(1):296–301

McCloskey KD (2006) Calcium currents in interstitial cells from the guinea-pig bladder. BJU Int 97(6):1338–1343. doi:10.1111/j.1464-410X.2006.06156.x

McCloskey KD (2010) Interstitial cells in the urinary bladder-localization and function. Neurourol Urodyn 29(1):82–87. doi:10.1002/nau.20739

McCloskey KD, Anderson UA, Davidson RA, Bayguinov YR, Sanders KM, Ward SM (2009) Comparison of mechanical and electrical activity and interstitial cells of Cajal in urinary bladders from wild-type and W/Wv mice. Br J Pharmacol 156(2):273–283. doi:10.1111/j.1476-5381.2008.00006.x

Miller C, Moczydlowski E, Latorre R, Phillips M (1985) Charybdotoxin, a protein inhibitor of single Ca2 + -activated K + channels from mammalian skeletal muscle. Nature 313(6000): 316–318

Nakajima T, Jo T, Meguro K, Oonuma H, Ma J, Kubota N, Imuta H, Takano H, Iida H, Nagase T, Nagata T (2008) Effect of dexamethasone on voltage-gated Na^{+} channel in cultured human bronchial smooth muscle cells. Life Sci 82(23–24):1210–1215. doi:10.1016/j.lfs.2008.04.007

Neild TO, Thomas RC (1973) New design for a chloride-sensitive micro-electrode. J Physiol 231 (1):7P–8P

Nelson MT, Quayle JM (1995) Physiological roles and properties of potassium channels in arterial smooth muscle. Am J Physiol 268(4 Pt 1):C799–C822

Ng SW, di Capite J, Singaravelu K, Parekh AB (2008) Sustained activation of the tyrosine kinase Syk by antigen in mast cells requires local Ca^{2+} influx through Ca^{2+} release-activated Ca^{2+} channels. J Biol Chem 283(46):31348–31355. doi:10.1074/jbc.M804942200

Nilius B, Droogmans G (2003) Amazing chloride channels: an overview. Acta Physiol Scand 177 (2):119–147

Parekh AB (2006) Cell biology: cracking the calcium entry code. Nature 441(7090):163–165

Platoshyn O, Remillard CV, Fantozzi I, Sison T, Yuan JX (2005) Identification of functional voltage-gated Na^{+} channels in cultured human pulmonary artery smooth muscle cells. Pflugers Arch 451(2):380–387. doi:10.1007/s00424-005-1478-3

Seki N, Suzuki H (1990) Electrical properties of smooth muscle cell membrane in renal pelvis of rabbits. Am J Physiol 259:F888–F894

Sergeant GP, Hollywood MA, McCloskey KD, Thornbury KD, McHale NG (2000) Specialised pacemaking cells in the rabbit urethra. J Physiol 526(Pt 2):359–366. doi:PHY_0872 [pii]

Shuba MF (1977) The effect of sodium-free and potassium-free solutions, ionic current inhibitors and ouabain on electrophysiological properties of smooth muscle of guinea-pig ureter. J Physiol 264(3):837–851

Suzuki M, Morita T, Iwamoto T (2006) Diversity of Cl^{-} channels. Cell Mol Life Sci 63(1):12–24. doi:10.1007/s00018-005-5336-4

Teramoto N, Brading AF (1996) Activation by levcromakalim and metabolic inhibition of glibenclamide-sensitive K channels in smooth muscle cells of pig proximal urethra. Br J Pharmacol 118(3):635–642

Toro L, Ramos-Franco J, Stefani E (1990) GTP-dependent regulation of myometrial KCa channels incorporated into lipid bilayers. J Gen Physiol 96(2):373–394

Uchida H, Shishido K, Nomiya M, Yamaguchi O (2005) Involvement of cyclic AMP-dependent and -independent mechanisms in the relaxation of rat detrusor muscle via beta-adrenoceptors. Eur J Pharmacol 518(2–3):195–202. doi:10.1016/j.ejphar.2005.06.029

Vaughan-Jones RD (1982) Chloride activity and its control in skeletal and cardiac muscle. Philos Trans R Soc Lond B Biol Sci 299(1097):537–548

Verkman AS, Galietta LJ (2009) Chloride channels as drug targets. Nat Rev Drug Discov 8(2): 153–171. doi:10.1038/nrd2780

Yamaguchi O, Chapple CR (2007) Beta3-adrenoceptors in urinary bladder. Neurourol Urodyn 26 (6):752–756

Yoshida M, Miyamae K, Iwashita H, Otani M, Inadome A (2004) Management of detrusor dysfunction in the elderly: changes in acetylcholine and adenosine triphosphate release during aging. Urology 63(3 Suppl 1):17–23

Young JS, Meng E, Cunnane TC, Brain KL (2008) Spontaneous purinergic neurotransmission in the mouse urinary bladder. J Physiol 586:5743–5755. doi:10.1113/jphysiol.2008.162040

Zhu HL, Brain KL, Aishima M, Shibata A, Young JS, Sueishi K, Teramoto N (2008) Actions of two main metabolites of propiverine (M-1 and M-2) on voltage-dependent L-Type Ca^{2+} currents and Ca^{2+} transients in murine urinary bladder myocytes. J Pharm Exp Ther 324:118–127. doi:10.1124/jpet.107.130021

Neuropeptides in Lower Urinary Tract Function

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Abstract Numerous neuropeptide/receptor systems including vasoactive intestinal polypeptide, pituitary adenylate cyclase-activating polypeptide, calcitonin gene-related peptide, substance P, neurokinin A, bradykinin, and endothelin-1 are expressed in the lower urinary tract (LUT) in both neural and nonneural (e.g., urothelium) components. LUT neuropeptide immunoreactivity is present in afferent and autonomic efferent neurons innervating the bladder and urethra and in the urothelium of the urinary bladder. Neuropeptides have tissue-specific distributions and functions in the LUT and exhibit neuroplastic changes in expression and function with LUT dysfunction following neural injury, inflammation, and disease. LUT dysfunction with abnormal voiding, including urinary urgency, increased voiding frequency, nocturia, urinary incontinence, and pain, may reflect a change in the balance of neuropeptides in bladder reflex pathways. LUT neuropeptide/receptor systems may represent potential targets for therapeutic intervention.

Keywords Calcitonin gene-related peptide · Nerve growth factor · Pituitary adenylate cyclase activating polypeptide · Substance P · Vasoactive intestinal polypeptide

Abbreviations

AA	Acetic acid
ATP	Adenosine triphosphate
BOO	Bladder outlet obstruction
CGRP	Calcitonin gene-related peptide
CYP	Cyclophosphamide
DRG	Dorsal root ganglia
DSD	Detrusor sphincter dyssynergia
EFS	Electric field stimulation
IC	Interstitial cystitis
ICI	Intercontraction interval
IL	Interleukin
IR	Immunoreactivity

LUT	Lower urinary tract
NGF	Nerve growth factor
NK	Neurokinin
NVCs	Nonvoiding contractions
OAB	Overactive bladder
PACAP	Pituitary adenylate cyclase activating polypeptide
BPS	Bladder pain syndrome
SCI	Spinal cord injury
SP	Substance P
VIP	Vasoactive intestinal polypeptide
VV	Void volume
WT	Wildtype

1 Introduction: Micturition Overview and Anatomy of Lower Urinary Tract

Micturition is regulated by neural circuits in the brain and spinal cord that coordinate the activity of the smooth and striated muscles of the lower urinary tract (LUT) (Fowler et al. 2008). These circuits act as on–off switches to shift the urinary tract between two modes of operation: storage and elimination. Bladder smooth muscle and the urethral outlet must function reciprocally for efficient elimination of urine (Fowler et al. 2008). The bladder smooth muscle must remain relaxed during storage mode to allow for filling, while the urethral outlet is contracted (Fowler et al. 2008). Elimination mode requires contraction of bladder smooth muscle and relaxation of the urethral outlet to allow urine flow (Andersson and Arner 2004). Precise coordination of the reciprocal functions of the urinary bladder and urethra and complex neural organization are required for normal function (Fowler et al. 2008).

The LUT, consisting of the urinary bladder, urethra, internal urethral sphincter, and external urethral sphincter, is composed of both striated and smooth muscle, and therefore, under both voluntary (somatic) and involuntary (autonomic) influence (Andersson and Arner 2004). The bladder consists of three layers: a urothelium on the lumenal surface, a lamina propria just deep to the urothelium that contains a suburothelial plexus of nerves and vasculature, and an outer muscle layer, named the detrusor, which contains both longitudinal and circular smooth muscles (Andersson and Arner 2004; Fig. 1). The urothelium in rodents is composed of at least three layers: the basal, intermediate, and superficial/umbrella layers. However, in higher mammals including humans, there are additional intermediate layers (Wu et al. 2009). The umbrella cells are connected by tight junctions and are covered on their apical surface by crystalline proteins, which assemble into hexagonal plaques [for review see, Wu et al. (2009)].

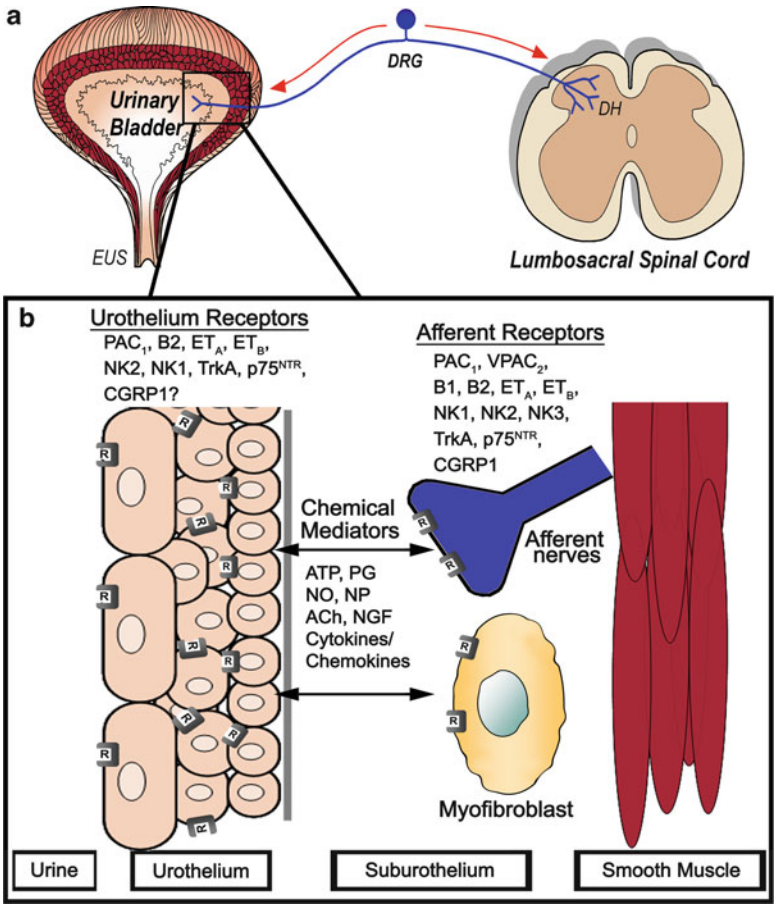

Fig. 1 Neuropeptide/receptor systems expression in micturition reflex pathways emphasizing bladder afferent and urothelium participation. (**a**) Diagram of afferent innervation of the urinary bladder. Numerous neuropeptide/receptor systems have been identified in bladder afferent (i.e., sensory) pathways with contributions to normal lower urinary tract function as well as that after neural injury, disease, or inflammation. (**b**) Potential model of possible reciprocal neuropeptide/receptor interactions among bladder afferent and efferent nerves (not shown), urothelial cells, myofibroblasts located in the suburothelium and detrusor smooth muscle that underlie physiological bladder reflex function as well as pathophysiology in bladder disease. Receptor activation and channel stimulation on urothelial cells can elicit secretion of chemical mediators that can affect adjacent tissues including bladder afferent nerves in the suburothelial plexus, myofibroblasts, and detrusor smooth muscle. Urothelial cells can also be responsive to neurotransmitters released from bladder nerves and other cell types including inflammatory cells. *Abbreviations*: *ATP* adenosine triphosphate; *TrkA* receptor tyrosine kinase A; $p75^{NTR}$ low affinity neurotrophin receptor; *PG* prostaglandin; *NO* nitric oxide; *NGF* nerve growth factor; *NP* neuropeptides; *Ach* acetylcholine; PAC_1 pituitary adenylate cyclase-activating polypeptide (PACAP) selective receptor; $VPAC_2$ receptor with equal and high affinity for vasoactive intestinal polypeptide and PACAP; *B1* bradykinin receptor 1; *B2* bradykinin receptor 2; ET_A endothelin receptor A; ET_B endothelin receptor B; *NK2* neurokinin receptor 2; *NK1* neurokinin receptor 2; *NK3* neurokinin receptor 3; *CGRP1* calcitonin gene-related receptor. See text for additional details. Figure modified from Arms et al. (2009)

1.1 Neural Control of Micturition

The storage and periodic elimination of urine requires a complex neural control system that coordinates the activities of a variety of effector organs including the smooth muscle of the urinary bladder and the smooth and striated muscle of the urethral sphincters (Kuru 1965; Klück 1980; de Groat and Steers 1990; Andersson and Arner 2004). Three neural pathways regulate the LUT (1) sacral parasympathetic (pelvic) nerves provide excitatory input to the bladder; (2) thoracolumbar sympathetic (hypogastric) nerves provide an inhibitory input to the bladder and an excitatory input to the bladder neck and urethra; and (3) sacral somatic (pudendal) nerves, which innervate the striated muscles of the sphincters and pelvic floor (Kuru 1965; Klück 1980; de Groat and Steers 1990; Middleton and Keast 2004). Each of these sets of nerves contains afferent (sensory) as well as efferent (motor) axons (Morrison 1987; Lincoln and Burnstock 1993).

The central neural pathways controlling the LUT exhibit "all-or-none" or "switch-like" characteristics reflecting the storage and elimination functions of the LUT (de Groat et al. 1993, 1997; de Groat and Kruse 1993). During urine storage, somatic and sympathetic pathways to the sphincters and sympathetic inhibitory inputs to the bladder are tonically active, whereas parasympathetic pathways are inactive (Kuru 1965; de Groat et al. 1993, 1997; de Groat and Kruse 1993). During reflex or voluntary micturition, the activity patterns are reversed such that parasympathetic pathways are excited and somatosympathetic pathways are inhibited, thereby promoting urine flow (Middleton and Keast 2004).

The LUT reflex mechanisms, organized at the level of the lumbosacral spinal cord, are modulated predominantly by supraspinal controls (Kuru 1965; de Groat 1975; de Groat et al. 1993, 1997; de Groat and Kruse 1993; Middleton and Keast 2004). These mechanisms can be summarized as follows (1) storage reflexes (parasympathetic and somatic) are organized at the spinal level; (2) elimination reflexes (parasympathetic) are organized at a supraspinal site in the pons; and (3) spinal storage reflexes are modulated by inputs from the rostral pons.

1.2 Neurochemistry and Morphology of Afferent and Spinal Pathways to the Urogenital Tract

Bladder afferent neurons travel in the hypogastric and pelvic nerves, and their cell bodies are located in dorsal root ganglia (DRG) at spinal segments T11–L2 and S2–S4 in humans and L1–L2 and L6–S1 in rats (Fowler et al. 2008; Fig. 1). Bladder afferent fibers consist of lightly myelinated Aδ fibers and unmyelinated C-fibers. Sensation of bladder filling is conveyed by Aδ fibers, the most important mechanoreceptors of the bladder. C-fibers are normally "silent," but they do respond to chemical or noxious stimuli, including extreme bladder pressure (Fowler et al. 2008). Aδ and C-fibers terminate in the urothelium, suburothelium, and smooth

muscle layers of the bladder (Kullmann et al. 2008). Most bladder afferent fibers project to lumbosacral spinal cord segments, and this is the most important region of the spinal cord relative to signaling the micturition reflex (Holstege 2005; Fig. 1). Most sensory nerves in the bladder are located in a dense suburothelial plexus just beneath the urothelium (Andersson and Wein 2004; Fig. 1).

Bladder afferent fibers in the pelvic nerve in rodents pass through the dorsal roots into Lissauer's tract at the apex of the dorsal horn and then give off collateral branches that extend ventromedially and ventrolaterally along the superficial layers of the dorsal horn to the dorsal commissure and to the area of the sacral parasympathetic nucleus (laminae V–VII) that contains preganglionic parasympathetic neurons that project to the periphery (de Groat et al. 1981, 1986, 1997; Donovan et al. 1983; Steers et al. 1991a; de Groat and Kruse 1993; Nadelhaft and Vera 1995; Marson 1997). The most prominent pathway is located in lamina I on the lateral edge of the dorsal horn in a region termed the lateral collateral pathway of Lissauer's tract. Afferent projections from the pudendal nerve and genital structures follow the medial edge of the dorsal horn into the dorsal commissure region, forming the medial collateral pathway (Kawatani et al. 1990). Bladder afferent fibers contain a variety of neuropeptides, including calcitonin-gene-related peptide (CGRP), substance P (SP), neurokinin A, neurokinin B, vasoactive intestinal polypeptide (VIP), cholecystokinin, and enkephalins (Donovan et al. 1983; de Groat et al. 1986, 1996; Keast 1991; Vizzard 2000d, 2001; Fig. 1). We have demonstrated (Vizzard 2000d) that bladder afferent cells express pituitary adenylate cyclase-activating polypeptide (PACAP) and that expression is increased after cyclophosphamide (CYP)-induced cystitis in rats. With the exception of CGRP, all of these substances are predominantly expressed in small (presumably C-fiber) afferents (Ek et al. 1977; Donovan et al. 1983; de Groat et al. 1986, 1996; Su et al. 1986; Keast and de Groat 1992; Vizzard et al. 1993a, b, 1994, 1995; Vizzard and de Groat 1996). The administration of capsaicin, which acts selectively on small diameter afferent fibers to deplete neurotransmitter stores to induce neuronal degeneration, reduces the levels of substance P, neurokinin A, and CGRP within the pelvic viscera but does not affect VIP or enkephalin expression (de Groat 1987). These findings are consistent with SP, related tachykinins, and CGRP expression in afferent pathways to the pelvic viscera (de Groat 1987).

Many bladder afferent fibers project to the sacral parasympathetic nucleus, synapsing with preganglionic parasympathetic neurons as well as interneurons (Morgan et al. 1981; Fowler et al. 2008). Primary bladder afferents from the pelvic and hypogastric nerves also project to the dorsal commissure and superficial dorsal horn (Fowler et al. 2008). The lumbosacral dorsal commissure, superficial dorsal horn, and parasympathetic nucleus all contain interneurons important to urinary bladder function (de Groat and Kruse 1993; Fowler et al. 2008). These interneurons project locally in the spinal cord or to the brain (Fowler et al. 2008). Some bladder afferents synapse with ascending pathways in the spinal cord that project to neuronal populations in the brain involved in micturition control, including the pontine micturition center (de Groat and Kruse 1993; Fowler et al. 2008).

1.3 Neurochemical Plasticity in the LUT with Bladder Inflammation, Neural Injury, or Disease

Neuroactive compounds, including neuropeptides, in the afferent pathways to the LUT exhibit either excitatory or inhibitory actions. Nonneural sources of peptides in the LUT include plasma, sites of tissue inflammation or injury, detrusor smooth muscle cells, bladder fibroblasts, and the urothelium. Pathology, neural injury, and target organ pathology (e.g., bladder inflammation) can alter the known balance of neuropeptides either in the periphery and/or central pathways, conceivably shifting the balance to a hyper- or hypoactive reflex state. Changes in micturition reflex function observed with urinary bladder inflammation (Vizzard 2000b, d, 2001), interstitial cystitis (IC)/bladder pain syndrome (BPS), spinal cord injury (SCI) (upper motoneuron injury) (Vizzard 2006; de Groat and Yoshimura 2009), overactive bladder (OAB) (Yoshimura et al. 2008), and detrusor overactivity secondary to bladder outlet obstruction (BOO) (Andersson 1999, 2006) may reflect a change in the balance of neuropeptides in LUT reflex pathways. Information presented in this review will address both direct and indirect effects of neuropeptides in the LUT. Due to neuropeptide/receptor expression and diversity of functions in the LUT and subsequent regulation with neural injury, disease, and bladder inflammation, neuropeptide/receptor systems may be potential targets for therapeutic intervention (Fig. 1).

The following sections will address the distribution, function, and regulation of specific neuropeptide/receptor systems in the LUT under normal and pathological LUT conditions.

2 PACAP/VIP and Associated Receptors Signaling in the LUT

A number of peptides have been demonstrated in the LUT and have demonstrated roles in regulating the micturition reflex. PACAP and VIP are members of the glucagon/secretin superfamily of hormones (Dickinson et al. 1999). PACAP and VIP share receptor subtypes coupled to different intracellular effectors; PACAP peptides exhibit high affinity for the PAC1 receptor, whereas VIP and PACAP have similar high affinities for the VPAC1 and VPAC2 receptors (Arimura 1998; Sherwood et al. 2000). Both PACAP- and VIP-immunoreactivity (IR) have been identified in urinary bladder (Fahrenkrug and Hannibal 1998; Mohammed et al. 2002). Widespread PACAP-IR exists in nerve fibers in rat LUT with the majority of the PACAP nerve fibers being derived from sensory neurons (Fahrenkrug and Hannibal 1998; Zvarova et al. 2005). PACAP receptors have been identified in various tissues of the micturition pathway including bladder detrusor smooth muscle, urothelium, and major pelvic ganglia (Table 1; Braas et al. 2006; Tompkins et al. 2010).

Table 1 Summary of VIP/PACAP receptor isoforms and tissue distribution in the lower urinary tract

Tissue	Receptor subtypes		
	PAC_1 receptor	$VPAC_1$ receptor	$VPAC_2$ receptor
Detrusor smooth muscle	+	−	+
Urothelium	+	−	−
Lumbosacral dorsal root ganglia	+	−	+
Lumbosacral spinal cord	+	−	+
Major pelvic ganglia	+	+	+

2.1 *PACAP/Receptors in Micturition Reflex Pathways*

Recent studies support roles for PACAP in micturition and suggest that inflammation-induced plasticity in PACAP expression in peripheral and central micturition pathways contribute to bladder dysfunction with cystitis. We have previously demonstrated facilitatory direct effects of PACAP on bladder smooth muscle contractility (Braas et al. 2006). PACAP increased bladder smooth muscle tone and potentiated electric field stimulation (EFS)-induced contractions (Braas et al. 2006). EFS-induced contractions were superimposed on spontaneous muscle contractions and were tetrodotoxin-insensitive, suggesting that the responses were direct detrusor smooth muscle effects (Braas et al. 2006). Excitatory effects of PACAP on the micturition reflex pathway are enhanced 2–4 weeks after SCI in the rat (Yoshiyama and de Groat 1997).

2.2 *PACAP Expression in Bladder Afferent Pathways and Regulation by CYP-Induced Cystitis*

PACAP is expressed in LUT pathways and is regulated by CYP-induced cystitis (Vizzard 2000d; Braas et al. 2006). In control rats, PACAP-IR was expressed in fibers in the superficial dorsal horn at all segmental levels examined (L1, L2, and L4–S1). Bladder afferent cells (40–45%) in the DRG (L1, L2, L6, and S1) from control animals also exhibited PACAP-IR (Vizzard 2000d). After chronic, CYP-induced cystitis, PACAP-IR increased dramatically in spinal segments and DRG (L1, L2, L6, and S1) involved in micturition reflexes (Vizzard 2000d). The density of PACAP-IR was increased in the superficial laminae (I–II) of the L1, L2, L6, and S1 spinal segments (Vizzard 2000d). Staining also increased dramatically in a fiber bundle extending ventrally from Lissauer's tract in lamina I along the lateral edge of the dorsal horn to the sacral parasympathetic nucleus in the L6–S1 spinal segments (lateral collateral pathway of Lissauer). After chronic cystitis, PACAP-IR in cells in the L1, L2, L6, and S1 DRG increased significantly, and the percentage of bladder afferent cells expressing PACAP-IR also increased significantly (70–85%) (Vizzard 2000d).

2.3 PACAP/VIP Receptor Expression in LUT and Modulation with Cystitis

2.3.1 Urinary Bladder

With acute CYP-induced cystitis, PAC1 receptor transcript exhibited a significant decrease in expression in both urothelium and detrusor; however, a significant increase in PAC1 receptor transcript expression in urothelium and detrusor smooth muscle was induced by intermediate and chronic CYP-induced cystitis (Girard et al. 2008).

2.3.2 Lumbosacral Dorsal Root Ganglia

CYP-induced inflammation of the urinary bladder only affected receptor transcript expression in L6–S1 DRG following acute (4 h) CYP-induced cystitis. PAC_1 and $VPAC_2$ receptor transcript expression significantly decreased in both L6 and S1 DRG with acute (4 h) CYP-induced cystitis. For $VPAC_1$ receptor transcript expression, a significant increase in expression was demonstrated in the S1 DRG after acute CYP-induced cystitis. No changes in PAC_1, $VPAC_1$, or $VPAC_2$ receptor expression in L6 or S1 DRG were demonstrated with intermediate (48 h) or chronic (8 day) CYP-induced cystitis. PACAP transcript expression significantly increased in the urothelium with intermediate (48 h) and chronic (8 days) CYP treatment, whereas no changes were observed with acute (4 h) CYP-induced cystitis in either urothelium or detrusor smooth muscle (Girard et al. 2008). Changes in PACAP transcript with CYP-induced cystitis mirrored those observed in the urinary bladder with acute CYP-induced cystitis decreasing and intermediate (48 h) and chronic (8 day) treatments significantly increasing PACAP transcript expression in both the L6 and S1 DRG (Girard et al. 2008). In a rat CYP-induced cystitis paradigm, intrathecal or intravesical administration of PAC_1 receptor antagonist, PACAP6-38, reduced cystitis-induced bladder hyperreflexia (Braas et al. 2006). These studies demonstrate that PACAP/receptor are modulated by CYP-induced cystitis in tissue-specific ways and that PACAP/receptor signaling plays a role in urinary bladder afferent pathways after urinary bladder inflammation.

2.4 PACAP in Micturition Reflexes and Modulation After Spinal Cord Injury

SCI rostral to the lumbosacral spinal cord alters the coordination between the urinary bladder and external urethral sphincter in many species and results in detrusor sphincter dyssynergia (DSD) that interferes with efficient voiding and results in urinary retention, bladder hypertrophy, increased voiding pressures,

increased bladder capacity, and numerous nonvoiding contractions (NVCs) during bladder filling (Vizzard 2006). PACAP is upregulated in micturition reflex pathways after SCI. These studies (Zvara et al. 2006) demonstrate that intrathecal (L6–S1) administration of the PAC_1 receptor antagonist, PACAP6-38 (10 nM), significantly reduced intermicturition interval, threshold and micturition pressures, and number and amplitude of NVCs after SCI (Zvara et al. 2006). In addition, PACAP6-38 increased voiding frequency (i.e., decreased bladder capacity) after SCI (Zvara et al. 2006). In contrast, intravesical administration of PACAP6-38 was without any effect, possibly due to the lack of penetration of the PAC_1 antagonist through the urothelium. The presence of PAC_1 receptor transcript in the lumbosacral spinal cord and DRG has been demonstrated (Girard et al. 2008). Thus, intrathecal administration of PACAP6-38 may act at the lumbosacral spinal cord or DRG. The effects of PACAP6-38 after SCI are consistent with PACAP-27 facilitation of micturition in rats (Ishizuka et al. 1995a). PACAP6-38 reduced the number and amplitude of NVCs after SCI (Zvara et al. 2006). This may be attributed to a reduction in DSD or through an effect on C-fiber bladder afferents (Cheng et al. 1995). An effect of PACAP6-38 on urinary bladder C-fiber afferents is consistent with previous studies that demonstrate that capsaicin depletes PACAP-IR in the LUT (Fahrenkrug and Hannibal 1998) and that PACAP-IR nerve fibers in the bladder express the vanilloid receptor (Zvarova et al. 2005). PACAP expression is upregulated in micturition pathways after SCI, and the present studies demonstrated improved bladder function after intrathecal PACAP antagonist administration. Additional studies addressing the role of PACAP in micturition reflexes suggest that after SCI, PACAP-38 activates spinal circuitry to facilitate the parasympathetic outflow to the urinary bladder and that the elimination of sympathetic pathways enhances this effect (Yoshiyama and de Groat 2008a, b).

2.5 PACAP and VIP Expression and Effects on Major Pelvic Ganglion Neurons

Tissue culture experiments modeling neuronal injury have shown PACAP expression regulation in the major pelvic ganglion (MPG), the ganglia supplying the majority of autonomic (both sympathetic and parasympathetic) innervations to the bladder, and other urogenital organs and components of the lower bowl. While PACAP expression was devoid in acute (4 h) cultures (Tompkins et al. 2010), both PACAP-IR and PACAP transcript levels increased significantly by day 3 (Girard et al. 2010a). PACAP was preferentially expressed in parasympathetic neurons (Girard et al. 2010a). Transcripts for VPAC1, VPAC2, and PAC1 were present by 4 h culture (Tompkins et al. 2010), but VPAC2 transcript levels alone increased by day 3 (Girard et al. 2010a). Local application of PACAP or maxadilan, a PAC1-selective agonist, decreased after hyperpolarization and increased neuronal excitability in a subpopulation of neurons (Tompkins et al. 2010). Therefore,

PACAP receptor signaling in the MPG may represent another mechanism for neuropeptide-modulated bladder function. Additionally, we have demonstrated VIP expression in the MPG, and local application of VIP results in a decreased afterhyperpolarization and increased neuronal excitability in a subpopulation of neurons (Tompkins et al. 2010). However, VIP expression did not increase with prolonged culture (3 days) (Girard et al. 2010a).

2.6 *PACAP Null Mice Exhibit Altered Bladder Function and Somatic Sensitivity*

PACAP contributions to micturition and somatic sensation were recently studied in PACAP knockout ($PACAP^{-/-}$), littermate heterozygote ($PACAP^{+/-}$), and wild-type (WT) mice using conscious cystometry with continuous intravesical saline or acetic acid (AA; 0.5%) instillation, urination patterns, somatic sensitivity testing of hindpaw and pelvic region with calibrated von Frey filaments, and morphological assessments of urinary bladder (May and Vizzard 2010). $PACAP^{-/-}$ mice exhibit increased bladder mass with fewer but larger urine spots (Fig. 2). In $PACAP^{-/-}$ mice, the lamina propria and detrusor smooth muscle are significantly thicker, whereas the urothelium is unchanged (Fig. 2). $PACAP^{-/-}$ mice exhibit increased bladder capacity, void volume (VV), and longer intercontraction interval (ICI) with significantly increased detrusor contraction duration and large residual volume (May and Vizzard 2010; Fig. 2). WT mice respond to AA (0.5%) with a reduction in VV and a decreased ICI, whereas $PACAP^{+/-}$ and $PACAP^{-/-}$ mice do not respond. $PACAP^{-/-}$ mice are less responsive to somatic stimulation. $PACAP^{+/-}$ also exhibit bladder dysfunction and somatic and visceral sensory abnormalities but to a lesser degree (May and Vizzard 2010). PACAP gene disruption contributes to changes in bladder morphology, bladder function, and somatic and visceral hypoalgesia (May and Vizzard 2010).

2.7 *VIP/Receptors and Micturition Reflex Pathways*

VIP exerts species-specific, excitatory or inhibitory actions in neural pathways controlling micturition, and these functions may be altered with neural injury, disease, or inflammation (Erol et al. 1992; Igawa et al. 1993; Uckert et al. 2002; Hernandez et al. 2006). A number of diverse and conflicting roles for VIP have been demonstrated in the urinary bladder from numerous species. VIP has been shown to relax urinary bladder from human (Uckert et al. 2002) or pig (Hernandez et al. 2006) and contract or produce no effects on urinary bladder from the rat (Erol et al. 1992; Igawa et al. 1993). These contradictory findings might be attributable to species differences (Uckert et al. 2002) and differential VIP receptor distribution

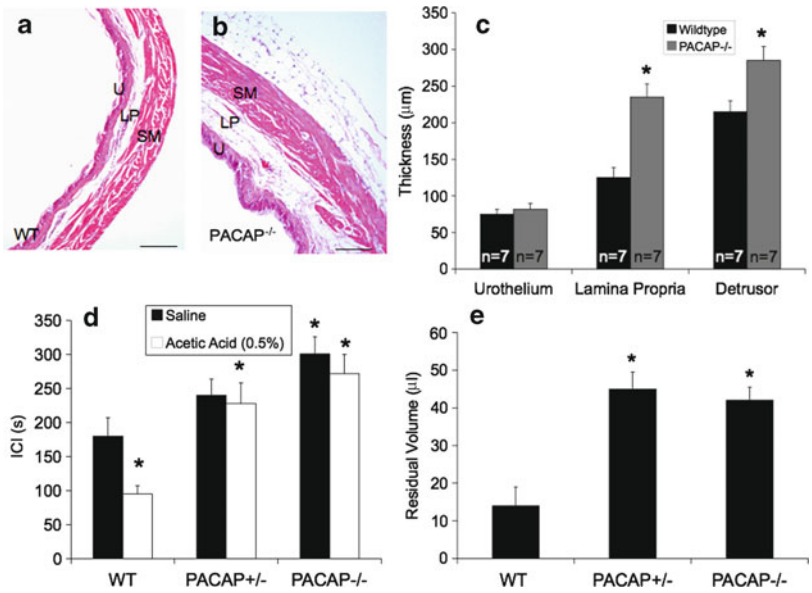

Fig. 2 PACAP contributions to bladder morphology and function. (**a**–**c**). Histological analyses of urinary bladders and bladder function among PACAP$^{-/-}$, PACAP$^{+/-}$ and WT mice. Representative hematoxylin and eosin stained cryostat bladder sections (15 μm) from WT (**a**) and PACAP$^{-/-}$ (**b**) mice that demonstrate significantly (*$p \leq 0.01$) increased thickness of lamina propria (*LP*) and detrusor smooth muscle (*SM*) supported by morphometric analyses (**c**). (**d**–**e**) Summary figures of the intercontraction interval (ICI, seconds (s); **d**) and residual volume (*RV*, μl; **e**) using conscious cystometry in conscious, unrestrained WT, PACAP$^{-/-}$ and PACAP$^{+/-}$ mice with continuous infusion of saline or AA (0.5%). (**d**) ICI was significantly ($p \leq 0.01$) longer in PACAP$^{-/-}$ compared to WT mice with instillation of saline and AA. No changes in ICI were detected with intravesical instillation of AA in PACAP$^{-/-}$ and PACAP$^{+/-}$ mice compared to saline instillation. ICI was significantly ($p \leq 0.01$) greater in PACAP$^{-/-}$ and PACAP$^{+/-}$ mice with AA instillation compared to WT. (**e**) RV was significantly ($p \leq 0.01$) increased in PACAP$^{+/-}$ and PACAP$^{-/-}$ mice compared to WT. Values represent mean ± S.E.M. for $n = 7$–10 animals in each group. *U* urothelium; *WT* wildtype. *Calibration bar* represents 120 μm in **a**, **b**. Figure modified from May and Vizzard (2010)

(Table 1). The majority of VIP in the LUT is located in postganglionic efferent neurons of the pelvic ganglia (Chapple et al. 1992; Smet et al. 1997; Wanigasekara et al. 2003). Surprisingly, VIP had no apparent effects on either bladder tone or EFS-stimulated contractions despite $VPAC_2$ receptor transcript expression in detrusor (Braas et al. 2006). However, as VIP innervation to the urinary bladder is minimal compared to that for PACAP (Fahrenkrug and Hannibal 1998), these results may be in keeping with suggestions that PACAP and PAC_1 signaling are more prominent regulators of rat bladder physiology (Braas et al. 2006). These VIP results are consistent with previous studies that demonstrated that VIP application to detrusor smooth muscle had no effect on spontaneous or carbachol-induced bladder contractions despite facilitation of micturition when VIP was administered

intrathecally or intraarterially close to the rat bladder (Igawa et al. 1993). Recent studies (Studeny et al. 2008) using $VIP^{-/-}$ mice reveal that $VIP^{-/-}$ mice exhibit increased bladder mass and fewer but larger urine spots on filter paper. $VIP^{-/-}$ mice exhibit increased void volumes and shorter ICIs with continuous intravesical infusion of saline (Studeny et al. 2008). No differences in transepithelial resistance or water permeability were demonstrated between $VIP^{-/-}$ and WT mice (Studeny et al. 2008). With the induction of bladder inflammation by acute administration of CYP, an exaggerated or prolonged bladder hyperreflexia was demonstrated in $VIP^{-/-}$ mice (Studeny et al. 2008). The changes in bladder hyperreflexia may reflect increased expression of neurotrophins and/or or proinflammatory cytokines in the urinary bladder (Studeny et al. 2008).

2.8 PACAP-Mediated ATP Release from Cultured, Rat Urothelium

Earlier studies have indicated that the urothelium releases ATP in response to various stimuli (Birder 2006). In addition, it has been suggested that adenosine triphosphate (ATP) released from the serosal surface of the urothelium during bladder filling stimulates receptors on suburothelial sensory nerve fibers and contributes to bladder filling sensation (Cockayne et al. 2000). ATP in the cell cytoplasm can be released extracellularly by several mechanisms including exocytosis of ATP-containing vesicles (Bodin and Burnstock 2001; Novak 2003). PACAP27, PACAP38, and VIP application evoked ATP release from rat urothelial cell cultures; however, ATP release was greatest with PACAP27 treatment and significantly blocked by the PAC_1 receptor selective antagonist, M65 (Girard et al. 2008). Current research supports the suggestion that PACAP and PAC_1 signaling are regulators of bladder physiology at the level of the urinary bladder and specifically the urothelium (Girard et al. 2008).

3 Tachykinins and Calcitonin-Gene Related Peptide in Micturition Reflex Pathways

A variety of neuropeptides are present in the somata and processes of urogenital DRG cells including urinary bladder and urethra, with CGRP and Substance P (SP) being the most widely distributed (Keast 1992). Tachykinins (SP, neurokinin A, neurokinin B) and CRGP are present in the LUT and act on neurokinin (NK)1, NK2, NK3, or CGRP receptors, respectively (Andersson 2002; Canda et al. 2006). NK1 and NK2 receptors have been reported in the detrusor smooth muscle, whereas NK2 receptors are present in the urothelium (Ishizuka et al. 1995b; Birder 2010). In broad terms, the sensory functions of the tachykinins include regulation of

micturition threshold, activation of cardiovascular reflexes, and perception of pain from the urinary bladder (Maggi 1995; Gu et al. 2000; Andersson 2002). Efferent functions of the tachykinins include regulation of local muscle cell activity, nerve excitability, plasma extravasation, and blood flow (Andersson 2002). Involvement of tachykinins located in supraspinal sites on micturition function has also been demonstrated (Gu et al. 2000).

3.1 *Tachykinins and CGRP in Micturition Reflex Pathways and Regulation with Inflammation, Injury, or Disease*

Expression of tachykinins and associated receptors in the LUT under basal conditions and alterations in expression has been reported in animal models of bladder inflammation and in the clinical syndrome of IC/BPS. Recent studies have demonstrated alterations in SP-immunoreactivity (IR) (Pang et al. 1995) and NK1 mRNA (Marchand et al. 1998) in bladder biopsies from patients with IC/BPS (Johansson and Fall 1994; Ho et al. 1997; Johansson et al. 1997). In addition, a study involving an acute rat model of urinary bladder inflammation (48 h following intravesical mustard oil treatment) has demonstrated significant increases in CGRP- and SP-IR in rostral (L1, L2 DRG) and caudal (L6, S1 DRG) bladder afferent neurons (Callsen-Cencic and Mense 1997). Furthermore, it has been demonstrated (Lecci et al. 1994) that intrathecal injection of SP antagonists reduces CYP-induced bladder hyperreflexia. Although numerous studies have demonstrated changes in CGRP and SP expression in sensory neurons following nerve injury (Hokfelt et al. 1994) or peripheral inflammatory states (Kataeva and Agro 1994; Luber-Narod et al. 1997; Traub et al. 1999; Hutchins et al. 2000), there are only a limited number of studies that have examined alterations in CGRP or SP expression following the induction of acute urinary bladder inflammation (Callsen-Cencic and Mense 1997; Luber-Narod et al. 1997). Increases in CGRP- and SP-IR in bladder afferent neurons in the lumbosacral DRG 48 h following the induction of cystitis with intravesical mustard-oil have been demonstrated (Callsen-Cencic and Mense 1997).

3.2 *SP and CGRP Plasticity in LUT Pathways with CYP-Induced Cystitis*

Additional plasticity of CGRP and SP expression in LUT pathways has been demonstrated in the CYP model of bladder inflammation in rats (Vizzard 2001). In control rats, CGRP- or SP-IR was expressed in fibers in the superficial dorsal horn in all segmental levels examined (L4–S1). Bladder afferent cells in the DRG (L6, S1) from control animals also exhibited CGRP- (41–55%) or SP-IR (2–3%). Following chronic, CYP-induced cystitis, CGRP- and SP-IR were dramatically

increased in spinal segments, and DRG (L6, S1) involved in micturition reflexes. The density of CGRP- and SP-IR was increased in the superficial laminae (I–II) and lateral collateral pathway of the L6 and S1 spinal segments. Following chronic cystitis, CGRP- and SP-IR in cells in the L6 and S1 DRG significantly increased, and the percentage of bladder afferent cells expressing CGRP- (76%) or SP-IR (11–18%) also significantly increased (Vizzard 2001).

The functional significance of an upregulation of CGRP or SP in bladder pathways following CYP-induced cystitis is not known, but the changes in neuropeptide expression and, presumably, the release at both central and/or peripheral projections of afferent pathways are possible. Several reports have suggested that neuropeptide-containing, capsaicin-sensitive bladder afferents may mediate urinary bladder hyperreflexia (Maggi 1991; Giuliani et al. 1993a, b; Ahluwalia et al. 1994, 1998). It has also been shown that intrathecal SP facilitates normal micturition, and SP antagonists delivered intrathecally depress normal micturition, indicating that the peptide may be involved as an excitatory transmitter in several types of bladder reflexes in the rat (Mersdorf et al. 1992; Lecci et al. 1993). CYP treatment in the rat induces cystitis that is characterized by increased frequency of voiding in awake rats and urinary bladder hyperreflexia in anesthetized rats (Maggi et al. 1992; Lecci et al. 1994; Lanteri-Minet et al. 1995). Furthermore, it has been demonstrated (Lecci et al. 1994) that intrathecal injection of SP antagonists reduces CYP-induced bladder hyperreflexia. Intrathecal administration of neurokinin receptor (NK)1 antagonists (RP 67580 and CP 96345) increase bladder capacity in normal conscious rats with no changes in voiding pressure, whereas NK2 receptor antagonists were ineffective (Lecci et al. 1994). Bladder hyperreflexia induced by capsaicin was reduced by an NK2 antagonist (SR 48965) with no effects on normal micturition (Lecci et al. 1994). Exogenous CGRP application or CRGP release from primary afferent nerves relaxes smooth muscle and produces relaxation, with bladder effects being more prominent in guinea pig and dog compared to rat and human (Maggi 1992; Andersson 1993). In addition to pharmacological demonstration of involvement of tachykinins/receptors systems in the LUT, studies with the preprotachykinin A (that encodes both SP and neurokinin A) null mouse have demonstrated involvement of tachykinins in the response to chemical irritation of the LUT as well as to the regulation of normal micturition activity (Kiss et al. 2001).

Recent studies with cizolirtine citrate, an inhibitor of CGRP and SP release at the spinal cord level, showed a significant reduction in the total number of voids per 24 h in patients with urinary incontinence secondary to OAB (Zat'ura et al. 2010). Further, cizolirtine citrate resulted in improvement in urinary incontinence and urgency in symptomatic outpatients with OAB and/or urodynamic diagnosis of detrusor overactivity (Martinez-Garcia et al. 2008). Therefore, it is possible that increased expression of CGRP- or SP-IR in bladder afferent cells and central and peripheral projections could contribute to this hyperreflexia. Changes in neuropeptide expression and release at central terminals could further result in a remodeling of spinal cord circuitry controlling micturition (Lecci et al. 1994). This remodeling may include changes (1) in the synaptic organization of spinal micturition reflexes;

(2) in the neurochemical coding of specific neuronal elements (primary afferent neurons, interneurons); and (3) in the organization of ascending and descending projections to spinal reflexes. Further, recent studies also demonstrate that SP released from nerve fibers or urothelial cells can act on urothelial receptors to release nitric oxide (Birder 2006, 2010). In response to stimulation of receptors on urothelial cells, SP and neurokinin A can be released from urothelial cells. Thus, neural as well as nonneural tachykinins may contribute to LUT pathways. Nonneural involvement of neuropeptides in the LUT has been recently addressed (Birder 2006, 2010).

The exact role(s) of tachykinins in urethral physiology is not known, but tachykinins induce urethral contraction in many species including humans (Maggi et al. 1988; Maggi 1992; Palea et al. 1996; Parlani et al. 1996; Canda et al. 2006, 2008). In patients with genitourinary prolapse with concomitant urinary incontinence, the density of SP-immunoreactive nerves in the perineal muscles was significantly decreased compared to a continent group suggestive of a role for tachykinins in urinary continence mechanisms (Busacchi et al. 2004). Additional research focused on the role of tachykinins in urethral physiology is necessary.

4 Role of Neurotrophic Factors in Neuropeptide Expression in Micturition Pathways after CYP-Induced Cystitis

Altered bladder neurotrophic factor content may underlie neurochemical (Vizzard and de Groat 1996; Vizzard 2000a, c, d, 2001) changes in bladder afferent neurons after cystitis. The occurrence of trophic interactions between nerve cells and target tissues is clearly demonstrated during embryonic and postnatal development (Lindsay et al. 1990; Oppenheim et al. 1991; Lapchak et al. 1992; Vantini and Skaper 1992). Recent experiments have demonstrated the influence of interactions between target organ and neurons in adult animals (Steers and de Groat 1988; Steers et al. 1991a, b; Tuttle and Steers 1992; Tuttle et al. 1994; Vizzard 2000b; Dupont et al. 2001). NGF is expressed under normal conditions in the urinary bladder, and part of its function is likely maintenance of sensory afferent fibers (Chao and Hempstead 1995; Chuang et al. 2001). We have demonstrated that chronic CYP-induced cystitis alters NGF expression and other neurotrophic factors in the bladder (Vizzard 2000b). The role(s) of NGF in micturition reflexes and sensation have been evaluated using a variety of addition and subtraction techniques (Dmitrieva and McMahon 1996; Clemow et al. 1998; Yoshimura et al. 2006). Importantly, exogenous NGF application to the rat bladder detrusor through an osmotic pump reduced bladder capacity and increased expression of CGRP in the lumbosacral spinal cord (Zvara and Vizzard 2007; Fig. 3).

Recent studies involving a novel NGF overexpressing (OE) mouse model (Cheppudira et al. 2008; Schnegelsberg et al. 2010) are being used to further define NGF-mediated changes in LUT structure and function and effects on

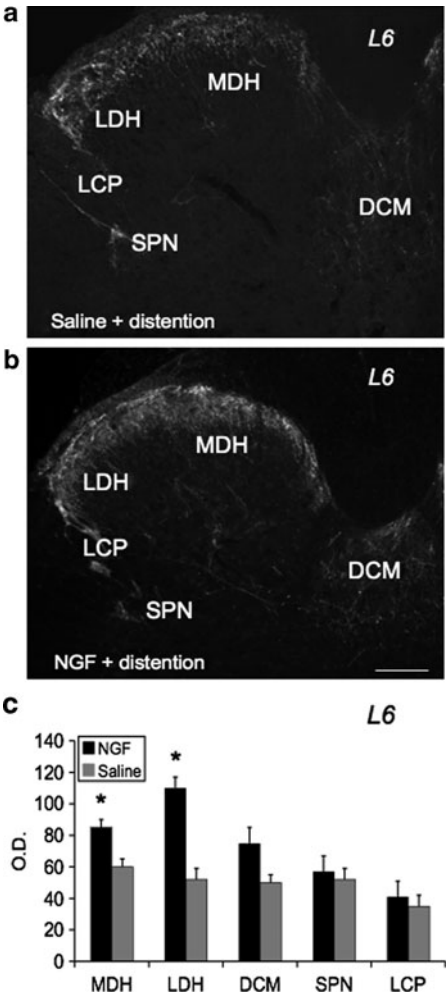

Fig. 3 CGRP spinal cord expression with exogenous NGF treatment. CGRP-IR increases in lumbosacral spinal cord with exogenous NGF treatment. CGRP-IR in the L6 (**a**, **b**) spinal segment in control (**a**) and NGF-treated (**b**) rats. A. Fluorescence photographs showing CGRP-IR in the L6 (**a**) spinal segment of control (saline) + bladder distention. (**b**) Fluorescence photographs showing CGRP-IR in the L6 (**b**) spinal segment with NGF treatment + bladder distention. Increased density of CGRP-IR was observed in the medial (MDH) to lateral (LDH) extent of the superficial laminae (I–II) of the dorsal horn (DH) with NGF treatment in L6 (**c**) segments. *Summary bar graph* of CGRP optical density (O.D.) as measured in specific regions of the L6 spinal cord (**c**). *Calibration bar* represents 125 μm. *SPN* sacral parasympathetic nucleus; *DCM* dorsal commissure; *LCP* lateral collateral pathway. *$p \leq 0.01$. Figure modified from Zvara and Vizzard (2007)

neuropeptide/receptor systems (Fig. 4). The urinary bladder in NGF OE mice exhibits marked hyperinnervation. To characterize the subpopulations of neurons contributing to the generalized hyperinnervation observed in NGF-OE transgenic

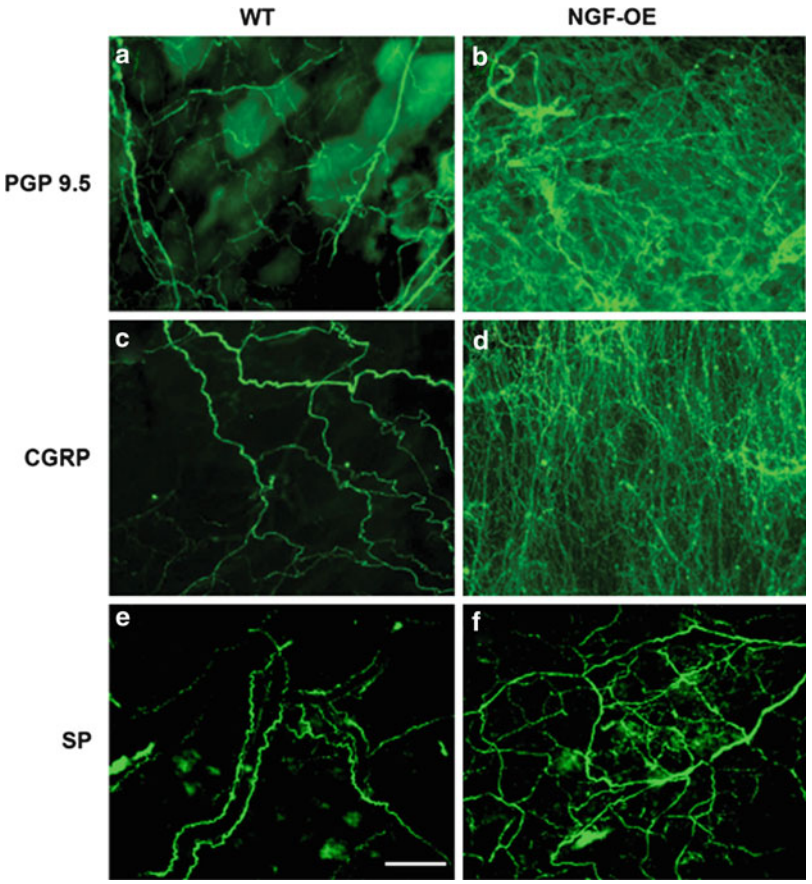

Fig. 4 Neuronal hyperinnervation in the urinary bladders of NGF-overexpressing (OE) transgenic mice. Representative fluorescence images of PGP 9.5 (**a**, **b**), CGRP (**c**, **d**), SP (**e**, **f**), immunoreactivity in the bladder neck region in urothelial whole-mount preparations of urinary bladders from 8- to 10-week-old female WT (**a**, **c**, **e**), and NGF-OE transgenic (**b**, **d**, **f**) mice from F0 line 23 (n = 3–5). *Scale bars*: **a**–**f**, 50 μm. Protein gene product (PGP 9.5), Calcitonin Gene-Related Protein (*CGRP*), Substance P (*SP*). Figure modified from Schnegelsberg et al. (2010)

mice, we have performed whole-mount immunostaining of bladder urothelium using a panel of neuronal markers. Using the pan-neuronal marker PGP 9.5, a marked increase in total nerve fiber density was seen in the urinary bladders of transgenic mice compared to WT controls (Fig. 4), consistent with the histology stains. This dense network was composed of CGRP-positive (Fig. 4) and substance P-positive (Fig. 4) unmyelinated C-fiber sensory afferents, NF 200-positive myelinated sensory afferents, and TH-positive, postganglionic sympathetic nerve fibers (Schnegelsberg et al. 2010). The increased nerve fiber density observed in transgenic mouse urinary bladders was evident in both the neck and the dome region of the urinary bladder. However, we observed a higher innervation density

within the urinary bladder neck region compared to the dome in both WT and transgenic mice. With CGRP immunostaining, we also observed CGRP-positive ganglia (5–9 CGRP-positive cells per ganglia) interspersed among CGRP-positive nerve fibers within the suburothelial plexus in transgenic mice. These CGRP-positive ganglia were not observed in whole-mount preparations of WT urinary bladder (Schnegelsberg et al. 2010). Studies are currently underway to examine effects of neuropeptide/receptor blockade on bladder function in NGF OE mice that exhibit bladder hyperreflexia with the presence of NVCs (Schnegelsberg et al. 2010).

Recent studies also demonstrate changes in PACAP/VIP and receptor expression in micturition pathways in NGF-OE mice (Girard et al. 2010b). Results demonstrate upregulation of PAC1 receptor transcript and PAC1-immunoreactivity in urothelium of NGF-OE mice, whereas PACAP transcript and PACAP-immunoreactivity were decreased in urothelium of NGF-OE mice (Girard et al. 2010b). In contrast, VPAC1 receptor transcript was decreased in both urothelium and detrusor smooth muscle of NGF-OE mice (Girard et al. 2010b). VIP transcript expression and immunostaining was not altered in urinary bladder of NGF-OE mice (Girard et al. 2010b). Changes in PACAP, VIP, and associated receptor transcripts and protein expression in micturition pathways resemble some, but not all, changes observed after induction of urinary bladder inflammation known to involve NGF production.

5 Bradykinin/Receptors System in Micturition Reflex Pathways

The bradykinin/receptors system plays an important role in normal micturition reflexes as well as in pathology (Lecci et al. 1995, 1999; Belichard et al. 1999; Meini et al. 2000; Chopra et al. 2005; Fabiyi and Brading 2006). Kinins are small peptides (8–10 amino acids) produced in plasma or in tissues at the sites of inflammation or tissue damage (Bhoola et al. 1992). Protease activation at the site of inflammation or tissue damage cleaves tissue/plasma kininogen precursors to release the nonapeptide, bradykinin (Dray and Perkins 1993). The biological effects of bradykinin are mediated by two different receptors, B1 and B2, with the bradykinin B2 receptor being constitutively expressed in various cell types and the B1 receptor being expressed *de novo* after inflammatory stimuli or tissue injury (Marceau et al. 1998; Lecci et al. 1999; Chopra et al. 2005). Recent studies (Chopra et al. 2005) have demonstrated B2 receptor expression in the detrusor smooth muscle and urothelium (apical and basal cells) consistent with reported bradykinin-evoked contractility of detrusor smooth muscle. It has been recently demonstrated that bradykinin-induced facilitation of micturition reflexes may be due to an increase in purinergic (P2) responsiveness (Chopra et al. 2005). Further, stimulation of the B2 receptor in cultured rat urothelial cells resulted in ATP release (Chopra et al. 2005). ATP release was reduced by the selective B2

receptor antagonist Hoe-140 and suggests that bradykinin may elicit bladder hyperreflexia indirectly through the release of ATP from the urothelium (Chopra et al. 2005). Direct effects of bradykinin on pelvic afferent nerves that evoke bladder hyperreflexia have also been demonstrated (Lecci et al. 1995). Thus, direct (neuronal) (Lecci et al. 1995) and indirect (urothelial secretion of ATP) (Chopra et al. 2005) actions of bradykinin on the LUT are likely to affect micturition reflexes.

5.1 Bradykinin/Receptors in Cystitis

The role of the bradykinin/receptor system in the LUT varies under control or pathological states. It is largely agreed that B1 receptor expression is undetectable in control urinary bladder (Ahluwalia and Perretti 1999; Chopra et al. 2005). Application of the selective B1 receptor agonist, des-Arg^9-bradykinin, exerts little or no effect on $[Ca^{2+}]_i$ or ATP release from cultured rat urothelial cells consistent with no or very low constitutive B1 receptor expression (Chopra et al. 2005). CYP-induced cystitis significantly upregulates B1 receptor expression in the detrusor smooth muscle and urothelium with acute (24 h) cystitis (Chopra et al. 2005). In cultured rat urothelial cells obtained from CYP (24 h)-treated rats, the B1 receptor agonist, des-Arg^9-bradykinin, evoked release of ATP and elevated calcium levels (Chopra et al. 2005). B1 receptor expression is also upregulated in bladder biopsies from patients with IC/BPS (Ruggieri et al. 1997). Cystometry performed on CYP (24 h)-treated rats demonstrated that the B1 receptor antagonist, des-Arg^{10}-Hoe-140, significantly reduced the frequency of nonvoiding bladder contractions (Chopra et al. 2005). In contrast, the B2 receptor antagonist, Hoe-140, decreased voiding frequency in CYP (24 h)-treated rats (Chopra et al. 2005). It is has been suggested that one explanation for these findings is the existence of B1-sensitive mechanisms/or afferent pathways underlying the emergence of nonvoiding contractions and B2 sensitive mechanisms underlying voiding contractions (Chopra et al. 2005). Additional studies are needed to address this possibility. Interestingly, chronic CYP-induced cystitis (8 days) resulted in a return to baseline of B1 receptor expression in the detrusor smooth muscle, whereas expression in the urothelium was similar to that observed with acute (24 h) CYP-induced cystitis (Chopra et al. 2005). Bradykinin/receptors systems may exert tissue-specific and inflammatory duration-dependent effects on LUT reflexes.

6 Endothelin/Receptors System in LUT Pathways

Endothelin-1 and endothelin receptors, ET_A and ET_B, contribute to LUT function under normal and pathological conditions (Ukai et al. 2006, 2008; Ogawa et al. 2008). In the LUT, endothelin-1 facilitates detrusor smooth muscle contraction in

various species including rabbits, rats, dogs, and humans and stimulates proliferation of the prostate and urinary bladder (Maggi et al. 1989; Garcia-Pascual et al. 1990; Persson et al. 1992; Langenstroer et al. 1997; Ogawa et al. 2004). Studies have demonstrated differential endothelin-1 contractile mechanisms depending on species (Persson et al. 1992). Differential endothelin receptor density has been revealed in the LUT, with the greatest density of ET_A receptors being present in the ureter (Latifpour et al. 1995; Saenz de Tejada et al. 1992; Afiatpour et al. 2003). In humans and other animals, ET_A expression is more dominant than ET_B in the bladder dome compared to the bladder base or urethra, which exhibits equal ET_A and ET_B receptor expression (Saenz de Tejada et al. 1992; Latifpour et al. 1995; Afiatpour et al. 2003). Endothelin expression has been detected in detrusor smooth muscle, urothelium, and vascular endothelium (Saenz de Tejada et al. 1992; Latifpour et al. 1995; Afiatpour et al. 2003).

6.1 Endothelin/Receptor System in Bladder Outlet Obstruction and Spinal Cord Transection

In animal models of BOO, modulation of endothelin-1 and ET_A and ET_B expression has been demonstrated in the LUT, and studies are researching the endothelin/receptor system as a potential therapeutic target for the treatment of bladder overactivity secondary to BOO (Khan et al. 1999; Ukai et al. 2008). BOO in rabbits is associated with increased expression of ET_A, ET_B, and endothelin-1 in detrusor smooth muscle (Khan et al. 1999; Ukai et al. 2008). The ET_A selective receptor antagonist, YM598, dose-dependently reduced the frequency of premicturition bladder contractions in BOO rats without effects on other voiding parameters (Ukai et al. 2008). Further, inhibition of the endothelin-converting enzyme, which metabolizes big endothelin to endothelin-1, decreased bladder overactivity in BOO rats (Schroder et al. 2004). Additional beneficial effects of repeated administration of endothelin-converting enzyme inhibitor in BOO rats included normalization of: bladder weight, micturition pressures, and voiding durations (Schroder et al. 2004). Suppression of ET_A receptors by intravenous infusion of ABT-627 in rats with complete spinal cord transection (thoracic 8–9) significantly decreased the amplitude and number of nonvoiding bladder contractions without changing bladder pressures, void volumes, or voiding efficiency (Ogawa et al. 2008). No effects on bladder function were observed when rats with spinal cord transection were similarly treated with an ET_B receptor antagonist, A-192621 (Ogawa et al. 2008). Thus, endothelin and ET_A receptors may be a novel therapeutic target to ameliorate the effects of bladder overactivity associated with BOO.

Additional studies are necessary to address the mechanisms by which YM598, an endothelin ETA receptor antagonist, suppresses the bladder overactivity observed in BOO rats; potentiation of postsynaptic norepinephrine effects and

endothelin-1 increase of interleukin-(IL) 6 have been suggested (Han et al. 2009). Interestingly, recent studies have demonstrated that endothelin-converting enzyme 1 promotes the recycling and resensitization of NK1 receptors and resensitization of the proinflammatory effects of SP (Cattaruzza et al. 2009). Thus, reductions in bladder overactivity secondary to BOO observed with endothelin-converting enzyme inhibition (described above) may, in part, be due to amelioration of the proinflammatory effects of SP (Cattaruzza et al. 2009).

6.2 Endothelin/Receptor Systems in the Urethra

There is very limited information concerning the distribution and function of endothelin/receptor systems in the urethra. ET receptors have been demonstrated in the urethral smooth muscle in several species (Latifpour et al. 1995; Afiatpour et al. 2003). ET_A-mediated smooth muscle contractions of the urethra in the rabbit have been demonstrated (Wada et al. 2000).

7 Perspectives

A large body of research supports a major role for neuropeptides in LUT function in health and disease, thereby identifying neuropeptide/receptor systems as potential novel therapeutic targets for the treatment of LUT disorders (Fig. 1). In animal models, pharmacological and/or genetic manipulation of SP, CGRP, VIP, PACAP, bradykinin, or endothelin function affects LUT function. Further, in animal models of LUT dysfunction or in clinical assessments, manipulation of neuropeptide/receptor systems improves void function. Furthermore, the field has become increasingly complex, with neuronal plasticity exhibited in pathophysiological situations where changes in neuropeptide expression patterns and receptor density are regularly observed. The study of neuropeptides in animal models has additionally revealed physiological and pathophysiological roles that in turn have led to the ongoing development of new drugs, through utilization predominantly of antagonist activities or blockade of release.

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References

Afiatpour P, Latifpour J, Takahashi W et al (2003) Developmental changes in the functional, biochemical and molecular properties of rat bladder endothelin receptors. Naunyn Schmiedebergs Arch Pharmacol 367:462–472

Ahluwalia A, Perretti M (1999) B1 receptors as a new inflammatory target. Could this B the 1? Trends Pharmacol Sci 20:100–104

Ahluwalia A, Maggi CA, Santicioli P et al (1994) Characterization of the capsaicin-sensitive component of cyclophosphamide-induced inflammation in the rat urinary bladder. Br J Pharmacol 111:1017–1022

Ahluwalia A, Giuliani S, Scotland R et al (1998) Ovalbumin-induced neurogenic inflammation in the bladder of sensitized rats. Br J Pharmacol 124:190–196

Andersson KE (1993) Pharmacology of lower urinary tract smooth muscles and penile erectile tissues. Pharmacol Rev 45:253–308

Andersson KE (1999) Advances in the pharmacological control of the bladder. Exp Physiol 84:195–213

Andersson KE (2002) Bladder activation: afferent mechanisms. Urology 59:43–50

Andersson KE (2006) Treatment-resistant detrusor overactivity-underlying pharmacology and potential mechanisms. Int J Clin Pract 60:8–16

Andersson KE, Arner A (2004) Urinary bladder contraction and relaxation: physiology and pathophysiology. Physiol Rev 84:935–986

Andersson KE, Wein AJ (2004) Pharmacology of the lower urinary tract: basis for current and future treatments of urinary incontinence. Pharmacol Rev 56:581–631

Arimura A (1998) Perspectives on pituitary adenylate cyclase activating polypeptide (PACAP) in the neuroendocrine, endocrine, and nervous systems. Jpn J Physiol 48:301–331

Arms LA, Girard BM, Vizzard MA (2009) In: Pontari M (ed) Role of the bladder urothelium in voiding dysfunction. Curr Bladder Dysfunct Rep 4:227–233

Belichard P, Luccarini JM, Defrene E et al (1999) Pharmacological and molecular evidence for kinin B-1 receptor expression in urinary bladder of cyclophosphamide-treated rats. Br J Pharmacol 128:213–219

Bhoola KD, Figueroa CD, Worthy K (1992) Bioregulation of kinins: kallikreins, kininogens, and kininases. Pharmacol Rev 44:1–80

Birder LA (2006) Urinary bladder urothelium: molecular sensors of chemical/thermal/mechanical stimuli. Vascul Pharmacol 45:221–226

Birder LA (2010) Urothelial signaling. Auton Neurosci 153:33–40

Bodin P, Burnstock G (2001) Evidence that release of adenosine triphosphate from endothelial cells during increased shear stress is vesicular. J Cardiovasc Pharmacol 38:900–908

Braas KM, May V, Zvara P et al (2006) Role for pituitary adenylate cyclase activating polypeptide in cystitis-induced plasticity of micturition reflexes. Am J Physiol Regul Integr Comp Physiol 290:R951–R962

Busacchi P, Perri T, Paradisi R et al (2004) Abnormalities of somatic peptide-containing nerves supplying the pelvic floor of women with genitourinary prolapse and stress urinary incontinence. Urology 63:591–595

Callsen-Cencic P, Mense S (1997) Expression of neuropeptides and nitric oxide synthase in neurones innervating the inflamed rat urinary bladder. J Auton Nerv Syst 65:33–44

Canda AE, Cross RL, Chapple CR (2006) Pharmacology of the lower urinary tract and management of the overactive bladder. J Turk German Gynecol Assoc 7:146–158

Canda AE, Cinar MG, Turna B et al (2008) Pharmacologic targets on the female urethra. Urol Int 80:341–354

Cattaruzza F, Cottrell GS, Vaksman N et al (2009) Endothelin-converting enzyme 1 promotes resensitization of neurokinin 1 receptor-dependent neurogenic inflammation. Br J Pharmacol 156:730–739

Chao MV, Hempstead BL (1995) p75 and Trk: a two-receptor system. Trends Neurosci 18:321–326
Chapple CR, Milner P, Moss HE et al (1992) Loss of sensory neuropeptides in the obstructed human bladder. Br J Urol 70:373–381
Cheng CL, Ma CP, de Groat WC (1995) Effect of capsaicin on micturition and associated reflexes in chronic spinal rats. Brain Res 678:40–48
Cheppudira BP, Girard BM, Malley SE et al (2008) Upregulation of vascular endothelial growth factor isoform VEGF-164 and receptors (VEGFR-2, Npn-1, and Npn-2) in rats with cyclophosphamide-induced cystitis. Am J Physiol Renal Physiol 295:F826–F836
Chopra B, Barrick SR, Meyers S et al (2005) Expression and function of bradykinin B1 and B2 receptors in normal and inflamed rat urinary bladder urothelium. J Physiol 562:859–871
Chuang YC, Fraser MO, Yu YB et al (2001) Analysis of the afferent limb of the vesicovascular reflex using neurotoxins, resiniferatoxin and capsaicin. Am J Physiol Regul Integr Comp Physiol 281:R1302–R1310
Clemow DB, Steers WD, McCarty R et al (1998) Altered regulation of bladder nerve growth factor and neurally mediated hyperactive voiding. Am J Physiol Regul Integr Comp Physiol 44: R1279–R1286
Cockayne DA, Hamilton SG, Zhu QM et al (2000) Urinary bladder hyporeflexia and reduced pain-related behaviour in P2X(3)-deficient mice. Nature 407:1011–1015
de Groat WC (1975) Nervous control of the urinary bladder of the cat. Brain Res 87:201–211
de Groat WC (1987) Neuropeptides in pelvic afferent pathways. Experientia 43:801–813
de Groat WC, Kruse MN (1993) Central processing and morphological plasticity in lumbosacral afferent pathways from the lower urinary tract. In: Mayer EA, Raybould HE (eds) Basic and clinical aspects of chronic abdominal pain. Pain research and clinical management, vol 9. Elsevier Science, Amsterdam, pp 219–235.
de Groat WC, Steers WD (1990) Autonomic regulation of the urinary bladder and sex organs. In: Loewy AD, Spyer KM (eds) Central regulation of autonomic functions. Oxford University Press, London, pp 310–333
de Groat WC, Yoshimura N (2009) Afferent nerve regulation of bladder function in health and disease. In: Canning BJ, Spina D (eds) Handbook of experimental pharmacology, Sensory nerves. Springer, Berlin, pp 91–138
de Groat WC, Nadelhaft I, Milne RJ et al (1981) Organization of the sacral parasympathetic reflex pathways to the urinary bladder and large intestine. J Auton Nerv Syst 3:135–160
de Groat WC, Kawatani M, Hisamitsu T et al (1986) Neural control of micturition: the role of neuropeptides. J Auton Nerv Syst suppl:369–387
de Groat WC, Booth AM, Yoshimura N (1993) Neurophysiology of micturition and its modification in animal models of human disease. In: Maggi CA (ed) The autonomic nervous system, vol 3. Harwood Academic, London, pp 227–290
de Groat WC, Vizzard MA, Araki I et al (1996) Spinal interneurons and preganglionic neurons in sacral autonomic reflex pathways. Prog Brain Res 107:97–111
de Groat WC, Kruse MN, Vizzard MA et al (1997) Modification of urinary bladder function after spinal cord injury. In: Seil FJ (ed) Advances in neurology, vol 72. Raven, New York, pp 347–364
Dickinson T, Mitchell R, Robberecht P et al (1999) The role of VIP/PACAP receptor subtypes in spinal somatosensory processing in rats with an experimental peripheral mononeuropathy. Neuropharmacology 38:167–180
Dmitrieva N, McMahon SB (1996) Sensitisation of visceral afferents by nerve growth factor in the adult rat. Pain 66:87–97
Donovan MK, Winternitz SR, Wyss JM (1983) An analysis of the sensory innervation of the urinary system of the rat. Brain Res Bull 11:321–324
Dray A, Perkins M (1993) Bradykinin and inflammatory pain. Trends Neurosci 16:99–104
Dupont MC, Spitsbergen JM, Kim KB et al (2001) Histological and neurotrophic changes triggered by varying models of bladder inflammation. J Urol 166:1111–1118

Ek A, Alm P, Andersson KE et al (1977) Adrenergic and cholinergic nerves of the human urethra and urinary bladder. Acta Physiol Scand 99:345–352
Erol K, Ulak G, Donmez T et al (1992) Effects of vasoactive intestinal polypeptide on isolated rat urinary bladder smooth muscle. Urol Int 49:151–153
Fabiyi AC, Brading AF (2006) The use of the isolated mouse whole bladder for investigating bladder overactivity. J Pharmacol Exp Ther 319:1386–1394
Fahrenkrug J, Hannibal J (1998) Pituitary adenylate cyclase activating polypeptide immunoreactivity in capsaicin-sensitive nerve fibres supplying the rat urinary tract. Neuroscience 83:1261–1272
Fowler CJ, Griffiths D, de Groat WC (2008) The neural control of micturition. Nat Rev Neurosci 9:453–466
Garcia-Pascual A, Larsson B, Andersson KE (1990) Contractile effects of endothelin-1 and localization of endothelin binding sites in rabbit lower urinary tract smooth muscle. Acta Physiol Scand 140:545–555
Girard BM, Wolf-Johnston A, Braas KM et al (2008) PACAP-mediated ATP release from rat urothelium and regulation of PACAP/VIP and receptor mRNA in micturition pathways after cyclophosphamide (CYP)-induced cystitis. J Mol Neurosci 36:310–320
Girard BM, Galli JR, Young BA, Vizzard MA, Parsons RL (2010a) PACAP expression in explant cultured mouse major pelvic ganglia. J Mol Neurosci. doi:10.1007/s12031-010-9359-4
Girard BM, Malley SE, Braas KM, May V, Vizzard MA (2010b) PACAP/VIP and receptor characterization in micturition pathways in mice with overexpression of NGF in urothelium. J Mol Neurosci. doi:10.1007/s12031-010-9384-3
Giuliani S, Patacchini R, Giachetti A et al (1993a) In vivo and in vitro activity of SR 48, 968, a non-peptide tachykinin NK-2 receptor antagonist. Regul Pept 46:314–316
Giuliani S, Santicioli P, Lippe IT et al (1993b) Effect of bradykinin and tachykinin receptor antagonist on xylene-induced cystitis in rats. J Urol 150:1014–1017
Gu BJ, Ishizuka O, Igawa Y et al (2000) Role of supraspinal tachykinins for micturition in conscious rats with and without bladder outlet obstruction. Naunyn Schmied Arch Pharmacol 361:543–548
Han JH, Lee MY, Myung SC (2009) The effect of endothelin-1 on the production of interleukin-6 in cultured human detrusor smooth muscle cells, and the effect of interleukin-6 on the contractile response of bladder smooth muscle strips from rats. BJU Int 104:707–712
Hernandez M, Barahona MV, Recio P et al (2006) Neuronal and smooth muscle receptors involved in the PACAP- and VIP-induced relaxations of the pig urinary bladder neck. Br J Pharmacol 149:100–109
Ho N, Koziol JA, Parsons CL (1997) Epidemiology of interstitial cystitis. In: Sant GR (ed) Interstitial cystitis. Lippincott-Raven, Philadelphia, pp 9–16
Hokfelt T, Zhang X, Wiesenfeld-Hallin Z (1994) Messenger plasticity in primary sensory neurons following axotomy and its functional implications. Trends Neurosci 17:22–30
Holstege G (2005) Micturition and the soul. J Comp Neurol 493:15–20
Hutchins B, Spears R, Hinton RJ et al (2000) Calcitonin gene-related peptide and substance P immunoreactivity in rat trigeminal ganglia and brainstem following adjuvant-induced inflammation of the temporomandibular joint. Arch Oral Biol 45:335–345
Igawa Y, Persson K, Andersson KE et al (1993) Facilitatory effect of vasoactive intestinal polypeptide on spinal and peripheral micturition reflex pathways in conscious rats with and without detrusor instability. J Urol 149:884–889
Ishizuka O, Alm P, Larsson B et al (1995a) Facilitatory effect of pituitary adenylate cyclase-activating polypeptide on micturition in normal, conscious rats. Neuroscience 66:1009–1014
Ishizuka O, Mattiasson A, Andersson KE (1995b) Tachykinin effects on bladder activity in conscious normal rats. J Urol 154:257–261
Johansson SL, Fall M (1994) Pathology of interstitial cystitis. In: Hanno PM (ed) Interstitial cystitis. Proc Urol Clin N Am 21:55–62
Johansson SL, Ogawa K, Fall M (1997) The pathology of interstitial cystitis. In: Sant GR (ed) Interstitial cystitis. Lippincott-Raven, Philadelphia, pp 143–152

Kataeva G, Agro A (1994) Substance-P-mediated intestinal inflammation: inhibitory effects of CP 96, 345 and SMS 201–995. Neuroimmunomodulation 1:350–356
Kawatani M, Takeshige C, de Groat W (1990) Central distribution of afferent pathways from the uterus of the cat. J Comp Neurol 302:294–304
Keast JR (1991) Patterns of co-existence of peptides and differences of nerve fibre types associated with noradrenergic and non-noradrenergic (putative cholinergic) neurons in the major pelvic ganglion of the male rat. Cell Tissue Res 266:405–415
Keast JR (1992) Location and peptide content of pelvic neurons supplying the muscle and lamina propria of the rat vas deferens. J Auton Nerv Syst 40:1–12
Keast JR, de Groat WC (1992) Segmental distribution and peptide content of primary afferent neurons innervating the urogenital organs and colon of male rats. J Comp Neurol 319:615–623
Khan MA, Dashwood MR, Thompson CS et al (1999) Up-regulation of endothelin-B (ETB) receptors and ETB receptor-mediated rabbit detrusor contraction in partial bladder outlet obstruction. BJU Int 84:714–719
Kiss S, Yoshiyama M, Cao YQ et al (2001) Impaired response to chemical irritation of the urinary tract in mice with disruption of the preprotachykinin gene. Neurosci Lett 313:57–60
Klück P (1980) The autonomic innervation of the human urinary bladder neck and urethra: a histochemical study. Anat Rec 198:439–447
Kullmann FA, Artim D, Birder LA et al (2008) Activation of muscarinic receptors in rat bladder sensory pathways alters reflex bladder activity. J Neurosci 28:1977–1987
Kuru M (1965) Nervous control of micturition. Physiol Rev 45:425–494
Langenstroer P, Tang R, Divish B et al (1997) Endothelins in canine genitourinary tissues. J Urol 157:1044–1048
Lanteri-Minet M, Bon K, de Pommery J et al (1995) Cyclophosphamide cystitis as a model of visceral pain in rats: model elaboration and spinal structures involved as revealed by the expression of c-Fos and Krox-24 proteins. Exp Brain Res 105:220–232
Lapchak PA, Araujo DM, Hefti F (1992) Neurotrophins in the central nervous system. Rev Neurosci 3:1–10
Latifpour J, Fukumoto Y, Weiss RM (1995) Regional differences in the density and subtype specificity of endothelin receptors in rabbit urinary tract. Naunyn Schmiedebergs Arch Pharmacol 352:459–468
Lecci A, Giuliani S, Garret C et al (1993) Evidence for a role of tachykinins as sensory transmitters in the activation of micturition reflex. Neuroscience 54:827–837
Lecci A, Giulani S, Santiciolo P et al (1994) Involvement of spinal tachykinin NK1 and NK2 receptors in detrusor hyperreflexia during chemical cystitis in anaesthetized rats. Eur J Pharmacol 259:129–135
Lecci A, Giuliani S, Meini S et al (1995) Pharmacological analysis of the local and reflex responses to bradykinin on rat urinary bladder motility in vivo. Br J Pharmacol 114:708–714
Lecci A, Meini S, Tramontana M et al (1999) Kinin B-1 receptor-mediated motor responses in normal or inflamed rat urinary bladder in vivo. Regul Pept 80:41–47
Lincoln J, Burnstock G (1993) Autonomic innervation of the urinary bladder and urethra. In: Maggi CA (ed) The autonomic nervous system, vol 3. Harwood Academic, London, pp 33–68
Lindsay RM, Shooter EM, Radeke MJ et al (1990) Nerve growth factor regulates expression of the nerve growth factor gene in adult sensory neurons. Eur J Neurosci 2:389–396
Luber-Narod J, Austin-Ritchie T, Hollins C et al (1997) Role of substance P in several models of bladder inflammation. Urol Res 25:395–399
Maggi CA (1991) The role of neuropeptides in the regulation of the micturition reflex: an update. Gen Pharmacol 22:1–24
Maggi CA (1992) The dual function of capsaicin-sensitive sensory nerves in the bladder and urethra. In: Maggi CA (Ed.) The autonomic nervous system. Nervous control of the urogenital system, vol. 2. Harwood Academic, London
Maggi CA (1995) Tachykinins and calcitonin gene-related peptide (CGRP) as co-transmitters released from peripheral endings of sensory nerves. Prog Neurobiol 45:1–98

Maggi CA, Santicioli P, Geppeti S et al (1988) Biochemical, anatomical and functional correlates of postnatal developments of the capsaicin-sensitive innervation of the rat urinary bladder. Dev Brain Res 43:183–190

Maggi CA, Giuliani S, Patacchini R et al (1989) Potent contractile activity of endothelin on the human isolated urinary bladder. Br J Pharmacol 96:755–757

Maggi CA, Lecci A, Santiciolo P et al (1992) Cyclophosphamide cystitis in rats: involvement of capsaicin-sensitive primary afferents. J Auton Nerv Syst 38:201–208

Marceau F, Hess JF, Bachvarov DR (1998) The B1 receptors for kinins. Pharmacol Rev 50:357–386

Marchand JE, Sant GR, Kream RM (1998) Increased expression of substance P receptor-encoding mRNA in bladder biopsies from patients with interstitial cystitis. Br J Urol 81:224–228

Marson L (1997) Identification of central nervous system neurons that innervate the bladder body, bladder base, or external urethral sphincter of female rats: a transneuronal tracing study using pseudorabies virus. J Comp Neurol 389:584–602

Martinez-Garcia R, Abadias M, Arano P et al (2008) Cizolirtine citrate, an effective treatment for symptomatic patients with urinary incontinence secondary to overactive bladder: a pilot dose-finding study. Eur Urol 56:184–192

May V, Vizzard MA (2010) Urinary bladder dysfunction and altered somatic sensitivity in pituitary adenylate cyclase activating polypeptide knockout (PACAP-/-) mice. J Urol 183: 772–779

Meini S, Patacchini R, Giuliani S et al (2000) Characterization of bradykinin B(2) receptor antagonists in human and rat urinary bladder. Eur J Pharmacol 388:177–182

Mersdorf A, Schmidt RA, Kaula N et al (1992) Intrathecal administration of substance P in the rat: the effect on bladder and urethral sphincteric activity. Urology 40:87–96

Middleton JW, Keast JR (2004) Artificial autonomic reflexes: using functional electrical stimulation to mimic bladder reflexes after injury or disease. Auton Neurosci 113:3–15

Mohammed H, Hannibal J, Fahrenkrug J et al (2002) Distribution and regional variation of pituitary adenylate cyclase activating polypeptide and other neuropeptides in the rat urinary bladder and ureter: effects of age. Urol Res 30:248–255

Morgan C, Nadelhaft I, de Groat WC (1981) The distribution of visceral primary afferents from the pelvic nerve within Lissauer's tract and the spinal gray matter and its relationship to the sacral parasympathetic nucleus. J Comp Neurol 201:415–440

Morrison JFB (1987) Reflex control of the lower urinary tract. In: Torrens M, Morrison JFB (eds) The physiology of the lower urinary tract. Springer, New York

Nadelhaft I, Vera PL (1995) Central nervous system neurons infected by pseudorabies virus injected into the rat urinary bladder following unilateral transection of the pelvic nerve. J Comp Neurol 359:443–456

Novak I (2003) ATP as a signaling molecule: the exocrine focus. News Physiol Sci 18:12–17

Ogawa T, Kamo I, Pflug BR et al (2004) Differential roles of peripheral and spinal endothelin receptors in the micturition reflex in rats. J Urol 172:1533–1537

Ogawa T, Sasatomi K, Hiragata S et al (2008) Therapeutic effects of endothelin-A receptor antagonist on bladder overactivity in rats with chronic spinal cord injury. Urology 71: 341–345

Oppenheim RW, Prevette D, Qin-Wei Y et al (1991) Control of embryonic motoneuron survival in vivo by ciliary neurotrophic factor. Science 251:1616–1618

Palea S, Corsi M, Artibani W et al (1996) Pharmacological characterization of tachykinin NK2 receptors on isolated human urinary bladder, prostatic urethra and prostate. J Pharmacol Exp Ther 277:700–705

Pang X, Marchand J, Sant GR et al (1995) Increased number of substance P positive nerve fibres in interstitial cystitis. Br J Urol 75:744–750

Parlani M, Conte B, Cirillo R et al (1996) Characterization of tachykinin NK2 receptor on dog proximal colon. Antagonism by MEN 10, 627 and SR 48, 968. Eur J Pharmacol 318: 419–424

Persson K, Garcia-Pascual A, Holmquist F et al (1992) Endothelin-1-induced contractions of isolated pig detrusor and vesical arterial smooth muscle: calcium dependence and phosphoinositide hydrolysis. Gen Pharmacol 23:445–453

Ruggieri MR, Wang J, Whitmore KE (1997) Expression of bradykinin 1 receptor subtype in interstitial cystitis bladder biopsies. J Urol 157:131–136

Saenz de Tejada I, Mueller JD, de Las MA et al (1992) Endothelin in the urinary bladder. I. Synthesis of endothelin-1 by epithelia, muscle and fibroblasts suggests autocrine and paracrine cellular regulation. J Urol 148:1290–1298

Schnegelsberg B, Sun TT, Cain G et al (2010) Overexpression of NGF in mouse urothelium leads neuronal hyperinnervation, pelvic sensitivity and changes in urinary bladder function. Am J Physiol Reg Integr Comp 298:R534–R547

Schroder A, Tajimi M, Matsumoto H et al (2004) Protective effect of an oral endothelin converting enzyme inhibitor on rat detrusor function after outlet obstruction. J Urol 172:1171–1174

Sherwood NM, Krueckl SL, McRory JE (2000) The origin and function of the pituitary adenylate cyclase-activating polypeptide (PACAP)/glucagon superfamily. Endocr Rev 21:619–670

Smet PJ, Moore KH, Jonavicius J (1997) Distribution and colocalization of calcitonin gene-related peptide, tachykinins, and vasoactive intestinal peptide in normal and idiopathic unstable human urinary bladder. Lab Invest 77:37–49

Steers WD, de Groat WC (1988) Effect of bladder outlet obstruction on micturition reflex pathways in the rat. J Urol 140:864–871

Steers WD, Ciambotti J, Etzel B et al (1991a) Alterations in afferent pathways from the urinary bladder of the rat in response to partial urethral obstruction. J Comp Neurol 310:1–10

Steers WD, Kolbeck S, Creedon D (1991b) Nerve growth factor in the urinary bladder of the adult regulates neuronal form and function. J Clin Invest 88:1709–1715

Studeny S, Cheppudira BP, Meyers S et al (2008) Urinary bladder function and somatic sensitivity in vasoactive intestinal polypeptide (VIP)-/- mice. J Mol Neurosci 36:175–187

Su HC, Polak JM, Mulderry PK et al (1986) Calcitonin gene-related peptide immunoreactivity in afferent neurons supplying the urinary tract: combined retrograde tracing and immunohistochemistry. Neuroscience 18:727–747

Tompkins JD, Girard BM, Vizzard MA, Parsons RL (2010) VIP and PACAP effects on mouse major pelvic ganglia neurons. J Mol Neurosci. doi:10.1007/s12031-010-9367-4

Traub RJ, Hutchcroft K, Gebhart GF (1999) The peptide content of colonic afferents decreases following colonic inflammation. Peptides 20:267–273

Tuttle JB, Steers WD (1992) Nerve growth factor responsiveness of cultured pelvic ganglion neurons from the adult rat. Brain Res 588:29–40

Tuttle JB, Steers WD, Albo M (1994) Neural input regulates tissue NGF and growth of the adult rat urinary bladder. J Auton Nerv Syst 49:147–158

Uckert S, Stief CG, Lietz B et al (2002) Possible role of bioactive peptides in the regulation of human detrusor smooth muscle – functional effects in vitro and immunohistochemical presence. World J Urol 20:244–249

Ukai M, Yuyama H, Noguchi Y et al (2006) Participation of endogenous endothelin and ETA receptor in premicturition contractions in rats with bladder outlet obstruction. Naunyn Schmiedebergs Arch Pharmacol 373:197–203

Ukai M, Yuyama H, Fujimori A et al (2008) In vitro and in vivo effects of endothelin-1 and YM598, a selective endothelin ET A receptor antagonist, on the lower urinary tract. Eur J Pharmacol 580:394–400

Vantini G, Skaper SD (1992) Neurotrophic factors: from physiology to pharmacology. Pharmacol Res 26:1–15

Vizzard MA (2000a) Alterations in spinal Fos protein expression induced by bladder stimulation following cystitis. Am J Physiol Regul Integr Comp 278:R1027–R1039

Vizzard MA (2000b) Changes in urinary bladder neurotrophic factor mRNA and NGF protein following urinary bladder dysfunction. Exp Neurol 161:273–284

Vizzard MA (2000c) Increased expression of spinal cord Fos protein induced by bladder stimulation after spinal cord injury. Am J Physiol Regul Integr Comp 279:R295–R305
Vizzard MA (2000d) Up-regulation of pituitary adenylate cyclase-activating polypeptide in urinary bladder pathways after chronic cystitis. J Comp Neurol 420:335–348
Vizzard MA (2001) Alterations in neuropeptide expression in lumbosacral bladder pathways following chronic cystitis. J Chem Neuroanat 21:125–138
Vizzard MA (2006) Neurochemical plasticity and the role of neurotrophic factors in bladder reflex pathways after spinal cord injury. Prog Brain Res 152:97–115
Vizzard MA, de Groat WC (1996) Increased expression of neuronal nitric oxide synthase (NOS) in bladder afferent pathways following chronic bladder irritation. J Comp Neurol 370:191–202
Vizzard MA, Erdman SL, de Groat WC (1993a) Localization of NADPH-diaphorase in bladder afferent and postganglionic efferent neurons of the rat. J Auton Nerv Syst 44:85–90
Vizzard MA, Erdman SL, de Groat WC (1993b) Localization of NADPH-diaphorase in pelvic afferent and efferent pathways of the rat. Neurosci Lett 152:72–76
Vizzard MA, Erdman SL, Förstermann U et al (1994) Differential distribution of nitric oxide synthase in neural pathways to the urogenital organs (urethra, penis, urinary bladder) of the rat. Brain Res 646:279–291
Vizzard MA, Erdman SL, de Groat WC (1995) Increased expression of neuronal nitric oxide synthase in dorsal root ganglion neurons after systemic capsaicin administration. Neuroscience 67:1–5
Wada Y, Latifpour J, Sanematsu H et al (2000) Age-related changes in contractile responses of rabbit lower urinary tract to endothelin. J Urol 164:806–813
Wanigasekara Y, Kepper ME, Keast JR (2003) Immunohistochemical characterisation of pelvic autonomic ganglia in male mice. Cell Tissue Res 311:175–185
Wu XR, Kong XP, Pellicer A et al (2009) Uroplakins in urothelial biology, function, and disease. Kidney Int 75:1153–1165
Yoshimura N, Bennett NE, Hayashi Y et al (2006) Bladder overactivity and hyperexcitability of bladder afferent neurons after intrathecal delivery of nerve growth factor in rats. J Neurosci 26:10847–10855
Yoshimura N, Kaiho Y, Miyazato M et al (2008) Therapeutic receptor targets for lower urinary tract dysfunction. Naunyn Schmiedebergs Arch Pharmacol 377:437–448
Yoshiyama M, de Groat WC (1997) Effects of intrathecal administration of pituitary adenylate cyclase-activating polypeptide (PACAP) on the lower urinary tract in the rat. Soc Neurosci Abstr 23:1523
Yoshiyama M, de Groat WC (2008a) Effects of intrathecal administration of pituitary adenylate cyclase activating polypeptide on lower urinary tract functions in rats with intact or transected spinal cords. Exp Neurol 211:449–455
Yoshiyama M, de Groat WC (2008b) The role of vasoactive intestinal polypeptide and pituitary adenylate cyclase-activating polypeptide in the neural pathways controlling the lower urinary tract. J Mol Neurosci 36:227–240
Zat'ura F, Vsetica J, Abadias M et al (2010) Cizolirtine citrate is safe and effective for treating urinary incontinence secondary to overactive bladder: a phase 2 proof-of-concept study. Eur Urol 57:145–152
Zvara P, Vizzard MA (2007) Exogenous overexpression of nerve growth factor in the urinary bladder produces bladder overactivity and altered micturition circuitry in the lumbosacral spinal cord. BMC Physiol 7:9
Zvara P, Braas KM, May V et al (2006) A role for pituitary adenylate cyclase activating polypeptide (PACAP) in detrusor hyperreflexia after spinal cord injury (SCI). Ann N Y Acad Sci 1070:622–628
Zvarova K, Dunleavy JD, Vizzard MA (2005) Changes in pituitary adenylate cyclase activating polypeptide expression in urinary bladder pathways after spinal cord injury. Exp Neurol 192:46–59

Cannabinoids: Potential Targets for Bladder Dysfunction

Michael R. Ruggieri, Sr

Contents

Abstract Cannabinoids are the active chemical components of *Cannabis sativa* (marijuana). The medical use of cannabis goes back over 5,000 years. Cannabinoids produce a very wide array of central and peripheral effects, some of which may have beneficial clinical applications. The discovery of cannabinoid receptors has spawned great interest within the pharmaceutical industry with the hopes of capitalizing on the beneficial effects of cannabis without the unwanted psychotropic effects on the central and peripheral nervous system. This chapter presents an overview of the pharmacology of cannabinoids and their derivatives. It reviews the current literature on central and peripheral cannabinoid receptors as related to effects on the lower urinary tract and the role of these receptors in normal and abnormal urinary tract function. An objective evaluation of the published results of clinical trials of cannabis extracts for the treatment of bladder dysfunction resulting from multiple sclerosis is also presented. It is clear that cannabinoid receptors are present in the lower urinary tract as well as spinal and higher centers involved in

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lower urinary tract control. Systemic cannabinoids have effects on the lower urinary tract that may be able to become clinically useful; however, a much greater understanding of the mechanisms of cannabinoid receptors in control of the human lower urinary tract is necessary to facilitate development of novel cannabinoid drugs for treatment of pelvic disorders.

Keywords Acrolein · Adenylyl cyclase · Bladder · Bladder contraction · Bladder spasticity · Cannabis · Cannabidiol · Cannabinoids · Capsazepine · Chemical cystitis · Cyclooxygenase · Cyclophosphamide · Endocannabinoids · Entourage lipids · G protein · Immunohistochemistry · Incontinence · Inflammation · In-situ hybridization · Intravesical turpentine · Leukotrienes · Marijuana · Micturition · Micturition volume threshold · Multiple sclerosis · Nerve growth factor · Nocturia · Overactive bladder · Pain · Pontine micturition center · Resiniferatoxin · Urinary frequency · Urinary hesitance · Urinary tract · Urinary urgency

1 Introduction

Natural products derived from plants have made a significant contribution to the development of modern drugs. It is estimated that approximately 25% of all the medicines on the market are derived from plant sources (De Smet 1997; Shu 1998). Preparations from the leaves of *Cannabis sativa* have been used to treat a variety of ailments since antiquity and the medical use dates back 5,000 years (Mechoulam and Fride 2001). Recreational use of cannabis has also been going on for millennia. It is the third most commonly used drug after tobacco and alcohol (Baker et al. 2003).

The major psychotropic component in *C. sativa* is Δ^9-tetrahydrocannabinol (Δ^9-THC) which is known to exert its biological effects primarily by action on two cannabinoid receptors, CB_1 and CB_2. Both are membrane bound, G-protein-coupled receptors. Activation results in pleiotropic G-protein signaling, primarily $G_{i/o}$-mediated reduction of adenylyl cyclase activity and subsequent inhibition of cAMP-mediated responses as well as a number of other signaling pathways such as activation of rho kinase and activation of large conductance, calcium activated potassium channels (Peters et al. 2009; Sade et al. 2006). Signaling through G_s-mediated increased intracellular cAMP has also been observed (Bonhaus et al. 1998).

The possibility that the orphan G-protein-coupled receptor GPR55 represents a third cannabinoid receptor is not certain because of conflicting pharmacological studies (Kapur et al. 2009). In addition some actions of cannabinoids may be mediated through activation of members of the intracellular peroxisome proliferator-activated receptor (PPAR) family (Mazzola et al. 2009; O'Sullivan 2007; O'Sullivan et al. 2009) and the transient receptor potential (TRP) family (Dinis et al. 2004a; Holzer 2004) of channels. CB_1 receptors are found primarily on central and peripheral nerves, whereas CB_2 receptors are located primarily on immune cells.

2 Cannabinoids and Their Receptors in the Urinary Tract

2.1 Endogenous Cannabinoids and Metabolism

Several endogenous eicosanoids have been identified that are cannabinoid receptor ligands including arachidonoylethanolamine (anandamide, AEA), 2-arachidonoylglycerol (2-AG), and 2-arachidonoylglycerol ether (noladin ether). The prototypic member of the endocannabinoids and the first to be discovered in mammalian brain and other tissues is anandamide (from the Sanskrit word ananda meaning "bliss"). AEA belongs to the 20:4, n-6 series of fatty acid amides (Devane et al. 1992). The other members of this series include docosatetraenoylethanolamine (22:4, n-6) and homo-γ-linolenoylethanolamine (20:3, n-6) (Hanus et al. 1993). Although the Nomenclature Committee of the International Union of Pharmacology (NC-IUPHAR) recommends naming receptors after their endogenous ligand, these receptors continue to be known as cannabinoid receptors because it is not yet completely clear which of these (and perhaps other) endogenous eicosanoids are the primary, or only, endogenous agonists for these receptors (Howlett et al. 2002). The endocannabinoids activate cannabinoid receptors; however, AEA is structurally related to the naturally occurring vanilloid capsaicin, a primary component of hot chili peppers (Caterina et al. 1997). Like capsaicin, AEA is a known agonist for TRP vanilloid-1 (TRPV1) receptors (Smart et al. 2000). However, unlike capsaicin, AEA does not evoke desensitization presumably due to a different allosteric modification of the TRPV1 receptor (Nagy et al. 2004).

The endogenous cannabinoids (endocannabinoids) are arachidonic acid metabolites that result from carboxylic acid modifications as opposed to the prostaglandins and the leukotrienes, which are side chain modifications of arachidonic acid. In contrast to the majority of hydrophilic neurotransmitters such as acetylcholine and norepinephrine, the lipophilic endocannabinoids are not stored in synaptic vesicles. AEA and 2-AG are synthesized by neurons and released on demand from membrane lipid precursors by distinct biochemical pathways involving first *N*-acetyltransferase then phospholipase C or D (Baker et al. 2003). As expected for their role as putative neurotransmitters or neuromodulators, they can undergo depolarization-induced release from neurons (Di Marzo et al. 1998). Both are inactivated after release by passive, diffusion-facilitated, endocannabinoid selective transporters. After uptake they are then hydrolyzed by the microsomal enzyme fatty-acid-amide hydrolyase (FAAH) (Ueda and Yamamoto 2000). 2-AG is also metabolized by monoacylglycerol lipase (Dinh et al. 2002) and arachidonoylglycerol ether is degraded by acylation (Fezza et al. 2002). The cannabinoid agonist effect can be potentiated by inhibitors of the uptake carrier mechanism including AM404 (Pertwee 2001) and VDM11 (Izzo et al. 2003; Mascolo et al. 2002).

AEA has also been reported to be metabolized by cyclooxygenase-2 into prostaglandin E_2 ethanolamine. This mechanism has been shown to cause contraction in the guinea pig trachea through activation of prostaglandin EP_1 receptors (Ross et al. 2002). In addition, FAAH degradation of AEA produces ethanolamine and the

cyclooxygenase substrate arachidonic acid which can lead to increased prostaglandin synthesis (Wahn et al. 2005). In the rat urinary bladder, inhibition of FAAH with URB597 reduces the contraction produced by AEA. This is consistent with the AEA-induced contraction being partly mediated indirectly through production of prostaglandins and activation of EP_1 receptors. Additional evidence for this includes the findings that the AEA-induced contraction of the rat bladder is not blocked by inhibition of CB_1 receptors with AM251 nor by inhibition of CB_2 receptors with AM5630 but nearly entirely abolished by the cyclooxygenase inhibitor indomethacin and partially attenuated with the EP_1 receptor antagonist ONO9130. Thus the contractile effect of AEA on the rat urinary bladder appears to be mediated partly by TRPV1 receptor activation, partly through production of prostaglandins and activation of EP1 receptors and perhaps by other, yet to be identified mechanisms but not by activation of CB_1 nor CB_2 receptors (Saitoh et al. 2007). There are no published reports of similar studies in human bladder muscle.

A series of three unsaturated *N*-acylethanolamines chemically related to AEA have been identified in cocoa powder, whose main target may be the brain endogenous cannabinoid system. One of these, the endocannabinoid AEA, can act as a direct cannabinoid mimetic and the other two (*N*-oleoylethanolamine and *N*-linoleoylethanolamine) inhibit the hydrolysis of AEA (di Tomaso et al. 1996). Because activation of the brain cannabinoid system is known to produce euphoria, it is possible that activation of the endogenous cannabinoid system may be involved in the pleasurable subjective feelings associated with eating chocolate. In addition cannabinoids might cooperate with other components of chocolate, including methylxanthines and biogenic amines, to intensify the sensory properties of chocolate and produce a transient feeling of well being which may produce a chocolate craving in susceptible persons.

2.2 Drugs Acting on Cannabinoid Receptors

Since the discovery of the CB_1 receptor which was first identified in 1988 (Devane et al. 1988) and then cloned and sequenced in 1990 (Matsuda et al. 1990) followed by the CB_2 receptor which was cloned and sequenced in 1993 (Munro et al. 1993), a large number of drugs acting on these receptors have been synthesized. There have been a number extensive reviews on this topic (Ashton and Ashton 2007; Ashton et al. 2007; Dewey 1986; Grotenhermen and Grotenhermen 2004, 2005; Howlett et al. 2002; Izzo and Coutts 2005; Machado Rocha et al. 2008). These compounds have been synthesized with the idea of generating compounds that are devoid of the unwanted central nervous system (CNS) mediated psychotropic effects of cannabis. If successful, these drugs may find clinical utility in treatment of a very wide variety of ailments ranging from pain, through gastrointestinal, cardiovascular, and chronic inflammatory conditions. Table 1, adapted, modified, and extended from Howlett et al. (2002), lists a number of these drugs including endogenous cannabinoids as well as some of the major cannabinoids isolated from *C. sativa*. This is not intended

Table 1 Selectivity of certain drugs at CB_1 and CB_2 receptors

Drug	Activity at CB_1 receptors	Activity at CB_2 receptors	CB_1/CB_2 selectivity ratio	References
Drugs with selectivity for CB_1 over CB_2 receptors				
2-Arachidonyl glyceryl ether	Agonist (endogenous)	Inactive[a]	>3,000	Hanus et al. (2001)
ACEA	Agonist	Inactive[a]	>2,000	Hillard et al. (1999)
O-1812	Agonist	Inactive[a]	1,138	Di Marzo et al. (2001)
SR14176A	Antagonist	Inactive[a]	1,119, 82 57, >1,000	Felder et al. (1995, 1998) Showalter et al. (1996), Rinaldi-Carmona et al. (1994)
AM281	Antagonist	Weak antagonist	350	Lan et al. (1999a)
ACPA	Agonist	Weak agonist	325	Hillard et al. (1999)
AM251	Antagonist	Weak antagonist	306	Lan et al. (1999b)
LY320135	Antagonist	Weak antagonist	106	Felder et al. (1998)
R-(+)-methanandamide	Agonist	Weak agonist	48, 41	Lin et al. (1998), Khanolkar et al. (1996)
Drugs without marked selectivity for CB_1 or CB_2 receptors				
Anandamide	Partial agonist (endogenous)	Partial agonist (endogenous)	32, 4, 4 4, 2	Lin et al. (1998), Showalter et al. (1996), Felder et al. (1995) Hillard et al. (1999), Mechoulam et al. (1995)
2-Arachidonyl glycerol	Partial agonist (endogenous)	Partial agonist (endogenous)	3, 2	Mechoulam et al. (1995), Ben-Shabat et al. (1998)
CT-3	Full agonist	Full agonist	9, 5	Dyson et al. (2005), Rhee et al. (1997)
HU-210	Full agonist	Full agonist	9, 2, 0.3	Felder et al. (1995), Rhee et al. (1997), Showalter et al. (1996)
AZ12646915	Full agonist	Full agonist	1	Walczak et al. (2009)
CP55940	Full agonist	Full agonist	0.4, 1, 1 1, 6	Ross et al. (1999), Felder et al. (1995), Rinaldi-Carmona et al. (1994), Showalter et al. (1996), Hillard et al. (1999)
Δ^9-THC	Partial agonist	Weak partial agonist	1, 1, 0.9 0.4, 0.1	Felder et al. (1995), Bayewitch et al. (1996), Showalter et al. (1996), Rhee et al. (1997), Rinaldi-Carmona et al. (1994)
Δ^8-THC	Partial agonist	Weak partial agonist	0.8	Busch-Petersen et al. (1996)
R-(+)-WIN55212	Full agonist	Full agonist	2, 0.3, 0.2 0.05, 0.03	Rinaldi-Carmona et al. (1994), Hillard et al. (1999), Showalter et al. (1996), Felder et al. (1995), Shire et al. (1996)

(*continued*)

Table 1 (continued)

Drug	Activity at CB_1 receptors	Activity at CB_2 receptors	CB_1/CB_2 selectivity ratio	References
Drugs with selectivity for CB_2 over CB_1 receptors				
JWH-015	Agonist	Agonist	4E-2	Showalter et al. (1996)
JWH-051	Agonist	Agonist	3E-2	Huffman et al. (1996)
L-768242	Agonist	Agonist	6E-3	Gallant et al. (1996)
JWH-139	Weak agonist	Agonist	6E-3	Huffman et al. (1999a)
AM 630	Antagonist	Agonist	6E-3	Ross et al. (1999)
JWH-133	Weak agonist	Agonist	5E-3	Huffman et al. (1999b)
L-759633	Weak agonist	Full agonist	6E-3,1E-3	Ross et al. (1999), Gareau et al. (1996)
L-759656	Inactive	Full agonist	2E-3,<5E-5	Ross et al. (1999), Gareau et al. (1996)
GP1a	Inactive	Agonist	1E-4	Murineddu et al. (2006)
HU-308	Weak agonist	Agonist	<1E-4	Hanus et al. (1999)
SR144528	Antagonist	Antagonist	1E-3,<1E-4	Rinaldi-Carmona et al. (1998), Ross et al. (1999)

[a]Inactive indicates that the compound has no detectable agonist or antagonist properties at the indicated receptor site

Abbreviations and synonyms – *ACEA* arachidonyl-2-chloroethylamide, *CT-3* Ajulemic acid (AJA, IP-751), *ACPA* arachidonylcyclopropylamide, SR14176A: Rimonabant™ (Acomplia®), *Δ^9-THC* Δ^9-tetrahydrocannabinol

to be an exhaustive list of all known compounds acting on cannabinoid receptors but rather to serve as a guide to recognizing the most widely used compounds in experimental studies. The CB_1/CB_2 selectivity ratio is based on the ratio of Ki values determined in radioligand binding studies for the in vitro displacement of [^{3}H]CP55940, [^{3}H]R-(+)-WIN55212, or [^{3}H]HU-243 from CB_1 and CB_2-specific binding sites. Based on this, the most selective CB_1 antagonist is SR14176A (Rimonabant™, Acomplia®) and the most selective CB_2 antagonist is SR144528. The most selective CB_1 agonist is the endocannabinoid 2-arachidonyl glyceryl ether, whereas the most selective full agonist at the CB_2 receptor is L-759656.

2.3 Cellular Location of Cannabinoid Receptors in the Urinary Tract and CNS Areas Involved in Control of Urinary Tract Function

The CNS distribution of CB receptors in many species including human, monkey, and rat has been investigated using autoradiography, in situ hybridization, and immunohistochemistry. These studies have been, for the most part, mutually confirmatory. Autoradiography studies with the mixed CB_1 and CB_2 receptor agonist [^{3}H]CP55,940 were carried out before cloning and sequencing of the CB_1 receptor made selective CB_1 receptor in situ hybridization and immunohistochemistry studies possible. [^{3}H]CP55,940 autoradiography did confirm the existence of high affinity cannabinoid receptors in areas of the brain predicted from the behavioral effects of cannabinoids, i.e., the cerebral cortex, hippocampus, basal ganglia, and cerebellum. The level of expression in these brain areas, which are associated with higher cognitive functioning, is often found to be greater than 1 pmol/mg. This level is higher than that of all other G-protein-coupled receptors and approaches the densities found for the common ionotropic receptors such as gamma amino butyric acid (GABA) and glutamate receptors (Bowery et al. 1987; Greenamyre et al. 1984). The higher CB_1 receptor expression in the amygdala and cingulate cortex of the human brain as compared to the receptor expression in the monkey or rat brain offers an explanation for the interspecies differences in the behavioral effects of cannabinoids (Herkenham et al. 1990). Much lower levels of CB_1 receptors are found in the hypothalamus and spinal cord. CB_1 receptors are nearly absent from the medullary nuclei that mediate respiratory and cardiovascular function, which seems to explain why cannabis overdose is rarely, if ever, fatal (Dewey 1986; Herkenham et al. 1990; Hollister 1986; Robson 2001). Cannabinoid receptor density as determined by [^{3}H]CP55,940 autoradiography in the area of the pontine micturition centers appears to be very low in both the rat (Herkenham et al. 1990) and human (Glass et al. 1997) brain. On the other hand, based on microinjection of CP55,940 into the ventrolateral aspect of the periaqueductal gray (PAG) area of the rat brain, this area appears to be important for the antinociceptive and cataleptic effects of the cannabinoids (Lichtman et al. 1996). Although this area of the PAG is

known to be involved in control of bladder function, it remains to be determined experimentally whether cannabinoids exert an effect on bladder function through actions on this area of the brain.

In situ hybridization studies largely confirmed the autoradiography results with the understanding that in situ hybridization identifies mRNA that is primarily located in cell bodies whereas autoradiography labels receptor protein that is distributed throughout the neuron. In general, brain areas show two patterns of distribution of CB_1 receptor transcript expressing neurons. A broad and uniform expression is found, for example, in the cerebellum where expression is observed for nearly all granule cells. In contrast, CB_1 receptor transcript expression is seen in only a few neurons at very high levels in areas such as hippocampus and cerebral cortex despite the labeling of nearly all cells in a given layer in the studies of receptor protein labeling by autoradiography (Mailleux et al. 1992; Matsuda et al. 1993; Westlake et al. 1994).

There have been several detailed immunohistochemical studies of CB_1 receptor protein expression in the rat brain that have confirmed the autoradiography and in situ hybridization studies (Egertova and Elphick 2000; Tsou et al. 1998). Electron microscopic immunohistochemistry has localized CB_1 receptors nearly exclusively on presynaptic terminals in the rat and human hippocampus (Hajos et al. 2000; Katona et al. 2000), and these are expressed on the entire presynaptic bouton (Rodriguez et al. 2001). The colocalization of CB_1 receptors on synaptic terminals that release GABA and cholecystokinin (CCK) indicates that CB_1 receptor activation may inhibit neurotransmission by decreasing GABA and CCK release (Katona et al. 1999; Tsou et al. 1999). In certain brain areas, CB_1 receptors and the endocannabinoid metabolizing enzyme FAAH are located on different neurons that form synaptic connections. For example in the hippocampus, the pyramidal neurons express FAAH but low levels of CB_1 receptors whereas the hippocampal interneurons that form synapses with pyramidal neurons express CB_1 receptors in high density but low levels of FAAH. A similar reciprocal relationship of CB_1 receptors and FAAH exists in the cerebellum between the Purkinje neurons that form synaptic connections with the cerebellar granule cells (Egertova et al. 1998; Tsou et al. 1998).

There have been far fewer studies of CB_2 receptor localization, and these indicate much lower density of CB_2 than CB_1 receptors in the CNS. Early immunohistochemistry studies were not able to detect the presence of CB_2 receptors in healthy brain (Galiegue et al. 1995; Griffin et al. 1999; Munro et al. 1993), whereas more recent studies, using more precise cannabinoid probes, have identified CB_2 receptors in neuronal and glial processes in a number of brain areas. In a number of these areas, CB_1 and CB_2 receptors may colocalize to the same brain structures but whether they may be colocalized on the same neurons has yet to be definitively determined (Gong et al. 2006; Onaivi et al. 2008). Investigation of CB_2 receptor localization in brain areas known to be involved control of micturition has not yet been reported. CB_2 receptors are known to be expressed primarily on cells of the immune system, and the spleen is often used as a positive tissue control in immunohistochemical studies (Hayn et al. 2008). This includes mast cells (Facci et al. 1995) which are known to increase with bladder inflammation.

For immunohistochemical studies, the specificity of the cellular localization of a given protein relies exclusively on the specificity of the antibody for the antigen under study, even more so than for Western blotting or immunoprecipitation techniques. For Western blots, proteins are first separated with an electrophoresis technique, usually based on their molecular weight. In this case, if the antisera contain antibodies that bind to proteins that are of a different molecular weight than the expected molecular weight of the protein under study, the staining of these bands can be ignored. For receptor immunoprecipitation, receptors can be radiolabeled with a ligand that selectively binds to the receptor; then after receptor solubilization, the radiolabeled receptors can be immunoprecipitated with the anti-receptor antibody; and the radioactivity in the immunoprecipitate can be used to determine receptor density. Nonspecific radiolabeling of proteins that do not bind to the antibody will not be detected. Likewise, nonspecific antibody binding to proteins that are not radiolabeled will also not be detected. This is not the case for staining of tissue sections because if the antisera also contain antibodies specific for antigenic determinants other than the receptor protein under study, these will also be observed, and these sites cannot be distinguished from specific staining of the intended receptor protein. This is the reason why controls are so critically necessary for immunohistochemical studies to be able to reliably reveal the true cellular location of a given protein.

One of the controls that is routinely reported in immunohistochemistry studies is omission of the primary antisera. While this determines the specificity of the secondary antibody and subsequent detection protocol, it reveals nothing about the specificity of the primary antisera. Another control that is less routinely reported is disappearance of labeling with preabsorption of the primary antisera with excess immunizing antigen. While this provides evidence that the binding of the primary antisera is being detected (immunologic specificity), it does not prove that the labeling is specific for antigenic determinants on the receptor of interest because if the antisera contains antibodies to nonreceptor determinants and these determinants are present in the tissue under study, then this staining will also disappear with antigen preabsorption. Antibodies to G-protein-coupled receptors are notorious for being nonspecific for their intended receptor sites in immunohistochemical assays (Bodei et al. 2009; Hamdani et al. 2009; Jensen et al. 2009; Jositsch et al. 2009; Lu et al. 2009; Pradidarcheep et al. 2009). The reason for this may be partly related to the relatively high degree of homology between both receptor subtypes within a given family as well as between different families. Because disappearance of staining in the presence of excess blocking antigen is considered insufficient to define specificity, the following more stringent criteria have been proposed: (1) inability to observe staining in animals with the target receptor knocked-out, (2) decreased staining with knock-down techniques such as siRNA or antisense treatment, (3) selectivity for the target receptor and not related subtypes in cells transfected with the target receptor, and (4) similar staining pattern observed with antibodies raised against different epitopes of the receptor (Michel et al. 2009). For CB_1 and CB_2 immunohistochemistry in tissues of the lower urinary tract, none of these more strict criteria for antisera specificity have been met.

The first immuno histofluorescent localization studies of CB_1 and CB_2 receptors in the bladder were carried out in the female rat bladder. Positive immunofluorescence staining was found for both subtypes, and staining was more pronounced in the urothelium suggesting localization in proximity to the afferent fibers present in the urothelium (Hayn et al. 2008). In this study, controls included absence of the primary antibody but controls for antisera specificity (blocking antisera binding to the tissue sections with excess immunizing antigen) were not included. These results were confirmed in the female mouse bladder for the CB_1 receptor using a different CB_1 antibody (Cayman Chemicals, Ann Arbor, MI as opposed to a CB_1 antibody from Santa Cruz Biotechnology, Santa Cruz, CA used in the previous study). Immunologic specificity of the staining in this mouse study was confirmed by substantially reduced immunofluorescence observed when tissue sections were incubated with a mixture of the antisera solution and the blocking peptide. This study found colocalization of CB_1 receptors with P_2X_3 receptors in the urothelial cells, primarily the umbrella cells exposed to the bladder lumen, as well as in some nerve fibers mainly in the muscular layer of the bladder wall (Walczak et al. 2009). The significance of the colocalization of CB_1 and P_2X_3 receptors on urothelial umbrella cells is that with urothelial stretching during bladder filling these umbrella cells release ATP (Apodaca et al. 2007; Wang et al. 2005) which can activate sensory nerves by activating P_2X receptors. Because most of the CB_1 positive nerve fibers were observed not to coexpress P_2X_3 receptors, these investigators concluded that any effects of cannabinoids on the purinergic sensory system would likely be mediated by reduction of urothelial ATP release as opposed to suppression of sensory nerve activity (Walczak et al. 2009). Similar colocalization studies of CB_1 and P_2X_3 receptors have not been reported for human urothelium.

Another report of immuno histofluorescent staining for CB_1 and CB_2 receptors in rat, monkey, and human bladders was carried out using antibodies from Sigma Chemical (St. Louis, MO). The Sigma Chemical catalog lists CB_1 and CB_2 receptor antibodies from both rabbit and goat hosts, and it was not reported which of these antibodies were used in this study. Controls were done only by omitting the primary antibodies and did not include antigen preabsorption controls; thus the immunologic specificity of these results for the CB_1 and CB_2 receptors is not completely certain. Positive CB_1 immunofluorescence was not observed in urothelial cells or in nerve structures but strong CB_2 immunoreactivity was visualized on the urothelial cells. In addition, sparse staining for CB_1 receptors was observed in cells having the appearance of immunocompetent cells between strands of smooth muscle cells and occasionally interspersed in the suburothelial region (Gratzke et al. 2009). This staining for CB_1 receptors on possible immune cells and CB_2 receptors on neuronal cells is the opposite of what has been reported by all other bladder immunohistochemical studies (Hayn et al. 2008; Tyagi et al. 2009; Walczak et al. 2009). Sections double stained with CB_2 receptor antibodies and antibodies to the vesicular acetylcholine transporter (VAChT) showed profiles labeled with both antibodies between strands of detrusor smooth muscle cells. Most of the CB_2 positive nerve fibers and varicosities were observed to also express calcitonin gene-related polypeptide (CGRP). In addition, slender nerve fibers that extended into the urothelium

stained positive for both CB_2 and TRPV1 receptors (Gratzke et al. 2009). Because both TRPV1 and CGRP labeled nerves are associated with sensory function, it was concluded that this apparent colabeling with CB_2 receptors suggests a possible role for cannabinoids in bladder afferent signaling.

Immunohistochemical localization of CB_1 and CB_2 receptors was determined in a study carried out entirely on human bladders obtained from male organ donors within 12 h after donor death using secondary antibodies labeled with horseradish peroxidase followed by diamino benzidine precipitation. This study used CB_1 and CB_2 receptor antibodies obtained from Abcam (Cambridge, MA), and controls included both omission of the primary antibodies as well as preabsorption of the primary antibodies with an excess of the control peptide. Dense staining for CB_1 receptors was observed in the urothelium and relatively less was observed in the detrusor cell layers. While CB_1 staining was reduced but not completely eliminated with antigen preabsorption, the CB_2 staining was only moderately reduced with preabsorption onto the control blocking peptide indicating insufficient specificity of this CB_2 antibody. Protein expression of CB_1 and CB_2 receptors determined using Western blot revealed that expression of both subtypes was greater in the urothelium than the detrusor muscle (Tyagi et al. 2009).

2.4 Effects of Cannabinoid Receptors on Normal Urinary Bladder Function

The first evidence for the presence of functional CB_1 receptors in the urinary bladder was from a study designed to determine whether cannabinoids inhibit electric field stimulated contractions of the mouse urinary bladder (Pertwee and Fernando 1996) as had previously been found for other innervated smooth muscle tissues including the mouse, rat and guinea pig vas deferens (Pacheco et al. 1991; Pertwee et al. 1992), and the mouse and guinea pig myenteric plexus-longitudinal small intestine muscle preparations (Pertwee et al. 1992, 1996). Electric field stimulation (EFS) of the isolated urinary bladder primarily causes contractions indirectly by evoking neurotransmitter release from nerve terminals because blocking action potentials with the sodium channel blocker tetrodotoxin nearly completely abolishes these contractions.

All cannabinoids tested including CP55,244, (−)-11-hydroxy-dimethylheptyl-Δ^8-THC, WIN55-212-2, Δ^9-THC, nabilone, and AEA caused a dose-dependent inhibition of EFS-induced (nerve evoked) mouse bladder contractions. The CB_1 selective antagonist SR14176A caused a dose dependent rightward displacement of these cannabinoid agonist concentration response curves; however, the CB_2 selective antagonist AM630 did not. Maximally effective concentrations of CP55,244 or Δ^9-THC had no effect on contractions induced by muscarinic receptor activation with acetylcholine or purinergic receptor activation with β,γ methylene ATP. The finding that the potency of SR14176A for inhibition of EFS-induced mouse bladder contractions is similar to its Ki for inhibition of [3H]CP55,940 binding to CB_1

receptors (Felder et al. 1995; Rinaldi-Carmona et al. 1994), and the ineffectiveness of the CB_2 selective antagonist AM630 is evidence for the involvement of CB_1 as opposed to CB_2 receptors in this cannabinoid effect (Pertwee and Fernando 1996). A similar study in the rat ventral prostate found that activation of epithelial CB_1 receptors causes inhibition of nerve evoked smooth muscle contraction via the cyclooxygenase pathway (Tokanovic et al. 2007).

These bladder findings were confirmed and extended in a later study in which the effects of cannabinoid agonists were determined on nerve evoked contractions of isolated muscle strips from rat, mouse, pig, dog, monkey, and human bladder (Martin et al. 2000). In both rat and mouse bladder, the rank order potency of cannabinoid receptor agonists for inhibition of electrically evoked contractions was CP55940 $\geq$ WIN55212 $>$ HU210 $>$ JWH015 $>$ AEA, which is consistent with that reported in cell lines transfected with human CB_1 receptors (Rinaldi-Carmona et al. 1998, Showalter et al. 1996). In the mouse bladder, the high potency of the CB_1 antagonist SR14176A (apparent $pK_B = 8.7$) and low potency of the CB_2 antagonist SR1445278 ($pK_B < 6.5$) for reversing the agonist-induced inhibition of nerve-evoked contractions are also consistent with CB_1 and not CB_2 receptors mediating this effect. In contrast, a high potency was found in the rat bladder for both SR14176A (apparent $pK_B = 8.4$) and SR1445278 ($pK_B = 8.0$) for inhibition of the effects of WIN55212-2 or JWH015. These results indicate that both CB_1 and CB_2 receptors are involved in the cannabinoid-induced inhibition of nerve evoked contractions in the isolated rat bladder.

In stark contrast to the findings in the mouse and rat bladder, nerve evoked contractions of muscle strips from dog, pig, primate, or human bladder were completely resistant to activation of cannabinoid receptors with up to 3 μM WIN55212-2 (Martin et al. 2000). This implies that cannabinoid effects on the in vitro mouse or rat bladder do not replicate effects on the isolated human bladder. It also seems to imply that any clinical benefit of cannabinoid therapy for bladder dysfunction is not likely to be mediated by interaction with presynaptic receptors in the bladder unless the bladder dysfunction causes induction of bladder presynaptic cannabinoid receptors. Other investigators reported that the CB_1 agonist ACEA and the CB_2 agonist GP1a seem to reduce nerve evoked contractions of human bladder muscle strips; however, the paucity of available human tissue precluded quantitative analysis of the data (Tyagi et al. 2009). Results from another laboratory report that AEA increases, but CP55940 decreases nerve evoked contractions of human, monkey, and rat bladder muscle strips (Gratzke et al. 2009). The AEA effect, in this instance, may be a result of its activation of TRPV1 receptors as opposed to its activity at cannabinoid receptors; however, there was no effect of AEA on baseline tonus of the human, monkey, or rat bladder strips (Gratzke et al. 2009), whereas AEA was previously reported to induce contraction of rat bladder strips through a CB_1 and CB_2 independent, possibly TRPV1-mediated mechanism (Saitoh et al. 2007).

The effect of activation of cannabinoid receptors on afferent nerve activity was reported in an ex-vivo mouse bladder-nerve preparation in the same report described above that colocalized CB_1 and P_2X_3 receptors in the urothelial umbrella cells (Walczak et al. 2009). Intravesical administration of the CB_1 and CB_2 agonist

AZ12646915 reduced the distension evoked activity of bladder afferents in the pelvic nerve. This inhibition was prevented by previous administration of the CB_1 selective antagonist AM251 implicating CB_1 receptor involvement in the peripheral modulation of bladder afferent signaling. It would be very informative to repeat this study in P_2X_3 knockout mice to determine whether the cannabinoid suppression of afferent nerve activity induced by bladder distension is truly mediated by P_2X_3 containing neurons. The mixed CP_1 and CP_2 agonist ajulemic acid has been reported to reduce the increased release of CGRP induced by capsaicin and ATP in the rat bladder. This effect was prevented by the CB_1 antagonist AM251 and the CB_2 antagonist AM630 (Hayn et al. 2008). Because nearly all bladder sensory fibers are immunoreactive for both the capsaicin receptor TRPV1 and CGRP (Avelino et al. 2002), capsaicin induced CGRP release serves as a marker for measuring bladder afferent sensory nerve activity.

2.5 Cannabinoid Effects on Inflamed Bladder

Intravesical infusion of a 50% solution of turpentine into the rat bladder is a well established model of bladder pain and inflammation. The most reproducible effect is a reduction in the volume of saline infused onto the bladder that is necessary to cause a bladder contraction (micturition volume threshold). This decrease is apparent soon after intravesical turpentine exposure and lasts for at least 24 h (McMahon 1988; McMahon and Abel 1987). Infusion of AEA but not palmitoylethanolamine (PEA) into the carotid artery before and 30 min into the 1 h intravesical turpentine exposure prevents this decrease if micturition volume threshold is reevaluated immediately after the turpentine exposure (Jaggar et al. 1998a). In a different report from this same laboratory, both AEA and PEA reversed the decrease in micturition threshold if administered 2 h after the intravesical turpentine exposure and allowing 1 h before reevaluation of micturition threshold (Jaggar et al. 1998b). Neither AEA nor PEA had any effect on inflammation-associated plasma extravasation of Evans Blue into the bladder tissue after intra arterial infusion indicating a lack of cannabinoid effect on this measure of bladder inflammation (Jaggar et al. 1998b). As described in more detail below, one explanation for the different effect of PEA in these two studies is that PEA may be enhancing the effects of endogenous endocannabinoids released as a result of the bladder inflammation, and this release and enhancement of their effects does not occur immediately.

The receptor responsible for the cellular action of PEA is not certain. Although an initial study reported that PEA displaces [3H]WIN55212-2 binding to RBL-2H3 cell membranes that are known to express CB_2 receptors (Facci et al. 1995), this was not replicated by several subsequent studies (Jacobsson and Fowler 2001; Lambert and Di Marzo 1999; Lambert et al. 2001; Showalter et al. 1996; Sugiura et al. 2000). It is now generally accepted that PEA does not bind to either CB_1 or CB_2 receptors. More recently it has become evident that certain substances that are not themselves endocannabinoids are cosynthesized and cosecreted with

endocannabinoids. These lipids can have enhancing effects on endocannabinoids through inhibition of hydrolysis (Mazzola et al. 2009), increasing the binding, or otherwise potentiating the biological effect of endocannabinoids (Ben-Shabat et al. 1998; Garcia et al. 2009; Ho et al. 2008; Jonsson et al. 2001; Smart et al. 2002). This facultative action has been named the entourage effect and has been described for several monoacylglycerols including oleamide (Lambert and Di Marzo 1999), some *N*-acyl dopamines (De Petrocellis et al. 2004), and a number of saturated *N*-acyl ethanolamines including PEA (Lambert and Di Marzo 1999; Smart et al. 2002). In addition PEA is known to activate the intracellular nuclear receptor PPAR-α (O'Sullivan 2007; O'Sullivan et al. 2009), and the antiinflammatory action of PEA is absent in PPAR-α null mice (Lo Verme et al. 2005). The exact relationship between the entourage effect of PEA on cannabinoid receptors and how endogenous PEA interacts with PPAR-α to regulate inflammation remains to be determined. However, it is clear that conclusions made by the earlier studies based on the use PEA as a selective CB_2 receptor agonist need to be reevaluated in light of these new concepts of the action of PEA and other entourage lipids.

The chemical cystitis produced by intravesical turpentine is reproduced by intravesical infusion of nerve growth factor (NGF), and this is reversed by NGF sequestration with a fusion protein made from the NGF receptor, trkA, coupled to the Fc portion of an immunoglobulin (Dmitrieva et al. 1997; Jaggar et al. 1999). The NGF-induced decrease in micturition volume threshold is prevented by intra carotid artery administration of AEA or PEA. The AEA effect is blocked by the CB_1 antagonist SR141716A or the CB_2 antagonist SR144528; however, the PEA effect is only blocked by the CB_2 and not the CB_1 antagonist (Farquhar-Smith et al. 2002). Based on this data alone, the entourage lipid PEA does seem to result in selective activation of the CB_2 receptor over the CB_1 receptor; however, both subtypes appear to be involved in the inflammation induced decrease in micturition volume threshold. Bladder inflammation is associated with an increase in the number of cells in the spinal cord expressing Fos, the protein product of the immediate early gene *c-fos* (Nazif et al. 2007). This is most evident in the L6 spinal level (Birder and de Groat 1992). Both AEA and PEA reduce this increase in Fos positive L6 spinal neurons following intravesical turpentine exposure. The AEA effect is not antagonized by the CB_1 antagonist SR141716A nor the CB_2 antagonist SR144528; however, the PEA effect is antagonized by the CB_2 but not the CB_1 antagonist (Farquhar-Smith et al. 2002). Thus AEA reduces bladder inflammation induced spinal Fos expression by an unidentified mechanism that appears to be independent of CB_1 and CB_2 receptors, whereas PEA reduces Fos expression by an action that involves selective activation of CB_2 receptors.

Intravesical turpentine instillation in the rat is also associated with hyperalgesia of the hind limbs as assessed by decreased limb withdrawal latency in response to a 46°C infrared beam applied to the foot pad. This is thought to be a result of the fact that both the hind limbs and the urinary bladder receive sensory innervation from the same spinal cord segments in the rat. This referred hind limb hyperalgesia is also reproduced by intravesical NGF exposure, which is blocked by the trkA–IgG fusion protein that sequesters NGF (Jaggar et al. 1999). The referred thermal

hyperalgesia induced by intravesical turpentine is attenuated by both AEA and PEA. The AEA effect is blocked by the CB_1 selective antagonist SR141716A but not by the CB_2 selective antagonist SR144528, whereas the PEA effect is blocked by the CB_2 but not the CB_1 antagonist (Farquhar-Smith et al. 2002). Therefore, in contrast to the intravesical NGF induced reduction in micturition volume threshold in which the AEA effect seems to involve both CB_1 and CB_2 receptors, and the AEA effect on bladder inflammation induced increase in spinal Fos expression which seems to be independent of both CB_1 and CB_2 receptors, the AEA effect on the referred thermal hyperalgesia of the hind limbs seems to involve only CB_1 and not CB_2 receptors. On the other hand, the effects of the entourage lipid PEA on these three effects of bladder inflammation are consistent in that the effect involves selective activation of CB_2 and not CB_1 receptors. It is not entirely clear how PEA, which is not thought to be able to bind to the CB_2 receptor, is able to cause selective activation of CB_2 receptors. There are no reports that the entourage effect of PEA which involves prevention of hydrolysis or increasing the binding of endogenous cannabinoids might be selective for CB_2 over CB_1 receptors. This possibility seems to be worth investigating. It also might be informative to repeat these studies using the recently identified selective CB_2 receptor agonists that are known to bind to the CB_2 receptor such as L768242, JHW051, or JHW015 as well as some of the other entourage lipids. Similar studies of the action of PEA in PPAR-α null mice in this model may shed light on whether an antiinflammatory effect of PEA is involved.

Another well established model of experimental bladder inflammation in the rat involves exposure of the bladder contents to acrolein either directly by intravesical infusion or indirectly by intraperitoneal injections of cyclophosphamide (CYP), which is metabolized to acrolein and excreted in the urine (Cox 1979). This is well known to induce a painful hemorrhagic cystitis associated with a decreased micturition volume threshold and increase in bladder reflex activity. These can be reversed by desensitizing bladder TRPV1 receptors with intravesical resiniferatoxin (Dinis et al. 2004b). Increased bladder reflex activity is assessed by monitoring bladder pressure increases during continuous bladder infusion through a suprapubic tube placed into the bladder dome. Application of the TRPV1 receptor antagonist capsazepine onto the serosal surface of the bladder reduces these increased volume-induced bladder contractions induced by intraperitoneal CYP. The bladder tissue AEA content measured by mass spectrometry was increased after CYP, reaching a peak 72 h after injection which exactly mirrors the peak in development of increased volume-induced bladder contractions. Serosal application of AEA onto the naïve bladder increases volume-induced bladder contractions in a concentration dependent fashion. This effect of AEA can be blocked acutely by the TRPV1 antagonist capsazepine or by previous TRPV1 desensitization with intravesical resiniferatoxin. Serosal application of the CB_1 antagonist SR141716A increases AEA potency in naïve but not CYP injected animals. Serosal application of an inhibitor of FAAH (palmitoylisopropylamide) increases these volume-induced bladder contractions in both naïve and CYP inflamed bladders. The FAAH inhibitor effect is blocked by capsazepine or resiniferatoxin pretreatment in naïve rats. Intravesical AEA causes increased spinal cord Fos expression which is attenuated by capsazepine or

resiniferatoxin pretreatment (Dinis et al. 2004a). These results indicate that the development of increased volume-induced bladder contractions and hyperalgesia following bladder inflammation in the rat involves endogenous AEA acting through TRPV1 receptors and a slight blunting of this effect through AEA action on CB_1 receptors. It is unknown whether similar events may occur in human bladder.

Ajulemic acid, a synthetic analog of Δ^9-THC, reduces the frequency of these volume-induced bladder contractions in normal rats and reduces the increased contraction frequency induced by previous intraperitoneal CYP injections or 30 min of intravesical infusion of 0.25% acetic acid. Administration of the CB_1 selective antagonist AM251 increases the contraction frequency in CYP injected rats and reverses the ajulemic acid induced decreased contraction frequency in normal, acetic acid infused, and CYP injected rats, whereas the CB_2 selective antagonist AM251 has no influence on the ajulemic acid induced effects (Hiragata et al. 2007). These results indicate that CYP-induced bladder inflammation in the rat is associated with an increased effect of endogenous cannabinoids on volume-induced bladder contractions and confirms the previous studies that exogenous cannabinoids can suppress the bladder contractile frequency induced by nociceptive stimuli through an action on CB_1 as opposed to CB_2 receptors. It is not known whether similar effects occur in human bladder.

The tissue concentration of CB_1 and CB_2 receptors in the rat bladder following acute or chronic intravesical exposure to acrolein has been determined using Western blot. Two days after a single intravesical exposure to 1 mM acrolein, CB_2 receptors are increased statistically significantly by approximately 50% in the bladder detrusor muscle but not the bladder mucosa, L5, L6, or S1 dorsal root ganglia, or spinal cord segments L3-5 or L6-S. Acute or chronic inflammation (three intravesical acrolein exposures every 3 days) has no statistically significant effect on CB_1 receptors in any of these tissues. Chronic inflammation causes a statistically significant increase in CB_2 receptors in the detrusor by 96% and in bladder mucosa by 39% but has no effect in the other tissues (Merriam et al. 2008). If similar effects occur in human bladder, these results suggest that the CB_2 receptor might be a viable target for treatment of bladder inflammation and associated pain. This is consistent with the previously described results of PEA reducing the effects of bladder inflammation through selective activation of CB_2 receptors. However, this does not seem to fit in with the in vivo studies described above indicating that CB_1 but not CB_2 receptors are involved in the effect of cannabinoids on inflammation induced increased bladder activity.

2.6 Clinical Studies of Cannabinoids for Control of Bladder Dysfunction in Multiple Sclerosis

Because of a very high placebo response rate in medical trials for treatment of multiple sclerosis (MS), the use of completely ineffective treatments is associated

with symptom improvements in 65–70% of patients (Sibley 1992). Therefore, reports of symptom improvement for a given treatment in less than 70% of participants may not truly represent an effective treatment. The results of a questionnaire given to 53 MS patients in the United Kingdom and 59 MS patients in the United States that was designed to document the claimed benefits of cannabis use by MS patients who use cannabis did indicate such a high level of improvement for the general categories of pain, spasticity, and tremor (>87%) but not for urinary urgency (64%), hesitancy (59%), or incontinence (55%) (Consroe et al. 1997). In an N-of-1 trial of four sequential 4 week treatments with Nabilone, placebo, Nabilone then placebo, there was a striking reduction of frequency of nocturia during each of the 4-week Nabilone treatments along with decreased severity of muscle spasm and improvement in mood (Martyn et al. 1995).

The first exploratory, placebo controlled, clinical trial of cannabis medical extract (CME) treatment was conducted in the United Kingdom with a mixed group of patients suffering from intractable neurogenic symptoms including 18 with MS, 4 with spinal cord injury, 1 with brachial plexus damage, and 1 patient with limb amputation due to neurofibromatosis (Wade et al. 2003). Because smoking was considered an inappropriate delivery method for a pharmaceutical product and oral bioavailability is unpredictable, the sublingual route was chosen. The test medications were whole plant extracts of Δ^9-THC-rich CME, cannabidiol (CBD)-rich CME, and a 1:1 mixture of the two (THC:CBD) supplied in a pump spray for oromucosal delivery with a peppermint flavor to disguise the taste of the CME. Cannabidiol was chosen because it is nonpsychoactive, has potent antioxidant and antiinflammatory properties, and may modulate unwanted THC effects. The first phase was a 2 week open labeled trial in which the patients could increase their daily dose up to 120 mg until an optimal dose was obtained. This was followed by an 8 week double blind crossover phase with four 2-week stages using placebo, THC:CBD, THC alone, or CBD alone. For ethical reasons, patients were supplied with a vial of THC:CBD for use as a "rescue" medication but were encouraged to use it sparingly. While pain relief associated with both THC and CBD was significantly superior to placebo and some individual patients reported benefits with bladder control, there were no statistically significant cannabinoid treatment related improvements in the bladder symptom visual analog scale (VAS) nor in the numerical symptom scales for incontinence frequency, incontinence severity, urinary urgency, or nocturia frequency. VAS is a method for measuring symptoms in which patients are asked to place a mark along a 100 mm line with one end of the line representing "no symptoms" and the other end representing the "worst symptoms ever." Thus symptoms can be measured "down to the millimeter" and relatively easy to apply parametric statistics can be used to assess the data.

In the cannabinoids for treatment of spasticity and other symptoms related to multiple sclerosis (CAMS) study, 630 MS patients were treated at 33 UK centers with oral cannabis extract ($n = 211$), Δ^9-tetrahydrocannabinol (Δ^9-THC; $n = 206$), or placebo ($n = 213$) during a 13 week treatment trial. This study found no treatment effect of cannabinoids on the primary outcome measure ($p = 0.40$), change in overall spasticity scores, using the Ashworth scale. Likewise,

no treatment effect on bladder symptoms was identified (Zajicek et al. 2003). A "substudy" of the above described CAMS study reviewed results for urge incontinence using a 3 day urinary diary at baseline and at the completion of treatment, week 13 (Freeman et al. 2006). While all three treatment groups showed a decrease in urge incontinence episodes from baseline to the end of treatment, there was a significant treatment effect for each of the cannabis groups over the placebo. The cannabis extract treated group had a 25% reduction ($p = 0.005$) and the THC treated group had a 19% reduction ($p = 0.039$) relative to placebo. Thus, in contrast to the negative finding of the CAMS study for the primary outcome measure of spasticity, the results do suggest a beneficial clinical effect of cannabis treatment on urge urinary incontinence episodes.

In an open label study of the effect on bladder dysfunction of these same sublingual spray preparations, data collected from 15 of the 21 recruited patients with advanced MS were sufficiently complete for evaluation. Patients were allowed to dose titrate up to 120 mg/day for the THC:CBD-CME over an 8 week period followed by THC-CME only for an additional 8 weeks. For both treatments, urinary urgency, number and volume of incontinence episodes, urinary frequency, and nocturia all decreased significantly. Improvements were also reported in patient self-assessment of pain, spasticity, and quality of life. Part of the improvement in urinary symptoms may have been related to total urinary output which decreased significantly during treatment indicating that the patients were drinking less fluid. Patients entering into the final long-term safety and efficacy extension part of the study all chose to take the THC only CME because they found it more effective for their bladder symptoms. This THC-CME preference over the THC:CBD-CME was confirmed during the dose titration portion of the study because patients took significantly less of the THC only preparation to achieve the same therapeutic effect (Brady et al. 2004). Because of few reports of troublesome side effects, it was concluded that cannabis-based medical extracts are a safe and effective treatment for the urinary dysfunction in patients with advanced MS.

A parallel group, double blind, randomized, multicenter, placebo-controlled trial has been carried out in 160 MS patients on the effect of the THC:CBD-CME (Sativex) oromucosal spray on the primary symptom that each patient reports (Wade et al. 2004). It should be noted that cannabis clinical trials are never truly double blind because most patients can recognize the CNS effects of cannabis, and thus they are completely aware of whether they are receiving the placebo or the active cannabis extract. For eligibility, MS patients of any type were required to volunteer one of the following primary symptoms at a severity of at least 50% for the most troublesome symptom on a 100 mm VAS: bladder problems, spasticity, spasms, tremors, or pain that was not obviously musculoskeletal. Of the 80 patients assigned to the active treatment and placebo control treatment groups, only 3 patients per group did not complete the 6 week treatment leaving 77 patients per treatment group completing the study. Although the primary symptom score on the VAS improved for both active and placebo treated groups, there was no statistically significant difference between groups. Patients with pain as the primary symptoms showed a large effect that was nearly identical in both active and placebo treatment

groups, and if the data is analyzed after removing the patients with pain as the primary symptom, then a statistically significant difference was obtained in the primary symptom score between active and placebo treatments. This study concluded that THC:CBD-CBE (Sativex) is an effective treatment for spasticity associated with MS. Although not statistically significant, a greater improvement was observed in VAS scores and diary data for bladder control for the active compared to the placebo treated group.

It should be noted that there is also evidence for a beneficial effect of cannabinoids in an animal model of MS. This model is known as Theiler's murine encephalomyelitis virus-induced demyelinating disease (TMEV-IDD) and is induced by intracerebral injection of the virus in mice. An increase in the concentration of the endocannabinoids 2-AG and PEA but not AEA is observed in the spinal cord 2 months after inducing the infection. If the TMEV-IDD animals are treated with PEA (5 mg/kg/day) for 10 days during days 60–70 postinfection, their performance on the rotarod test improved significantly from the untreated group to become not statistically significantly different from the sham infected controls. In addition their vertical and horizontal activity improved indicating a significant reduction in the motor disability of the animals subjected to this model of MS (Loria et al. 2008).

3 Conclusions

Development of cannabinoid drugs for treatment of lower urinary tract disorders is currently at a very early stage, and thus the above review raises many more questions than answers. Answers to many of these questions is expected to significantly facilitate development of therapeutic cannabinoids. Many of the findings in animal models need to be confirmed in human tissue. For example, while CB_1 but not CB_2 receptor activation in both mouse and rat inhibits nerve evoked bladder contractions, neither subtype affects nerve evoked human bladder contractions. While CYP-induced bladder inflammation in the rat induces an increased effect of endocannabinoids and exogenous CB_1 but not CB_2 agonists can suppress the bladder overactivity induced by nociceptive stimuli, it is not known whether similar events occur in the inflamed human bladder. While relatively normal human bladder specimens are available from organ transplant donors as well as from nonaffected bladder tissue obtained from cancer cystectomy surgeries, inflamed human bladder is not readily available for laboratory study. Microtechnique are beginning to be applied to the study of the in vitro contractility of human bladder muscle biopsy tissue (Fry et al. 2010), and this type approach may be able to address these issues using biopsy material from inflamed or otherwise diseased human bladder.

The following is a list of some of these currently unanswered questions whose answers can be expected to greatly facilitate development of cannabinoid drugs for treatment of lower urinary tract disorders:

What is the mechanism of action of AEA in human bladder and what is the relative role of direct CB_1, CB_2, or TRPV1 receptor activation vs. metabolism to PgE_2-ethanolamine and activation of EP_1 receptors?

Are P_2X_3 receptors colocalized with CB_1 receptors in human urothelium as they are in the rat and what is the effect of bladder inflammation on this?

Are the CB_1 receptors in the PAG the primary target of the antinociceptive action of cannabinoids in human bladder pain or are cannabinoid receptors in the spinal cord and bladder afferent nerves also involved?

How does PEA cause selective CB_2 receptor activation when it does not bind to CB_2 receptors? Does PEA selectively increase the binding of endocannabinoids to CB_2 over CB_1 receptors? What is the role of PPAR-α in this mechanism?

Which cannabinoid receptor subtype is most important in inflammation induced increased human bladder activity?

What is the effect of CME in a clinical trial specifically designed to investigate effects on urinary urge incontinence or pelvic pain?

References

Apodaca G, Balestreire E, Birder LA (2007) The uroepithelial-associated sensory web. Kidney Int 72(9):1057–1064

Ashton JC, Ashton JC (2007) Cannabinoids for the treatment of inflammation. Curr Opin Invest Drugs 8(5):373–384

Ashton JC, Smith PF, Ashton JC, Smith PF (2007) Cannabinoids and cardiovascular disease: the outlook for clinical treatments. Curr Vasc Pharmacol 5(3):175–185

Avelino A, Cruz C, Nagy I, Cruz F (2002) Vanilloid receptor 1 expression in the rat urinary tract. Neuroscience 109(4):787–798

Baker D, Pryce G, Giovannoni G, Thompson AJ (2003) The therapeutic potential of cannabis. Lancet Neurol 2(5):291–298

Bayewitch M, Rhee MH, Avidor-Reiss T, Breuer A, Mechoulam R, Vogel Z (1996) (−)-Delta9-tetrahydrocannabinol antagonizes the peripheral cannabinoid receptor-mediated inhibition of adenylyl cyclase. J Biol Chem 271(17):9902–9905

Ben-Shabat S, Fride E, Sheskin T, Tamiri T, Rhee MH, Vogel Z et al (1998) An entourage effect: inactive endogenous fatty acid glycerol esters enhance 2-arachidonoyl-glycerol cannabinoid activity. Eur J Pharmacol 353(1):23–31

Birder LA, de Groat WC (1992) Increased c-fos expression in spinal neurons after irritation of the lower urinary tract in the rat. J Neurosci 12(12):4878–4889

Bodei S, Arrighi N, Spano P, Sigala S, Bodei S, Arrighi N et al (2009) Should we be cautious on the use of commercially available antibodies to dopamine receptors? Naunyn Schmiedebergs Arch Pharmacol 379(4):413–415

Bonhaus DW, Chang LK, Kwan J, Martin GR (1998) Dual activation and inhibition of adenylyl cyclase by cannabinoid receptor agonists: evidence for agonist-specific trafficking of intracellular responses. J Pharmacol Exp Ther 287(3):884–888

Bowery NG, Hudson AL, Price GW (1987) GABAA and GABAB receptor site distribution in the rat central nervous system. Neuroscience 20(2):365–383

Brady CM, DasGupta R, Dalton C, Wiseman OJ, Berkley KJ, Fowler CJ (2004) An open-label pilot study of cannabis-based extracts for bladder dysfunction in advanced multiple sclerosis. [see comment]. Mult Scler 10(4):425–433

Busch-Petersen J, Hill WA, Fan P, Khanolkar A, Xie XQ, Tius MA et al (1996) Unsaturated side chain beta-11-hydroxyhexahydrocannabinol analogs. J Med Chem 39(19):3790–3796

Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D (1997) The capsaicin receptor: a heat-activated ion channel in the pain pathway. [see comment]. Nature 389 (6653):816–824

Consroe P, Musty R, Rein J, Tillery W, Pertwee R (1997) The perceived effects of smoked cannabis on patients with multiple sclerosis. Eur Neurol 38(1):44–48

Cox PJ (1979) Cyclophosphamide cystitis – identification of acrolein as the causative agent. Biochem Pharmacol 28(13):2045–2049

De Petrocellis L, Chu CJ, Moriello AS, Kellner JC, Walker JM, Di Marzo V et al (2004) Actions of two naturally occurring saturated N-acyldopamines on transient receptor potential vanilloid 1 (TRPV1) channels. Br J Pharmacol 143(2):251–256

De Smet PA (1997) The role of plant-derived drugs and herbal medicines in healthcare. Drugs 54(6):801–840

Devane WA, Dysarz FA 3rd, Johnson MR, Melvin LS, Howlett AC (1988) Determination and characterization of a cannabinoid receptor in rat brain. Mol Pharmacol 34(5):605–613

Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G et al (1992) Isolation and structure of a brain constituent that binds to the cannabinoid receptor. [see comment]. Science 258(5090):1946–1949

Dewey WL (1986) Cannabinoid pharmacology. Pharmacol Rev 38(2):151–178

Di Marzo V, Melck D, Bisogno T, De Petrocellis L (1998) Endocannabinoids: endogenous cannabinoid receptor ligands with neuromodulatory action. Trends Neurosci 21(12):521–528 [erratum appears in Trends Neurosci 1999 Feb;22(2):80]

Di Marzo V, Bisogno T, De Petrocellis L, Brandi I, Jefferson RG, Winckler RL et al (2001) Highly selective CB(1) cannabinoid receptor ligands and novel CB(1)/VR(1) vanilloid receptor "hybrid" ligands. Biochem Biophys Res Commun 281(2):444–451

di Tomaso E, Beltramo M, Piomelli D (1996) Brain cannabinoids in chocolate. Nature 382 (6593):677–678 [see comment]

Dinh TP, Freund TF, Piomelli D, Dinh TP, Freund TF, Piomelli D (2002) A role for monoglyceride lipase in 2-arachidonoylglycerol inactivation. Chem Phys Lipids 121 (1–2):149–158

Dinis P, Charrua A, Avelino A, Yaqoob M, Bevan S, Nagy I et al (2004a) Anandamide-evoked activation of vanilloid receptor 1 contributes to the development of bladder hyperreflexia and nociceptive transmission to spinal dorsal horn neurons in cystitis. J Neurosci 24(50):11253–11263

Dinis P, Charrua A, Avelino A, Cruz F, Dinis P, Charrua A et al (2004b) Intravesical resiniferatoxin decreases spinal c-fos expression and increases bladder volume to reflex micturition in rats with chronic inflamed urinary bladders. BJU Int 94(1):153–157

Dmitrieva N, Shelton D, Rice AS, McMahon SB (1997) The role of nerve growth factor in a model of visceral inflammation. Neuroscience 78(2):449–459

Dyson A, Peacock M, Chen A, Courade JP, Yaqoob M, Groarke A et al (2005) Antihyperalgesic properties of the cannabinoid CT-3 in chronic neuropathic and inflammatory pain states in the rat. Pain 116(1–2):129–137

Egertova M, Elphick MR (2000) Localisation of cannabinoid receptors in the rat brain using antibodies to the intracellular C-terminal tail of CB. J Comp Neurol 422(2):159–171

Egertova M, Giang DK, Cravatt BF, Elphick MR (1998) A new perspective on cannabinoid signalling: complementary localization of fatty acid amide hydrolase and the CB_1 receptor in rat brain. Proc R Soc Lond Ser B Biol Sci 265(1410):2081–2085

Facci L, Dal Toso R, Romanello S, Buriani A, Skaper SD, Leon A (1995) Mast cells express a peripheral cannabinoid receptor with differential sensitivity to anandamide and palmitoylethanolamide. Proc Natl Acad Sci USA 92(8):3376–3380

Farquhar-Smith WP, Jaggar SI, Rice AS, Farquhar-Smith WP, Jaggar SI, Rice ASC (2002) Attenuation of nerve growth factor-induced visceral hyperalgesia via cannabinoid CB(1) and CB(2)-like receptors. Pain 97(1–2):11–21

Felder CC, Joyce KE, Briley EM, Mansouri J, Mackie K, Blond O et al (1995) Comparison of the pharmacology and signal transduction of the human cannabinoid CB_1 and CB_2 receptors. Mol Pharmacol 48(3):443–450

Felder CC, Joyce KE, Briley EM, Glass M, Mackie KP, Fahey KJ et al (1998) LY320135, a novel cannabinoid CB1 receptor antagonist, unmasks coupling of the CB1 receptor to stimulation of cAMP accumulation. J Pharmacol Exp Ther 284(1):291–297

Fezza F, Bisogno T, Minassi A, Appendino G, Mechoulam R, Di Marzo V et al (2002) Noladin ether, a putative novel endocannabinoid: inactivation mechanisms and a sensitive method for its quantification in rat tissues. FEBS Lett 513(2–3):294–298

Freeman RM, Adekanmi O, Waterfield MR, Waterfield AE, Wright D, Zajicek J (2006) The effect of cannabis on urge incontinence in patients with multiple sclerosis: a multicentre, randomised placebo-controlled trial (CAMS-LUTS). Int Urogynecol J 17(6):636–641

Fry CH, Byliss M, Pirolo JS, Ikeda Y, Hussain M (2010) The influence of age, gender and bladder dysfunction on the contractile properties of isolated human detrusor smooth muscle. BJU Int in press, first published online: 11 Nov 2010, doi: 10.1111/j.1464-410X.2010.09845.x

Galiegue S, Mary S, Marchand J, Dussossoy D, Carriere D, Carayon P et al (1995) Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. Eur J Biochem 232(1):54–61

Gallant M, Dufresme C, Girard Y, Gual D, Leblanc Y, Prasit P et al (1996) New class of potent ligands for the human peripheral cannabinoid receptor. Bioorg Med Chem Lett 6(19):2263–2268

Garcia MdC, Adler-Graschinsky E, Celuch SM (2009) Enhancement of the hypotensive effects of intrathecally injected endocannabinoids by the entourage compound palmitoylethanolamide. Eur J Pharmacol 610(1–3):75–80

Gareau Y, Dufresne C, Gallant M, Rochette C, Sawyer N, Slipetz DM et al (1996) Structure activity relationships of tetrahydrocannabinol analogues on human cannabinoid receptors. Bioorg Med Chem Lett 6(2):189–194

Glass M, Dragunow M, Faull RL (1997) Cannabinoid receptors in the human brain: a detailed anatomical and quantitative autoradiographic study in the fetal, neonatal and adult human brain. Neuroscience 77(2):299–318

Gong JP, Onaivi ES, Ishiguro H, Liu QR, Tagliaferro PA, Brusco A et al (2006) Cannabinoid CB2 receptors: immunohistochemical localization in rat brain. Brain Res 1071(1):10–23

Gratzke C, Streng T, Park A, Christ G, Stief CG, Hedlund P et al (2009) Distribution and function of cannabinoid receptors 1 and 2 in the rat, monkey and human bladder. J Urol 181 (4):1939–1948

Greenamyre JT, Young AB, Penney JB (1984) Quantitative autoradiographic distribution of L-[3H]glutamate-binding sites in rat central nervous system. J Neurosci 4(8):2133–2144

Griffin G, Wray EJ, Tao Q, McAllister SD, Rorrer WK, Aung MM et al (1999) Evaluation of the cannabinoid CB2 receptor-selective antagonist, SR144528: further evidence for cannabinoid CB2 receptor absence in the rat central nervous system. Eur J Pharmacol 377(1):117–125

Grotenhermen F, Grotenhermen F (2004) Pharmacology of cannabinoids. Neuroendocrinol Lett 25(1–2):14–23

Grotenhermen F, Grotenhermen F (2005) Cannabinoids. Curr Drug Targets CNS Neurol Disord 4(5):507–530

Hajos N, Katona I, Naiem SS, MacKie K, Ledent C, Mody I et al (2000) Cannabinoids inhibit hippocampal GABAergic transmission and network oscillations. Eur J Neurosci 12 (9):3239–3249

Hamdani N, van der Velden J, Hamdani N, van der Velden J (2009) Lack of specificity of antibodies directed against human beta-adrenergic receptors. Naunyn Schmiedebergs Arch Pharmacol 379(4):403–407 [Erratum appears in Naunyn Schmiedebergs Arch Pharmacol. 2009 Jun;379(6):637]

Hanus L, Gopher A, Almog S, Mechoulam R (1993) Two new unsaturated fatty acid ethanolamides in brain that bind to the cannabinoid receptor. J Med Chem 36(20):3032–3034

Hanus L, Breuer A, Tchilibon S, Shiloah S, Goldenberg D, Horowitz M et al (1999) HU-308: a specific agonist for CB(2), a peripheral cannabinoid receptor. Proc Natl Acad Sci USA 96(25):14228–14233

Hanus L, Abu-Lafi S, Fride E, Breuer A, Vogel Z, Shalev DE et al (2001) 2-arachidonyl glyceryl ether, an endogenous agonist of the cannabinoid CB1 receptor. Proc Natl Acad Sci USA 98 (7):3662–3665

Hayn MH, Ballesteros I, de Miguel F, Coyle CH, Tyagi S, Yoshimura N et al (2008) Functional and immunohistochemical characterization of CB1 and CB2 receptors in rat bladder. Urology 72(5):1174–1178

Herkenham M, Lynn AB, Little MD, Johnson MR, Melvin LS, de Costa BR et al (1990) Cannabinoid receptor localization in brain. Proc Natl Acad Sci USA 87(5):1932–1936

Hillard CJ, Manna S, Greenberg MJ, DiCamelli R, Ross RA, Stevenson LA et al (1999) Synthesis and characterization of potent and selective agonists of the neuronal cannabinoid receptor (CB1). J Pharmacol Exp Ther 289(3):1427–1433

Hiragata S, Ogawa T, Hayashi Y, Tyagi P, Seki S, Nishizawa O et al (2007) Effects of IP-751, ajulemic acid, on bladder overactivity induced by bladder irritation in rats. Urology 70 (1):202–208

Ho WS, Barrett DA, Randall MD, Ho WSV (2008) 'Entourage' effects of N-palmitoylethanolamide and N-oleoylethanolamide on vasorelaxation to anandamide occur through TRPV1 receptors. Br J Pharmacol 155(6):837–846

Hollister LE (1986) Health aspects of cannabis. Pharmacol Rev 38(1):1–20

Holzer P (2004) TRPV1 and the gut: from a tasty receptor for a painful vanilloid to a key player in hyperalgesia. Eur J Pharmacol 500(1–3):231–241

Howlett AC, Barth F, Bonner TI, Cabral G, Casellas P, Devane WA et al (2002) International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. Pharmacol Rev 54(2):161–202

Huffman JW, Yu S, Showalter V, Abood ME, Wiley JL, Compton DR et al (1996) Synthesis and pharmacology of a very potent cannabinoid lacking a phenolic hydroxyl with high affinity for the CB2 receptor. J Med Chem 39(20):3875–3877

Huffman JW, Yu S, Liddle J, Wiley JL, Abood M, Martin BR et al (eds) (1999) 1-Deoxy-1_, 1_dimethylalkyl-_8-THC derivatives: selective ligands for the CB2 receptor. In: 1999 Symposium on the Cannabinoids 1998. International Cannabinoid Research Society, Acapulco, Mexico, Burlington, Vermont

Huffman JW, Liddle J, Yu S, Aung MM, Abood ME, Wiley JL et al (1999b) 3-(1′, 1′-Dimethylbutyl)-1-deoxy-delta8-THC and related compounds: synthesis of selective ligands for the CB2 receptor. Bioorg Med Chem 7(12):2905–2914

Izzo AA, Coutts AA (2005) Cannabinoids and the digestive tract. Handb Exp Pharmacol 168:573–598

Izzo AA, Capasso F, Costagliola A, Bisogno T, Marsicano G, Ligresti A et al (2003) An endogenous cannabinoid tone attenuates cholera toxin-induced fluid accumulation in mice. Gastroenterology 125(3):765–774 [see comment]

Jacobsson SO, Fowler CJ (2001) Characterization of palmitoylethanolamide transport in mouse Neuro-2a neuroblastoma and rat RBL-2H3 basophilic leukaemia cells: comparison with anandamide. Br J Pharmacol 132(8):1743–1754

Jaggar SI, Sellaturay S, Rice AS (1998a) The endogenous cannabinoid anandamide, but not the CB2 ligand palmitoylethanolamide, prevents the viscero-visceral hyper-reflexia associated with inflammation of the rat urinary bladder. Neurosci Lett 253(2):123–126

Jaggar SI, Hasnie FS, Sellaturay S, Rice AS (1998b) The anti-hyperalgesic actions of the cannabinoid anandamide and the putative CB2 receptor agonist palmitoylethanolamide in visceral and somatic inflammatory pain. Pain 76(1–2):189–199

Jaggar SI, Scott HC, Rice AS (1999) Inflammation of the rat urinary bladder is associated with a referred thermal hyperalgesia which is nerve growth factor dependent. Br J Anaesthiol 83(3):442–448

Jensen BC, Swigart PM, Simpson PC, Jensen BC, Swigart PM, Simpson PC (2009) Ten commercial antibodies for alpha-1-adrenergic receptor subtypes are nonspecific. Naunyn Schmiedebergs Arch Pharmacol 379(4):409–412

Jonsson KO, Vandevoorde S, Lambert DM, Tiger G, Fowler CJ (2001) Effects of homologues and analogues of palmitoylethanolamide upon the inactivation of the endocannabinoid anandamide. Br J Pharmacol 133(8):1263–1275

Jositsch G, Papadakis T, Haberberger RV, Wolff M, Wess J, Kummer W et al (2009) Suitability of muscarinic acetylcholine receptor antibodies for immunohistochemistry evaluated on tissue sections of receptor gene-deficient mice. Naunyn Schmiedebergs Arch Pharmacol 379(4):389–395

Kapur A, Zhao P, Sharir H, Bai Y, Caron MG, Barak LS et al (2009) Atypical responsiveness of the orphan receptor GPR55 to cannabinoid ligands. J Biol Chem 284(43):29817–29827

Katona I, Sperlagh B, Sik A, Kafalvi A, Vizi ES, Mackie K et al (1999) Presynaptically located CB1 cannabinoid receptors regulate GABA release from axon terminals of specific hippocampal interneurons. J Neurosci 19(11):4544–4558

Katona I, Sperlagh B, Magloczky Z, Santha E, Kofalvi A, Czirjak S et al (2000) GABAergic interneurons are the targets of cannabinoid actions in the human hippocampus. Neuroscience 100(4):797–804

Khanolkar AD, Abadji V, Lin S, Hill WA, Taha G, Abouzid K et al (1996) Head group analogs of arachidonylethanolamide, the endogenous cannabinoid ligand. J Med Chem 39(22):4515–4519

Lambert DM, Di Marzo V (1999) The palmitoylethanolamide and oleamide enigmas: are these two fatty acid amides cannabimimetic? Curr Med Chem 6(8):757–773

Lambert DM, Vandevoorde S, Diependaele G, Govaerts SJ, Robert AR (2001) Anticonvulsant activity of N-palmitoylethanolamide, a putative endocannabinoid, in mice. Epilepsia 42(3):321–327

Lan R, Gatley J, Lu Q, Fan P, Fernando SR, Volkow ND et al (1999a) Design and synthesis of the CB1 selective cannabinoid antagonist AM281: a potential human SPECT ligand. AAPS Pharmsci 1(2):E4

Lan R, Liu Q, Fan P, Lin S, Fernando SR, McCallion D et al (1999b) Structure-activity relationships of pyrazole derivatives as cannabinoid receptor antagonists. J Med Chem 42(4):769–776

Lichtman AH, Cook SA, Martin BR (1996) Investigation of brain sites mediating cannabinoid-induced antinociception in rats: evidence supporting periaqueductal gray involvement. J Pharmacol Exp Ther 276(2):585–593

Lin S, Khanolkar AD, Fan P, Goutopoulos A, Qin C, Papahadjis D et al (1998) Novel analogues of arachidonylethanolamide (anandamide): affinities for the CB1 and CB2 cannabinoid receptors and metabolic stability. J Med Chem 41(27):5353–5361

Lo Verme J, Fu J, Astarita G, La Rana G, Russo R, Calignano A et al (2005) The nuclear receptor peroxisome proliferator-activated receptor-alpha mediates the anti-inflammatory actions of palmitoylethanolamide. Mol Pharmacol 67(1):15–19

Loria F, Petrosino S, Mestre L, Spagnolo A, Correa F, Hernangomez M et al (2008) Study of the regulation of the endocannabinoid system in a virus model of multiple sclerosis reveals a therapeutic effect of palmitoylethanolamide. Eur J Neurosci 28(4):633–641

Lu X, Bartfai T, Lu X, Bartfai T (2009) Analyzing the validity of GalR1 and GalR2 antibodies using knockout mice. Naunyn Schmiedebergs Arch Pharmacol 379(4):417–420

Machado Rocha FC, Stefano SC, De Cassia HR, Rosa Oliveira LM, Da Silveira DX, Rosa Oliveira LMQ (2008) Therapeutic use of Cannabis sativa on chemotherapy-induced nausea and vomiting among cancer patients: systematic review and meta-analysis. Eur J Cancer Care 17 (5):431–443

Mailleux P, Parmentier M, Vanderhaeghen JJ (1992) Distribution of cannabinoid receptor messenger RNA in the human brain: an in situ hybridization histochemistry with oligonucleotides. Neurosci Lett 143(1–2):200–204

Martin RS, Luong LA, Welsh NJ, Eglen RM, Martin GR, MacLennan SJ (2000) Effects of cannabinoid receptor agonists on neuronally-evoked contractions of urinary bladder tissues isolated from rat, mouse, pig, dog, monkey and human. Br J Pharmacol 129(8):1707–1715

Martyn CN, Illis LS, Thom J (1995) Nabilone in the treatment of multiple sclerosis. Lancet 345 (8949):579

Mascolo N, Izzo AA, Ligresti A, Costagliola A, Pinto L, Cascio MG et al (2002) The endocannabinoid system and the molecular basis of paralytic ileus in mice. FASEB J 16(14):1973–1975

Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI (1990) Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature 346(6284):561–564 [see comment]

Matsuda LA, Bonner TI, Lolait SJ (1993) Localization of cannabinoid receptor mRNA in rat brain. J Comp Neurol 327(4):535–550

Mazzola C, Medalie J, Scherma M, Panlilio LV, Solinas M, Tanda G et al (2009) Fatty acid amide hydrolase (FAAH) inhibition enhances memory acquisition through activation of PPAR-alpha nuclear receptors. Learn Mem 16(5):332–337

McMahon SB (1988) Neuronal and behavioural consequences of chemical inflammation of rat urinary bladder. Agents Actions 25(3–4):231–233

McMahon SB, Abel C (1987) A model for the study of visceral pain states: chronic inflammation of the chronic decerebrate rat urinary bladder by irritant chemicals. Pain 28(1):109–127

Mechoulam R, Fride E (2001) Physiology. a hunger for cannabinoids. Nature 410(6830):763 [comment]

Mechoulam R, Ben-Shabat S, Hanus L, Ligumsky M, Kaminski NE, Schatz AR et al (1995) Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. Biochem Pharmacol 50(1):83–90

Merriam FV, Wang ZY, Guerios SD, Bjorling DE, Merriam FV, Z-y W et al (2008) Cannabinoid receptor 2 is increased in acutely and chronically inflamed bladder of rats. Neurosci Lett 445 (1):130–134

Michel MC, Wieland T, Tsujimoto G, Michel MC, Wieland T, Tsujimoto G (2009) How reliable are G-protein-coupled receptor antibodies? Naunyn Schmiedebergs Arch Pharmacol 379 (4):385–388

Munro S, Thomas KL, Abu-Shaar M (1993) Molecular characterization of a peripheral receptor for cannabinoids. Nature 365(6441):61–65 [see comment]

Murineddu G, Lazzari P, Ruiu S, Sanna A, Loriga G, Manca I et al (2006) Tricyclic pyrazoles. 4. Synthesis and biological evaluation of analogues of the robust and selective CB2 cannabinoid ligand 1-(2′, 4′-dichlorophenyl)-6-methyl-N-piperidin-1-yl-1, 4-dihydroindeno[1, 2-c]pyrazole-3-carboxamide. J Med Chem 49(25):7502–7512

Nagy I, Santha P, Jancso G, Urban L, Nagy I, Santha P et al (2004) The role of the vanilloid (capsaicin) receptor (TRPV1) in physiology and pathology. Eur J Pharmacol 500(1–3):351–369

Nazif O, Teichman JM, Gebhart GF, Nazif O, Teichman JMH (2007) Neural upregulation in interstitial cystitis. Urology 69(4 Suppl):24–33

O'Sullivan SE (2007) Cannabinoids go nuclear: evidence for activation of peroxisome proliferator-activated receptors. Br J Pharmacol 152(5):576–582

O'Sullivan SE, Kendall DA, Randall MD, O'Sullivan SE, Kendall DA, Randall MD (2009) Time-dependent vascular effects of endocannabinoids mediated by peroxisome proliferator-activated receptor gamma (PPARgamma). PPAR Res 2009:425289

Onaivi ES, Ishiguro H, Gong JP, Patel S, Meozzi PA, Myers L et al (2008) Functional expression of brain neuronal CB2 cannabinoid receptors are involved in the effects of drugs of abuse and in depression. Ann NY Acad Sci 1139:434–449

Pacheco M, Childers SR, Arnold R, Casiano F, Ward SJ (1991) Aminoalkylindoles: actions on specific G-protein-linked receptors. J Pharmacol Exp Ther 257(1):170–183

Pertwee RG (2001) Cannabinoids and the gastrointestinal tract. Gut 48(6):859–867

Pertwee RG, Fernando SR (1996) Evidence for the presence of cannabinoid CB1 receptors in mouse urinary bladder. Br J Pharmacol 118(8):2053–2058

Pertwee RG, Stevenson LA, Elrick DB, Mechoulam R, Corbett AD (1992) Inhibitory effects of certain enantiomeric cannabinoids in the mouse vas deferens and the myenteric plexus preparation of guinea-pig small intestine. Br J Pharmacol 105(4):980–984

Pertwee RG, Fernando SR, Nash JE, Coutts AA (1996) Further evidence for the presence of cannabinoid CB1 receptors in guinea-pig small intestine. Br J Pharmacol 118(8):2199–2205

Peters MF, Scott CW, Peters MF, Scott CW (2009) Evaluating cellular impedance assays for detection of GPCR pleiotropic signaling and functional selectivity. J Biomol Screen 14(3):246–255

Pradidarcheep W, Stallen J, Labruyere WT, Dabhoiwala NF, Michel MC, Lamers WH et al (2009) Lack of specificity of commercially available antisera against muscarinergic and adrenergic receptors. Naunyn Schmiedebergs Arch Pharmacol 379(4):397–402

Rhee MH, Vogel Z, Barg J, Bayewitch M, Levy R, Hanus L et al (1997) Cannabinol derivatives: binding to cannabinoid receptors and inhibition of adenylylcyclase. J Med Chem 40 (20):3228–3233

Rinaldi-Carmona M, Barth F, Heaulme M, Shire D, Calandra B, Congy C et al (1994) SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. FEBS Lett 350(2–3):240–244

Rinaldi-Carmona M, Barth F, Millan J, Derocq JM, Casellas P, Congy C et al (1998) SR 144528, the first potent and selective antagonist of the CB2 cannabinoid receptor. J Pharmacol Exp Ther 284(2):644–650

Robson P (2001) Therapeutic aspects of cannabis and cannabinoids. Br J Psychiatry 178:107–115 [see comment]

Rodriguez JJ, Mackie K, Pickel VM (2001) Ultrastructural localization of the CB1 cannabinoid receptor in mu-opioid receptor patches of the rat Caudate putamen nucleus. J Neurosci 21(3):823–833

Ross RA, Brockie HC, Stevenson LA, Murphy VL, Templeton F, Makriyannis A et al (1999) Agonist-inverse agonist characterization at CB1 and CB2 cannabinoid receptors of L759633, L759656, and AM630. Br J Pharmacol 126(3):665–672

Ross RA, Craib SJ, Stevenson LA, Pertwee RG, Henderson A, Toole J et al (2002) Pharmacological characterization of the anandamide cyclooxygenase metabolite: prostaglandin E2 ethanolamide. J Pharmacol Exp Ther 301(3):900–907

Sade H, Muraki K, Ohya S, Hatano N, Imaizumi Y, Sade H et al (2006) Activation of large-conductance, Ca2+-activated K+ channels by cannabinoids. Am J Physiol Cell Physiol 290(1): C77–C86

Saitoh C, Kitada C, Uchida W, Chancellor MB, de Groat WC, Yoshimura N et al (2007) The differential contractile responses to capsaicin and anandamide in muscle strips isolated from the rat urinary bladder. Eur J Pharmacol 570(1–3):182–187

Shire D, Calandra B, Rinaldi-Carmona M, Oustric D, Pessegue B, Bonnin-Cabanne O et al (1996) Molecular cloning, expression and function of the murine CB2 peripheral cannabinoid receptor. Biochim Biophys Acta 1307(2):132–136

Showalter VM, Compton DR, Martin BR, Abood ME (1996) Evaluation of binding in a transfected cell line expressing a peripheral cannabinoid receptor (CB2): identification of cannabinoid receptor subtype selective ligands. J Pharmacol Exp Ther 278(3):989–999

Shu YZ (1998) Recent natural products based drug development: a pharmaceutical industry perspective. J Nat Prod 61(8):1053–1071

Sibley WA (1992) Therapeutic claims in multiple sclerosis, 3rd edn. Demos Medical, New York

Smart D, Gunthorpe MJ, Jerman JC, Nasir S, Gray J, Muir AI et al (2000) The endogenous lipid anandamide is a full agonist at the human vanilloid receptor (hVR1). Br J Pharmacol 129 (2):227–230

Smart D, Jonsson KO, Vandevoorde S, Lambert DM, Fowler CJ, Smart D et al (2002) 'Entourage' effects of N-acyl ethanolamines at human vanilloid receptors. Comparison of effects upon anandamide-induced vanilloid receptor activation and upon anandamide metabolism. Br J Pharmacol 136(3):452–458

Sugiura T, Kondo S, Kishimoto S, Miyashita T, Nakane S, Kodaka T et al (2000) Evidence that 2-arachidonoylglycerol but not N-palmitoylethanolamine or anandamide is the physiological ligand for the cannabinoid CB2 receptor. Comparison of the agonistic activities of various cannabinoid receptor ligands in HL-60 cells. J Biol Chem 275(1):605–612

Tokanovic S, Malone DT, Ventura S (2007) Stimulation of epithelial CB1 receptors inhibits contractions of the rat prostate gland. Br J Pharmacol 150(2):227–234

Tsou K, Brown S, Sanudo-Pena MC, Mackie K, Walker JM (1998) Immunohistochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. Neuroscience 83(2):393–411

Tsou K, Mackie K, Sanudo-Pena MC, Walker JM (1999) Cannabinoid CB1 receptors are localized primarily on cholecystokinin-containing GABAergic interneurons in the rat hippocampal formation. Neuroscience 93(3):969–975

Tyagi V, Philips BJ, Su R, Smaldone MC, Erickson VL, Chancellor MB et al (2009) Differential expression of functional cannabinoid receptors in human bladder detrusor and urothelium. J Urol 181(4):1932–1938

Ueda N, Yamamoto S (2000) Anandamide amidohydrolase (fatty acid amide hydrolase). Prostaglandins Other Lipid Mediat 61(1–2):19–28

Wade DT, Robson P, House H, Makela P, Aram J (2003) A preliminary controlled study to determine whether whole-plant cannabis extracts can improve intractable neurogenic symptoms. Clin Rehabil 17(1):21–29

Wade DT, Makela P, Robson P, House H, Bateman C (2004) Do cannabis-based medicinal extracts have general or specific effects on symptoms in multiple sclerosis? A double-blind, randomized, placebo-controlled study on 160 patients. Mult Scler 10(4):434–441 [see comment]

Wahn H, Wolf J, Kram F, Frantz S, Wagner JA, Wahn H et al (2005) The endocannabinoid arachidonyl ethanolamide (anandamide) increases pulmonary arterial pressure via cyclooxygenase-2 products in isolated rabbit lungs. Am J Physiol Heart Circ Physiol 289(6): H2491–H2496

Walczak JS, Price TJ, Cervero F (2009) Cannabinoid CB1 receptors are expressed in the mouse urinary bladder and their activation modulates afferent bladder activity. Neuroscience 159(3):1154–1163

Wang EC, Lee JM, Ruiz WG, Balestreire EM, von Bodungen M, Barrick S et al (2005) ATP and purinergic receptor-dependent membrane traffic in bladder umbrella cells. J Clin Invest 115(9):2412–2422

Westlake TM, Howlett AC, Bonner TI, Matsuda LA, Herkenham M (1994) Cannabinoid receptor binding and messenger RNA expression in human brain: an in vitro receptor autoradiography and in situ hybridization histochemistry study of normal aged and Alzheimer's brains. Neuroscience 63(3):637–652

Zajicek J, Fox P, Sanders H, Wright D, Vickery J, Nunn A et al (2003) Cannabinoids for treatment of spasticity and other symptoms related to multiple sclerosis (CAMS study): multicentre randomised placebo-controlled trial. Lancet 362(9395):1517–1526 [see comment]

Vasopressin Receptors in Voiding Dysfunction

Sailaja Pisipati and Hashim Hashim

Contents

Abstract Arginine vasopressin (AVP), also known as vasopressin or anti-diuretic hormone, is a neuropeptide produced in the hypothalamus. It is primarily responsible for osmoregulation and thus maintains body fluid homeostasis. It is also a potent vasoconstrictor, may have a role in higher cognitive functions and affects metabolism. All the biological and cellular effects of vasopressin are mediated by the interaction of this hormone with three G-protein-coupled receptors – V_{1a}, V_{1b} and V_2.

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Urological applications are based on the rationale that V_2 receptors mediate water conservation and increase urine osmolality. Due to their anti-diuretic properties mediated by the V_2 receptors, synthetic vasopressin agonists, such as desmopressin, are now commonly used for the treatment of nocturnal polyuria, central diabetes insipidus and nocturnal enuresis and potentially in urinary incontinence. Desmopressin has been licenced worldwide for haematological indications of haemophilia and von Willebrand disease. Vasopressin receptor antagonists correct hyponatremia by blocking the activation of the V_2 receptor and induce a free water diuresis without an accompanying natriuresis or kaliuresis; an effect termed 'aquaresis'. Interfering with vasopressin signalling by administering vasopressin antagonists may have clinical benefits in acute and chronic heart failure.

Keywords Aquaporins · Desmopressin · Receptors · Vasopressin

1 Introduction

Arginine Vasopressin (AVP), also known as vasopressin or anti-diuretic hormone (ADH), is a peptide prohormone synthesised in the perikarya of magnocellular neurons in the supraoptic and paraventricular nuclei of the hypothalamus of all mammals, except pigs. The most important function of vasopressin is to maintain fluid homeostasis by increasing water reabsorption in the distal convoluted tubule and collecting ducts, thus increasing the urine osmolality. It also has other diverse functions such as regulation of metabolism and hormone secretion (Michell et al. 1979).

The structure of vasopressin was determined in the early 1950s (Du Vigneaud 1954; Acher et al. 1958; Turner et al. 1951), but it was not until the late 1960s that the first vasopressin analogue was synthesised (Kimbrough et al. 1963; Studer and Cash 1963; Vavra et al. 1974). The aim was to have a metabolically stable and potent anti-diuretic. Genes encoding vasopressin-like peptides evolved more than 700 million years ago. Therefore, it is not surprising that vasopressin acts at sites in the nephron other than the collecting duct and on tissues other than kidney (Jackson 2006).

Vasopressin is a potent vasopressor; hence the derivation of its name. The vasopressins are peptides consisting of nine amino acids (nonapeptides). Its molecular formula is $C_{46}H_{65}N_{15}O_{12}S_2$. The amino acid sequence of AVP is Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Arg-Gly, with the cysteine residues forming a sulphur bridge. The structure of vasopressin is shown in Fig. 1. The alteration and substitution of amino acids at certain positions results in various vasopressin analogues. Vasopressin is a neurotransmitter; it has a role in the secretion of adreno-corticotrophic hormone (ACTH) and in the regulation of the cardiovascular system, temperature and other visceral functions. Vasopressin may play a role in haemostasis by promoting the release of coagulation factors by the vascular endothelium and increasing platelet aggregation (Jackson 2006).

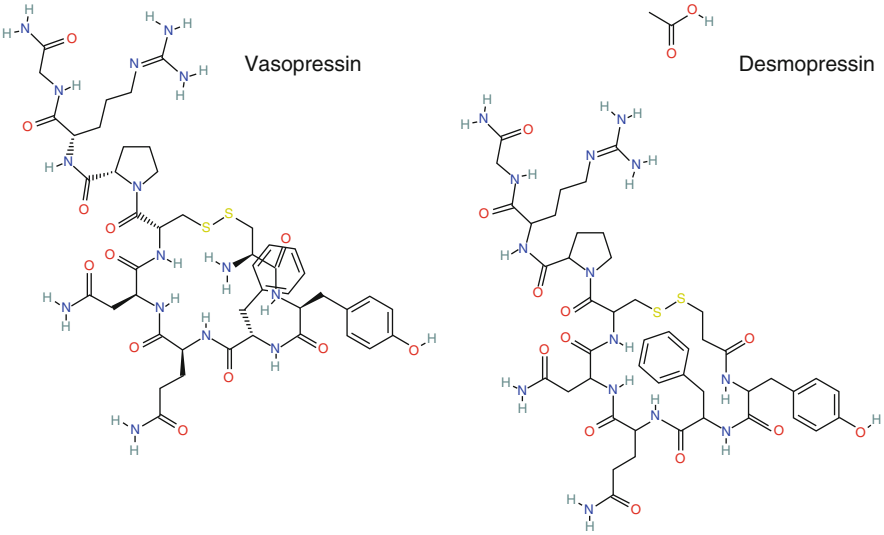

Fig. 1 Structure of vasopressin and desmopressin. Vasopressin – $C_{46}H_{65}N_{15}O_{12}S_2$; Desmopressin – $C_{48}H_{68}N_{14}O_{14}S_2$

2 Vasopressin Receptors

2.1 Types of Receptors

The biological and cellular effects of vasopressin are mediated mainly by the interaction of the hormone with three types of receptors – V_{1a}, V_{1b} (also known as V_3) and V_2 (Fig. 2; Thbonnier et al. 2002). Although all the three receptors are G-protein-coupled receptors (GPCRs), activation of AVP Receptor type 1a (AVPR1a) and AVP Receptor type 1b (AVPR1b) stimulates phospholipase C via Gq/11, whilst activation of AVP Receptor type 2 (AVPR2) stimulates adenylyl cyclase by interacting with Gs protein (Birnbaumer 2000). Although originally defined by pharmacological criteria, vasopressin receptors are now defined by their primary amino acid sequences. The cloned vasopressin receptors are typical heptahelical GPCRs. Manning et al. (1999) have synthesised novel hypotensive vasopressin peptide agonists that do not interact with V_{1a}, V_{1b} or V_2 receptors and may stimulate a putative vasopressin vasodilatory receptor. Finally, two additional putative receptors for vasopressin have been cloned. The first of these two is a vasopressin-activated Ca^{2+} – mobilising receptor with one transmembrane domain that binds vasopressin and increases intracellular Ca^{2+} (Serradeil-Le Gal et al. 2002b). The second is a dual angiotensin II – vasopressin heptahelical receptor that activates adenylyl cyclase in response to both angiotensin II and vasopressin (Serradeil-Le Gal et al. 2002b). The physiological roles of these putative vasopressin receptors are unclear (Jackson 2006).

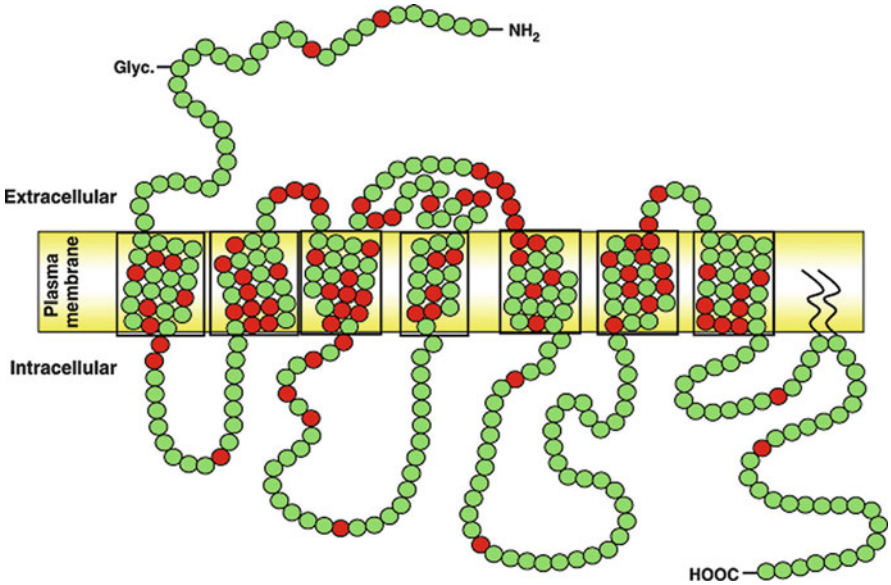

Fig. 2 Schematic structure of the vasopressin V_2 receptor [Adapted with permission from Insel et al. (2007)]. Each amino acid residue is shown as a *grey dot*; amino acid residues that are mutated in patients with nephrogenic diabetes insipidus are shown as *black dots*

2.2 Distribution

V_{1a} receptor is the most widely spread subtype, found in the vascular smooth muscle (Caldwell et al. 2008), hepatocytes, platelets, renal medullary interstitial cells, vasa recta in the renal microcirculation, epithelial cells in the renal cortical collecting-duct, the adrenal gland, myometrium, bladder, adipocytes, central nervous system (CNS) structures, spleen and testes.

V_{1b} receptors, also known as V_3 receptors, have a more limited distribution. They are expressed in the cells of the anterior pituitary (Antoni et al. 1984), where it stimulates the release of ACTH and several other brain regions, particularly in the pyramidal neurons of the hippocampal CA2 field (Hernando et al. 2001; Young et al. 2006), in the pancreas and in the adrenal medulla.

V_2 receptors are predominantly located in the distal convoluted tubule and the collecting ducts of the kidney, but are also found on the epithelial cells in the thick ascending limb of the loop of Henle and on vascular endothelial cells. They are also located in extra-renal tissues, such as the foetal lung and lung cancer. On stimulation of V_2 receptors, a variety of clotting factors are released into the circulation.

3 Basic Pharmacology

3.1 V_1 Receptor Effector Coupling

Binding of 8-AVP to vasopressin receptors V_1 stimulates several membrane-bound phospholipases. Activation of the G_q–PLCβ pathway results in the formation of inositol triphosphate (IP_3) and diacylglycerol, which in turn increase the intracellular Ca^{2+} concentration, mobilise the intracellular Ca^{2+} and activate protein kinase C. Activation of V_1 receptors also causes influx of extracellular Ca^{2+} by an unknown mechanism. Activated protein kinase C and Ca^{2+}/calmodulin-activated protein kinases phosphorylate cell-type-specific proteins leading to cellular responses causing specific biological effects. This mitogenic effect involves activation of the AP-1 transcription factor and its c-fos and c-jun subunits. A further component of the AVP response derives from the production of eicosanoids secondary to the activation of PLA_2; the resulting mobilisation of arachidonic acid (AA) provides substrate for eicosanoid synthesis via the cyclo-oxygenase (COX) and lipoxygenase (LOX) pathways, leading to local production of prostaglandins (PG), thromboxanes (TX) and leukotrienes (LT), which may activate a variety of signalling pathways, including those linked to G_S and G_q. Biological effects mediated by the V_1 receptor include vasoconstriction, glycogenolysis, platelet aggregation, ACTH release and growth of vascular smooth muscle cells (Fig. 3). The effects of vasopressin on cell growth involve transcriptional regulation via the FOS/JUN AP-1 transcription complex (Jackson 2006).

3.2 V_2 Receptor Effector Coupling

Principal cells in the renal collecting duct have V_2 receptors on their basolateral membranes. Binding of AVP to the V_2 receptor activates the G_S – adenylyl cyclase pathway. The resulting increase in cellular cyclic AMP content and protein kinase A (PKA) activity triggers an increased rate of insertion of water channel-containing vesicles (WCVs) into the apical membrane and a decreased rate of endocytosis of WCVs from the apical membrane (Snyder et al. 1992). The distribution of WCVs between the cytosolic compartment and the apical membrane compartment is thus shifted in favour of the apical membrane compartment (Nielsen et al. 1999). Because WCVs contain preformed functional water channels (aquaporin 2), their net shift into apical membranes in response to V_2-receptor stimulation greatly increases the water permeability of the apical membrane. Although phosphorylation of serine 256 of aquaporin 2 is involved in V_2 receptor signalling, other proteins located both in the WCVs and the apical membrane of the cytoplasm also may be involved (Fig. 4) (Jackson 2006).

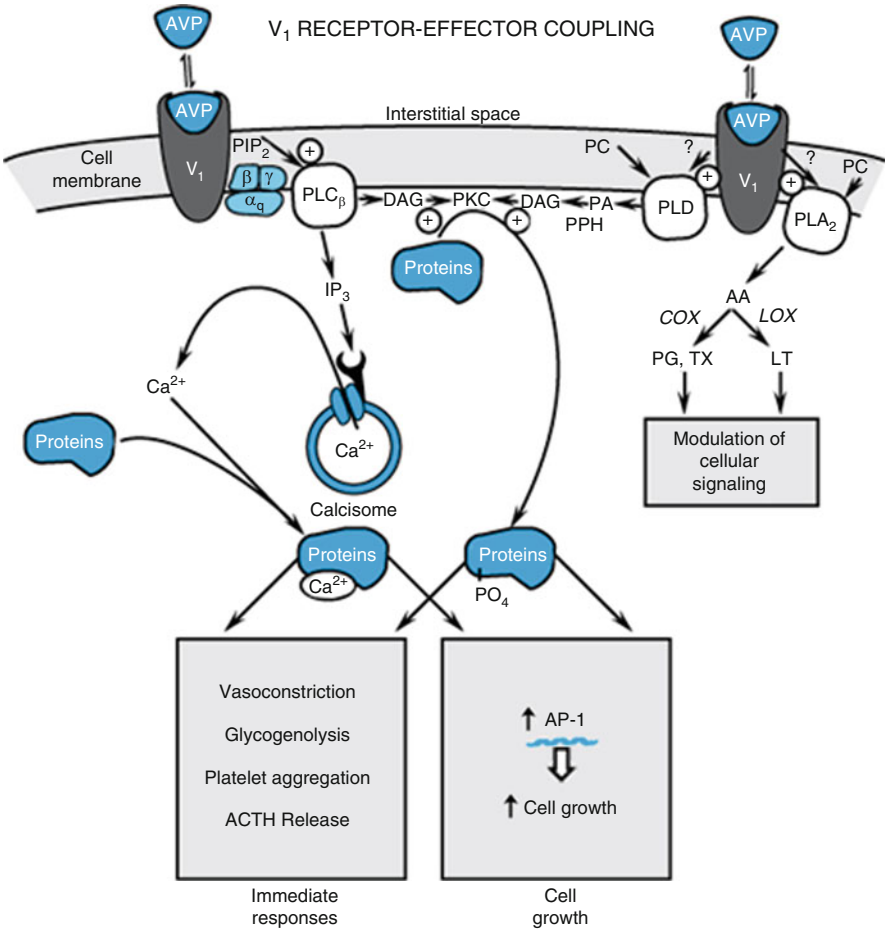

Fig. 3 V_1 receptor effector coupling (Adapted with permission from Goodman and Gilman's Pharmacological Basis of Therapeutics)

3.2.1 Aquaporins

The pioneering discovery by Agre et al. (1993), of a family of water channel proteins, the aquaporins (AQP) around 1989–1991, clarified the mechanism through which water crosses biological membranes and provided insight into the physiology of water homeostasis and the pathophysiology of water imbalance (Nielsen et al. 1999). The AQP are a family of small (24–30 kDa) pore-forming integral membrane proteins. So far 11 different AQPs have been identified in vertebrates (Kruse et al. 2006). Seven of these AQPs are known to be present in the kidney at distinct sites along the nephron and the collecting duct (Nielsen et al. 2002). AQPs have six membrane-spanning domains, both the amino- and carboxyl-terminals are intracellular, and have tandem repeats that, presumably, are due to an

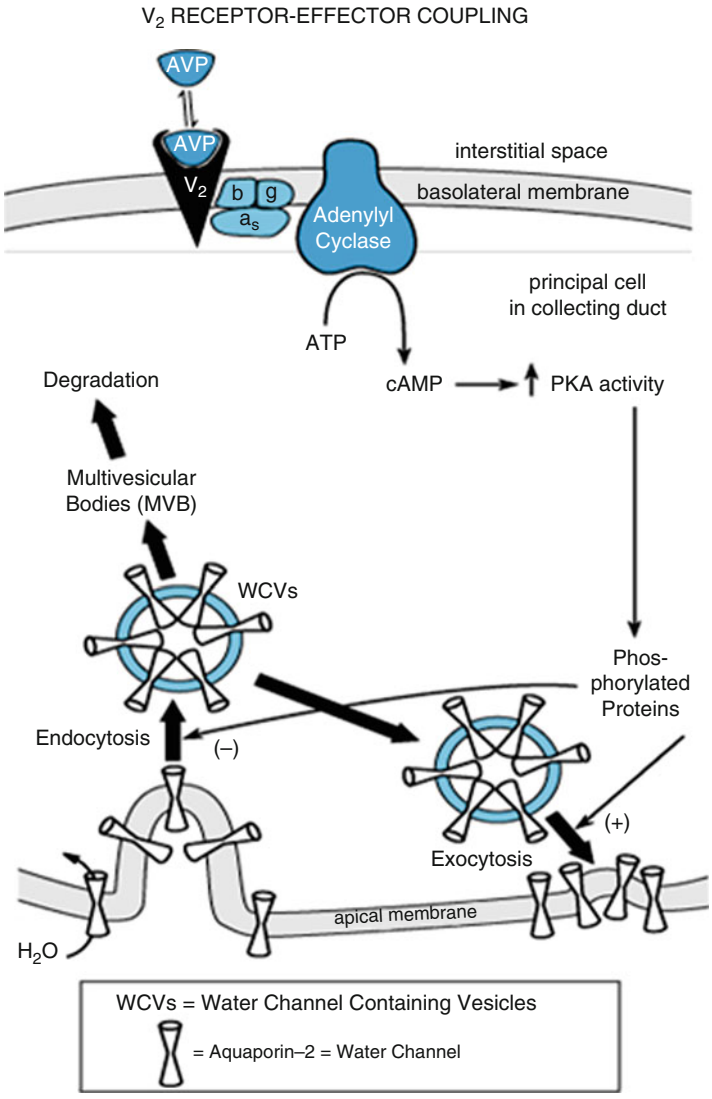

Fig. 4 V_2 receptor effector coupling (Adapted from Goodman and Gilman's Pharmacological Basis of Therapeutics)

ancient gene duplication (Agre et al. 1998). The six domains are interconnected by five loops (A–E). The tandem repeat structures with asparagine–proline–alanine (NPA) sequences in loop B and E have been proposed to form tight turn structures that interact in the membrane to form the pathway for translocation of water across the plasma membrane. Of the five loops in AQP1, the B and E loops dip into the lipid bilayer, and it has been proposed that they form hemi-channels that connect between the leaflets to form a single pathway within a symmetric structure that

resembles an 'hourglass' (Fig. 5) (Nielsen et al. 1999). The current model of AQP1 described above was further supported by the Fourier transform infrared spectroscopy, the results of which revealed that six closely associated α-helixes span the lipid membrane (Cabiaux et al. 1997). Moreover, the three-dimensional structure of AQP1 was determined at 6A resolution by cryoelectron microscopy (Walz et al. 1997). Each AQP1 monomer has six tilted, bilayer spanning α-helixes, which form a right-handed bundle surrounding a central density (Walz et al. 1997). These studies also confirmed the organisation of the tetrameric complex in the membrane (Walz et al. 1997). The three-dimensional structure of AQP1 was also reported at 7A resolution by other investigators (Cheng et al. 1997).

Studies by Brown and colleagues using Chinese hamster ovary cells transfected with AQP1 through AQP5 have indicated that AQP2, 3 and 5 may also form tetramers in the membrane (Yang et al. 1996; Van Hoek et al. 1998). Not all AQPs appear to assemble in the plasma membrane as tetramers. Recently, several studies revealed that AQP4 forms larger multimeric structures in the plasma membrane.

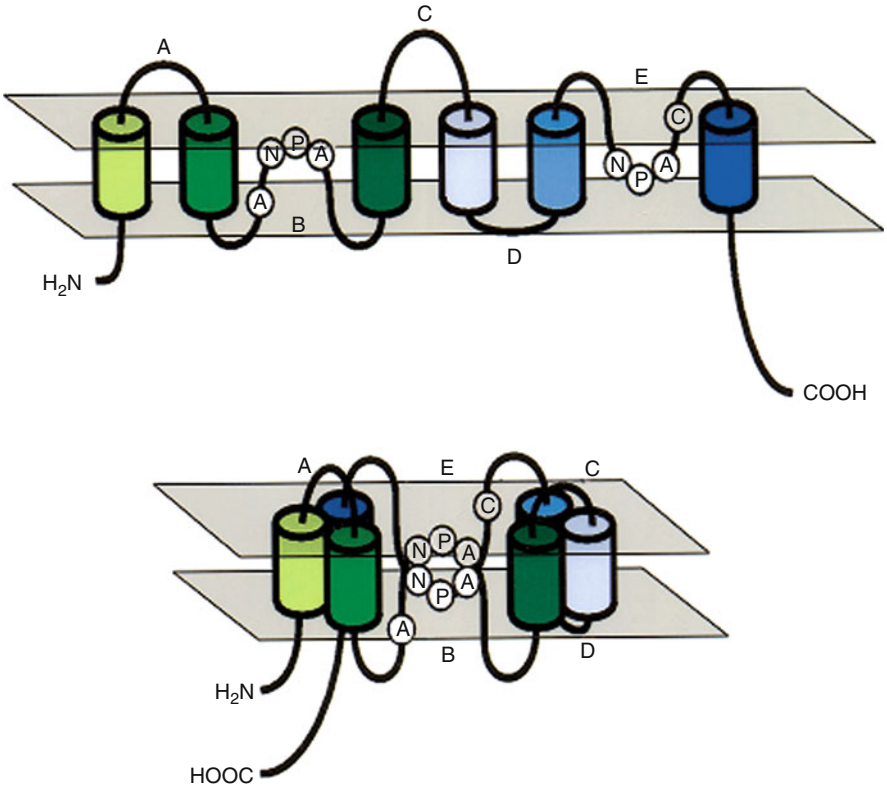

Fig. 5 Structure of aquaporin [Adapted with permission from Nielsen et al. (1999)]

Aquaporin Localisation in the Kidney

Absorption of water out of the renal tubule depends on the osmotic driving force for water reabsorption and the equilibration of water across the tubular epithelium (Knepper 1997). A series of studies over the past few years by Agre et al. (1993) has made it clear that osmotic water transport across the tubular epithelium is predominantly dependent on AQP water channels. At least seven AQPs are expressed in the kidney (Table 1, Fig. 6).

Table 1 Distribution of the renal aquaporins

Renal aquaporins	Number of amino acids	Location in the kidney	Extra-renal location
AQP1	269	Proximal convoluted tubules, descending thin, limb of loop of Henle	Multiple organs
AQP2	271	Collecting duct, principal cells	Testis
AQP3	292	Collecting duct, principal cells	Multiple organs
AQP4	301	Medullary collecting duct, principal cells	Brain and multiple organs
AQP6	276	Intercalated cells	Cerebellum, salivary glands
AQP7	269	Proximal convoluted tubule (straight)	Testis, adipocytes
AQP8	263	Cortex and medulla	Testis, epididymis, pancreas, liver, colon, heart, placenta

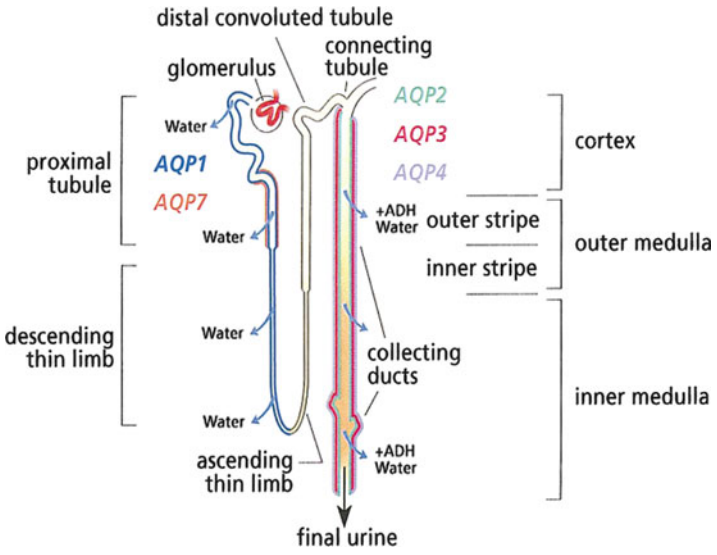

Fig. 6 Localisation of different aquaporins in the nephron and collecting duct system (Adapted from Nielsen et al. (2002)]

AQP2 has been identified to be the principal target by which vasopressin regulates renal collecting duct water permeability and hence renal regulation of body water homeostasis (Nielsen et al. 1993, 1999; Knepper et al. 1994). Two additional AQP are expressed in the collecting duct principal cells, AQP3 and AQP4 (Ecelbarger et al. 1995; Echevarria et al. 1994; Terris et al. 1995, 1996). Both of these channels are present in the basolateral plasma membranes and are likely to represent exit pathways for water entering the cells through AQP2. AQP5 and AQP9 are extra-renal AQPs identified in rats and humans, respectively. AQP5 is constituted of 265 amino acids and found predominantly in salivary glands, lungs and eyes. AQP9 has a sequence of 295 aminoacids and has a much widespread extrarenal distribution. It is present in hepatocytes, leucocytes, lungs, spleen, brain, epididymides and testes.

Urea permeability is enhanced by 400% in the terminal parts of the inner medullary collecting ducts as a result of activation of V_2 receptors. This is mediated by the activation of a vasopressin-regulated urea transporter, labelled as VRUT, via PKA-induced phosphorylation (Sands 2003). In addition to increasing the water permeability of the collecting duct and the urea permeability of the inner medullary collecting duct, activation of the V_2-receptor also results in an increased Na^+ transport in the thick ascending limb and collecting duct. This is mediated by three mechanisms that affect the Na^+–K^+–$2Cl^-$ symporter, i.e. rapid phosphorylation of the symporter, translocation of the symporter into the luminal membrane and increased expression of symporter protein (Ecelbarger et al. 2001; Giminz and Forbush 2003).

4 Functions of Vasopressin

The primary role of vasopressin is to maintain the fluid homeostasis in our body. Vasopressin is released under conditions of dehydration, thus allowing the collecting ducts of the kidneys to reabsorb water, resulting in a concentrated, low volume urine. It also induces moderate vasoconstriction in higher concentrations, thus elevating the blood pressure in hypovolemic states.

4.1 Renal Actions of Vasopressin

Vasopressin has three effects by which it contributes to increased urine osmolarity and decreased urine excretion, which are:

1. It increases the permeability of the distal convoluted tubules and collecting tubule of the nephrons to water and thus allows water reabsorption and excretion of a smaller volume of concentrated urine – antidiuresis. This occurs through insertion of additional AQP2s into the apical membrane of the tubules/collecting duct epithelial cells. V_2 receptors, GPCRs coupled to G_s, on the basolateral membrane of the cells lining the distal convoluted tubules and conducting

tubules (in the nephron) have an active site for AVP. The G protein, which is in contact with the V_2 receptor inside the cell, move to adenylyl cyclase, triggering adenylyl cyclase to convert ATP into cAMP, plus two inorganic phosphates. The cAMP cascade then triggers the insertion of AQP2 water pores by exocytosis of storage vesicles.

The repressor protein that regulates the gene for PKA has a binding site for cAMP, causing the repressor protein to change its shape and leave the operator region of the gene. This allows for transcription of the gene for PKA. PKA then signals ATP to dephosphorylate, providing energy for vesicles (which contain AQP channel proteins in their membranes) to fuse with the apical membrane of the cell. Calcium ions may also be required in this process; therefore, it may be possible that PLC (phospholipase C-β) has an associated role. It should be noted that PLC can be activated by a GPCR.

2. Vasopressin increases the permeability of the papillary portion of the collecting duct to urea, allowing increased reabsorption of urea into the medullary interstitium, down the concentration gradient created from the removal of water in the cortical collecting duct.
3. Vasopressin stimulates sodium reabsorption in the thick ascending loop of Henle by increasing the activity of the Na^+–K^+–$2Cl^-$-cotransporter.

Due to their anti-diuretic properties mediated by the V_2 receptors, synthetic vasopressin agonists are now commonly used for the treatment of nocturnal polyuria, central diabetes insipidus, nocturnal enuresis and potentially urinary incontinence and overactive bladder (OAB) syndrome (Robinson et al. 2004; Hashim et al. 2009).

4.2 Non-Renal Actions of Vasopressin

4.2.1 Cardiovascular System

At high concentrations, vasopressin acts as a potent vasoconstrictor, thus helping to maintain the arterial blood pressure in conditions of severe hypovolemia or hypotension. This action is mediated by the V_{1a} receptors. It can also contribute to ischemic heart disease due to reduced cardiac output as a result of coronary vasoconstriction, reduced coronary blood flow and alterations in sympathetic and vagal tone, all these leading to progression of heart failure.

4.2.2 Central Nervous System

Vasopressin acts as a neurotransmitter and its CNS effects seem to be mediated via the V_1 receptors. It may have a role in higher cognitive functions (Dantzer and Bluthé 1993; Young et al. 1998) and in the pathogenesis of specific psychiatric disorders such as depression (Scott and Dinan 2002). Cridland and Kasting (1992)

demonstrated that vasopressin may have a critical role in thermoregulation in bacterial infections. Vasopressin may provide for sustained activation of the hypothalamic–pituitary–adrenal axis during chronic stress (Aguilera and Rabadan-Diehl 2000). Egashira et al. (2009) reported that V_{1a} and V_{1b} receptors may be involved in the regulation of social interaction and prepulse inhibition of the startle reflex. Moreover, they have also demonstrated that V_{1a} receptors control anxiety-related behaviour and spatial memory. Hence, the V_{1a} or V_{1b} receptor related drugs have become the focus of intense interest as the first in this novel class of drugs for the treatment of psychiatric disorders and memory disturbance.

4.2.3 Coagulation

Activation of extra-renal V_2 receptors by desmopressin or vasopressin increases circulating levels of procoagulant factor VIII and of von Willebrand factor (Bernat et al. 1997).

4.2.4 Nociception

Vasopressin shows analgesic effects through an undetermined mechanism (Koshimizu and Tsujimoto 2009). In their review, Honda and Takano concluded that supraspinal V_{1b} receptors contribute to an increase in the perception for noxious thermal stimuli, whereas spinal V_{1a} receptors may contribute to a decrease in the perception of noxious thermal stimuli. V_{1b} receptors are involved in inhibiting the morphine-induced analgesic response. V_{1b} receptors are likely to be involved in the neural network of the mesolimbic dopamine system, which plays a crucial role in the control of morphine-induced hyperlocomotion. V_{1b} receptors play a role in the regulation of the hypothermic effect of morphine (Honda and Takano 2009).

4.2.5 Metabolism and Other Effects

Vasopressin can stimulate platelet aggregation and maintains glucose homeostasis (Zerbe et al. 1979) via the V_1 receptors. It also induces the release of aldosterone from adrenal gland cells via the V_{1a} receptors in mice (Birumachi et al. 2007). Vasopressin-stimulated glucagon and insulin release are mediated by the V_{1b} receptors (Abu-Basha et al. 2002; Oshikawa et al. 2004). It can modulate the lipid metabolism by its antilipolytic action and synthesis of bile acid via the V_{1a} receptor (Hiroyama et al. 2007a, b). AVP plays a physiological role via the V_{1a} receptor in regulating both protein catabolism and glucose homeostasis in V_{1a} receptor deficient mice (Hiroyama et al. 2007a, b).

5 Vasopressin: Like Peptides and Their Clinical Utility

Naturally occurring and synthetic vasopressin – like peptides are listed in Table 2. All are nonapeptides, containing cysteine residues in positions 1 and 6, and have an intramolecular disulfide bridge between the two cysteine residues (essential for agonist activity), have additional conserved amino acids in positions 5, 7 and 9 (asparagine, proline and glycine, respectively), contain a basic amino acid in position 8 and are amidated on the carboxyl terminus. Many vasopressin analogues were synthesised with the goal of increasing the duration of action and selectivity for vasopressin receptor subtypes (V_1 vs. V_2 vasopressin receptors, which mediate pressor responses and antidiuretic responses, respectively). Substitution of valine for glutamine in position 4 increases the antidiuretic selectivity, and the antidiuretic-to-vasopressor ratio for deamino [Val^4, D-Arg^8] AVP is approximately 11,000 times greater than that for vasopressin (Jackson 2006). Nakamura and colleagues synthesised a non-peptide V_2-receptor agonist OPC-51803 (Nakamura et al. 2000).

Only two antidiuretic peptides are available for clinical use in the United States. (1) Vasopressin (synthetic 8-L-arginine vasopressin or Pitressin); (2) Desmopressin acetate (synthetic 1-deamino-8-D-arginine vasopressin or DDAVP). The therapeutic uses of vasopressin and its congeners can be divided into two main categories according to the type of vasopressin receptor involved.

Table 2 Vasopressin-like peptides

A. Naturally occurring vasopressin-like peptides
I. Vertebrates
1. Mammals
Arginine vasopressin – humans & other mammals
Lypressin – pigs, marsupials
Phenypressin – macropodids
2. Non-mammalian vertebrates
Vasotocin
II. Invertebrates
Arginine conopressin
Lysine conopressin
Locus suboesophageal ganglia peptide
B. Synthetic vasopressin peptides
I. V_1 – selective agonists
1. V_{1a} – selective agonist AVP
2. V_{1b} – selective agonist Deamino AVP
II. V_2 – selective agonists
1. Desmopressin (DDAVP)
2. Deamino AVP
C. Non-peptide agonist
I. OPC-51803

5.1 Urological Applications

Urological applications are based on the rationale that V_2 receptors mediate water conservation and increase urine osmolality. Central but not nephrogenic diabetes insipidus (NDI) can be treated with V_2-receptor agonists, and polyuria and polydipsia usually are well controlled. Some patients experience transient DI (e.g. in head injury or surgery in the area of the pituitary); however, therapy for most patients with DI is lifelong. They are also being increasingly advocated in the treatment of nocturnal polyuria, nocturnal enuresis and urinary incontinence.

5.1.1 Desmopressin

Desmopressin is a synthetic V_2 receptor agonist with the molecular formula $C_{46}H_{64}N_{14}O_{12}S_2$ and a molecular weight 1069.22 g/mol. The basic amino acid D-arginine in position 8 confers the antidiuretic activity. Desmopressin was first registered for use in nocturnal enuresis in 1977–1978 (Dimson 1977; Tuvemo 1978). In the early 1980s, it was found that there was a lack of nocturnal vasopressin in bedwetting children (Puri 1980; Norgaard et al. 1985) and, from then on, desmopressin became an established treatment for nocturnal enuresis in children. Its role subsequently extended to the treatment of nocturia, nocturnal polyuria, overactive bladder syndrome (OAB) and is remotely used in acute ureteric colic, although there is currently minimal evidence available to support this.

The pharmacological effects of desmopressin have resulted because of two structural alterations. Deamination at position 1 increases duration of action and increases the antidiuretic activity without increasing vasopressor activity. Substitution of D-arginine for L-arginine greatly reduces vasopressor activity without reducing antidiuretic activity. Thus the antidiuretic-to-vasopressor ratio for DDAVP is approximately 3,000 times greater than that for vasopressin, and desmopressin now is the preferred drug for the treatment of nocturnal polyuria and central diabetes insipidus (Jackson 2006).

Desmopressin can be administered intravenously, subcutaneously, intramuscularly, intranasally and orally as tablets or sublingual lypophilisates. The pharmacokinetics of the different formulations are summarised in Table 3. The duration of effect from a single intranasal dose is from 6 to 20 h; twice-daily dosing is effective in most patients. There is considerable variability in the intranasal dose of desmopressin required to maintain normal urine volume, and the dosage must be tailored individually. In adults, the usual intranasal dose is 10–40 μg/day administered either as a single dose or divided into two or three doses. In view of the high cost of the drug and the importance of avoiding water intoxication, the schedule of administration should be adjusted to the minimal amount required. An initial dose of 2.5 μg can be used, with therapy first directed towards the control of nocturia. An equivalent or higher morning dose controls daytime polyuria in most patients,

Table 3 Pharmacokinetics of the different desmopressin formulations (adapted from Summary of Product Characteristics, http://www.ferring.co.uk)

	Intravenous	Intranasal	Oral tablet	Oral lypophilisate
Maximum daily doses	0.001–0.004 mg	0.01 mg; 0.02 mg; 0.04 mg	0.1 mg; 0.2 mg; 0.4 mg	0.06 mg; 0.12 mg; 0.24 mg
Peak levels	Biphasic	Biphasic	Monophasic	Monophasic
$t_{1/2}$	<10 min (fast) 51–158 min (slow)	7.8 min (fast) 75.5 (slow)	1.5–2.5 h	2.8–3 h
Tmax	N/A	1.5 h	0.9–1.5 h	0.5–2.0 h
Terminal half-life	N/A	3 h	2–3.11 h	2.8 h
Bioavailability	N/A	5–10%	0.08–0.16%	0.28%

although a third dose occasionally may be needed in the afternoon (Jackson 2006). The intranasal formulation has now been withdrawn from several markets around the world due to its variable absorption and has been replaced by newer formulations. Subcutaneous administration of 1–2 μg daily of desmopressin is also effective in central DI.

Oral administration of desmopressin in doses 10–20 times the intranasal dose provides adequate blood levels of desmopressin to control polyuria. Desmopressin enters plasma 15–30 min following oral administration (Fjellestad-Paulsen et al. 1993). The bioavailability of the oral route ranges (Fjellestad-Paulsen et al. 1993) between 0.1 and 1.0% (Vilhardt and Lundin 1986). The bioavailability of oral tablets is about 5% compared to intranasal desmopressin, about 0.16% compared to intravenous desmopressin and 0.26% for the sublingual formulation. The median time to reach maximum concentration (Tmax) after oral administration is 1.5 h. The rate and extent of absorption of desmopressin is reduced by 40% if concomitantly administered with food and delayed if administered within 90 min of having a meal, but the antidiuretic action of the drug is not affected, at least for the first 3 h following desmopressin administration (Rittig et al. 1998). Desmopressin does not enter the intracellular compartment; therefore, its apparent volume of distribution is relatively small, and it does not cross the blood–brain barrier (Stegner et al. 1983; Sorensen et al. 1984). It is excreted in the urine (Fjellestad-Paulsen et al. 1993) with a renal clearance of 0.8 ml/min/kg. Sixty-five percent of the amount of desmopressin absorbed after oral administration can be recovered in the urine within 24 h, whilst the remaining 35% is metabolised by enzymatic degradation. There is a significant increase of urine osmolality and decrease in urine production with desmopressin administration (Fjellestad-Paulsen et al. 1993). Prolonged administration for 12–44 months does not reduce the potency of the drug, and there are no reports of antibodies detected. The number of nocturnal voids and nocturnal diuresis was half that with placebo. The time to the first nocturnal void is almost doubled compared with placebo (Hvistendahl et al. 2005).

Nocturnal Enuresis

Desmopressin has a proven pharmacological effect in most enuretic patients, although a clinical response is not seen in all patients. Numerous questions about the current treatment status of desmopressin include the specific anti-enuretic effect of desmopressin, the effect of desmopressin on sleep and the use of desmopressin as a possible cure for enuresis. The Swedish Enuresis Trial has produced some very positive results on the long-term use of desmopressin, showing a 61% response rate (>50% reduction in wet nights). Desmopressin has proven to be highly effective when used in combination with other treatments, including the alarm and oxybutinin, and after urotherapy (Vogt et al. 2010; Hjalmas 1999).

Primary efficacy outcomes following desmopressin treatment are more favourable in monosymptomatic nocturnal enuresis than non-monosymptomatic nocturnal enuresis. Desmopressin administered with adjunct measures achieves superior outcomes compared to monotherapy, especially in non-monosymptomatic nocturnal enuresis (Alloussi et al. 2010). Desmopressin appears to be an effective and well-tolerated treatment even in adult men with monosymptomatic nocturnal enuresis. Higher response rate can be predicted if the nocturnal urine output exceeds functional bladder capacity for each individual (Burgu et al. 2009). Insufficient response to desmopressin is attributable to various factors, including differences in the primary nocturnal enuresis definition, underlying bladder dysfunction and/or desmopressin pharmacokinetic characteristics. A recent study has demonstrated that some patients were poorly compliant with medication even at study initiation and only 71% were fully compliant with long-term treatment. Decreased compliance was associated with a lower response rate (van Herzeele et al. 2009).

Patients should be encouraged to comply fully with treatment to achieve an optimal outcome. Desmopressin in all formulations has a good safety profile in children with monosymptomatic nocturnal enuresis, provided that treatment is properly prescribed and monitored; improving the training of doctors and patients in the dose–response kinetics of the drug, teaching appropriate restriction of fluid intake and by encouraging the use of desmopressin within a narrow dose range (10–20 μg spray, 120–240 μg melt and 200–400 μg tablet) when used in primary care settings. Titrating higher doses in therapy-resistant patients should probably be carried out in a specialised enuresis centre and only after documenting adequate morning urinary diluting capacity. The risk of hyponatraemia is exacerbated by misuse of the drug rather than an inherent danger associated with the drug, which should be addressed with better education rather than withdrawal of a medication that has the potential to benefit children with nocturnal enuresis (Van de Walle et al. 2010). In selected patients who have undergone a staged reconstruction of the exstrophy–epispadias complex, desmopressin is effective in improving nocturnal dryness, with no significant side-effects (Caione et al. 1999).

Nocturia and Nocturnal Polyuria

In the early 1980s, desmopressin was used off-licence in the treatment of adult nocturia (Mansson et al. 1980). It was not until 1991 that desmopressin was first licenced in the United Kingdom for the treatment of nocturnal polyuria and nocturia in patients with multiple sclerosis (Hilton et al. 1983; Valiquette et al. 1992, 1996; Eckford et al. 1994, 1995; Fredrikson 1996; Ferreira and Letwin 1998). It has also been used for nocturia in patients suffering from Parkinson's Disease (Suchowersky et al. 1995). Desmopressin has undergone an extensive clinical trials program known as the NOCTUPUS studies and in 2001, it became first licenced in Panama and Finland for the treatment of nocturia and since then desmopressin has become licenced for these two indications in many countries around the world. Desmopressin is currently licenced for the treatment of nocturia in the oral 'Melt' form in 25 countries around the world and in the oral tablet form in 68 countries including Canada, New Zealand, France, Scandinavian countries and other European, Middle-Eastern, Asian and South American countries. In the United States, it is still undergoing trials and in the UK further trials are required to show cost-benefit before licencing. Desmopressin should be considered as first-line therapy for patients with nocturia where nocturnal polyuria is present (van Kerrebroeck et al. 2010). Its effectiveness, if taken during the day, in providing patients with up to 6 h during which they were untroubled by urinary frequency, without any rebound night-time frequency, has been demonstrated by a number of small studies (Hoverd and Fowler 1998; Tubridy et al. 1999). After intake before sleeping, urine excretion during the night decreases; therefore, the desire to void is postponed, and the number of voids at night is reduced (Rembratt et al. 2004; Hvistendahl et al. 2005). The clinical effects – in terms of urine volume decrease and an increase in urine osmolality – last for approximately 8–12 h (Rembratt et al. 2004). Maximal bladder capacity is a valuable predictor of response to desmopressin in patients with multiple sclerosis and neurogenic detrusor overactivity (Zahariou et al. 2008). Desmopressin in combination with anticholinergics might improve the efficacy of the treatment of nocturia in neurogenic lower urinary tract dysfunction (Chancellor et al. 1994; Valiquette et al. 1996).

The majority of clinical trials have used desmopressin in an oral formulation. A dose-finding study showed that the nocturnal urine volume/nocturnal diuresis was more reduced by oral desmopressin 0.2 mg than 0.1 mg; however, this study also showed that a 0.4 mg dose taken once before sleeping had no additional effects on the nocturnal diuresis compared to a 0.2 mg dose (Asplund et al. 1998). In the pivotal clinical trials, the drug was titrated from 0.1 to 0.4 mg according to the individual clinical response. Desmopressin significantly reduced nocturnal diuresis by approximately 0.6–0.8 ml/min (−40%), decreased the number of nocturnal voids by approximately 0.8–1.3 (−40%) (−2 in the long-term open-label trial) and extended the time until the first nocturnal void by approximately 1.6 h (−2.3 in the long-term open-label trial) (Table 4). Furthermore, desmopressin significantly reduced night-time urine volume as well as the percentage of urine volume excreted at

Table 4 Clinical trials with desmopressin in men with nocturnal polyuria (Oelke et al. 2010)

Trials	Duration (weeks)	Treatment, i.e. oral daily dose before bedtime unless otherwise indicated	No. of pts (n)	Change nocturnal urine volume (ml/min)	Change nocturnal voids (n)	Time to first void (h)	Level of evidence
Asplund et al. (1998)	3	1 × 0.1 mg	23	−0.5 (−31%)	–	–	2b
		1 × 0.2 mg	23	−0.7 (−44%)	–	–	
		2 × 0.2 mg	23	−0.6 (−38%)	–	–	
Cannon et al. (1999)	6	Placebo 1 × 20 μg	20	–	+0.1 (+3%)	–	1b
		intranasal 1 × 40 μg	20	–	−0.3 (−10%)	–	
		intranasal	20	–	−0.7 (−23%)[a]	–	
Asplund et al. (1999)	2	Placebo 1 × 0.1–0.4 mg	17	−0.2 (−11%)	−0.2 (−11%)	+ 0.2	1b
			17	−0.8 (−44%)[a]	−0.8 (−42%)[a]	+1.6	
Chancellor et al. (1999)	12	1 × 20–40 μg intranasal	12	–	−1.8 (−50%)	–	2b
Mattiasson et al. (2002)	3	Placebo 1 × 0.1–0.4 mg	65	−0.2 (−6%)	−0.5 (−12%)	+ 0.4	1b
			86	−0.6 (−36%)[a]	−1.3 (−43%)[a]	+1.8[a]	
Kuo 2002	4	1 × 0.1 mg	30	–	−2.72 (−48.5)	–	2b
Rembratt et al. (2003)	0.5	1 × 0.2 mg	72	−0.5	−1.0	+1.9	2b
van Kerrebroeck et al. (2007)	3	Placebo 1 × 0.1–0.4 mg	66	–	−0.4 (−15%)	+ 0.55	1b
			61	–	−1.25 (−39%)[a]	+1.66[a]	
Lose et al. (2004)	52	1 × 0.1–0.4 mg	132	–	−2	+2.3	2b

[a]Significant compared to placebo

night (Cannon et al. 1999; Mattiasson et al. 2002). The clinical effects of desmopressin were more pronounced in patients with more severe nocturnal polyuria and bladder capacity within the normal range at baseline. The 24-h diuresis remained unchanged during desmopressin treatment (Asplund et al. 1999). The clinical effects were stable over a follow-up period of 10–12 months and returned to baseline values after trial discontinuation (Lose et al. 2004). A significantly higher proportion of patients felt fresh in the morning after desmopressin use (odds ratio 2.71). Compared with placebo, nocturnal voiding frequency is reduced, duration of the first sleep period is increased and sleep quality may be improved (van Kerrebroeck et al. 2007). Desmopressin has level 1 evidence and grade A recommendation for use in nocturia of polyuric origin.

The absolute number of adverse events associated with desmopressin treatment was higher compared to placebo but usually mild in nature. The most frequent adverse events in short-term (up to 3 weeks) and long-term studies (12 months) were headache, nausea, diarrhoea, abdominal pain, dizziness, dry mouth and hyponatraemia. These events were comparable with the established safety profile of desmopressin in the treatment of polyuria due to other conditions. Peripheral edema (2%) and hypertension (5%) were reported in the long-term treatment trial (Lose et al. 2004). Hyponatremia (serum sodium concentration <130 mmol/l) was observed mainly in patients aged 65 years or older and seemed to occur less frequently in men compared to women of the same age (Hvistendahl et al. 2005). Hyponatremia of all degrees, not necessarily associated with symptoms, occurs in approximately 5–7.6% (Rembratt et al. 2006) of patients (Weatherall 2004) early after treatment initiation. The risk of developing hyponatremia significantly increases with age (odds ratio 1.16 per year of age), lower serum sodium concentration at baseline (odds ratio 0.76) and higher basal 24-h urine volume per bodyweight (odds ratio 1.09) (Rembratt et al. 2006). The chance of developing hyponatremia in patients younger than 65 years is less than 1%, whereas the risk for older patients increases to 8% with normal sodium concentration and up to 75% in patients with low sodium concentration at baseline (Rembratt et al. 2006). Therefore, the treatment of patients aged 65 years or older should not be initiated without monitoring the serum sodium concentration. At the time of treatment initiation or dose change, older patients with normal values of serum sodium should be monitored by serum sodium measurement at day 3 and day 7 of treatment as well as at 1 month later. If serum sodium concentration has remained normal and no dose adjustment is intended, serum sodium should be monitored every 3–6 months thereafter (Bae et al. 2007). Furthermore, patients should be informed about the prodromal symptoms of hyponatremia, such as headache, nausea or insomnia.

Overactive Bladder (OAB) and Urinary Incontinence

Antimuscarinics are the mainstay of OAB medication but may cause dry mouth, blurred vision or constipation. It is, therefore, crucial that new treatment modalities are sought to help with this potentially debilitating condition. Antidiuresis, using

desmopressin, forms a potential candidate for a novel treatment. As the bladder fills with urine, symptoms of OAB are experienced by patients. It would be reasonable to hypothesise that if the rate of bladder filling is reduced then so would the symptoms of OAB and urinary incontinence. Desmopressin reduces the production of urine by the kidneys, therefore reducing the amount of urine in the bladder and, therefore, the symptoms of OAB. Desmopressin has been used previously in small single centre trials in neurogenic OAB patients with some success but recently two multi-centre, multi-national randomised placebo controlled trials using this concept have been completed in idiopathic OAB sufferers and reported in the literature (Robinson et al. 2004; Hashim et al. 2009). The results were quite promising although there were minor side effects. These trials suggest that this potential novel treatment modality for OAB sufferers might avoid the necessity for invasive treatments, such as botulinum toxin, neuromodulation or surgery, in some instances. These trials also open the way to combination therapy with current treatment modalities of OAB (Hashim and Abrams 2007).

In a 2-week, multi-national, multi-centre, phase IIb, double-blind, placebo-controlled, prospective, randomised, cross-over study using 0.2 mg of oral desmopressin in adults suffering with OAB, time to first void was 8-min later on the drug than on placebo ($p = 0.27$). However, the drug led to one less void (3.2 vs. 4.2) in the same period ($p < 0.001$). There was an increase in the time to first urgency episode with a decrease in the number of urgency episodes in the drug days compared to placebo ($p < 0.003$). There was a subjective improvement in frequency and urgency and overall quality of life. Antidiuresis, using oral desmopressin tablets, is a novel, feasible and safe (short-term basis) method of treatment for adults with OAB and could be considered in the armamentarium of drugs available for the treatment of OAB (Hashim et al. 2009).

In another multi-centre, multi-national, randomised, double-blind, placebo-controlled, proof-of-concept study, administered desmopressin intra-nasally (Robinson et al. 2004), in a dose of 0.04 mg, to 64 women with urinary incontinence over 10 days (7 days of active drug and 3 days of placebo). The trial was completed by 57 women and the primary efficacy endpoint was the number of periods with no leakage for 4 h after dosing. Women were instructed to take the drug at a time of their choosing, but at least 4 h before bedtime. Secondary efficacy variables included the time to first void or incontinence episode, volume leaked per incontinence episode, total volume voided and number of periods with no leakage. Leakage was measured by weighing incontinence pads. The results showed that patients taking desmopressin had a significantly higher mean incidence of periods with no leakage during the first 4 and 8 h after taking desmopressin, but a similar incidence of leakage thereafter. The volume leaked on desmopressin was less than placebo, and the time to the first incontinence episode was longer on desmopressin. There was also a higher frequency of dry days on desmopressin than on placebo, and the volume voided on desmopressin, for 24 h following administration, was consistently lower on desmopressin. These effects seemed to be the same on the three main types of incontinence (stress, mixed and urgency). There was no hyponatremia or fluid retention reported by patients in the trial.

Desmopressin, based on the results of the previous two trials, can be used as a 'designer drug' on an 'as-required basis', thus helping with symptoms when patients want to go out and have limited access to toilets or if they feel embarrassed by going too often to the toilet to pass urine (Level 2 evidence, Grade C recommendation). However, daily usage of desmopressin in OAB and incontinence is not recommended until further trials have been conducted to look at this concept.

Ureteric Colic

Desmopressin is not commonly used in the treatment of acute ureteric colic. It has been demonstrated in experimental studies in rats that desmopressin has a powerful anti-diuretic effect and has reduced intraureteral pressures (Moro et al. 1999). The mechanism of analgesic action of desmopressin in renal colic is uncertain. At the peripheral level, desmopressin may alleviate the acute renal colic through its potent antidiuretic effect or by relaxing the renal pelvic and ureteral smooth muscles. The central analgesic effect of desmopressin by stimulating the release of the hypothalamic β-endorphin is proposed. Desmopressin acts rapidly and seems to be effective in both single and combined therapy with diclofenac; it decreases the need for a second treatment and increases the analgesic effect of diclofenac. Desmopressin may be used to treat renal colic either alone or in combination, increasing the analgesic effect of other drugs (Lopes et al. 2001). Recently, a double-blind controlled study on a combination of intranasal desmopressin spray and diclofenac sodium suppository on acute renal colic compared with diclofenac sodium suppository alone revealed that the combination therapy caused prompt pain relief with significant decreases in pain scores after 15 and 30 min (Roshani et al. 2010). However, this is not advocated in clinical practise, and the potential side-effects, particularly of fluid retention, need to be taken into consideration. More studies are needed to validate and confirm the results; it would also be useful to determine factors that may identify the subgroup of patients who respond quickly and with almost complete pain relief.

5.2 Non-Urological Applications of Vasopressin and Agonists

A non-urological, V_2 receptor-mediated therapeutic application is the use of desmopressin in bleeding disorders. Like vasopressin, desmopressin can increase the concentrations of Factor VIII:C, Factor VIII:Ag and Plasminogen Activator. However, the dosages required to stimulate the coagulation factors are ten times higher than the antidiuretic ones. Desmopressin in higher dosages has been licenced worldwide for haematological indications of haemophilia and von Willebrand disease.

V_1-receptor-mediated contraction of gastrointestinal smooth muscle has been used to treat postoperative ileus and abdominal distension. V_1-receptor-mediated vasoconstriction of the splanchnic arterial vessels reduces blood flow to the portal system and thereby attenuates pressure and bleeding in oesophageal varices (Burroughs 1998). Hence, V_1-receptor agonists can be used in an emergency setting until endoscopy can be performed (Serradeil-Le Gal et al. 2002b). Also, V_1-receptor agonists have been used during abdominal surgery in patients with portal hypertension to diminish the risk of haemorrhage during the procedure. Finally, V_1-receptor-mediated vasoconstriction has been used to reduce bleeding during acute hemorrhagic gastritis, burn wound excision, cyclophosphamide-induced hemorrhagic cystitis, liver transplant, caesarean sections and uterine myoma resection (Jackson 2006). Terlipressin has increased safety over vasopressin and is hence preferred for bleeding esophageal varices (Vlavianos and Westaby 2001). Terlipressin is effective in patients with hepatorenal syndrome, particularly when combined with albumin (Ortega et al. 2002).

6 Vasopressin Antagonists

A vasopressin antagonist is an agent which interferes with action at the vasopressin receptors. They can be used in the treatment of hyponatremia, especially in patients with congestive heart failure or liver cirrhosis (Palm et al. 2006). Demeclocycline, a tetracycline antibiotic, was used in the past to block the action of vasopressin in the kidney in hyponatremia due to inappropriate high secretion of vasopressin (syndrome of inappropriate ADH secretion or SIADH), when fluid restriction has failed (Padfield et al. 1978). This has now been succeeded by a new class of drugs called ‘vaptans’ which act by inhibiting the action of vasopressin on its receptors. The vaptan class of drugs (Table 5) contains a number of compounds with varying selectivity, several of which are either already in clinical use or in clinical trials as of 2009 (Serradeil-Le Gal et al. 2002a, b; Lemmens-Gruber and Kamyar 2006; Decaux et al. 2008). Peptide antagonists have limited oral activity, and the potency of peptide V_2 antagonists is species-dependent. Also, with prolonged infusion, peptide V_2 antagonists have significant agonist activity (Jackson 2006).

6.1 Clinical Utility of Vasopressin Receptor Antagonists

Disorders of vasopressin secretion frequently cause imbalances of body water: deficient vasopressin secretion can cause hyperosmolality as a result of inadequate renal water conservation, and excess or inappropriate AVP secretion can cause hypo-osmolality due to impaired renal water excretion (Kronenberg et al. 2002).

Hypo-osmolality usually manifests as hyponatremia, a very common electrolyte disorder associated with a variety of underlying conditions that are usually caused

Table 5 Vasopressin receptor antagonists

A. Peptide antagonists
I. V_1 selective antagonists
1. V_{1a} selective antagonist – $d(CH_2)_5[Tyr(Me)^2]AVP$
2. V_{1b} selective antagonist – dP $[Tyr(Me)^2]AVP$
II. V_2 selective antagonists
1. des Gly-$NH_2{}^9$-$d(CH_2)_5$[D-Ile^2, Ile^4]AVP
2. $d(CH_2)_5$[D-Ile^2, Ile^4, Ala-$NH_2{}^9$]AVP
B. Non-peptide antagonists
I. V_{1a} selective antagonists
1. OPC – 21268
2. SR 49059 (Relcovaptan)
II. V_{1b} selective antagonists
1. SSR 149415 (Nelivaptan)
III. V_2 selective antagonists
1. SR 121463A (Satavaptan)
2. VPA – 985 (Lixivaptan)
3. OPC – 31260 (Mozavaptan)
4. OPC – 41061 (Tolvaptan)
IV. V_{1a}/V_2 selective antagonists
1. YM – 471
2. YM 087 (Conivaptan)
3. JTV – 605
4. CL – 385004

by SIADH (Janicic and Verbalis 2003). In addition to SIADH, patients with edema-forming states such as congestive heart failure and cirrhosis, as well as patients treated with diuretics, also often have elevated plasma levels of vasopressin and hyponatremia (Schrier 2006; Sonnenblick et al. 1993; Greenberg 2000). Vasopressin receptor antagonists correct hyponatremia by blocking the activation of the V_2 receptor. These agents induce a free water diuresis without an accompanying natriuresis or kaliuresis; this effect has been termed 'aquaresis' to differentiate it from the effect produced by traditional diuretic agents. All data to date indicate that the vasopressin antagonists are highly effective in producing a safe and predictable aquaresis, thereby increasing serum sodium levels in hyponatremic patients. Several investigational oral vasopressin receptor antagonists are in late-stage clinical trials and hold promise for long-term therapy of chronic hyponatremia (Verbalis 2006). The V_2-receptor antagonists mozavaptan, lixivaptan, satavaptan and tolvaptan induce a highly hypotonic diuresis without substantially affecting the excretion of electrolytes. Conivaptan is the first V_{1a}/V_2 receptor antagonist to receive Food and Drug Administration (FDA) approval, specifically for intravenous administration to hospitalised patients with euvolemic or hypervolemic hyponatremia (Arai et al. 2009). Relcovaptan is a selective V_{1a}-receptor antagonist, which has shown initial positive results in the treatment of Raynaud's disease, dysmenorrhoea and tocolysis. SSR-149415 is a selective V_{1b}-receptor antagonist, which may have beneficial effects in the treatment of psychiatric disorders (Tsujimoto and Takano 2009).

Vasopressin has multiple actions, mediated through the V_{1a} and V_2 receptors, which could contribute to the progression of heart failure. Interfering with vasopressin signalling may have clinical benefits in acute and chronic heart failure. Facilitation of diuresis, a safe diuresis, and normalisation of serum sodium are potential mechanisms of benefit of V_2 antagonism in heart failure. Selective interference with only one set of receptors, could in theory, trigger counter-productive increased signalling at the other sites. Combined V_{1a} and V_2 antagonism might therefore be preferable as a therapeutic strategy, especially in the chronic setting, but this hypothesis has yet to be tested clinically (Goldsmith 2006).

7 Mutations

All members of the vasopressin receptor family belong to the superfamily of rhodopsin-like GPCRs. Since the V_2 receptor is critical to fluid homeostasis, mutations in the receptor are of great consequence. The V_2 receptor DNA encodes a protein of 371 amino acids. In most cases, V_2 receptor mutations interfere with protein synthesis and result in a reduced number or complete lack of receptors on the cell surface (Prasad 2001). These mutations are most often single amino acid changes, called missense mutations (Klug and Cummings 2000). These variations in the primary structure of the protein result in incorrectly formed secondary and tertiary structures. Perhaps the most widely studied missense mutations occur on amino acid residues 204, 205 and 206 in the C-terminal part of the second extracellular loop of the receptor (O'Toole 1992).

NDI is a well-studied monogenic disorder of a GPCR, the AVP receptor 2, in which mutations cause congenital NDI in around 90% of patients via an X-linked recessive inheritance (Sands and Bichet 2006). To date, more than 280 families with a history of NDI have been shown to have more than 180 putative disease-causing mutations in V_2 receptor (Fig. 2 and Sands and Bichet 2006). In most cases, these mutations lead to the intracellular trapping of the V2 receptors, such that few receptors reach the plasma membrane to trigger the activation of Gs and adenylyl cyclase and thereby the generation of cAMP (Insel et al. 2007). Therapeutic approaches are under investigation that involve the use of non-peptide V2 receptor antagonists to bind intracellular receptors as what have been termed 'pharmacochaperones' that will facilitate their folding, insertion and function in the plasma membrane (Wuller et al. 2004).

In their study on the role of V_2 receptors in adaptations to limited water supply, Böselt et al. (2009) identified over 103 missense mutations in the V_2 receptor genes in patients with NDI. Detailed analysis of their results revealed six major findings (Böselt et al. 2009). First, there were several length variations within the extracellular and intracellular loops, with the length variation in the third intracellular loop (ICL3) being the most prominent. Second, the N-terminus is the most diverse in its length and amino acid sequence between mammalian orthologs clearly indicating less specific relevance in ligand binding and in receptor activity. Third, the

C-terminus of the human V_2 receptor contains two Cys residues (Cys^{341}, Cys^{342}) which allow anchoring via palmitoylation and forming a fourth intracellular loop (ICL4). It was demonstrated by mutagenesis of the two Cys residues that palmitoylation of V_2 receptor is important for intracellular trafficking and/or sequestration/internalisation. Fourth, a proposed glutamate/dileucine motif equivalent ($E^{335}LRSLL^{340}$ in human V_2 receptor gene) in the C terminus (Schulein et al. 1998) is highly preserved during mammalian V_2 receptor evolution. Fifth, most rhodopsin-like GPCR possess an evolutionarily conserved Asp-Arg-Tyr (DRY) motif in the C-terminal region of TMD3. Mutations of the first two residues within this motif usually alter receptor function and, when they occur naturally, can even cause diseases. In V_2 receptor, the DRY motif is a DRH which is highly conserved in mammals and only the manatee has DRQ. Variation of the Tyr residue in the DRY motif appears to be functionally tolerated since this position is variable in many GPCR and substitution of Tyr has no or marginal effects in most cases (Rovati et al. 2007). And finally, phosphorylation plays a pivotal role in the regulation of GPCR function.

8 Conclusion

Vasopressin is mainly involved in the osmoregulation of the body. The biological effects of vasopressin are mediated by the aforementioned vasopressin receptors V_{1a}, V_{1b} and V_2. Although little is known about the clinical utility of vasopressin analogue, various reports were published on the newly discovered AQPs and on the polymorphisms and mutations of the receptors. Desmopressin – a synthetic peptide analogue of vasopressin is increasingly used in the treatment of nocturnal polyuria and nocturnal enuresis with potential use in OAB and urinary incontinence in the future. Due to their actions via V_1 receptors on the cardiovascular, gastrointestinal, haematological and central nervous system, its analogues and antagonists have gained widespread clinical demand over the last few years. Research is ongoing on the potential development of other non-peptide analogues and antagonists.

References

Abu-Basha EA, Yibchok-anun S, Hsu WH (2002) Glucose dependency of arginine vasopressin-induced insulin and glucagon release from the perfused rat pancreas. Metabolism 51:1184–1190

Acher R, Light A, Du Vigneaud V (1958) Purification of oxytocin and vasopressin by way of a protein complex. J Biol Chem 233(1):116–120

Agre P, Preston GM, Smith BL, Jung JS, Raina S, Moon C, Guggino WB, Nielsen S (1993) Aquaporin CHIP: the archetypal molecular channel. Am J Physiol 265:F463–F476

Agre P, Bonhivers M, Borgnia MJ (1998) The aquaporins, blueprints for cellular plumbing systems. J Bio Chem 273:14659–14662

Aguilera G, Rabadan-Diehl C (2000) Vasopressinergic regulation of the hypothalamic–pituitary–adrenal axis: implications for stress adaptation. Regul Pept 96:23–29

Alloussi SH, Mürtz G, Lang C, Madersbacher H, Strugala G, Seibold J, Schwentner C, Stenzl A, Alloussi S. Desmopressin treatment regimens in monosymptomatic and nonmonosymptomatic enuresis: a review from a clinical perspective. J Pediatr Urol. 2010 [Epub] Accessed 01st August 2010

Antoni FA, Holmes MC, Makara GB, Kárteszi M, László FA (1984) Evidence that the effects of arginine-8-vasopressin (AVP) on pituitary corticotropin (ACTH) release are mediated by a novel type of receptor. Peptides 5(3):519–522

Arai Y, Fujimori A, Sasamata M, Miyata K (2009) New topics in vasopressin receptors and approach to novel drugs: research and development of Conivaptan hydrochloride (YM087), a drug for the treatment of hyponatremia. J Pharmacol Sci 109:53–59

Asplund R, Sundberg B, Bengtsson P (1998) Desmopressin for the treatment of nocturnal polyuria in the elderly: a dose titration study. Br J Urol 82(5):642–646, Level 2b

Asplund R, Sundberg B, Bengtsson P (1999) Oral desmopressin for nocturnal polyuria in elderly subjects: a double-blind, placebo-controlled randomized exploratory study. BJU Int 83:591–595 (Level 1b)

Bae JH, Oh MM, Shim KS, Cheon J, Lee JG, Kim JJ, Moon DG (2007) The effects of long-term administration of oral desmopressin on the baseline secretion of antidiuretic hormone and serum sodium concentration for the treatment of nocturia: a circadian study. J Urol 178(1):200–203

Bernat A, Hoffmann T, Dumas A et al (1997) V_2 receptor antagonism of DDAVP-induced release of hemostatis factors in conscious dogs. J Pharmacol Exp Ther 282:597–602

Birnbaumer M (2000) Vasopressin receptors. Trends Endocrinol Metab 11(10):406–410

Birumachi JI, Hiroyama M, Fujiwara Y, Aoyagi T, Sanbe A, Tanoue A (2007) Impaired arginine-vasopressin-induced aldosterone release from adrenal gland cells in mice lacking the vasopressin V1A receptor. Eur J Pharmacol 566:226–230

Böselt I, Römpler H, Hermsdorf T, Thor D, Busch W et al (2009) Involvement of the V2 vasopressin receptor in adaptation to limited water supply. PLoS One 4(5):e5573. doi:10.1371/journal.pone.0005573. Accessed online on 26th July, 2009

Burgu B, Gökce MI, Gücük A, Soygür T (2009) Prospective evaluation of factors affecting the response and relapse rates to desmopressin therapy in male monosymptomatic enuretic adults. Urology 74(4):915–919

Burroughs AK (1998) Pharmacological treatment of acute variceal bleeding. Digestion 59:28–36

Cabiaux V, Oberg KA, Pancoska P, Walz T, Agre P, Engel A (1997) Secondary structures comparison of aquaporin-1 and bacteriorhodopsin: a Fourier transform infrared spectroscopy study of two-dimensional membrane crystals. Biophys J 73:406–417

Caione P, Nappo S, De Castro R, Prestipino M, Capozza N (1999) Low-dose desmopressin in the treatment of nocturnal urinary incontinence in the exstrophy–epispadias complex. BJU Int 84 (3):329–334

Caldwell HK, Lee HJ, Macbeth AH, Young WS (2008) Vasopressin: behavioral roles of an 'original' neuropeptide. Prog Neurobiol 84(1):1–24

Cannon A, Carter PG, McConnell AA, Abrams P (1999) Desmopressin in the treatment of nocturnal polyuria in the male. BJU Int 84:20–24, Level 1b

Chancellor MB, Rivas DA, Staas WE Jr (1994) DDAVP in the urological management of the difficult neurogenic bladder in spinal cord injury: preliminary report. J Am Paraplegia Soc 17 (4):165–167

Chancellor MB, Atan A, Rivas DA, Watanabe T, Tai HL, Kumon H (1999) Beneficial effect of intranasal desmopressin for men with benign prostatic hyperplasia and nocturia: preliminary results. Tech Urol 5(4):191–194 (Level 2b)

Cheng A, van Hoek AN, Yeager M, Verkman AS, Mitra AK (1997) Three-dimensional organization of a human water channel. Nature 387:627–630

Cridland RA, Kasting NW (1992) A critical role for central vasopressin in regulation of fever during bacterial infection. Am J Physiol 263:R1235–R1240

Dantzer R, Bluthé RM (1993) Vasopressin and behaviour: from memory to olfaction. Regul Pept 45:121–125
Decaux G, Soupart A, Vassart G (2008) Non-peptide arginine-vasopressin antagonists: the vaptans. Lancet 371(9624):1624–1632
Dimson SB (1977) Desmopressin as a treatment for enuresis. Lancet 1(8024):1260
Du Vigneaud V (1954) Hormones of the posterior pituitary gland: oxytocin and vasopressin. Harvey Lect 50:1–26
Ecelbarger CA, Terris J, Frindt G, Echevarria M, Marples D, Nielsen S, Knepper MA (1995) Aquaporin-3 water channel localization and regulation in rat kidney. Am J Physiol Renal Fluid Electrolyte Physiol 269:F663–F672
Ecelbarger CA, Kim GH, Wade JB, Knepper MA (2001) Regulation of the abundance of renal sodium transporters and channels by vasopressin. Exp Neurol 171:227–234
Echevarria M, Windhager EE, Tate SS, Frindt G (1994) Cloning and expression of AQP3, a water channel from the medullary collecting duct of rat kidney. Proc Natl Acad Sci USA 91:10997–11001
Eckford SD, Swami KS, Jackson SR, Abrams PH (1994) Desmopressin in the treatment of nocturia and enuresis in patients with multiple sclerosis. Br J Urol 74(6):733–735
Eckford SD, Carter PG, Jackson SR, Penney MD, Abrams P (1995) An open, in-patient incremental safety and efficacy study of desmopressin in women with multiple sclerosis and nocturia. Br J Urol 76(4):459–463
Egashira N, Mishima K, Iwasaki K, Oishi R, Fujiwara M (2009) New topics in vasopressin receptors and approach to novel drugs: Role of the vasopressin receptors in psychological and cognitive functions. J Pharmacol Sci 109:44–49
Ferreira E, Letwin SR (1998) Desmopressin for nocturia and enuresis associated with multiple sclerosis. Ann Pharmacother 32(1):114–116
Fjellestad-Paulsen A, Hoglund P, Lundin S, Paulsen O (1993) Pharmacokinetics of 1-deamino-8-D-arginine vasopressin after various routes of administration in healthy volunteers. Clin Endocrinol (Oxf) 38(2):177–182
Fredrikson S (1996) Nasal spray desmopressin treatment of bladder dysfunction in patients with multiple sclerosis. Acta Neurol Scand 94(1):31–34
Giminz I, Forbush B (2003) Short-term stimulation of the renal Na–K–Cl cotransporter (NKCC2) by vasopressin involves phosphorylation and membrane translocation of the protein. J Biol Chem 278:26946–26951
Goldsmith S (2006) Vasopressin receptor antagonists: mechanisms of action and potential effects in heart failure. Cleve Clin J Med 73(2):S20–S23
Greenberg A (2000) Diuretic complications. Am J Med Sci 319:10
Hashim H, Abrams P (2007) Novel uses for antidiuresis. Int J Clin Pract Suppl 155:32–36
Hashim H, Malmberg L, Graugaard-Jensen C, Abrams P (2009) Desmopressin, as a 'designer-drug', in the treatment of overactive bladder syndrome. Neurourol Urodyn 28(1):40–46
Hernando F, Schoots O, Lolait SJ, Burbach JP (2001) Immunohistochemical localization of the vasopressin V_{1b} receptor in the rat brain and pituitary gland: anatomical support for its involvement in the central effects of vasopressin. Endocrinology 142(4):1659–1668
Hilton P, Hertogs K, Stanton SL (1983) The use of desmopressin (DDAVP) for nocturia in women with multiple sclerosis. J Neurol Neurosurg Psychiatr 46(9):854–855
Hiroyama M, Aoyagi T, Fujiwara Y, Oshikawa S, Sanbe A, Endo F et al (2007a) Hyperammonemia in V1a vasopressin receptor knockout mice caused by the promoted proteolysis and reduced intrahepatic blood volume. J Physiol 581:1183–1192
Hiroyama M, Aoyagi T, Fujiwara Y, Birumachi J, Shigematsu Y, Kiwaki K et al (2007b) Hypermetabolism of fat in V1a vasopressin receptor knockout mice. Mol Endocrinol 21:247–258
Hjalmas K (1999) Desmopressin treatment: current status. Scand J Urol Nephrol Suppl 202:70–72
Honda K, Takano Y (2009) New topics in vasopressin receptors and approach to novel drugs: Involvement of vasopressin V1a and V1b receptors in nociceptive responses and morphine-induced effects. J Pharmacol Sci 109:38–43

Hoverd PA, Fowler CJ (1998) Desmopressin in the treatment of daytime urinary frequency in patients with multiple sclerosis. J Neurol Neurosurg Psychiatr 65:778–780

Hvistendahl GM, Riis A, Norgaard JP, Djurhuus JC (2005) The pharmacokinetics of 400 microg of oral desmopressin in elderly patients with nocturia, and the correlation between the absorption of desmopressin and clinical effect. BJU Int 95(6):804–809

Insel PA, Tang CM, Hahntow I, Michel MC (2007) Impact of GPCRs in clinical medicine: monogenic diseases, genetic variants and drug targets – a review. Biochimica Biophysica Acta 1768:994–1005

Jackson EK (2006). Vasopressin and other agents affecting the renal conservation of water. In: Goodman and Gilman's The Pharmacological Basis of Therapeutics. 11th edition. The McGraw-Hill companies. United States of America

Janicic N, Verbalis JG (2003) Evaluation and management of hypo-osmolality in hospitalized patients. Endocrinol Metab Clin North Am 32:459–481

Kimbrough RDJ, Cash WD, Branda LA, Chan WY, Du Vigneaud V (1963) Synthesis and biological properties of 1-desamino-8-lysine-vasopressin. J Biol Chem 238:1411–1414

Klug WS, Cummings MR (2000) Concepts of genetics, 6th edn. Prentice Hall, Upper Saddle River

Knepper MA (1997) Molecular physiology of urinary concentrating mechanism: regulation of aquaporin water channels by vasopressin. Am J Physiol Renal Physiol 272:F3–F12

Knepper MA, Nielsen S, Chou CL, Digiovanni SR (1994) Mechanism of vasopressin action in the renal collecting duct. Semin Nephrol 14:302–321

Koshimizu A, Tsujimoto G (2009) New topics in vasopressin receptors and approach to novel drugs: Vasopressin and pain perception. J Pharmacol Sci 109:33–37

Kronenberg HM, Melmed S, Polonsky KS (eds) (2002) Textbook of endocrinology, 10th edn. Elsevier, Philadelphia

Kruse E, Uehlein N, Kaldenhoff R (2006) The aquaporins. Protein family review. Genome Biol 7 (2):206.1–206.6

Kuo HC (2002) Efficacy of desmopressin in treatment of refractory nocturia in patients older than 65 years. Urology 59:485–489 (Level 2b)

Lemmens-Gruber R, Kamyar M (2006) Vasopressin antagonists. Cell Mol Life Sci 63 (15):1766–1779

Lopes T, Dias JS, Marcelino J, Varela J, Ribeiro S, Dias J (2001) An assessment of the clinical efficacy of intranasal desmopressin spray in the treatment of renal colic. BJU Int 87(4):322–325

Lose G, Mattiasson A, Walter S, Lalos O, van Kerrebroeck P, Abrams P, Freeman R (2004) Clinical experiences with desmopressin for long-term treatment of nocturia. J Urol 172 (3):1021–1025

Manning M, Stoev S, Cheng LL et al (1999) Discovery and design of novel vasopressin hypotensive peptide agonists. J Recept Signal Transduct Res 19:631–644

Mansson W, Sundin T, Gullberg B (1980) Evaluation of a synthetic vasopressin analogue for treatment of nocturia in benign prostatic hypertrophy. A double-blind study. Scand J Urol Nephrol 14(2):139–141

Mattiasson A, Abrams P, Van Kerrebroeck P, Walter S, Weiss J (2002) Efficacy of desmopressin in the treatment of nocturia: a double-blind placebo-controlled study in men. BJU Int 89 (9):855–862 (Level 1b)

Michell RH, Kirk CJ, Billah MM (1979) Hormonal stimulation of phosphatidylinositol breakdown with particular reference to the hepatic effects of vasopressin. Biochem Soc Trans 7:861–865

Moro U, De Stefani S, Crisci A, De Antoni P, Scott CA, Selli C (1999) Evaluation of the effects of desmopressin in acute ureteral obstruction. Urol Int 62(1):8–11

Nakamura S, Hirano T, Tsujimae K et al (2000) Antidiuretic effects of a nonpeptide vasopressin V_2-receptor agonist OPC-51803 administered orally to rats. J Pharmacol Exp Ther 295:1005–1011

Nielsen S, Digiovanni SR, Christensen EI, Knepper MA, Harris HW (1993) Cellular and subcellular immunolocalization of vasopressin regulated water channel in rat kidney. Proc Natl Acad Sci USA 90:11663–11667

Nielsen S, Kwon TH, Christensen BM, Promeneur D, Frokier J, Marples D (1999) Physiology and pathophysiology of renal aquaporins. J Am Soc Nephrol 10:647–663
Nielsen S, Frokier J, Marples D, Kwon TH, Age P, Knepper M (2002) Aquaporins in the kidney: from molecules to medicine. Physiol Rev 82:205–244
Norgaard JP, Pedersen EB, Djurhuus JC (1985) Diurnal anti-diuretic-hormone levels in enuretics. J Urol 134(5):1029–1031
O'Toole M (ed) (1992) Miller-Keane encyclopedia & dictionary of medicine, nursing & allied health, 5th edn. Saunders, Philadelphia
Oelke M, Bachmann A, Descazeaud A, Emberton M, Gravas S, Michel MC, N'Dow J, Nordling J, de la Rosette JJ (2010) Guidelines on conservative treatment of non-neurogenic male LUTS. EAU Guidelines 3.5:23–25
Ortega R, Ginès P, Uriz J et al (2002) Terlipressin therapy with and without albumin for patients with hepatorenal syndrome: results of a prospective, nonrandomized study. Hepatology 36:941–948
Oshikawa S, Tanoue A, Koshimizu T, Kitagawa Y, Tsujimoto G (2004) Vasopressin stimulates insulin release from islet cells through V1b receptors: a combined pharmacological/knockout approach. Mol Pharmacol 65:623–629
Padfield PL, Hodsman GP, Morton JJ (1978) Demeclocycline in the treatment of the syndrome of inappropriate antidiuretic hormone release: with measurement of plasma ADH. Postgrad Med J 54(635):623–627
Palm C, Pistrosch F, Herbrig K, Gross P (2006) Vasopressin antagonists as aquaretic agents for the treatment of hyponatremia. Am J Med 119(7 Suppl 1):S87–S92
Prasad S (2001). The role of the V_2 vasopressin receptor in nephrogenic diabetes insipidus. Stanislaus Journal of biochemical reviews. http://wwwchem.csustan.edu/chem4400/sjbr/Prasad01.htm. Accessed on 26th July, 2009
Puri VN (1980) Urinary levels of antidiuretic hormone in nocturnal enuresis. Indian Pediatr 17 (8):675–676
Rembratt A, Norgaard JP, Andersson KE (2003) Desmopressin in elderly patients with nocturia: short-term safety and effects on urine output, sleep and voiding patterns. BJU Int 91 (7):642–646 (Level 2b)
Rembratt A, Graugaard-Jensen C, Senderovitz T, Norgaard JP, Djurhuus JC (2004) Pharmacokinetics and pharmacodynamics of desmopressin administered orally versus intravenously at daytime versus night-time in healthy men aged 55–70 years. Eur J Clin Pharmacol 60 (6):397–402 (Level 2b)
Rembratt A, Riis A, Norgaard JP (2006) Desmopressin treatment in nocturia; an analysis of risk factors for hyponatremia. Neurourol Urodyn 25(2):105–109 (Level 1b)
Rittig S, Jensen AR, Jensen KT, Pedersen EB (1998) Effect of food intake on the pharmacokinetics and antidiuretic activity of oral desmopressin (DDAVP) in hydrated normal subjects. Clin Endocrinol (Oxf) 48(2):235–241
Robinson D, Cardozo L, Akeson M, Hvistendahl G, Riis A, Norgaard JP (2004) Antidiuresis: a new concept in managing female daytime urinary incontinence. BJU Int 93(7):996–1000
Roshani A, Falahatkar S, Khosropanah I, Roshan ZA, Zarkami T, Palizkar M, Emadi SA, Akbarpour M, Khaki N (2010) Assessment of clinical efficacy of intranasal desmopressin spray and diclofenac sodium suppository in treatment of renal colic versus diclofenac sodium alone. Urology 75(3):540–542
Rovati GE, Capra V, Neubig RR (2007) The highly conserved DRY motif of class A G protein-coupled receptors: beyond the ground state. Mol Pharmacol 71:959–964
Sands JM (2003) Molecular mechanisms of urea transport. J Membr Biol 191:149–163
Sands JM, Bichet DG (2006) Nephrogenic diabetes insipidus. Ann Intern Med 144:186–194
Schrier RW (2006) Water and sodium retention in oedematous disorders: role of vasopressin and aldosterone. Am J Med 119(7 Suppl 1):S47–S53
Schulein R, Hermosilla R, Oksche A, Dehe M, Wiesner B et al (1998) A dileucine sequence and an upstream glutamate residue in the intracellular carboxyl terminus of the vasopressin V2 receptor are essential for cell surface transport in COS. M6 cells. Mol Pharmacol 54:525–535

Scott LV, Dinan TG (2002) Vasopressin as a target for antidepressant development: an assessment of the available evidence. J Affect Disord 72:113–124

Serradeil-Le Gal C, Wagnon J, Valette G et al (2002a) Nonpeptide vasopressin receptor antagonists: development of selective and orally active V_{1a}, V_2 and V_{1b} receptor ligands. Prog Brain Res 139:197–210

Serradeil-Le Gal C, Wagnon J, Valette G, Garcia G, Pascal M, Maffrand JP, Le Fur G (2002b) Nonpeptide vasopressin receptor antagonists: development of selective and orally active V1a, V2 and V1b receptor ligands. Prog Brain Res 139:197–210

Snyder HM, Noland TD, Breyer MD (1992) cAMP-dependent protein kinase mediates hydroosmotic effect of vasopressin in collecting duct. Am J Physiol 263:C147–C153

Sonnenblick M, Friedlander Y, Rosin AJ (1993) Diuretic-induced severe hyponatremia. Review and analysis of 129 reported patients. Chest 103:601–606

Sorensen PS, Vilhardt H, Gjerris F, Warberg J (1984) Impermeability of the blood–cerebrospinal fluid barrier to 1-deamino-8-D-arginine-vasopressin (DDAVP) in patients with acquired, communicating hydrocephalus. Eur J Clin Invest 14(6):435–439

Stegner H, Artman HG, Leake RD, Fisher DA (1983) Does DDAVP (1-desamino-8-D-arginine-vasopressin) cross the blood–CSF barrier? Neuroendocrinology 37(4):262–265

Studer RO, Cash WD (1963) Synthesis of 1-acetyl-8-argininevasopressin and a study of its effects in the rat pressor assay. J Biol Chem 238:657–659

Suchowersky O, Furtado S, Rohs G (1995) Beneficial effect of intranasal desmopressin for nocturnal polyuria in Parkinson's disease. Mov Disord 10(3):337–340

Terris J, Ecelbarger CA, Marples D, Knepper MA, Nielsen S (1995) Distribution of aquaporin-4 water channel expression within rat kidney. Am J Physiol Renal Fluid Electrolyte Physiol 269: F775–F785

Terris J, Ecelbarger CA, Nielsen S, Knepper MA (1996) Long-term regulation of four renal aquaporins in rats. Am J Physiol Renal Physiol 271:F414–F422

Thbonnier M, Coles P, Thibionnier A, Shoham M (2002) Molecular pharmacology and modelling of vasopressin receptors. Prog Brain Res 139:179–196

Tsujimoto G, Takano Y (2009) New topics in vasopressin receptors and approach to novel drugs: preface. J Pharmacol Sci 109:32

Tubridy N et al (1999) Long term use of desmopressin for urinary symptoms in multiple sclerosis. Mult Scler 5:416–417

Turner RA, Pierce JG, Du Vigneaud V (1951) The purification and the amino acid content of vasopressin preparations. J Biol Chem 191(1):21–28

Tuvemo T (1978) DDAVP in childhood nocturnal enuresis. Acta Paediatr Scand 67(6):753–755

Valiquette G, Abrams GM, Herbert J (1992) DDAVP in the management of nocturia in multiple sclerosis. Ann Neurol 31(5):577

Valiquette G, Herbert J, Maede-D'Alisera P (1996) Desmopressin in the management of nocturia in patients with multiple sclerosis. A double-blind, crossover trial. Arch Neurol 53 (12):1270–1275

Van de Walle J, Van Herzeele C, Raes A (2010) Is there still a role for desmopressin in children with primary monosymptomatic nocturnal enuresis?: a focus on safety issues. Drug Saf 33 (4):261–271

Van Herzeele C, Alova I, Evans J, Eggert P, Lottmann H, Nørgaard JP, Vande Walle J (2009) Poor compliance with primary nocturnal enuresis therapy may contribute to insufficient desmopressin response. J Urol 182(4 Suppl):2045–2049

Van Hoek AN, Yang B, Kirmiz S, Brown D (1998) Freeze-fracture analysis of plasma membranes of CHO cells stably expressing aquaporins 1–5. J Membr Biol 165:243–254

van Kerrebroeck P, Rezapour M, Cortesse A, Thüroff J, Rijs A, Norgaard JP (2007) Desmopressin in the treatment of nocturia: a double blind placebo-controlled study. Eur Urol 52(1):221–229 (Level 1b)

van Kerrebroeck P, Hashim H, Holm-Larsen T, Robinson D, Stanley N (2010) Thinking beyond the bladder: antidiuretic treatment of nocturia. Int J Clin Pract 64(6):807–816

Vavra I, Machova A, Krejci I (1974) Antidiuretic action of 1-deamino-(8-D-arginine)-vasopressin in unanesthetized rats. J Pharmacol Exp Ther 188(1):241–247
Verbalis JG (2006) AVP receptor antagonists as aquaretics: review and assessment of clinical data. Cleve Clin J Med 73(3):S24–S33
Vilhardt H, Lundin S (1986) Biological effect and plasma concentrations of DDAVP after intranasal and peroral administration to humans. Gen Pharmacol 17(4):481–483
Vlavianos P, Westaby D (2001) Management of acute variceal haemorrhage. Eur J Gastroenterol Hepatol 13:335–342
Vogt M, Lehnert T, Till H, Rolle U (2010) Evaluation of different modes of combined therapy in children with monosymptomatic nocturnal enuresis. BJU Int 105(10):1456–1459
Walz T, Hirai T, Murata K, Heymann JB, Mitsuoka K, Fujiyoshi Y, Smith BL, Agre P, Engel A (1997) The three-dimensional structure of aquaporin-1. Nature 387:624–627
Weatherall M (2004) The risk of hyponatremia in older adults using desmopressin for nocturia: a systematic review and meta-analysis. Neurourol Urodyn 23(4):302–305 (Level 1a)
Wuller S, Wiesner B, Loffler A, Furkert J, Krause G, Hermosilla R, Schaefer M, Schulein R, Rosenthal W, Oksche A (2004) Pharmacochaperones post-translationally enhance cell surface expression by increasing conformational stability of wild-type and mutant vasopressin V2 receptors. J Biol Chem 279:47254–47263
Yang B, Brown D, Verkman AS (1996) The mercurial insensitive water channel (AQP-4) forms orthogonal arrays in stably transfected Chinese hamster ovary cells. J Biol Chem 271:4577–4580
Young LJ, Wang Z, Insel TR (1998) Neuroendocrine bases of monogamy. Trends Neurosci 21:71–75
Young WS, Li J, Wersinger SR, Palkovits M (2006) The vasopressin 1b receptor is prominent in the hippocampal area CA2 where it is unaffected by restraint stress or adrenalectomy. Neuroscience 143(4):1031–1039
Zahariou A, Karamouti M, Karagiannis G, Papaioannou P (2008) Maximal bladder capacity is a positive predictor of response to desmopressin treatment in patients with MS and nocturia. Int Urol Nephrol 40(1):65–69
Zerbe RL, Vinicor F, Robertson GL (1979) Plasma vasopressin in uncontrolled diabetes mellitus. Diabetes 28:503–508

ATP and P2X Purinoceptors in Urinary Tract Disorders

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Contents

Abstract The pharmacological concept of specifically targeting purinoceptors (receptors for ATP and related nucleotides) has emerged over the last two decades in the quest for novel, differentiated therapeutics. Investigations from many laboratories have established a prominent role for ATP in the functional regulation of most tissue and organ systems, including the urinary tract, under normal and pathophysiological conditions. In the particular case of the urinary tract, ATP signaling via P2X1 receptors participates in the efferent control of detrusor smooth muscle excitability, and this function may be heightened in disease and aging. Perhaps of greater interest, ATP also appears to be involved in bladder sensation, operating via activation of P2X3-containing receptors on sensory afferent neurones,

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both on peripheral terminals within the urinary tract tissues (e.g., ureters, bladder) and on central synapses in the dorsal horn of the spinal cord. Such findings are based on results from classical pharmacological and localization studies in nonhuman and human tissues, gene knockout mice, and studies using recently identified pharmacological antagonists – some of which have progressed as candidate drug molecules. Based on recent advances in this field, it is apparent that the development of selective antagonists for these receptors will occur that could lead to therapies offering better relief of storage, voiding, and sensory symptoms for patients, while minimizing the systemic side effects that curb the clinical effectiveness of current urologic medicines.

Keywords Afferent · ATP · Hyperreflexia · P2X1 · P2X2/3 · P2X3 · Purinergic · Purinoceptor · Reflex bladder · Sensitization

Abbreviations

ADP	Adenosine 5′-diphosphate
AMP	Amplitude of contractions
ATP	Adenosine 5′-triphosphate
BPH	Benign prostatic hyperplasia
BPS/IC	Bladder pain syndrome/interstitial cystitis
BzATP	2′(3′)-O-(4-benzoylbenzoyl) adenosine-5′-triphosphate
DRG	Dorsal root ganglion
FLIPR	Fluorometric imaging plate reader
FREQ	Frequency of contractions
HTS	High throughput screening
IB4	Isolectin B4
LS	Lumbosacral
LUT	Lower urinary tract
LUTS	Lower urinary tract symptoms
NANC	Nonadrenergic, noncholinergic
OAB	Overactive bladder
PPADS	Pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid
TL	Thoracolumbar
TNP-ATP	2′,3′-O-(2,4,6-trinitrophenyl)adenosine-5′-triphosphate
TRPV1	Transient receptor potential vanilloid-1 receptor
TV	Threshold volume
UTI	Urinary tract infection
UTP	Uridine 5′-triphosphate
α,βmeATP	α,β-methyleneATP

1 Introduction

Our current appreciation of the large and diverse family of nucleotide [adenosine 5′-triphosphate (ATP), adenosine 5′-diphosphate (ADP), and uridine 5′-triphosphate (UTP)]-activated receptors is based on the pioneering work of Burnstock and collaborators, who had first carved out evidence for "purinergic" transmission in a variety of tissues and organs (Abbracchio and Burnstock 1994; Burnstock et al. 1970; Burnstock 1972, 1976, 2001b). Over the last 15 years we have learned that these so-called P2 purinoceptors vary greatly in their form, function, cellular distribution, and pharmacological properties (North 1996a, b, 2002; North and Barnard 1997; North and Surprenant 2000), and one of these receptors, the P2Y12 receptor, transpired to be the target of existing chemical therapeutics (the antiplatelet agent, clopidogrel). It is now apparent that ATP and related nucleotides are frequent participants in disease pathogenesis and that many more P2 purinoceptors represent rational potential targets for novel, differentiated medicines for a wide range of conditions. Indeed, in a few cases, significant discovery progress has been made to that end. In the context of the urinary tract, the focus of research discoveries within the P2 family has been more limited, but a strong body of data has emerged implicating ATP activity *especially* in pathological situations, inviting speculation as to the benefits of selective pharmacological intervention.

The current medicinal landscape offers medicines for the vast population plagued by symptoms of overactive bladder (OAB) and benign prostatic hyperplasia (BPH) that impart only marginal clinical effectiveness, largely due to the broad physiological function of the specific target substrates. Meanwhile, other highly bothersome disorders such as bladder pain syndrome/interstitial cystitis (BPS/IC), chronic prostatitis, and renal colic are without clinically meaningful, convenient therapeutic options. In this light, interventions targeting ATP signaling mechanisms may represent the next best hope for differentiated, effective, safe, and well tolerated new medicines for many of these urologic disorders. Our goal is to provide the context for such an aspiration.

In this chapter, selected examples from a large body of data in the purinergic receptor field are discussed to provide an overall picture of the current medicinal opportunity in urologic disorders, followed by an update on the status of pharmaceutical targeting of purinoceptors. The chapter focuses almost exclusively on members of the ionotropic (P2X) class of P2 receptors and most extensively on P2X3-containing receptors, as these have provided both a stronger scientific rationale in the context of urinary tract function and pathology and have surfaced as being – at least to date – more amenable to chemical intervention. Most attention is placed on evidence supporting a role for P2X3-containing receptors in the function of the urinary bladder and ureters, as this area of research has generated a great deal of interest and invited speculation that heightened ATP signaling may be an important component of urological dysfunction. It is also clear that many of the facets of purinergic function described for the urinary tract seem to have parallels in

other visceral structures (e.g., airways, bowel) and so inferential extension to structures such as urethra, prostate, and kidney may be worthy of consideration.

1.1 Purinergic Efferent and Afferent Function

The wiring control of urinary tract function has received much attention over the last two decades, and a diagrammatic view of the circuitry of afferent and efferent control pathways is illustrated in Fig. 1 as a reference for some of the concepts discussed below. One perhaps fundamental concept that has emerged is that of "the reflex bladder" (as coined by de Groat), a concept based on the notion that involuntary, local segmental spinal circuits that dominate postnatal urinary storage and voiding function become silenced by descending inhibition as voluntary control of urine storage

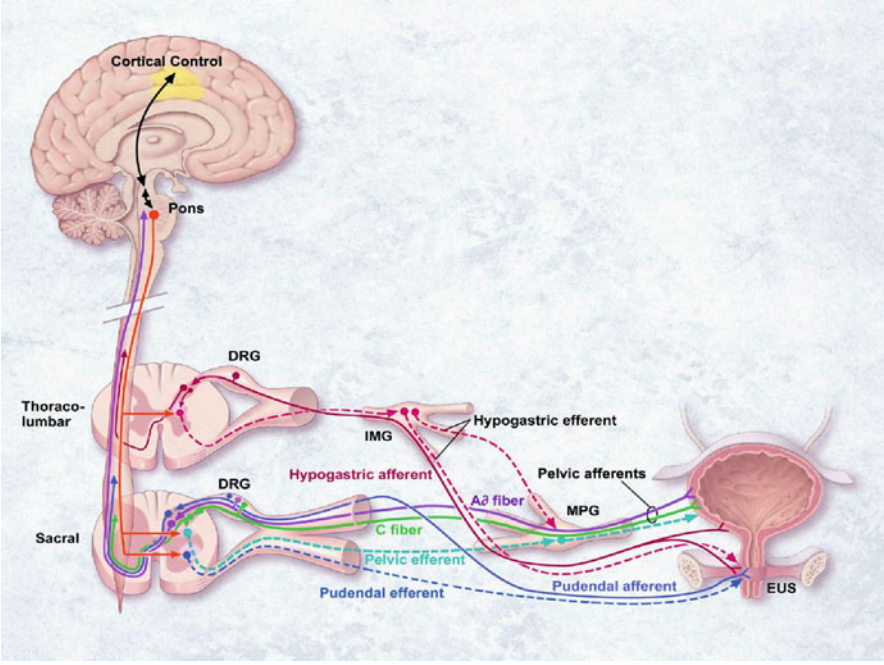

Fig. 1 Schematic diagram of the neural circuits controlling continence and micturition. The majority of Aδ- and C-afferents that innervate the urinary bladder and urethra are found in pelvic nerves, which also contain parasympathetic efferent fibers originating from the sacral spinal cord. The remaining bladder afferents are carried by mostly hypogastric nerves, which also contain sympathetic efferent fibers originating from the TL spinal cord. Sacral somatic afferent and efferent innervation to the external urethral sphincter is via pudendal nerves. Under normal physiological conditions in adults, the micturition reflex is controlled predominantly by Aδ afferents communicating via the spinal cord to supraspinal centers in the pons and cortex. Under pathophysiological conditions or with aging, spinal reflex mechanisms mediated by C-fiber afferents may become dominant

emerges in early years and then resurface in the context of aging and injury. Although a little simplistic, this concept imputes a dual pathway view to the wiring physiology and pathophysiology in the urinary tract, and a neurological underpinning for lower urinary tracts symptoms (LUTS) in many clinical syndromes and their translation from animal models. This concept may be analogous to sensory dysregulation outside of the urinary tract, for example in pain conditions, and bowel and respiratory hyperreactivity, and may suggest a sensory basis to conditions such as OAB, BPH, and BPS/IC. As these concepts are significantly covered elsewhere in this book, they will not be a significant focus of discussion in this chapter.

A sizeable body of evidence now places extracellular ATP at the heart of the sensory hypothesis of LUTS, both within the cellular milieu of the urinary tract as well as in the spinal cord, at central terminals of primary sensory afferents. The background context and data from selected studies supporting this hypothesis are described in this chapter.

1.2 ATP: The Ubiquitous Signaling Factor

ATP is of course the cellular currency of fuel, and every cell thus employs complex mechanisms for maintaining synthesis and supporting demand for its use in every biochemical process. It is estimated that approximately 0.25 kg of ATP is present in our bodies, with a daily turnover approximately equivalent to one's weight (Tornroth-Horsefield and Neutze 2008); as such, ATP is a highly available chemical. However, ATP is now fully recognized not just as cellular fuel and a cofactor for a multitude of enzymatic processes, but as a ubiquitous signaling factor – a local messenger (autacoid) and neurotransmitter, with paracrine and autocrine modulatory functions in all tissues and cells. The breadth of intracellular and extracellular metabolic and messenger roles of ATP is the quintessential reflection of nature's capacity to harness key substances in a multiplicity of functions, with the abundant target receptors providing cellular and signaling specificity for the fine-tuning of diverse functions in tissues.

Although the first proposal of an extracellular signaling role for ATP surfaced over 80 years ago (Drury and Szent-Györgyi 1929), the concept itself was surprisingly contested for the next 60 years, despite increasing evidence of ATP release from cells and data illustrating the extent of ATP effects on a variety of cells and tissues. A full appreciation of this function became evident in the early-to-mid 1990s as molecular cloning studies revealed the existence of a diverse range of cell surface receptors for ATP and other nucleotides, providing definitive proof that targets for ATP signaling were abundantly expressed on all cell types. Great progress has been made in elaborating the structure, localization, regulation, and function of these P2 receptors, and this has been extensively reviewed elsewhere (Burnstock 2008; Burnstock and Knight 2004; Khakh 2001).

The evidence for specific and relatively nonspecific ATP release from cells surfaced from two distinct lines of investigation. Specific release of ATP from

nerves was extensively described by Burnstock (Burnstock 1972; Burnstock et al. 1978; Dean and Downie 1978), who provided evidence for vesicular storage of ATP in sympathetic and parasympathetic nerve varicosities innervating vascular and visceral tissues. ATP was clearly released from such "purinergic" nerves, and the discovery that these nerves also stored and released other transmitters (e.g., acetylcholine, noradrenaline) formed the basis of neural cotransmission (Banks et al. 2010; Burnstock 1976; Hoyle 1996), a phenomenon, although greeted with some skepticism, is now acknowledged to be a fundamental property of all neurones (Burnstock 2009; Khakh 2001; Khakh and North 2006).

The identification of nonneuronal release of ATP from cells arose in the context of extensive investigations of candidate factors released from endothelial cells, smooth muscle cells, and tissue segments in response to sheer stress, pressure fluctuations, and chemical provocation within the vascular system (Coutts et al. 1981; Pearson and Gordon 1979). ATP was identified as being stored within cells in high concentrations (based on high levels of quinacrine staining), where it could be released in response to a variety of chemical and physical stimuli (Bodin et al. 1991, 1992; Yang et al. 1994). Subsequent studies demonstrated that ATP released from cells was capable of mediating several downstream actions on the same (autocrine) and nearby (paracrine) cells by acting on specific receptive substrates that were responding to ATP as well as its breakdown products (e.g., ADP and adenosine) (White et al. 1985; Windscheif et al. 1994).

The mechanisms by which cells release ATP are varied. In some cells (e.g., glia, epithelial, endothelial, merkel cells, taste buds, and myocytes), mechanical stimulation, pressure, stretch, and chemical activation results in the release of ATP via transfer through specialized protein complexes ("conductive" release through anion channels) that may be associated with gap junctions. This route of ATP release is thought to be via co-called hemi-channels, connexins (or connexons), and pannexins (Faigle et al. 2008; Suadicani et al. 2000; Iglesias et al. 2009; Kang et al. 2008; Thuringer 2004), which when transfected into cells have been shown to markedly increase ATP extrusion (Barbe et al. 2006; Cotrina et al. 1998). While in some of these cells ATP release does not appear to be stimulated by either an increase in inositol trisphosphate (InsP3) or Ca^{2+}, in many others, including neurones, epithelial, and inflammatory cells (e.g., mast cells), ATP release occurs via vesicle-plasmalemma fusion and degranulation involving SNAP (soluble NSF attachment protein) receptors and SNARE (soluble NSF attachment protein receptors) proteins, typically employing a phosphoinositide 3-kinase (PI3-kinase)-dependent mechanism that is sensitive to inhibitors of vesicular exocytosis such as monensin (Orriss et al. 2009). Clearly, many cell types may release ATP using both gap junction (conductive) and vesicular (exocytotic) mechanisms, and it is possible that the vesicular fusion pathway may distribute more conductive channels into the plasma membrane, allowing for more sustained changes in nucleotide release.

In addition to these more "specialized" release processes, a third type of liberation is the passive spillage of ATP (with many other factors) from damaged and dying cells, a mechanism that could also contribute significantly to the extracellular

microenvironment, especially in the context of inflammation, injury, infection, and other pathological influences. There is evidence that some cell types, especially tumor cells, synthesize and release more copious amounts of ATP (Maehara et al. 1987; Pellegatti et al. 2008; Wink et al. 2003), supporting the idea that ATP signaling mechnisms may be important under certain pathophysiological conditions. Likewise, various tissues and fluid matrices isolated from patients with common disease conditions, such as airway fluids in chronic obstructive pulmonary disease (COPD) (Lommatzsch et al. 2010; Mortaz et al. 2009, 2010) and synovial fluid in arthritis (Kumahashi et al. 2004, 2010; Ryan et al. 1991), have been shown to contain much higher ATP levels than normal tissues. This property is discussed below in the context of urological indications.

ATP is present within cells at concentrations in the range of ~10 mM (DiVirgilio 2005). It therefore seems reasonable to suggest that the amount of ATP released from highly concentrated vesicular sources (tens to hundreds of mM) into the extracellular space could be considerable – perhaps hundreds of micromolar – and would likely be high enough to permit activation of even the least sensitive of the P2X receptor family members such as P2X7 which, at least in vitro, requires supramicromolar concentrations of ATP for activation. In keeping with other released transmitters, mechanisms also exist for ATP disposition from sites of release, including spontaneous and enzymatic hydrolytic degradation via a large, ubiquitous family of ectonucleotidases (Colgan et al. 2006) and diffusion down concentration gradients. While these mechanisms are capable of rapidly eliminating the released ATP, the tremendous cellular capacity to synthesize and replenish ATP storage compartments, and thus fuel further release, means that the elevations in ATP concentrations under chronically stressed or inflamed conditions can lead to persistence of elevated purinoceptor activation.

An excellent review and commentary was recently published (Corriden and Insel 2010) on the ubiquitous autocrine and paracrine mechanisms of ATP-mediated cellular regulation that covers many of the diverse aspects of ATP release under baseline and activated conditions. It is clear that far from representing an expensive spillage of precious cellular fuel, ATP release and detection by specific cellular receptors has evolved tremendously from simple eukaryotic organisms, such as slime-molds (dicytiostelium discoides), where signals for osmoregulation appear to be modulated by *intracellular* ATP acting via homologs of mammalian P2X receptors (Fountain et al. 2007), and yellow algae (Bavan et al. 2009; Ludlow et al. 2009), to more complex organisms, where these mechanisms have evolved to the family of cell surface receptors mediating many of the cell–cell and messenger–sensor functions of extracellularly released ATP.

1.3 P2X Receptors

Purinergic signaling, as will be discussed further, regulates both afferent and efferent pathways controlling urinary tract function (Figs. 1 and 2). The scope of

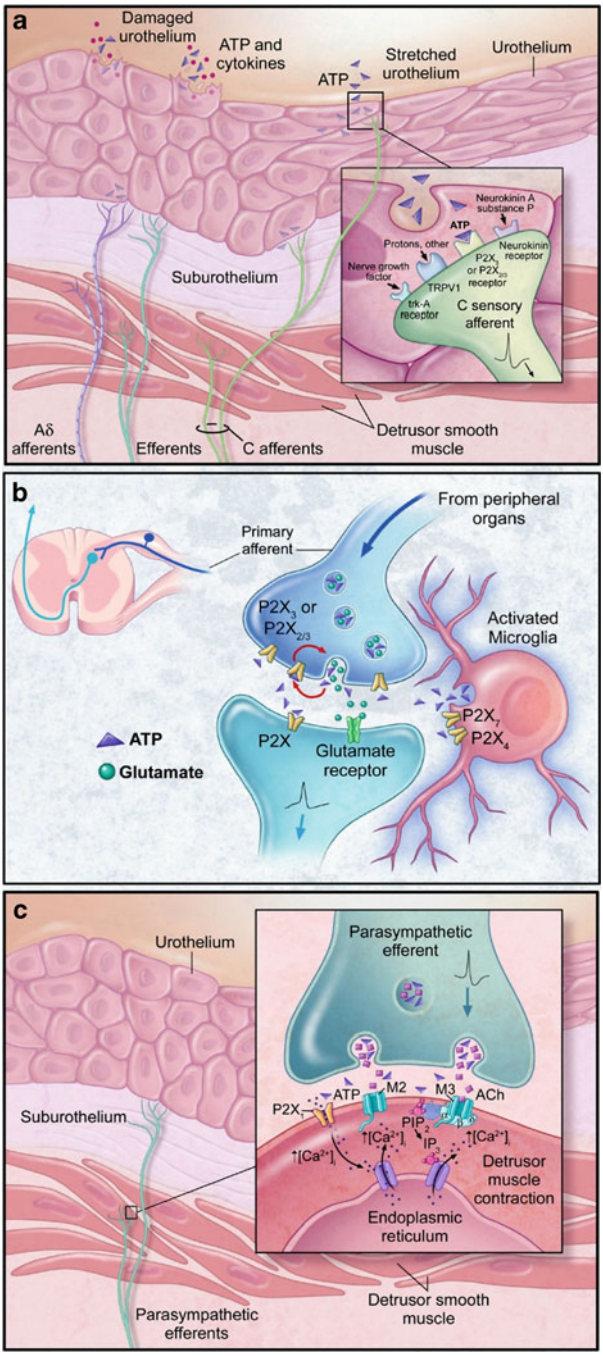

Fig. 2 Schematic diagrams of key afferent and efferent signaling mechanisms controlling the micturition reflex pathway. (**a**) Mechanical distension or damage to the urothelium causes release of ATP, and this release is augmented in disease states such as interstitial cystitis, benign prostate

purinergic signaling within the lower urinary tract (LUT) involves several receptors for ATP (and other purine and pyrimidine nucleotides) which are members of the distinct P2Y and P2X receptor families (Burnstock 2000).

Currently, eight metabotropic or heptahelical G-protein coupled (GPCRs) P2Y receptors (which are not the primary topic of this chapter) and seven P2X (P2X1-7) receptor subunits that coassemble to form ionotropic ATP receptors have been identified and characterized functionally and pharmacologically (Abbracchio et al. 2003; North 2002; North and Surprenant 2000). Of these, the P2X family of ATP-gated ion channels is rather unique among the larger population of cell surface receptors and channels; key milestones in the discovery and elucidation of this receptor family are shown in Fig. 3. P2X receptors exist in their native conformation as trimers (Nicke et al. 1998, 2005; Nicke 2008) and can form either homotrimeric or heterotrimeric receptors. Recently, the crystal structure for one of these receptors, the P2X4 homotrimer, was elucidated (Kawate et al. 2009).

The pharmacology of the individual native P2X channels has been extensively characterized and reviewed and correlates well with detailed assessments of the various recombinant channels when heterologously expressed in mammalian cells (Gever et al. 2006). Sequence homology across the seven P2X receptor subunits is only moderate, which combined with data using prototypical pharmacological probes suggests the reasonable possibility of selective pharmacological targeting. In contrast, subunit homology across species is fairly high and, to date, there are only limited examples of interspecies variation in P2X receptor pharmacology. One notable exception is the potency of pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid (PPADS), one of the prototypical polyanionic antagonists, which shows much weaker activity at rat vs. human P2X4 receptors due to an amino acid switch that obviates a Schiff's base formation in the rat receptor. A second example relates to the P2X7 receptor, where selective antagonists developed from multiple chemical series show excellent potency and affinity against the human receptor but low potency against rodent or guinea pig receptors (Michel et al. 2009). Otherwise, compounds with potency and affinity at the rodent and guinea pig channels show good translation to their human counterparts.

Fig. 2 (continued) hyperplasia, or spinal cord injury. ATP acts on P2X3 and P2X2/3 receptors on the peripheral terminals of Aδ- and C-bladder afferents, where it may convey mechanosensory and nociceptive information to the spinal cord. (**b**) At the central terminals of primary sensory afferents within the dorsal horn of the spinal cord, ATP may be coreleased with glutamate. P2X receptors are expressed on both presynaptic and postsynaptic membranes. Presynaptic P2X3 and P2X2/3 receptors are thought to be important in facilitating glutamate release. In addition, P2X4 and P2X7 receptors present on microglia may mediate inflammatory responses, thus contributing to hyperexcitability at these synapses. (**c**) Excitation of parasympathetic efferents causes corelease of ATP with acetylcholine from the nerve terminal. These neurotransmitters act on P2X1 and muscarinic (M_3, and possibly M_2) receptors respectively, present on the postjunctional membrane to cause detrusor smooth muscle contraction

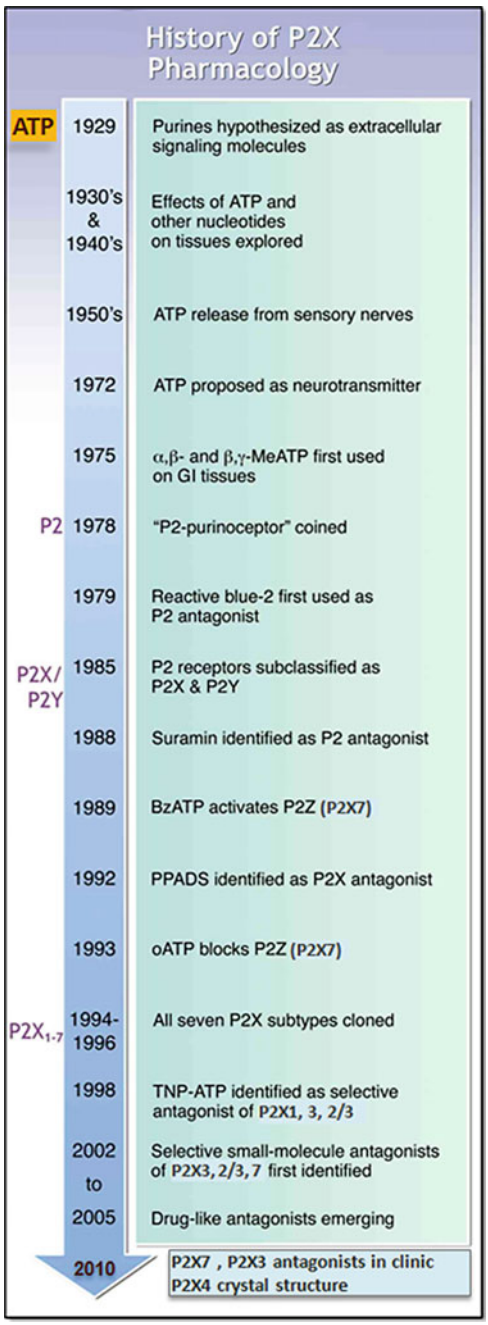

Fig. 3 The discovery of ATP signaling and P2X receptor subunits: major milestones

All P2X trimers appear to respond to the cooperative binding of three molecules of ATP with the opening of an intrinsic cation pore that is permeable to calcium and sodium ions. Other nucleotides and nucleosides are essentially inactive or weakly active; however, the nonhydrolysable analog α,β-methyleneATP (α,β-meATP) is able to activate P2X1- and P2X3-containing trimers, while 2′(3′)-O-(4-benzoylbenzoyl) adenosine-5′-triphosphate (BzATP) is often used as a more potent agonist at the P2X7 homotrimer (although it is far from specific and actually a more potent agonist at P2X3). Prototypic antagonists are polyanions and mostly nonselective as in the case of suramin, PPADS, and reactive blue 2, although some exhibit a limited degree of selectivity such as 2′,3′-O-(2,4,6-trinitrophenyl) adenosine-5′-triphosphate (TNP-ATP) for P2X1- and P2X3-containing receptors. While useful initial probes, all of these molecules offer limited pharmacological utility for in vitro and in vivo studies due to nonselectivity, poor stability, or suboptimal pharmacokinetic properties. Many chemical classes of novel antagonists have emerged in the last decade, based almost exclusively on leads identified from high throughput screening (HTS) efforts in pharmaceutical companies, and in most cases, these efforts have resulted in the generation of molecules with high selectivity for one or two of the P2X receptor forms. The P2X7, P2X3, and P2X2/3 receptors have witnessed the greatest medicinal chemistry progress, with patents published on multiple compound series and a selection of these compounds having progressed into early clinical studies (see Gunosewoyo and Kassiou 2010).

Earlier reviews have addressed and speculated upon the role of these diverse receptor subtypes in LUT function (Andersson and Wein 2004; Burnstock 2000; Ford et al. 2006; Rapp et al. 2005; Ruggieri 2006), although with limited benefit from recent developments in the area of P2X medicinal chemistry. In this review, we extend this discussion in light of such progress.

2 ATP, Urinary Function, and Dysfunction

2.1 Evidence of a Role for ATP in the Urinary Tract

One of several key papers that triggered heightened interest in the role of ATP in urinary tract signaling came from Ferguson et al. (1997), who examined the release of ATP from pieces of rabbit bladder, in which the tissue strip was intact or the epithelial or smooth muscle layers were studied alone. Figure 4 (taken from this paper) illustrates that ATP was readily liberated from bladder tissue strips upon electrical field stimulation, and that the source of this release was predominantly the epithelial tissue layer, prompting speculation that this release might occur as part of a local sensing mechanism. Subsequently, this putative sensing mechanism was nicely studied using an isolated bladder-pelvic nerve preparation from rat (Namasivayam et al. 1999) where recordings from pelvic sensory nerves were able

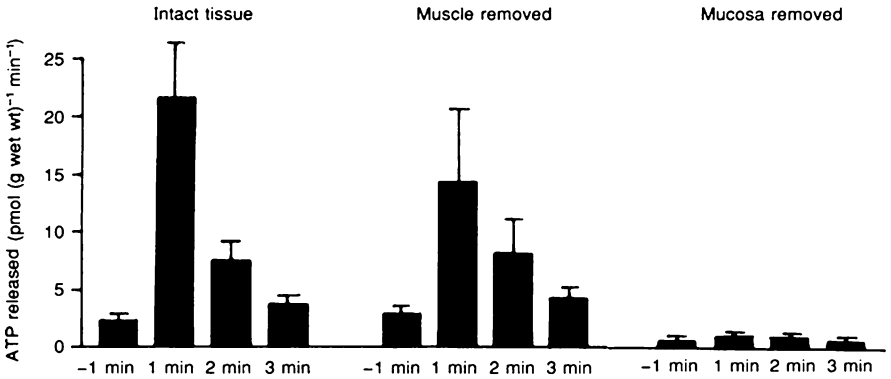

Fig. 4 Identification of the source of electrically stimulated ATP release in rabbit bladder. Release of ATP was induced by electrical stimulation, using the same protocol as in Figs. 5 and 6. Release from full thickness strips of bladder wall (intact tissue) was compared with urothelial strips with the smooth muscle layer removed (muscle removed) or with the smooth muscular strips with the urothelium removed (mucosa removed). These data suggest that the urothelial layer is the source of the released ATP [reproduced with permission from Ferguson et al. (1997)]

to detect filling of the bladder, with firing of these nerves mimicked by ATP application and inhibited by antagonism of P2X receptors.

Using a guinea pig ureter preparation, Knight and colleagues (Knight et al. 2002) further demonstrated the extent to which distension of the epithelium causes release of ATP and suggested that the consequence of this release was most probably an effect on the activity of subepithelial sensory nerve fibers and not on smooth muscle function or peristalsis. Subsequently, this was confirmed in studies using an isolated ureter pelvic/hypogastric nerve preparation from guinea pig (Rong and Burnstock 2004). More recently, in strips of human ureter, Calvert et al. (2008) demonstrated that P2X3 protein expression was detectable on suburothelial afferent nerve fibers, and they were able to show that distension of ureter strips gave rise to approximately tenfold elevations in released ATP (Fig. 5). Most intriguingly, they contended that the pressures used to generate these effects in isolated ureters were similar in magnitude to intraureteric pressures that had given rise to flank pain (typified in renal and ureteric colic) when applied to a cohort of women in a fascinating clinical investigation conducted over 50 years ago (Risholm 1954).

Coincident with this, interest has also surfaced over the last 15 years on the potential impact of the relatively high local, extracellular ATP levels found in a variety of target tissues and organs, including joints, airways, alimentary tract, urinary tract, and vasculature from patients and healthy individuals. In the context of urological tissues, access to bladder tissue is not uncommon in investigative urological procedures, and bladder tissue is also available from post mortem samples. Urine is a readily accessible matrix that has also been explored for the presence of various bioactive substances. However, analysis of urine samples should be approached with caution as measurements might reflect renal activity

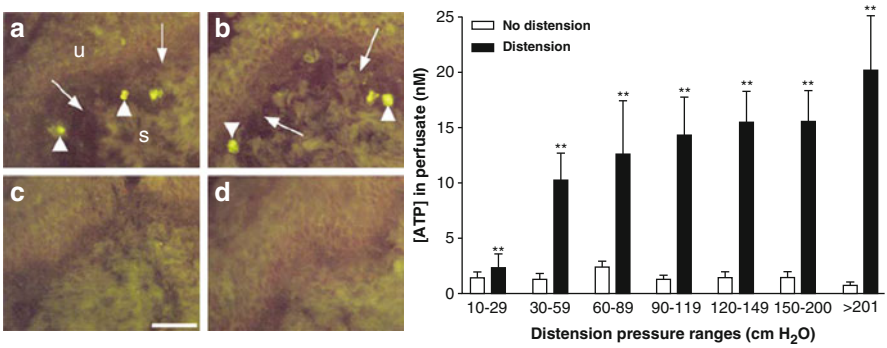

Fig. 5 ATP release and P2X3 expression in human ureter. *Left*: Immunofluorescent staining of human suburothelial ureteric nerves: (**a**) P2X3 receptor, (**b**) Capsaicin receptor (TRPV1), (**c**) P2X3 receptor preabsorption control, (**d**) no primary antibody control. Scale bar = 50 μm for each figure. *U* urothelium, *S* submucosa, *arrows* indicate suburothelial layer, *arrowheads* indicate sensory nerves. *Right*: [ATP] in perfusate immediately before and after distension of human ureter in vitro, grouped in pressure ranges. The mean [ATP] after distension is significantly greater than before distension in each pressure range (**$P < 0.01$; $n \geq 7$) for each group, error bars represent SEM [reproduced with permission from Calvert et al. (2008)]

in addition to changes emanating from the urinary bladder, and findings can be easily confounded by the presence of contaminating cells (e.g., blood cells) and pathogens present within the bladder.

In this respect, although there are now a number of recent studies that have quantified ATP concentrations in the context of various LUT disorders, it is not long ago that routine measurement of urinary ATP concentrations and ratios of ATP to excreted creatinine were touted as sensitive and useful diagnostic markers for urinary tract infection (UTI) (Gill et al. 2010; Osterberg et al. 1991): ATP measurements have also been proposed as a means of improving the detection of bacterial pathogens in the bronchoalveolar lavage fluid of patients with airways disease (Laupland et al. 2003). These bioluminescence determinations were investigated under the initial premise that ATP concentrations in urine (or other fluids) were not just a direct result of bacteruria, but that the pathogen was the source. It is claimed that such an approach could accelerate diagnosis and treatment vs. standard culture approaches. Accordingly, it has been suggested, for example, that urinary ATP concentrations of <10 nM were indicative of abacteruria, whereas ATP concentrations >50 nM were a marker of infection, with analyses showing fairly impressive sensitivity and specificity (Osterberg et al. 1991).

Whereas these diagnostic studies made the assumption that ATP in urine comes largely from invading pathogens, more recent studies suggest that the ATP is emanating from the bladder wall itself. Unfortunately, nature failed to provide us with a uniquely eukaryotic form of ATP with which to make this determination, and even then the contribution from the bladder wall would be difficult to resolve in situations where there is also pyuria. Likewise, as the evidence now seems strong that ATP is released in large amounts from urothelial cells in response to distention,

stress, and inflammation, it is reasonable to consider that exposure to bacterial endotoxin would cause release of ATP, that while perhaps not of bacterial origin would be an important response to bacterial infection, and one which sets in motion a heightened state of sensory activation with accompanied urgency, frequency, and urethral irritation, as part of the defense reaction to void the infection. It seems that further investigation is warranted to clarify the precise cellular origins of ATP under these conditions.

More recent studies addressing the contribution of ATP to LUT function have focused less on associations with UTI and more on associations with the symptomatic manifestations of otherwise idiopathic LUT dysfunctions, such as BPS/IC, OAB, and BPH. Numerous human and nonhuman studies have reported that ATP release from the bladder urothelium is augmented under these pathological conditions. Urothelial cells from cats with a naturally occurring cystitis (feline interstitial cystitis or FIC) exhibit enhanced hypotonic-evoked release of ATP (Birder et al. 2003). ATP release from urothelial cells is also markedly increased in rats subjected to spinal cord injury, in which bladder hyperreflexia develops (Khera et al. 2004), and in rats following chemically induced bladder inflammation (Smith et al. 2005). Following spinal cord injury in rats, an increase in basal and bladder stimulation-evoked ATP release has also been observed within the lumbosacral (LS) spinal cord by microdialysis (Salas et al. 2005, 2007), suggesting a heightened influence of ATP released from the central terminals of primary sensory afferents in the dorsal horn of the spinal cord.

It has also been demonstrated that in patients with BPS/IC (Sun et al. 2001a, b; Sun and Chai 2002), or LUTS associated with BPH (Sun et al. 2002), stretch-activated ATP release from bladder urothelial cells is augmented compared to age-matched controls. Recent data from the Chai group (Sun et al. 2009) have also shown that ATP can stimulate ATP release from urothelial cells and that this response is heightened in BPS/IC. Interestingly, this group also showed that the inflammatory mediators epidermal growth factor and the putative "antiproliferative factor" (APF) could similarly stimulate ATP release from bladder epithelial cells and increase P2X3 receptor expression. Several more groups have now extended these findings and shown correlations between urinary ATP levels and lowered thresholds for sensing urgency in patients with detrusor overactivity and BPS/IC (Cheng et al. 2009, 2010). OAB patients with exacerbated symptoms and significant pyuria have also been shown to liberate greater amounts of ATP from urinary epithelial cells (Contreras-Sanz et al. 2009).

The mechanism(s) by which urothelial ATP release is augmented under pathophysiological conditions is not entirely clear, although it apparently occurs in isolated urothelial cultures and so reflects an intrinsic change in epithelial signaling. Intravesical treatment with Botox can inhibit the augmented urothelial release of ATP following spinal cord injury or chemically induced cystitis, suggesting that vesicular release may be altered (Khera et al. 2004; Smith et al. 2005) and supporting the notion that Botox action is not limited to modulation of efferent neuronal transmitter release. In mice lacking the TRPV1 receptor, distension- or hypotonicity-evoked release of ATP from the bladder urothelium is significantly

reduced, further raising the possibility that activation of TRPV1 receptors may be a mechanosensory stimulus involved in distension-evoked release of ATP during bladder filling (Birder et al. 2002).

2.2 P2X Receptors and Urinary Tract Function

Lee et al. (2000) studied the distribution of P2X receptors in rat urinary bladder and ureter by immunohistochemical staining and demonstrated the presence of multiple P2X subunits, including P2X1 and P2X2 in smooth muscle, P2X5 and P2X7 in basal and squamous epithelium, respectively, and P2X3 in submucosal sensory neurones. There has been very little additional examination of the expression and/or roles of P2X5 and P2X7 in the urinary tract, although a potential involvement in epithelial cell differentiation (P2X5) and epithelial activation and/or apoptosis (P2X7) would be reasonable speculation based on studies in other epithelial tissues including skin (Greig et al. 2003a, b, c, d; Shabbir and Burnstock 2009). P2X7 and P2X4 receptors have also been proposed as targets for specific antagonism in various pain conditions (especially neuropathic) based on findings from gene knockout and rodent pharmacological studies that have demonstrated their involvement in spinal microglial activation consequent to peripheral nerve injury and possibly contributing to central sensitization pathways (Chessell et al. 2005; Clark et al. 2010; Inoue et al. 2004; Jarvis 2010; Ulmann et al. 2008, 2010). These approaches have not been reported in the context of sensory regulation in urological disorders, although the availability of selective P2X7 receptor antagonists, including compounds that have progressed into Phase 1 and Phase 2 clinical studies, makes the potential for examination in pelvic pain syndromes a reasonable possibility.

2.2.1 P2X1 Receptors: Efferent Regulation of Urinary Function

The cholinergic contribution to parasympathetically mediated detrusor smooth muscle contraction is well established. However, it is also recognized that in most mammalian species, part of the bladder contraction response evoked by transmural nerve stimulation is atropine-resistant and mediated mostly by ATP (purinergic; see Fig 2c). The concept of purinergic signaling in the LUT emerged through the pioneering work of Burnstock (Burnstock et al. 1972), who demonstrated that ATP was the neurotransmitter involved in atropine-resistant, nonadrenergic, noncholinergic (NANC) contractions in the guinea pig urinary bladder. Although broad acceptance of this idea coincided with the cloning of receptors for ATP in the mid-1990s, a substantial body of evidence was amassed in the intervening years to support the idea of purinergic signaling in the urinary tract (Burnstock 2000).

Some key early findings included the demonstration that NANC-mediated detrusor smooth muscle contractions could be mimicked by ATP (Burnstock et al. 1972; Burnstock et al. 1978; Dean and Downie 1978) and the more stable ATP analog α,β-meATP (Kasakov and Burnstock 1983; Hoyle and Burnstock 1985). NANC- and ATP-mediated detrusor smooth muscle contractions could also be suppressed by desensitization with α,β-meATP or by various nonselective purinergic antagonists, such as quinidine, reactive blue 2, suramin, and PPADS, without depressing responses to acetylcholine (Dean and Downie 1978; Kasakov and Burnstock 1983; Hoyle and Burnstock 1985; Brading and Williams 1990; Ziganshin et al. 1993; Tong et al. 1997; King et al. 1997). Ecto-ATPase inhibitors were shown to potentiate NANC and ATP responses in the guinea pig bladder (Hourani and Chown 1989; Westfall et al. 1997), and release of ATP in response to transmural stimulation of NANC nerves was demonstrated (Burnstock et al. 1978; Tong et al. 1997). Electrophysiological recordings from isolated detrusor smooth muscle cells from guinea pig, rabbit, and pig also showed that ATP and α,β-meATP elicited concentration-dependent, membrane depolarization and inward currents that showed rapid desensitization (Fujii 1988; Inoue and Brading 1990, 1991). Similar to the contractile response of detrusor smooth muscle strips, desensitization with α,β-meATP blocked ATP-induced currents in isolated myocytes. These findings are consistent with ATP being an excitatory neurotransmitter in the urinary bladder.

Although NANC-mediated detrusor smooth muscle contractions are clearly identifiable in some species, the purinergic contribution to nerve-mediated bladder contraction varies with species, age, and frequency of stimulation (Andersson and Wein 2004). The purinergic component in bladder strips can vary from being dominant in cat, mouse, and rabbit, to moderate in guinea pig, rat, and dog, and less pronounced compared to cholinergic responses in pig and human (Sibley 1984; Levin et al. 1990; Andersson 1993; Wust et al. 2002). In the normal human bladder, atropine-resistant, nerve-mediated contractions have been observed by some investigators (Sjogren et al. 1982; Bayliss et al. 1999; Cowan and Daniel 1983) but not by others (Kinder and Mundy 1987; Sibley 1984). Atropine-resistant responses may reflect only a small portion of the contraction of the human bladder under normal physiological conditions. However, many studies have shown that the purinergic component of human bladder contraction is significantly increased with age and under various pathological conditions of the LUT (see below).

Data supporting purinergic involvement in nerve-mediated detrusor contraction have also come from whole organ and in vivo studies measuring bladder pressure changes in response to stimulation. In vitro whole bladder studies in rabbit and cat demonstrated that ATP and transmural nerve stimulation, in the presence of atropine, produced transient rises in intravesical pressure (Levin and Wein 1982; Levin et al. 1990; Chancellor et al. 1992). In a conscious rat cystometry model, arterial administration of ATP and α,β-meATP close to the bladder produced rapid, phasic contractions that were desensitized by α,β-meATP (Igawa et al. 1993). In a pithed rat model, spinal electrical stimulation (L6-S2) evoked an increase in intravesical pressure that was sensitive to PPADS, thus demonstrating the importance of

peripheral, purinergic neurotransmission in the bladder reflex (Hegde et al. 1998). In an anesthetized rat model, contractile responses of the bladder to pelvic nerve stimulation were further characterized as consisting of a phasic, purinergic component predominating at low stimulation frequencies, followed by a tonic, cholinergic component at higher stimulation frequencies (Nunn and Newgreen 1999).

The availability of P2X receptor antagonists with improved subtype selectivity, and P2X1 gene knockout mice, subsequently confirmed the involvement of P2X1 receptors in atropine-resistant detrusor contractions (Heppner et al. 2009; Mulryan et al. 2000; Vial and Evans 2000). In a distension-evoked micturition reflex model in anesthetized rats, intravenous administration of the P2X1, P2X3 receptor antagonist di-inosine pentaphosphate (IP5I), or a novel P2X1 receptor antagonist RO-1 (also reported as RO116-6446), caused a significant attenuation of phasic, isovolumetric bladder contractions without affecting the volume or pressure thresholds for evoking the micturition reflex (King et al. 2004). Moreover, in P2X1-deficient mice, P2X receptor-mediated inward currents were abolished in detrusor smooth muscle cells (Vial and Evans 2000). Neurogenic bladder contractions in these mice were also reduced by ~70% compared to P2X1 wild-type mice, while carbachol-mediated responses were unaffected. Supporting these findings, dense P2X1-like immunoreactivity is found in the detrusor smooth muscle (Elneil et al. 2001; Lee et al. 2000; Vial and Evans 2001) and in close apposition to motor nerve varicosities in the rat detrusor (Hansen et al. 1998). Quantitative mRNA studies have also shown that P2X1 is the most abundant P2X receptor subtype in the adult human bladder (O'Reilly et al. 2001). Collectively, these data confirm that P2X1 receptors (probably homomultimeric channels) are responsible for NANC-mediated detrusor smooth muscle contraction, and that these receptors play a substantial role in parasympathetic neuronal control of urinary bladder function, although to varying extents across species from rodent to man.

In several reports on the efferent and afferent mechanisms controlling LUT function, it has been suggested that not just ATP but specifically P2X receptor-mediated responses are heightened in pathological situations. Changes in purinergic responses associated with efferent control of the micturition reflex have been observed in various bladder disease states, as well as in aging where conditions associated with LUTS are common. For example, an increased atropine-resistant component (up to ~50%) of the bladder contraction response to neurogenic stimulation was observed in tissues from men with BPH and detrusor overactivity (Sjogren et al. 1982) and in women with BPS/IC (Palea et al. 1993) or idiopathic detrusor instability (O'Reilly et al. 2002). Enhanced atropine-resistant detrusor contractions have also been observed in other studies of patients with BPH and detrusor overactivity (Nergardh and Kinn 1983; Sibley 1984; Chapple and Smith 1994). Likewise, among subsets of patients with unstable bladder, atropine-resistant responses were present in those with idiopathic detrusor instability or detrusor instability secondary to obstruction, but not in patients with neurogenic detrusor instability (Bayliss et al. 1999).

Recent studies have explored the basis for the enhanced purinergic neurotransmission that emerges in certain unstable bladder conditions. Studies by Fry and

coworkers have suggested that purinergic-mediated contractions are not just due to altered sensitivities of the detrusor smooth muscle cells to ATP or cholinergic agonists (Wu 1999), but instead may result from reduced extracellular hydrolysis of ATP (Fry et al. 2002; Harvey 2002). They found that ATP is significantly more potent at generating detrusor contractions in diseased bladder biopsies compared to control stable bladders, and that this is perhaps due to the fact that ATPase activity in unstable bladders is ~50% of that measured in stable bladder biopsies (Harvey 2002). This would explain the heightened efferent nerve response to electrical field stimulation but not to exogenous agonists such as α,β-meATP. Studies of age-related changes in detrusor contractility have also shown a significant positive correlation between age and the purinergic component of detrusor contraction (Lieu et al. 1997; Yoshida et al. 2001), while aging was negatively correlated with cholinergic neurotransmission and decreased release of ACh from isolated detrusor smooth muscle tissue. Consistent with this, aging has been associated with increased release of ATP from isolated detrusor smooth muscle tissue (Yoshida et al. 2004). A recent report has further extended these observations to patients with outflow obstruction associated with BPH and found that isolated bladder myocytes from these patients had a significantly greater sensitivity to ATP compared to myocytes isolated from OAB patients (Bishara et al. 2009).

2.2.2 P2X3 Receptors: Afferent Regulation of Urinary Function

A sensory role for ATP can be traced back 50 years (Holton 1959) to studies showing that ATP released from sensory nerves during antidromic stimulation caused vasodilation in the rabbit ear artery. It is possible that multiple purinergic pathways and receptors are involved in the sensory actions of ATP. However, since their discovery in 1995 (Chen et al. 1995; Lewis et al. 1995), a crucial role has been proposed for receptors containing P2X3 subunits (homotrimeric P2X3 and heterotrimeric P2X2/3 receptors) in mediating the primary sensory effects of ATP (Burnstock 2001a; Ford et al. 2006; Jarvis 2003). P2X3 and P2X2/3 receptors are predominantly localized on small-to-medium diameter C-fiber and Aδ sensory neurones within the dorsal root ganglion (DRG) and other sensory ganglia (Bradbury et al. 1998; Vulchanova et al. 1997; Dunn et al. 2001) and on peripheral nerve terminals in tissues including the urinary bladder (see Fig. 2a) (Cockayne et al. 2000). P2X3 and P2X2/3 receptors are also present on the central projections of primary sensory neurones within the dorsal horn of the spinal cord (see Fig 2b), where they may play a role in modulating glutamate release (Gu and MacDermott 1997; Vulchanova et al. 1998; Nakatsuka et al. 2003; Nakatsuka and Gu 2001).

Several reports over the last ~35 years have suggested the involvement of ATP in pain, including the early clinical demonstration that ATP applied to a blister base in healthy human volunteers was associated with heightened pain sensation (Collier et al. 1966; Bleehen et al. 1976; Bleehen and Keele 1977). ATP applied to

forearm skin by iontophoresis also caused mild painful responses that were enhanced by sensitization with UV irradiation or intradermal capsaicin (Hamilton et al. 2000). Intracutaneous injection of ATP (Hilliges et al. 2002), or its direct infusion into skeletal muscle (Mork et al. 2003), also caused pain in human volunteers. Studies in animals using more selective pharmacological and genetic tools have further established a crucial role for P2X3 and P2X2/3 receptors in both peripheral and centrally mediated pain facilitation. Studies using the P2X1, P2X3, and P2X2/3 selective antagonist TNP-ATP (Tsuda et al. 1999a, b; Jarvis et al. 2001; Honore et al. 2002b; Ueno et al. 2003), and the P2X3, P2X2/3 selective antagonist A-317491 (Jarvis et al. 2002; McGaraughty et al. 2003; Wu et al. 2004), have shown that peripheral and spinal P2X3 and P2X2/3 receptors are involved in persistent, chronic neuropathic, and inflammatory pain. Mice deficient in P2X3, P2X2, or both receptor subunits (Cockayne et al. 2000, 2005; Souslova et al. 2000), as well as animals treated with P2X3-selective antisense (Barclay et al. 2002; Honore et al. 2002a; Inoue et al. 2003) or short interfering RNA (siRNA) (Dorn et al. 2004), revealed comparable findings. These data provide strong preclinical evidence that P2X3 and P2X2/3 receptors are important in pain circuitry in vivo and suggest that antagonism of P2X3 and/or P2X2/3 receptors may have potential therapeutic utility in the management of chronic pain conditions.

An important role for ATP and P2X3-containing receptors in the mechanosensory regulation of urinary bladder function has also emerged in recent years (Burnstock 2001a). Sensory afferent innervation is essential for the normal control of urinary bladder function, coordinating compliance and excitation during the storage and elimination phases of the micturition reflex. The urinary bladder is innervated by the pelvic and hypogastric/thoracolumbar (TL) splanchnic nerves with cell bodies in the LS and TL DRG, respectively (Fig. 1). Under normal physiological conditions, it is believed that the predominant sensory afferents involved in detecting bladder volume changes are the Aδ pelvic nerve afferents which convey information about the state of bladder fullness to spinal and supraspinal centers coordinating the micturition reflex (Habler et al. 1993; de Groat et al. 1999; Andersson and Wein 2004). In contrast, the normally silent pelvic afferent C-fibers are thought to assume a prominent role under pathological conditions, where they become hyperexcitable and convey augmented information about noxious, inflammatory, or painful stimuli, and evoke reflex contractions mainly through a localized segmental spinal reflex (the "reflex bladder" mentioned earlier) (Habler et al. 1990; de Groat et al. 1998; Yoshimura and de Groat 1999). C-fiber afferents within the hypogastric/lumbar splanchnic nerve can also facilitate the effects of noxious chemical irritation within the urinary bladder (Mitsui et al. 2001). Thus, hyperexcitability of C-fibers in different functional pathways may contribute to the underlying pathophysiology of LUTS, including increased urinary frequency, sensations of urgency and pain (Yoshimura et al. 2002).

Anatomically, the urinary bladder is innervated by sensory nerve fibers that project into the suburothelial lamina propria, urothelium, and detrusor smooth muscle (Fig. 2a). P2X3 immunoreactivity has been found on many of these nerve fibers and also on bladder epithelial cells (Cockayne et al. 2000; Lee et al. 2000;

Elneil et al. 2001; Vlaskovska et al. 2001; Yiangou et al. 2001; Birder et al. 2004; Wang et al. 2005), thus a role for P2X3-containing receptors in regulating sensory functions of these cells is reasonable. Numerous studies have shown that ATP is released from the bladder urothelium in response to distension (Ferguson et al. 1997; Vlaskovska et al. 2001; Wang et al. 2005), and these findings can be mimicked in isolated urothelial cell cultures (Sun and Chai 2002; Birder et al. 2003). Studies using an isolated bladder-pelvic nerve preparation in either rats (Namasivayam et al. 1999) or mice (Vlaskovska et al. 2001; Rong et al. 2002) have also shown that distension leads to increased afferent nerve activity that is mimicked by ATP and/or α,β-meATP. Intravesical infusion of ATP or α,β-meATP can directly stimulate bladder overactivity in conscious rats, in a manner that is concentration-dependent and sensitive to TNP-ATP (Pandita and Andersson 2002). Conversely, intravesical infusion of suramin or PPADS can inhibit nonvoiding bladder contractions in bladder outlet-obstructed rats and increase bladder capacity in normal conscious rats (Cova et al. 1999; Velasco et al. 2003).

Studies in P2X3- and P2X2-deficient mice have been instrumental in demonstrating the importance of homomultimeric P2X3 and heteromultimeric P2X2/3 receptors in regulating bladder reflex excitability. Urinary bladder reflexes in response to filling are reduced in anesthetized P2X3, P2X2, and P2X2/P2X3 double knockout mice (Cockayne et al. 2000, 2005), despite normal levels of distension-evoked ATP release from the bladder urothelium (Vlaskovska et al. 2001). Bladder pelvic afferents from P2X-deficient mice also display altered electrophysiological responses, as measured by an increased volume threshold for activation in response to bladder distension (Vlaskovska et al. 2001; Cockayne et al. 2000, 2005). Single unit activity recordings confirmed the reduced afferent mechanosensitivity in P2X-deficient mice; however, it remains to be determined whether these deficits reflected changes in the sensitivity of Aδ and/or C-fiber afferents. Supporting these findings, several studies have demonstrated that labeled rat and mouse bladder sensory afferents projecting via the pelvic nerve express both P2X3 and P2X2/3 receptors (see Fig. 6), and electrophysiological recordings from these lumbosacral pelvic afferents (LS DRG) showed that ~80–90% responded to ATP and α,β-meATP with persistent, slowly desensitizing currents characteristic of the P2X2/3 receptor (Zhong et al. 2003; Dang et al. 2005, 2008; Chen and Gebhart 2010). Rat and mouse bladder afferents projecting via the hypogastric/lumbar splanchnic nerve (TL DRG) also contain currents consistent with P2X3 and P2X2/3 receptors (Dang et al. 2005, 2008; Chen and Gebhart 2010), although species differences exist in the proportion of these receptors. Interestingly, in these same studies, Gebhart and colleagues (Dang et al. 2008; Chen and Gebhart 2010) also demonstrated that cyclophosphamide-induced bladder inflammation resulted in an increase in P2X receptor expression and an increase in the relative contributions of P2X2 (sustained) and P2X2/3 (slowly desensitizing), or P2X3 (fast-inactivating) and P2X2/3 currents on lumbosacral pelvic and thoracolumbar splanchnic bladder afferents, respectively.

Several studies have also investigated the role of C-fibers in models of ATP-induced bladder overactivity. Selective deletion of nonpeptidergic C-fibers (i.e.,

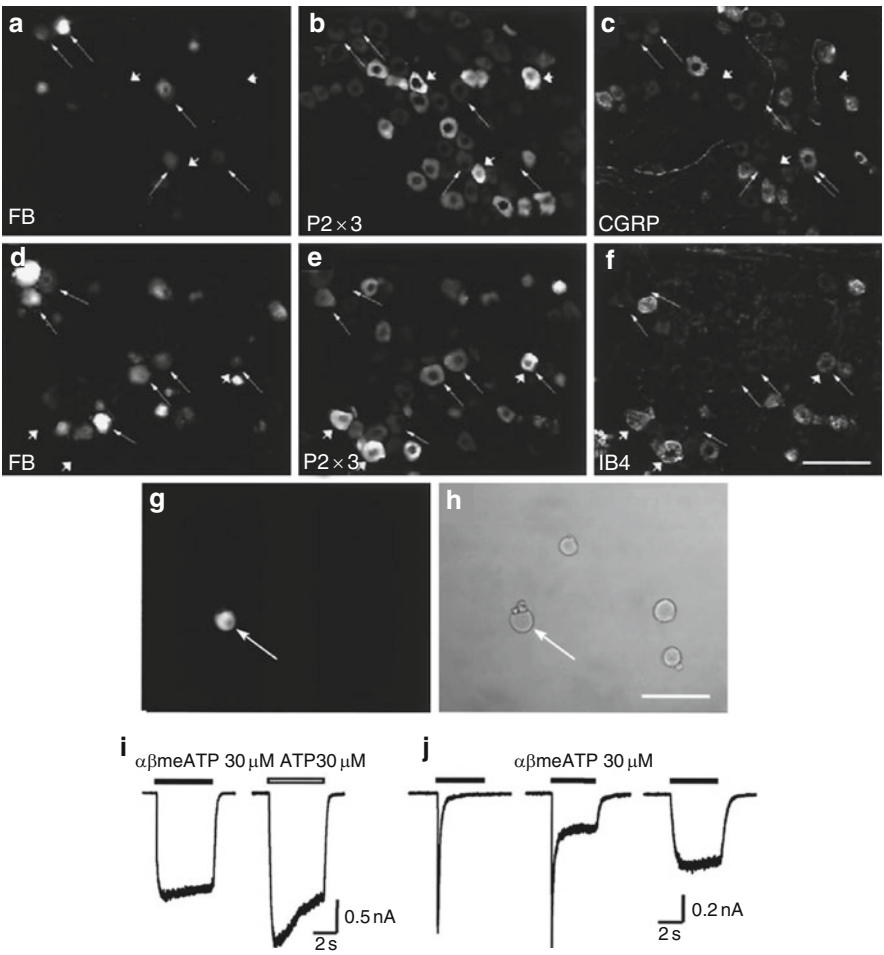

Fig. 6 P2X-containing receptors in bladder and cutaneous tissues from rat. *Above*: Colocalization of P2X3-IR with CGRP or IB4 in bladder and cutaneous sensory neurones of adult rats. Fast Blue (FB)-positive bladder sensory neurones (*long arrows*, **a**–**c**) displayed only weak to moderate P2X3-IR, while strongly P2X3-positive cells (*short arrows*, **b**) did not contain FB. Some bladder afferents were CGRP-positive (*double long arrows*, **c**). In contrast, the P2X3-IR in cutaneous sensory neurones (*long arrows*, **d**–**f**) ranged from weak to intense. One intensely P2X3-positive cutaneous afferent neurone was shown (**e**). Scale bar = 100 μm. *Below*: Patch-clamp electrophysiological phenotype of agonist responses in bladder and cutaneous sensory neurones of adult rats. Fluorescence (**g**) and phase contrast (**h**) images showing a Fast Blue (FB)-containing neurone (*arrow*) in an acutely dissociated L6 and S1 DRG preparation. Scale bar = 100 μm. (**i**) Representative traces of responses from 88% of bladder sensory neurones to α,β-meATP and ATP (30 μM). (**j**) Representative traces of P2X responses in cutaneous sensory neurones. α,β-meATP (30 μM) evoked transient, biphasic, and persistent responses in approximately 34%, 32%, and 24% of neurones, respectively. The membrane potential was held at −60 mV. The *bars* above the traces indicate the duration of agonist application [reproduced from Zhong et al. (2003)]

P2X3-expressing C-fibers) via intrathecal administration of an isolectin-B4 (IB4)-conjugated saporin molecule reduced both ATP- and capsaicin-induced bladder overactivity in conscious rats (Nishiguchi et al. 2004). Two reports further demonstrated the importance of spinal endogenous ATP and P2X receptors in chemical irritation-induced (Masuda et al. 2005) or spinal cord injury-induced (Salas et al. 2005) bladder overactivity in rats. It has also been shown that in patients with neurogenic detrusor overactivity, who were successfully treated with resiniferatoxin (RTX, a highly potent, desensitizing TRPV1 antagonist), that the density of P2X3-immunoreactive nerve fibers in the bladder was significantly reduced compared with that observed in nonresponder patients (Brady et al. 2004). Collectively, these data suggest that P2X3-containing receptors are present on sensory fibers within the urinary bladder and at central synapses in the spinal cord, and that increased expression and function of the P2X pathway may play a role in mediating bladder hyperexcitability, at least under certain experimental or pathologic conditions associated with LUT dysfunction.

A mechanosensory transduction pathway supporting the micturition reflex is therefore postulated wherein ATP released from the urothelium activates P2X3 and/or P2X2/3 receptors on submucosal primary afferents (Fig. 2a). ATP and α,β-meATP have been shown to not only activate low- and high-threshold bladder afferents directly but also to sensitize their mechanosensory responses (Vlaskovska et al. 2001; Rong et al. 2002). Under pathophysiological conditions, bladder inflammation can further sensitize and enhance P2X receptor expression/function on pelvic visceral and lumbar splanchnic afferents in the LS and TL DRG (Dang et al. 2005, 2008; Chen and Gebhart 2010). Bladder epithelial cells also demonstrate increased ATP release and P2X3 receptor expression under pathophysiological conditions (Sun and Chai 2004; Sun et al. 2001a, b, 2009), further augmenting the purinergic contribution to altered bladder sensory function. Thus, P2X3 and P2X2/3 receptors may be important in sensing volume changes during normal bladder filling and may participate in lowering the threshold for C-fiber activation under pathophysiological conditions.

3 P2X Receptors as Therapeutic Targets for Urinary Tract Disorders

Given these well-described roles of ATP, modulation of P2X receptor activity has surfaced as a potential point of therapeutic intervention in diseases of the urinary tract. Among the P2X receptor class, antagonism of P2X1, P2X3, and P2X2/3 receptors appears to be the most biologically reasonable. However, exploiting the full therapeutic potential of the purinoceptor family will require more than sound biological rationale. Novel medicines must possess the right combination of potency and selectivity with suitable drug-like properties, such as oral bioavailability, metabolic stability, and optimal distribution characteristics. The prototypical

ligands originally used to discover and delineate the purinoceptor family (e.g., suramin, PPADS, and reactive blue 2) represent very poor starting points for drug discovery, and although significant advances have been made in the last decade in modifying these ligands to attain increased potency and selectivity at some P2 receptors (Jacobson et al. 2004), little progress has been reported on "drug-like" ligands.

Indeed, most of the ligands highlighted in purinoceptor medicinal chemistry reports up until ~5 years ago violate more than one of the so-called Lipinski rules, a standard that provides a rough guide to drug likeness within pharmaceutical discovery (Lipinski 2000). Such a paucity of attractive chemical leads directed efforts at many pharmaceuticals research units toward novel lead discovery using proprietary or commercially available compound libraries. Since calcium passes through open P2X channels, functional activation of these channels can be easily quantitated using a fluorescence change evoked by cytosolic calcium flux in the presence of the calcium-sensitive dye, Fluo-3, and measured using a fluorometric imaging plate reader (FLIPR) (Jaime-Figueroa et al. 2005). In this manner, FLIPR-based HTS campaigns were conducted at Roche Palo Alto targeting homomultimeric hP2X1, hP2X2, hP2X2/3, and rP2X3 receptors to identify compounds capable of inhibiting α,β-meATP (P2X1, P2X2/3, and P2X3)- or ATP (P2X2)-evoked cytosolic calcium flux, without interfering with a secondary calcium flux produced by the calcium ionophore, ionomycin. In contrast to the P2X1, P2X2/3, and P2X3 HTS screening campaigns, the HTS screen targeting P2X2 resulted in no chemically tractable small molecule leads. It therefore remains to be seen whether P2X2 is a feasible target for medicinal intervention.

3.1 P2X1 Receptor Antagonism

There has been some limited effort to identify novel, selective "drug-like" antagonists for P2X1 receptors, on the principle that if purinergic efferent transmission is considerably heightened in urinary tract tissues from patients with storage and voiding disorders, then its blockade may improve symptoms by attenuating detrusor hyperexcitability. This approach would be somewhat analogous to antimuscarinic use, an accepted therapeutic for OAB, with the potential differentiator being the purported targeting of a heightened "pathological" response that seems to be associated with symptom development in urologic disorders. Some reasoned attempts were made to identify selective P2X1 receptor antagonists from prototypical antagonists, including NF-279 with modest preference toward P2X1 and P2X7 receptors and NF-864 a potent and selective P2X1 antagonist (Hausmann et al. 2006; Horner et al. 2005; Rettinger et al. 2000; Soto et al. 1999). However, although more potent and selective than their predecessors, these molecules all retained their chemical features (especially polyanionic nature) that offered little satisfaction of the "Lipinski rules" and could not be considered "drug-like."

A comparison with the antimuscarinics is important in the context of P2X1 blockade, as one limiting feature of the former class of agents relates to the abundant roles of muscarinic receptors, including those specifically involved in urinary bladder function (M3 and M2 receptors) which also have important roles in most tissues and organs. Tissue specificity is not seen with these agents, and the impact and limitation of selective muscarinic blockade can be seen in the array of safety and tolerability issues (dry mouth and eyes, visual impairment, CNS effects, constipation, tachycardia) that limit efficacious dosing and lead to marginal overall benefit and persistence with therapy. By analogy, the fact that P2X1 receptors have physiological roles in many tissues and organs also makes it somewhat likely that safety and tolerability issues may be a limiting factor for selective antagonists. P2X1 receptors have functional roles in all smooth muscle tissues, as well as functions in vascular, gastrointestinal, renal, CNS, and reproductive systems all of which could represent risks to successful medicinal development (Harrington et al. 2007; Heppner et al. 2009; Inscho et al. 2003, 2004; Lamont et al. 2006; Mulryan et al. 2000; Vial and Evans 2000).

The P2X1 HTS screening campaign at Roche Palo Alto resulted in the discovery of an antagonist from a series of dipeptide compounds, prepared initially as potential renin inhibitors (Jaime-Figueroa et al. 2005). The lead was subsequently optimized to RO-1 (also published as RO1166446), a novel small molecule antagonist of moderate potency (pIC_{50} ~5.5) with selectivity over homomultimeric P2X2 and P2X3, and heteromultimeric P2X2/3 receptors ($IC_{50} > 100$ μM at all three receptors). RO-1 (10 μM) nearly abolished calcium responses evoked by 1 μM ATP in dissociated rat bladder smooth muscle cells and also greatly reduced rat tail artery ring contractions evoked by electrical field stimulation (Ford et al. 2006; Gever et al. 2006). In tissue bath studies examining NANC responses, contractions evoked by β,γ-meATP in rat detrusor smooth muscle strips, or by ATP in rat tail artery rings, were significantly reduced with as little as 0.1 μM RO-1 and almost completely abolished by 10 μM RO-1 (Ford et al. 2006; Gever et al. 2006). In a distension-evoked micturition reflex model in anesthetized rats, the magnitude of phasic, isovolumetric bladder contractions was significantly attenuated by intravenous administration of 1 or 10 μmol/kg of RO-1 (King et al. 2004), a finding consistent with the decreased detrusor contraction observed in tissue bath studies. These results illustrate the feasibility of identifying P2X1 receptor antagonists that are not nucleotides or polyanionic compounds and provide increased confidence that this target can be successfully exploited to identify therapeutically attractive molecules. However, despite apparent selectivity, optimization of potency did not progress successfully, and the effort was abandoned. It thus remains to be seen whether a more potent P2X1 receptor antagonist can be developed that will allow for a more effective modulation of efferent purinergic mechanisms. Moreover, in vivo studies examining the effects of selective P2X1 receptor antagonists on other smooth muscle preparations (especially vascular) that contain P2X1 receptors would be necessary to determine whether safe and tolerable antagonism of P2X1 receptors can be imparted to alter LUT function.

3.2 P2X3 and P2X2/3 Receptor Antagonism

In 2002, data were published for the first time on a selective dual P2X3, P2X2/3 small molecule antagonist, A-317491 (Jarvis et al. 2002; Fig. 7e), that was identified following random HTS screening of Abbott's chemical library. Activation of recombinant and native P2X3 and P2X2/3 receptors was inhibited by submicromolar concentrations of A-317491, and efficacy was demonstrated in several

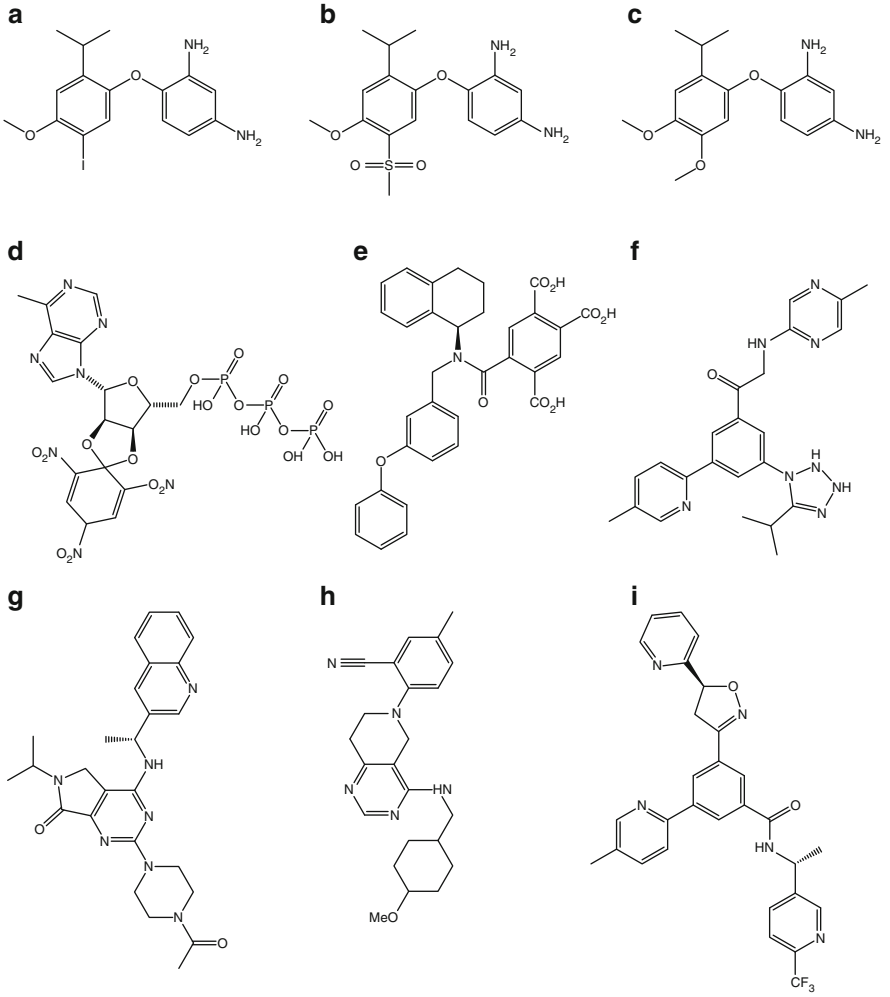

Fig. 7 Chemical structures of selective antagonists for P2X3-containing receptors. (**a–c**) diaminopyrimidines AF-353, AF-130, and AF-010 (Afferent/Roche), (**d**) nucleotide 2′,3′-O-(2,4,6-trinitrophenyl)-ATP (TNP-ATP), (**e**) A-317491 (Abbott), (**f**) benzamides (Afferent/Roche), (**g**) pyrrolopyrimidin-7-ones (Astra-Zeneca), (**h**) tetrahydropyrido-pyrimidines (Renovis/Evotec), and (**i**) biaryl benzamides (Merck)

models of chronic inflammatory and neuropathic pain. A-317491 dose-dependently decreased nociceptive responses evoked by intraplantar injection of formalin or complete Freund's adjuvant with ED_{50} values of 50 and 30 μmol/kg, s.c., respectively (McGaraughty et al. 2003). Reports on the use of this antagonist to study LUT function are few; however, one report examined the effects of intravenous administration of A-317491 in a rat spinal cord injury model (Lu et al. 2002, 2007). The antagonist produced a dose-dependent inhibition of nonmicturition bladder contractions and increased intermicturition interval and bladder capacity without influencing the amplitude of voiding contractions.

Such a mechanistic pattern of effects would seem fairly desirable if translated into a clinical setting, where inappropriate sensitivity to low volume filling and frequency/urgency could be potentially minimized while leaving untouched the force of detrusor contraction needed to maintain voiding efficiency. The relevance of the spinal transection model for human disease can be debated, but one of the purported consequences is that descending inhibitory pontine control of voiding reflexes is lost and so local segmental ("reflex bladder") circuits are awakened. Accordingly, and as reported above, this model is associated with increased ATP release from bladder epithelial cells and increased levels of ATP in the dorsal horn of the spinal cord (Khera et al. 2007; Salas et al. 2007; Smith et al. 2008). A-317491 was also studied, along with PPADS, in a cystometric model in rats pretreated (2 days prior) with cyclophosphamide (Ito et al. 2008) to produce a typical inflammatory hyperreflexia. Both antagonists produced similar responses as seen in the spinal model, with increased intervals between voids, reduced nonmicturition bladder contractions, and amelioration of cyclophosphamide-induced residual volumes, all without impact on the amplitude of voiding contractions.

While the discovery of this molecule has provided some advance over the large, nonselective, "polyanionic" antagonists described to this point (e.g., suramin, PPADS, reactive blue 2), the poor pharmacokinetic properties of A-317491 (a tricarboxylic acid with no oral bioavailability, high protein binding, and limited tissue distribution) may make it unattractive for medicinal development. The polyanionic characteristics seem to remain a requisite for competitive antagonists and lead to challenging medicinal chemistry starting points.

The P2X3 and P2X2/3 HTS screening campaigns at Roche Palo Alto resulted in the discovery of many distinct chemical series with apparently selective antagonistic action, a few of which have been chemically optimized with excellent success. The first and most medicinally advanced in the class of P2X3 antagonists is a series of diaminopyrimidine-containing molecules derived from a chemical lead related in structure to the antibacterial drug trimethoprim. Subsequent optimization resulted in a number of small molecule dual P2X3, P2X2/3 antagonists, exemplified by AF-353, AF-130, and AF-010 (Fig. 7a–c; shown alongside several P2X3 selective chemotypes that have surfaced in the patent literature) (Brotherton-Pleiss et al. 2010; Carter et al. 2009; Gever et al. 2010; Jahangir et al. 2009; Gunosewoyo and Kassiou 2010). AF-353 (also reported previously as RO-4) is a potent inhibitor of human and rat homomultimeric P2X3 (pIC_{50} = 8.0–8.5) and heteromultimeric P2X2/3 (pIC_{50} = 7.4–7.7) receptors. These potency estimates were confirmed

using patch-clamp electrophysiology of rat dorsal root (P2X3 $pIC_{50} = 8.5$) and nodose (P2X2/3 $pIC_{50} = 7.6$) ganglion neurones. AF-353 shows selectivity for P2X3 and P2X2/3 over all other functional homomultimeric P2X receptors ($IC_{50} \gg 10$ μM at P2X1, 2, 4, 5, 7), despite the relatively low molecular weight (MW ~350 Da). Additionally, two general screens of selectivity, one comprising 75 receptors, channels, enzymes, and transporters (Cerep), and a second one covering more than 100 kinases (Ambit) established AF-353 to be a highly selective molecule. Finally, because of the structural similarity of AF-353 to well-known bacterial dihydrofolate reductase inhibitors (trimethoprim itself), AF-353 was also tested for its ability to inhibit the human isoform of this enzyme and found to be only weakly active.

Based on this selectivity profile, the pharmacological effects observed with AF-353 in preclinical models can be reasonably attributed to P2X3 and P2X2/3 receptor antagonism. Most importantly, given concerns about drug-like properties, AF-353 and related diaminopyrimidines all satisfy Lipinski's so-called "rule of five" and have been shown to have attractive physicochemical and pharmacokinetic properties for in vivo utility (Gever et al. 2010; Kaan et al. 2010). The most advanced lead molecule from this chemical series, AF-219, has been in clinical studies since 2007 and is progressing into patient studies according to information available on the website of Afferent Pharmaceuticals (www.afferentpharma.com).

Data have been published on several of these diaminopyrimidine molecules and indicate that, as predicted, selective dual P2X3, P2X2/3 antagonists are effective at reducing nocifensive responses, hyperalgesia and allodynia in a range of animal models, including those arising from neuropathic, inflammatory, and cancer (osteolytic) interventions (Kaan et al. 2009, 2010). In urological models, data so far also look very intriguing. AF-353 was studied in a closed cystometric model ("refill VIBC") in urethane-anesthetized rats, where a slow infusion of saline is introduced into the bladder and at the point where reflex contractions are elicited, the volume is held constant to allow 5–6 further phasic contractions to occur. Bladders are emptied and "rested" for 30 min before repeating the cycle, which over a course of 2–3 h yields a reproducible pattern of responses, allowing estimates of threshold volume (TV) for eliciting contraction, contraction frequency (FREQ), and contraction amplitude (AMP) (Cefalu et al. 2007). When increasing doses of antagonist were given as an intravenous bolus, a dose-dependent effect was observed where TV increased by up to 50–70%, frequency was slightly reduced, but no appreciable change in amplitude was seen. Similar observations were reported in some of the earlier studies with A-317491, although these had been observed in rats subjected to cyclophosphamide inflammation or spinal transection, whereas the refill-VIBC model was performed in anesthetized but otherwise normal rats. Such findings are shown for AF-353 in Fig. 8.

As AF-353 is easily able to penetrate the blood brain barrier (Gever et al. 2010), it is not clear whether the effects described above were the result of P2X3 antagonism at peripheral terminals within the bladder wall, or alternatively at central terminals in the spinal cord dorsal horn, where it has been shown that ATP is released and can act on P2X3 receptors to enhance central glutamate transmission

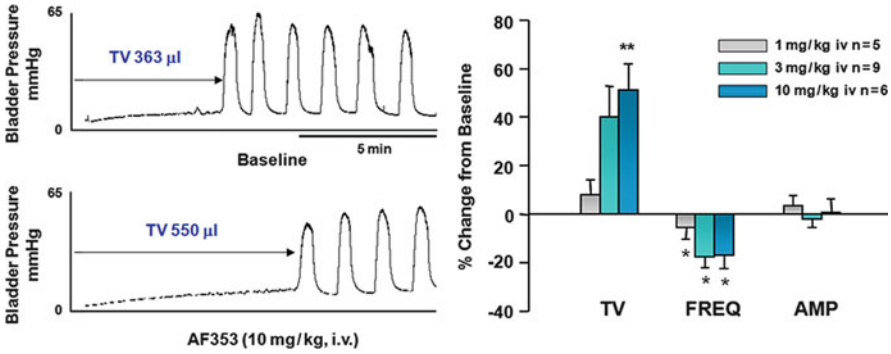

Fig. 8 The effect of P2X3 antagonists on cystometric reflexes in anesthetized rats. P2X3 blockade raises urinary volume thresholds in a "Refill" cystometry model of volume-induced bladder contractions (VIBC) in urethane anesthetized rats. The potent and selective P2X3, P2X2/3 antagonist AF-353 dose-dependently increases threshold volume (TV) in rats. In this model, filling responses are under control of local segmental (spinal) reflexes, mostly via capsaicin-sensitive C-fiber. Measured parameters are threshold volume (TV), frequency (FREQ), and amplitude of contractions (AMP). *Left panel*: representative trace from one rat; *right panel*: effect of multiple doses of AF-353

(Salas et al. 2007; Bardoni et al. 1997; Gu and MacDermott 1997). Two separate studies using distinct members of the diaminopyrimidine class helped to elucidate the question of central vs. peripheral mode of effect. In the first study, the Refill-VIBC method as described above was used in normal, urethane-anesthetized rats to examine antagonist effects on TV, FREQ, and AMP of contractions after peripheral intravenous administration. In a second study, a slight modification was introduced in that once reproducible reflex contractions were achieved on bladder filling, the volume was kept constant (isovolumetric) for up to 3 h during which time the contractions demonstrate good reproducibility in magnitude and frequency. This allowed study of the effects of direct intrathecal injection of test compounds.

Two antagonists with very similar P2X3 and P2X2/3 potencies and plasma free fraction estimations, but which differed markedly in CNS penetration (see Fig. 9a), were compared in these models. AF-010, like AF-353, has a low polar surface area (PSA) and as a result crosses the blood brain barrier readily, whereas AF-130, with a sulfone substitution, has a much higher PSA, is a modest substrate for efflux pumps, and as a result crosses the blood brain barrier very poorly, as reflected by low brain to plasma ratios. When dosed intravenously, the more CNS penetrant antagonist was significantly more potent at raising threshold volumes in the refill model (Fig. 9b), whereas intrathecal dosing (Fig. 9c, d) showed almost equivalent potencies in prolonging the intercontraction interval (ICI). These data suggest that in noninflamed models, a large component of the urinary hyporeflexic response to P2X3 antagonism is mediated at the level of the spinal cord/central terminals. Given the studies reported above which show that intravenous A-317491 (which also does not penetrate the CNS) produces significant suppression of hyper-reflexic responses following spinal injury or cyclophosphamide-induced bladder

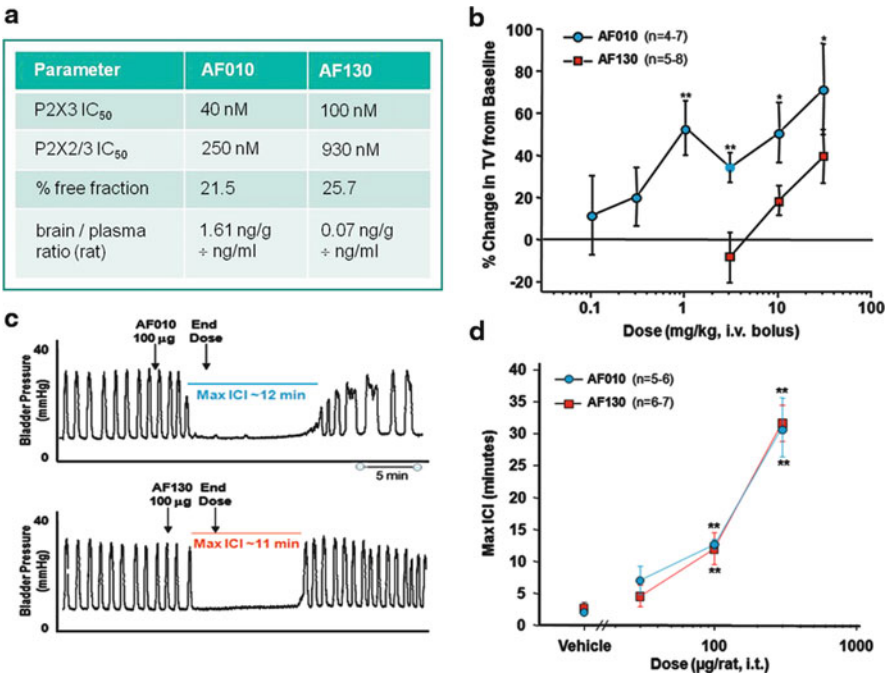

Fig. 9 Peripheral vs. central role of P2X3-containing receptors in micturition. (**a**) Comparison of the properties of the P2X3 and P2X2/3 antagonists AF-010 and AF-130. (**b**) Effect of the two antagonists when administered intravenously on threshold volume in the Refill-VIBC cystometry model in urethane-anesthetized rats. (**c** and **d**) Effect of each antagonist when administered intrathecally in the isovolumetric VIBC cystometry model in urethane-anesthetized rats

inflammation, it is possible that peripheral components of P2X3 receptor blockade are only clearly demonstrable when there is damage to the bladder or associated reflex circuitry.

A second, more mechanistic examination was performed using AF-792, one of the most potent members of the P2X3 blocking diaminopyrimidines and most closely related to AF-353 in chemical and pharmacokinetic terms (Kaan et al. 2010). Using intrathecal administration, but with a compound that due to increased potency offered a greater range of doses for examination, it was again shown that intercontraction intervals were greatly prolonged in an isovolumetric model, an effect mimicked marginally by the prototypical antagonists PPADS and TNP-ATP and fully replicated by local desensitization of P2X3-containing receptors using the agonist α,β-meATP. Subsequently, a high intrathecal dose of AF-792 was shown to prevent the increase in pERK activation in the outer laminae of the spinal cord dorsal horn that was produced by acetic acid instillation into the bladder (which irritates bladder afferents). Thus, presynaptic P2X3 and/or P2X2/3 receptors on the central terminals of bladder primary afferents are able to facilitate both normal and noxious input from the micturition reflex. Whether the potential for therapeutic

impact in urinary tract disorders such as OAB, BPH-LUTS, PBS/IC, or renal/ureteric colic requires blockade of peripheral, central, or all P2X3-containing receptors will be a matter for further exploration.

There are now several pharmaceutical companies (including Renovis/Evotec AG, Shionogi, Astra-Zeneca, and Merck) that have published patent applications covering novel chemical classes of antagonists with selectivity and high affinity for P2X3-containing receptors. Some key exemplary structures are shown in Fig. 7 and have been recently discussed in an excellent review on the topic (Gunosewoyo and Kassiou 2010). So far, however, there have been no peer-reviewed publications that reveal useful information on the in vivo activity, selectivity, or drug-likeness of these chemical classes, and thus data are awaited with great interest.

4 Conclusions

The impact and significance of ATP and purinergic signaling in urinary tract function – and its potential relevance to disease – has become greatly substantiated in recent years, particularly with the development of gene targeting approaches in mice and the emergence of novel pharmacological probes. Efforts focused on antagonism of P2X3 and P2X2/3 receptors represent hypothesis-driven approaches, and good progress to clinic has been made in these areas. Gene knockout and pharmacological data for other P2 receptors (e.g., P2X4 and P2X7) may also offer hints at opportunities in visceral organ diseases associated with inflammation and pain; however, the role of these P2X receptors in urinary tract function has not yet been established. The path from pharmacological tool to therapeutic probe and then to novel, differentiated, and safe medicines is a complex and challenging one, and to our knowledge, we are aware of one novel P2X3 antagonist that is currently in clinical trials and poised for assessment in urinary tract disorders. Thus, the time is now approaching when therapeutic potential will begin to be resolved.

A perpetual concern of course is that there are multiple receptors for ATP (P2X and P2Y) and many other factors released onto afferent neurones during urinary filling, and there may well be sufficient redundancy built into targeted biological systems such that blockade at any one receptor or ion channel will be too subtle to have clear clinical impact. P2X3 and P2X2/3 regulation of sensory mechanisms in the LUT appears to be an attractive purinergic opportunity, and advances have been made in the identification of chemical entities with properties suitable for medicinal optimization. To date, focus has been somewhat limited to P2X3 and P2X2/3 receptor involvement in afferent mechanisms *within* peripheral target tissues. However, attention has noticeably turned also to the role of ATP and P2X3-containing receptors at central synapses in the spinal cord where central sensitization develops (Kaan et al. 2010; Masuda et al. 2005; Salas et al. 2005). The latter evidence may indicate that the P2X3 mechanism is not just part of the divergent soup of activators in the periphery, but also part of the convergence of the primary

afferent input into the CNS, modulating signaling at the first synapse – regardless of the nature of the receptive field sensitizer.

Moving forward, a greater focus is warranted on the function of these receptors across visceral sensory pathways (e.g., hypogastric and pudendal in addition to pelvic afferents) to gain a more comprehensive insight into the integrated function of the kidney, ureters, bladder, urethra, prostate, and sphincters (Mitsui et al. 2001; Yoshimura et al. 2003; Dang et al. 2005). Lastly, it is well known that C-fibers are heterogeneous, and that the population with the highest level of P2X3 expression is the "nonpeptidergic" subpopulation that binds the isolectin-B4 (IB4) and has its central projections mostly within inner lamina II of the dorsal horn. Exactly how these different subfamilies of afferents differentially affect sensory signaling and sensitization is still poorly understood.

In general, there has been a clear shift in perspective regarding the causes of urinary tract dysfunction and to this end various novel therapeutic mechanisms for intervention have arisen, with many, like P2X3 antagonists, targeting parts of the afferent limb of the micturition reflex (e.g., TRPV1 antagonists, NGF antibodies). "Afferent neurourology" is a term that has been coined recently (Clemens 2010a, b) that reflects this shift. In unraveling these biological mechanisms and developing appropriate chemical and biological tools, we are hopefully ready to tackle the most bothersome and poorly managed aspects of functional urologic disorders – notably, urgency and pain – and bring a step change in therapeutic options to millions of patients.

References

Abbracchio MP, Burnstock G (1994) Purinoceptors: are there families of P2X and P2Y purinoceptors? Pharmacol Ther 64:445–475

Abbracchio MP, Boeynaems JM, Barnard EA, Boyer JL, Kennedy C, Miras-Portugal MT, King BF, Gachet C, Jacobson KA, Weisman GA, Burnstock G (2003) Characterization of the UDP-glucose receptor (re-named here the P2Y14 receptor) adds diversity to the P2Y receptor family. Trends Pharmacol Sci 24:52–55

Andersson KE (1993) Pharmacology of lower urinary tract smooth muscles and penile erectile tissues. Pharmacol Rev 45:253–308

Andersson KE, Wein A (2004) Pharmacology of the lower urinary tract: basis for current and future treatments of urinary incontinence. Pharmacol Rev 56:581–631

Banks FC, Calvert RC, Burnstock G (2010) Changing P2X receptor localization on maturing sperm in the epididymides of mice, hamsters, rats, and humans: a preliminary study. Fertil Steril 93:1415–1420

Barbe MT, Monyer H, Bruzzone R (2006) Cell-cell communication beyond connexins: the pannexin channels. Physiology (Bethesda) 21:103–114

Barclay J, Patel S, Dorn G, Wotherspoon G, Moffatt S, Eunson L, Abdel'al S, Natt F, Hall J, Winter J, Bevan S, Wishart W, Fox A, Ganju P (2002) Functional downregulation of $P2X_3$ receptor subunit in rat sensory neurons reveals a significant role in chronic neuropathic and inflammatory pain. J Neurosci 22:8139–8147

Bardoni R, Goldstein PA, Lee CJ, Gu JG, MacDermott AB (1997) ATP P_{2X} receptors mediate fast synaptic transmission in the dorsal horn of the rat spinal cord. J Neurosci 17:5297–5304

Bavan S, Straub VA, Blaxter ML, Ennion SJ (2009) A P2X receptor from the tardigrade species Hypsibius dujardini with fast kinetics and sensitivity to zinc and copper. BMC Evol Biol 9:17

Bayliss M, Wu C, Newgreen D, Mundy AR, Fry CH (1999) A quantitative study of atropine-resistant contractile responses in human detrusor smooth muscle, from stable, unstable and obstructed bladders. J Urol 162:1833–1839

Birder LA, Nakamura Y, Kiss S, Nealen ML, Barrick S, Kanai AJ, Wang E, Ruiz G, de Groat WC, Apodaca G, Watkins S, Caterina MJ (2002) Altered urinary bladder function in mice lacking the vanilloid receptor TRPV1. Nat Neurosci 5:856–860

Birder LA, Barrick SR, Roppolo JR, Kanai AJ, de Groat WC, Kiss S, Buffington CA (2003) Feline interstitial cystitis results in mechanical hypersensitivity and altered ATP release from bladder urothelium. Am J Physiol Renal Physiol 285:F423–F429

Birder LA, Ruan HZ, Chopra B, Xiang Z, Barrick S, Buffington CA, Roppolo JR, Ford APDW, de Groat WC, Burnstock G (2004) Alterations in P2X and P2Y purinergic receptor expression in urinary bladder from normal cats and cats with interstitial cystitis. Am J Physiol Renal Physiol 287:F1084–F1091

Bishara S, Gao Y, Malone-Lee M, Clark S, Lunawat R, Khan S, Khasriya R, King B, Malone-Lee J (2009) Increased ATP mediated contraction of isolated detrusor cells of patients with outflow obstruction. Neurourol Urodyn 28:746–747

Bleehen T, Keele CA (1977) Observations on the algogenic actions of adenosine compounds on the human skin blister base preparation. Pain 3:367–377

Bleehen T, Hobbiger F, Keele CA (1976) Identification of algogenic substances in human erythrocytes. J Physiol (Lond) 262:131–149

Bodin P, Bailey D, Burnstock G (1991) Increased flow-induced ATP release from isolated vascular endothelial cells but not smooth muscle cells. Br J Pharmacol 103:1203–1205

Bodin P, Milner P, Winter R, Burnstock G (1992) Chronic hypoxia changes the ratio of endothelin to ATP release from rat aortic endothelial cells exposed to high flow. Proc Biol Sci 247:131–135

Bradbury EJ, Burnstock G, McMahon SB (1998) The expression of $P2X_3$ purinoreceptors in sensory neurons: effects of axotomy and glial-derived neurotrophic factor. Mol Cell Neurosci 12:256–268

Brading AF, WIlliams JH (1990) Contractile responses of smooth muscle strips from rat and guinea-pig urinary bladder to transmural stimulation: effects of atropine and alpha, beta-methylene ATP. Br J Pharmacol 99:493–498

Brady CM, Apostolidis A, Yiangou Y, Baecker PA, Ford AP, Freeman A, Jacques TS, Fowler CJ, Anand P (2004) $P2X_3$-immunoreactive nerve fibres in neurogenic detrusor overactivity and the effect of intravesical resiniferatoxin. Eur Urol 46:247–253

Brotherton-Pleiss CE, Dillon MP, Ford AP, Gever JR, Carter DS, Gleason SK, Lin CJ, Moore AG, Thompson AW, Villa M, Zhai Y (2010) Discovery and optimization of RO-85, a novel drug-like, potent, and selective P2X3 receptor antagonist. Bioorg Med Chem Lett 20:1031–1036

Burnstock G (1972) Purinergic nerves. Pharmacol Rev 24:509–581

Burnstock G (1976) Do some nerve cells release more than one transmitter? Neuroscience 1:239–248

Burnstock G (2000) Purinergic signalling in lower urinary tract. In: Abbracchio MP, Williams M (eds) Handbook of experimental pharmacology. Springer, Berlin, pp 423–515

Burnstock G (2001a) Purine-mediated signalling in pain and visceral perception. Trends Pharmacol Sci 22:182–188

Burnstock G (2001b) Purinergic signalling. Springer, Berlin, pp 89–127

Burnstock G (2008) Unresolved issues and controversies in purinergic signalling. J Physiol (Lond) 586:3307–3312

Burnstock G (2009) Autonomic neurotransmission: 60 years since Sir Henry Dale. Annu Rev Pharmacol Toxicol 49:1–30

Burnstock G, Knight GE (2004) Cellular distribution and functions of P2 receptor subtypes in different systems. Int Rev Cytol 240:31–304

Burnstock G, Campbell G, Satchell DG, Smythe A (1970) Evidence that adenosine triphosphate or a related nucleotide is the transmitter substance released by non-adrenergic inhibitory nerves in the gut. Br J Pharmacol 40:668–688

Burnstock G, Dumsday B, Smythe A (1972) Atropine resistant excitation of the urinary bladder: the possibility of transmission via nerves releasing a purine nucleotide. Br J Pharmacol 44:451–461

Burnstock G, Cocks T, Crowe R, Kasakov L (1978) Purinergic innervation of the guinea-pig urinary bladder. Br J Pharmacol 63:125–138

Calvert RC, Thompson CS, Burnstock G (2008) ATP release from the human ureter on distension and P2X(3) receptor expression on suburothelial sensory nerves. Purinergic Signal 4:377–381

Carter DS, Alam M, Cai H, Dillon MP, Ford AP, Gever JR, Jahangir A, Lin C, Moore AG, Wagner PJ, Zhai Y (2009) Identification and SAR of novel diaminopyrimidines. Part 1: the discovery of RO-4, a dual P2X(3)/P2X(2/3) antagonist for the treatment of pain. Bioorg Med Chem Lett 19:1628–1631

Cefalu JS, Zhu QM, Eggers AC, Kaan TK, Ho MJ, Jett MF, Cockayne DA, Ford AP, Nunn PA (2007) Effects of the selective prostacyclin receptor antagonist RO3244019 on the micturition reflex in rats. J Urol 178:2683–2688

Chancellor MB, Kaplan SA, Blaivas JG (1992) The cholinergic and purinergic components of detrusor contractility in a whole rabbit bladder model. J Urol 148:906–909

Chapple CR, Smith D (1994) The pathophysiological changes in the bladder obstructed by benign prostatic hyperplasia. Br J Urol 73:117–123

Chen X, Gebhart GF (2010) Differential purinergic signaling in bladder sensory neurons of naive and bladder-inflamed mice. Pain 148:462–472

Chen CC, Akopian AN, Sivilotti L, Colquhoun D, Burnstock G, Wood JN (1995) A P2X purinoceptor expressed by a subset of sensory neurons. Nature 377:428–431

Cheng Y, Allan W, Walsh C, Mansfield KJ, Burcher E, Moore KH (2009) ATP release during cystometry in women with detrusor overactivity and painful bladder syndrome: contributiion to "urgency"? Neurourol Urodyn 28:838–839

Cheng Y, Mansfield KJ, Allen W, Walsh CA, Burcher E, Moore KH (2010) Does adenosine triphosphate released into voided urodynamic fluid contribute to urgency signaling in women with bladder dysfunction? J Urol 183:1082–1086

Chessell IP, Hatcher JP, Bountra C, Michel AD, Hughes JP, Green P, Egerton J, Murfin M, Richardson J, Peck WL, Grahames CB, Casula MA, Yiangou Y, Birch R, Anand P, Buell GN (2005) Disruption of the P2X7 purinoceptor gene abolishes chronic inflammatory and neuropathic pain. Pain 114:386–396

Clark AK, Staniland AA, Marchand F, Kaan TK, McMahon SB, Malcangio M (2010) P2X7-dependent release of interleukin-1beta and nociception in the spinal cord following lipopolysaccharide. J Neurosci 30:573–582

Clemens JQ (2010a) Afferent neurourology: a novel paradigm. Neurourol Urodyn 29(Suppl 1): S29–S31

Clemens JQ (2010b) Afferent neurourology: an epidemiological perspective. J Urol 184:432–439

Cockayne DA, Hamilton SG, Zhu QM, Dunn PM, Zhong Y, Novakovic S, Malmberg AB, Cain G, Berson A, Kassotakis L, Hedley L, Lachnit WG, Burnstock G, McMahon SB, Ford AP (2000) Urinary bladder hyporeflexia and reduced pain-related behaviour in P2X3-deficient mice. Nature 407:1011–1015

Cockayne DA, Dunn PM, Zhong Y, Rong W, Hamilton SG, Knight GE, Ruan HZ, Ma B, Yip P, Nunn P, McMahon SB, Burnstock G, Ford APDW (2005) $P2X_2$ knockout mice and $P2X_2/P2X_3$ double knockout mice reveal a role for the $P2X_2$ receptor subunit in mediating multiple sensory effects of ATP. J Physiol 567:621–639

Colgan SP, Eltzschig HK, Eckle T, Thompson LF (2006) Physiological roles for ecto-5′-nucleotidase (CD73). Purinergic Signal 2:351–360

Collier HO, James GW, Schneider C (1966) Antagonism by aspirin and fenamates of bronchoconstriction and nociception induced by adenosine-5′-triphosphate. Nature 212:411–412

Contreras-Sanz A, Kennedy-Lydon T, Bishara S, Lunawat R, Khasriya R, Taylor K, Malone-Lee J, Wildman SS (2009) Altered ATP signalling in urothelium of OAB patients with exacerbated symptoms. Neurourol Urodyn 28:935

Corriden R, Insel PA (2010) Basal release of ATP: an autocrine-paracrine mechanism for cell regulation. Sci Signal 3:re1

Cotrina ML, Lin JH, Alves-Rodrigues A, Liu S, Li J, Azmi-Ghadimi H, Kang J, Naus CC, Nedergaard M (1998) Connexins regulate calcium signaling by controlling ATP release. Proc Natl Acad Sci USA 95:15735–15740

Coutts AA, Jorizzo JL, Eady RA, Greaves MW, Burnstock G (1981) Adenosine triphosphate-evoked vascular changes in human skin: mechanism of action. Eur J Pharmacol 76:391–401

Cova R, Guarneri L, Angelico P, Velasco C, Leonardi A, Testa R (1999) Effects of infravesical suramin and PPADS on cystometrographic parameters in conscious rats. Urodinamica 9:162–168

Cowan WD, Daniel EE (1983) Human female bladder and its noncholinergic contractile function. Can J Physiol Pharmacol 61:1236–1246

Dang K, Bielefeldt K, Gebhart GF (2005) Differential responses of bladder lumbosacral and thoracolumbar dorsal root ganglion neurons to purinergic agonists, protons, and capsaicin. J Neurosci 25:3973–3984

Dang K, Lamb K, Cohen M, Bielefeldt K, Gebhart GF (2008) Cyclophosphamide-induced bladder inflammation sensitizes and enhances P2X receptor function in rat bladder sensory neurons. J Neurophysiol 99:49–59

de Groat WC, Araki I, Vizzard MA, Yoshiyama M, Yoshimura N, Sugaya K, Tai C, Roppolo JR (1998) Developmental and injury induced plasticity in the micturition reflex pathway. Behav Brain Res 92:127–140

de Groat WC, Downie JW, Levin RM, Lin ATL, Morrison JFB, Nishizawa O, Steers WD, Thor KB (1999) Basic neurophysiology and neuropharmacology. In: Abrams P, Khoury S, Wein A (eds) Incontinence: 1st international consultation on incontinence, June 28–July 1, 1998, Monaco. Health Publication, St. Helier, Jersey, UK, pp 105–154

Dean DM, Downie JW (1978) Contribution of adrenergic and "purinergic" neurotransmission to contraction in rabbit detrusor. J Pharmacol Exp Ther 207:431–445

DiVirgilio F (2005) Purinergic mechanism in the immune system: a signal of danger for dendritic cells. Purinergic Signal 1:205–209

Dorn G, Patel S, Wotherspoon G, Hemmings-Mieszczak M, Barclay J, Natt FJC, Martin P, Bevan S, Fox A, Ganju P, Wishart W, Hall J (2004) siRNA relieves chronic neuropathic pain. Nucleic Acids Res 32:e49

Drury AN, Szent-Györgyi A (1929) The physiological activity of adenine compounds with especial reference to their action upon mammalian heart. J Physiol 68:213–237

Dunn PM, Zhong Y, Burnstock G (2001) P2X receptors in peripheral neurons. Prog Neurobiol 65:107–134

Elneil S, Skepper JN, Kidd EJ, Williamson JG, Ferguson DR (2001) Distribution of $P2X_1$ and $P2X_3$ receptors in the rat and human urinary bladder. Pharmacology 63:120–128

Faigle M, Seessle J, Zug S, El Kasmi KC, Eltzschig HK (2008) ATP release from vascular endothelia occurs across Cx43 hemichannels and is attenuated during hypoxia. PLoS One 3:e2801

Ferguson DR, Kennedy I, Burton TJ (1997) ATP is released from rabbit urinary bladder epithelial cells by hydrostatic pressure changes – a possible sensory mechanism? J Physiol 505:503–511

Ford APDW, Gever JR, Nunn PA, Zhong Y, Cefalu JS, Dillon MP, Cockayne DA (2006) Purinoceptors as therapeutic targets for lower urinary tract dysfunction. Br J Pharmacol 147: S132–S143

Fountain SJ, Parkinson K, Young MT, Cao L, Thompson CR, North RA (2007) An intracellular P2X receptor required for osmoregulation in *Dictyostelium discoideum*. Nature 448:200–203

Fry CH, Skennerton D, Wood D, Wu C (2002) The cellular basis of contraction in human detrusor smooth muscle from patients with stable and unstable bladders. Urology 59:3–12

Fujii K (1988) Evidence for adenosine triphosphate as an excitatory transmitter in guinea-pig, rabbit and pig urinary bladder. J Physiol 404:39–52
Gever JR, Cockayne DA, Dillon MP, Burnstock G, Ford APDW (2006) Pharmacology of P2X channels. Pflugers Arch 452:513–537
Gever JR, Soto R, Henningsen RA, Martin RS, Hackos DH, Panicker S, Rubas W, Oglesby IB, Dillon MP, Milla ME, Burnstock G, Ford AP (2010) AF-353, a novel, potent and orally bioavailable P2X3/P2X2/3 receptor antagonist. Br J Pharmacol 160:1387–1398
Gill K, Jeyakumar A, Brenton T, Khasriya R, Kupelian A, Malone-Lee J (2010) Urinary ATP – a test to replace dipsticks and microscopy for pyuria. Neurourol Urodyn 29 (ICS/IUGA meeting abstracts – Toronto, Canada: Aug 2010):Abstract 337
Greig AV, James SE, McGrouther DA, Terenghi G, Burnstock G (2003a) Purinergic receptor expression in the regenerating epidermis in a rat model of normal and delayed wound healing. Exp Dermatol 12:860–871
Greig AV, Linge C, Cambrey A, Burnstock G (2003b) Purinergic receptors are part of a signaling system for keratinocyte proliferation, differentiation, and apoptosis in human fetal epidermis. J Invest Dermatol 121:1145–1149
Greig AV, Linge C, Healy V, Lim P, Clayton E, Rustin MH, McGrouther DA, Burnstock G (2003c) Expression of purinergic receptors in non-melanoma skin cancers and their functional roles in A431 cells. J Invest Dermatol 121:315–327
Greig AV, Linge C, Terenghi G, McGrouther DA, Burnstock G (2003d) Purinergic receptors are part of a functional signaling system for proliferation and differentiation of human epidermal keratinocytes. J Invest Dermatol 120:1007–1015
Gu JG, MacDermott AB (1997) Activation of ATP P2X receptors elicits glutamate release from sensory neuron synapses. Nature 389:749–753
Gunosewoyo H, Kassiou M (2010) P2X purinergic receptor ligands: recently patented compounds. Expert Opin Ther Pat 20:625–646
Habler HJ, Janig W, Koltzenburg M (1990) Activation of unmyelinated afferent fibres by mechanical stimuli and inflammation of the urinary bladder in the cat. J Physiol 425:545–562
Habler HJ, Janig W, Koltzenburg M (1993) Myelinated primary afferents of the sacral spinal cord responding to slow filling and distension of the cat urinary bladder. J Physiol 463:449–460
Hamilton SG, Warburton J, Bhattacharjee A, Ward J, McMahon SB (2000) ATP in human skin elicits a dose-related pain response which is potentiated under conditions of hyperalgesia. Brain 123:1238–1246
Hansen MA, Balcar VJ, Barden JA, Bennett MR (1998) The distribution of single P2x1-receptor clusters on smooth muscle cells in relation to nerve varicosities in the rat urinary bladder. J Neurocytol 27:529–539
Harrington LS, Evans RJ, Wray J, Norling L, Swales KE, Vial C, Ali F, Carrier MJ, Mitchell JA (2007) Purinergic 2X1 receptors mediate endothelial dependent vasodilation to ATP. Mol Pharmacol 72:1132–1136
Harvey R (2002) The contractile potency of adenosine triphosphate and ecto-adenosine triphosphatase activity in guinea pig detrusor and detrusor from patients with a stable, unstable or obstructed bladder. J Urol 168:1235–1239
Hausmann R, Rettinger J, Gerevich Z, Meis S, Kassack MU, Illes P, Lambrecht G, Schmalzing G (2006) The suramin analog 4,4′,4″,4‴-(carbonylbis(imino-5, 1, 3-benzenetriylbis (carbonylimino)))tetra-kis-benzenesulfonic acid (NF110) potently blocks P2X3 receptors: subtype selectivity is determined by location of sulfonic acid groups. Mol Pharmacol 69:2058–2067
Hegde SS, Mandel DA, Wilford MR, Briaud S, Ford APDW, Eglen RM (1998) Evidence for purinergic neurotransmission in the urinary bladder of pithed rats. Eur J Pharmacol 349:75–82
Heppner TJ, Werner ME, Nausch B, Vial C, Evans RJ, Nelson MT (2009) Nerve-evoked purinergic signalling suppresses action potentials, Ca^{2+} flashes and contractility evoked by muscarinic receptor activation in mouse urinary bladder smooth muscle. J Physiol 587:5275–5288
Hilliges M, Weidner C, Schmelz M, Schmidt R, Orstavik K, Torebjork E, Handwerker H (2002) ATP responses in human C nociceptors. Pain 98:59–68

Holton P (1959) The liberation of adenosine triphosphate on antidromic stimulation of sensory nerves. J Physiol 145:494–504

Honore P, Kage K, Mikusa J, Watt AT, Johnston JF, Wyatt JR, Faltynek CR, Jarvis MF, Lynch K (2002a) Analgesic profile of intrathecal $P2X_3$ antisense oligonucleotide treatment in chronic inflammatory and neuropathic pain states in rats. Pain 99:11–19

Honore P, Mikusa J, Bianchi B, McDonald H, Cartmell J, Faltynek C, Jarvis MF (2002b) TNP-ATP, a potent $P2X_3$ receptor antagonist, blocks acetic acid-induced abdominal constriction in mice: comparison with reference analgesics. Pain 96:99–105

Horner S, Menke K, Hildebrandt C, Kassack MU, Nickel P, Ullmann H, Mahaut-Smith MP, Lambrecht G (2005) The novel suramin analogue NF864 selectively blocks $P2X_1$ receptors in human platelets with potency in the low nanomolar range. Naunyn Schmiedebergs Arch Pharmacol 372:1–13

Hourani SM, Chown JA (1989) The effects of some possible inhibitors of ectonucleotidases on the breakdown and pharmacological effects of ATP in the guinea-pig urinary bladder. Gen Pharmacol 20:413–416

Hoyle CHV (1996) Purinergic cotransmission: parasympathetic and enteric nerves. Semin Neurosci 8:207–215

Hoyle CHV, Burnstock G (1985) Atropine-resistant excitatory junction potentials in rabbit bladder are blocked by α,β-methylene ATP. Eur J Pharmacol 114:239–240

Igawa Y, Mattiasson A, Andersson K-E (1993) Functional importance of cholinergic and purinergic neurotransmission for micturition contraction in the normal, unanaesthetized rat. Br J Pharmacol 109:473–479

Iglesias R, Dahl G, Qiu F, Spray DC, Scemes E (2009) Pannexin 1: the molecular substrate of astrocyte "hemichannels". J Neurosci 29:7092–7097

Inoue R, Brading AF (1990) The properties of the ATP-induced depolarization and current in single cells isolated from the guinea-pig urinary bladder. Br J Pharmacol 100:619–625

Inoue R, Brading AF (1991) Human, pig and guinea-pig bladder smooth muscle cell generate similar inward currents in response to purinoceptor activation. Br J Pharmacol 103:1840–1841

Inoue K, Tsuda M, Koizumi S (2003) ATP induced three types of pain behaviors, including allodynia. Drug Dev Res 59:56–63

Inoue K, Tsuda M, Koizumi S (2004) Chronic pain and microglia: the role of ATP. Novartis Found Symp 261:55–64

Inscho EW, Cook AK, Imig JD, Vial C, Evans RJ (2003) Physiological role for $P2X_1$ receptors in renal microvascular autoregulatory behavior. J Clin Invest 112:1895–1905

Inscho EW, Cook AK, Imig JD, Vial C, Evans RJ (2004) Renal autoregulation in P2X1 knockout mice. Acta Physiol Scand 181:445–453

Ito K, Iwami A, Katsura H, Ikeda M (2008) Therapeutic effects of the putative P2X3/P2X2/3 antagonist A-317491 on cyclophosphamide-induced cystitis in rats. Naunyn Schmiedebergs Arch Pharmacol 377:483–490

Jacobson KA, Costanzi S, Ohno M, Joshi BV, Besada P, Xu B, Tchilibon S (2004) Molecular recognition at purine and pyrimidine nucleotide (P2) receptors. Curr Top Med Chem 4:805–819

Jahangir A, Alam M, Carter DS, Dillon MP, Bois DJ, Ford AP, Gever JR, Lin C, Wagner PJ, Zhai Y, Zira J (2009) Identification and SAR of novel diaminopyrimidines. Part 2: the discovery of RO-51, a potent and selective, dual P2X(3)/P2X(2/3) antagonist for the treatment of pain. Bioorg Med Chem Lett 19:1632–1635

Jaime-Figueroa S, Greenhouse R, Padilla F, Dillon MP, Gever JR, Ford APDW (2005) Discovery and synthesis of a novel and selective drug-like $P2X_1$ antagonist. Bioorg Med Chem Lett 15:3292–3295

Jarvis MJ (2003) Contributions of $P2X_3$ homomeric and heteromeric channels to acute and chronic pain. Expert Opin Ther Targets 7:513–522

Jarvis MF (2010) The neural-glial purinergic receptor ensemble in chronic pain states. Trends Neurosci 33:48–57

Jarvis MF, Wismer CT, Schweitzer E, Yu H, van Biesen T, Lynch KJ, Burgard EC, Kowaluk EA (2001) Modulation of BzATP and formalin induced nociception: attenuation by the P2X receptor antagonist, TNP-ATP and enhancement by the P2X3 allosteric modulator, cibacron blue. Br J Pharmacol 132:259–269

Jarvis MF, Burgard EC, McGaraughty S, Honore P, Lynch K, Brennan TJ, Subieta A, Van Biesen T, Cartmell J, Bianchi B, Niforatos W, Kage K, Yu H, Mikusa J, Wismer CT, Zhu CZ, Chu K, Lee CH, Stewart AO, Polakowski J, Cox BF, Kowaluk E, Williams M, Sullivan J, Faltynek C (2002) A-317491, a novel potent and selective non-nucleotide antagonist of $P2X_3$ and $P2X_{2/3}$ receptors, reduces chronic inflammatory and neuropathic pain in the rat. Proc Natl Acad Sci USA 99:17179–17184

Kaan TKY, Yip PK, Zhong Y, Nunn PA, Ford APDW, Malcangio M, McMahon SB (2009) Novel P2X3 and P2X2/3 receptor antagonist prevents and reverses bone cancer-induced pain behaviour in rats. Soc for Neurosci Abstracts (39th Annual Meeting, Chicago USA)

Kaan TK, Yip PK, Grist J, Cefalu JS, Nunn PA, Ford AP, Zhong Y, McMahon SB (2010) Endogenous purinergic control of bladder activity via presynaptic P2X3 and P2X2/3 receptors in the spinal cord. J Neurosci 30:4503–4507

Kang J, Kang N, Lovatt D, Torres A, Zhao Z, Lin J, Nedergaard M (2008) Connexin 43 hemichannels are permeable to ATP. J Neurosci 28:4702–4711

Kasakov L, Burnstock G (1983) The use of the slowly degradable analog, α,β-methylene ATP, to produce desensitisation of the P2-purinoceptor: Effect on non-adrenergic, non-cholinergic responses of the guinea-pig urinary bladder. Eur J Pharmacol 86:291–294

Kawate T, Michel JC, Birdsong WT, Gouaux E (2009) Crystal structure of the ATP-gated P2X(4) ion channel in the closed state. Nature 460:592–598

Khakh BS (2001) International union of pharmacology. XXIV. Current status of the nomenclature and properties of P2X receptors and their subunits. Pharmacol Rev 53:107–118

Khakh BS, North R (2006) P2X receptors as cell-surface ATP sensors in health and disease. Nature 442:527–532

Khera M, Somogyi GT, Kiss S, Boone TB, Smith CP (2004) Botulinum toxin A inhibits ATP release from bladder urothelium after chronic spinal cord injury. Neurochem Int 45:987–993

Khera M, Boone TB, Salas N, Jett MF, Somogyi GT (2007) The role of the prostacyclin receptor antagonist RO3244019 in treating neurogenic detrusor overactivity after spinal cord injury in rats. BJU Int 99:442–446

Kinder RB, Mundy AR (1987) Pathophysiology of idiopathic detrusor instability and detrusor hyper-reflexia. An in vitro study of human detrusor muscle. Br J Urol 60:509–515

King JA, Huddart H, Staff WG (1997) Purinergic modulation of rat urinary bladder detrusor smooth muscle. Gen Pharmacol 29:597–604

King BF, Knowles ID, Burnstock G, Ramage AG (2004) Investigation of the effects of P2 purinoceptor ligands on the micturition reflex in female urethane-anaesthetized rats. Br J Pharmacol 142:519–530

Knight GE, Bodin P, De Groat WC, Burnstock G (2002) ATP is released from guinea pig ureter epithelium on distension. Am J Physiol Renal Physiol 282:F281–F288

Kumahashi N, Ochi M, Kataoka H, Uchio Y, Kakimaru H, Sugawara K, Enomoto K (2004) Involvement of ATP, increase of intracellular calcium and the early expression of c-fos in the repair of rat fetal articular cartilage. Cell Tissue Res 317:117–128

Kumahashi N, Naitou K, Nishi H, Oae K, Watanabe Y, Kuwata S, Ochi M, Ikeda M, Uchio Y (2010) Correlation of changes in pain intensity with synovial fluid adenosine triphosphate levels after treatment of patients with osteoarthritis of the knee with high-molecular-weight hyaluronic acid. Knee [June 18, epub ahead of print]

Lamont C, Vial C, Evans RJ, Wier WG (2006) P2X1 receptors mediate sympathetic postjunctional Ca^{2+} transients in mesenteric small arteries. Am J Physiol Heart Circ Physiol 291:H3106–H3113

Laupland KB, Church DL, Gregson DB (2003) Evaluation of a rapid bacterial ATP assay for screening BAL samples from ICU patients submitted for quantitative bacterial cultures. Diagn Microbiol Infect Dis 47:465–469

Lee HY, Bradini M, Burnstock G (2000) Distribution of P2X receptors in the urinary bladder and the ureter of the rat. J Urol 163:2002–2007

Levin RM, Wein AJ (1982) Response of the in vitro whole bladder (rabbit) preparation to autonomic agonists. J Urol 128:1087–1090

Levin RM, Longhurst PA, Kato K, McGuire EJ, Elbadawi A, Wein AJ (1990) Comparative physiology and pharmacology of the cat and rabbit urinary bladder. J Urol 143:848–852

Lewis C, Neidhart S, Holy C, North RA, Buell G, Surprenant A (1995) Coexpression of $P2X_2$ and $P2X_3$ receptor subunits can account for ATP-gated currents in sensory neurons. Nature 377:432–435

Lieu PK, Sa'adu A, Orugun EO, Malone-Lee JG (1997) The influence of age on isometric and isotonic rat detrusor contractions. J Gerontol A Biol Sci Med Sci 52:M94–M96

Lipinski CA (2000) Drug-like properties and the causes of poor solubility and poor permeability. J Pharmacol Toxicol Methods 44:235–249

Lommatzsch M, Cicko S, Muller T, Lucattelli M, Bratke K, Stoll P, Grimm M, Durk T, Zissel G, Ferrari D, Di VF, Sorichter S, Lungarella G, Virchow JC, Idzko M (2010) Extracellular adenosine triphosphate and chronic obstructive pulmonary disease. Am J Respir Crit Care Med 181:928–934

Lu S, Fraser M, Chancellor M, Sun L, de Groat WC (2002) Evaluation of voiding dysfunction and purinergic mechanism in awake long-term spinal cord injured rats: comparison of metabolism cage and cystometrogram measurements. J Urol 167:276

Lu SH, Groat WC, Lin AT, Chen KK, Chang LS (2007) Evaluation of purinergic mechanism for the treatment of voiding dysfunction: a study in conscious spinal cord-injured rats. J Chin Med Assoc 70:439–444

Ludlow MJ, Durai L, Ennion SJ (2009) Functional characterization of intracellular *Dictyostelium discoideum* P2X receptors. J Biol Chem 284:35227–35239

Maehara Y, Kusumoto H, Anai H, Kusumoto T, Sugimachi K (1987) Human tumor tissues have higher ATP contents than normal tissues. Clin Chim Acta 169:341–343

Masuda H, Chancellor M, Kihara K, de Groat W, Yoshimura N (2005) Evidence for the involvement of spinal endogenous ATP and P2X receptors in detrusor overactivity caused by acetic acid, acrolein or cyclophosphamide. Neurourol Urodyn 24:401–598

McGaraughty S, Wismer CT, Zhu CZ, Mikusa J, Honore P, Chu KL, Lee CH, Faltynek CR, Jarvis MF (2003) Effects of A-317491, a novel and selective $P2X_3/P2X_{2/3}$ receptor antagonist, on neuropathic, inflammatory and chemogenic nociception following intrathecal and intraplantar administration. Br J Pharmacol 140:1381–1388

Michel AD, Ng SW, Roman S, Clay WC, Dean DK, Walter DS (2009) Mechanism of action of species-selective P2X7 antagonists. Br J Pharmacol 156:1312–1325

Mitsui T, Kakizaki H, Matsuura S, Ameda K, Yoshioka M, Koyanagi T (2001) Afferent fibers of the hypogastric nerves are involved in the facilitating effects of chemical bladder irritation in rats. J Neurophysiol 86:2276–2284

Mork H, Ashina M, Bendtsen L, Olesen J, Jensen R (2003) Experimental muscle pain and tenderness following infusion of endogenous substances in humans. Eur J Pain 7:145–153

Mortaz E, Braber S, Nazary M, Givi ME, Nijkamp FP, Folkerts G (2009) ATP in the pathogenesis of lung emphysema. Eur J Pharmacol 619:92–96

Mortaz E, Folkerts G, Nijkamp FP, Henricks PA (2010) ATP and the pathogenesis of COPD. Eur J Pharmacol 638:1–4

Mulryan K, Gitterman DP, Lewis CJ, Vial C, Leckie BJ, Cobb AL, Brown JE, Conley EC, Buell G, Pritchard CA, Evans RJ (2000) Reduced vas deferens contraction and male infertility in mice lacking $P2X_1$ receptors. Nature 403:86–89

Nakatsuka T, Gu JG (2001) ATP P2X receptor-mediated enhancement of glutamate release and evoked EPSCs in dorsal horn neurons of the rat spinal cord. J Neurosci 21:6522–6531

Nakatsuka T, Tsuzuki K, Ling JX, Sonobe H, Gu JG (2003) Distinct roles of P2X receptors in modulating glutamate release at different primary sensory synapses in rat spinal cord. J Neurophysiol 89:3243–3252

Namasivayam S, Eardley I, Morrison JFB (1999) Purinergic sensory neurotransmission in the urinary bladder: an in vitro study in the rat. Br J Urol Int 84:854–860
Nergardh A, Kinn AC (1983) Neurotransmission in activation of the contractile response in the human urinary bladder. Scand J Urol Nephrol 17:153–157
Nicke A (2008) Homotrimeric complexes are the dominant assembly state of native P2X7 subunits. Biochem Biophys Res Commun 377:803–808
Nicke A, Baeumert HG, Rettinger J, Eichele A, Lambrecht G, Mutschler E, Schmalzing G (1998) $P2X_1$ and $P2X_3$ receptors form stable trimers: a novel structural motif of ligand-gated ion channels. EMBO J 17:3016–3028
Nicke A, Kerschensteiner D, Soto F (2005) Biochemical and functional evidence for heteromeric assembly of $P2X_1$ and $P2X_4$ subunits. J Neurochem 92:925–933
Nishiguchi J, Sasaki K, Seki S, Chancellor MB, Erickson KA, de Groat WC, Kumon H, Yoshimura N (2004) Effects of isolectin B4-conjugated saporin, a targeting cytotoxin, on bladder overactivity induced by bladder irritation. Eur J Neurosci 20:474–482
North RA (1996a) Families of ion channels with two hydrophobic segments. Curr Opin Cell Biol 8:474–483
North RA (1996b) P2X purinoceptor plethora. Sem Neurosci 8:187–194
North RA (2002) Molecular physiology of P2X receptors. Physiol Rev 82:1013–1067
North RA, Barnard EA (1997) Nucleotide receptors. Curr Opin Neurobiol 7:346–357
North RA, Surprenant A (2000) Pharmacology of cloned P2X receptors. Annu Rev Pharmacol Toxicol 40:563–580
Nunn PA, Newgreen DT (1999) An investigation into the bladder responses induced via pelvic nerve stimulation in the anesthetized rat. Br J Pharmacol 126:227P
O'Reilly BA, Kosaka AH, Chang TK, Ford AP, Popert R, McMahon SB (2001) A quantitative analysis of purinoceptor expression in the bladders of patients with symptomatic outlet obstruction. BJU Int 87:617–622
O'Reilly BA, Kosaka AH, Knight GF, Chang TK, Ford APDW, Rymer JM, Popert R, Burnstock G, McMahon SB (2002) P2X receptors and their role in female idiopathic detrusor instability. J Urol 167:157–164
Orriss IR, Knight GE, Utting JC, Taylor SE, Burnstock G, Arnett TR (2009) Hypoxia stimulates vesicular ATP release from rat osteoblasts. J Cell Physiol 220:155–162
Osterberg E, Hallander HO, Kallner A, Lundin A, Aberg H (1991) Evaluation of the adenosine triphosphate test in the diagnosis of urinary tract infection. Eur J Clin Microbiol Infect Dis 10:70–73
Palea S, Artibani W, Ostardo E, Trist DG, Pietra C (1993) Evidence for purinergic neurotransmission in human urinary bladder affected by interstitial cystitis. J Urol 150:2007–2012
Pandita RK, Andersson K-E (2002) Intravesical adenosine triphosphate stimulates the micturition reflex in awake, freely moving rats. J Urol 168:1230–1234
Pearson JD, Gordon JL (1979) Vascular endothelial and smooth muscle cells in culture selectively release adenine nucleotides. Nature 281:384–386
Pellegatti P, Raffaghello L, Bianchi G, Piccardi F, Pistoia V, Di VF (2008) Increased level of extracellular ATP at tumor sites: in vivo imaging with plasma membrane luciferase. PLoS One 3:e2599
Rapp DE, Lyon MB, Bales GT, Cook SP (2005) A role for the P2X receptor in urinary tract physiology and in the pathophysiology of urinary dysfunction. Eur Urol 48:303–308
Rettinger J, Schmalzing G, Damer S, Muller G, Nickel P, Lambrecht G (2000) The suramin analogue NF279 is a novel and potent antagonist selective for the $P2X_1$ receptor. Neuropharmacology 39:2044–2053
Risholm L (1954) Studies on renal colic and its treatment by posterior splanchnic block. Acta Chir Scand Suppl 184:5–64
Rong W, Burnstock G (2004) Activation of ureter nociceptors by exogenous and endogenous ATP in guinea pig. Neuropharmacology 47:1093–1101

Rong W, Spyer KM, Burnstock G (2002) Activation and sensitisation of low and high threshold afferent fibres mediated by P2X receptors in the mouse urinary bladder. J Physiol 541:591–600
Ruggieri MR (2006) Mechanisms of disease: role of purinergic signaling in the pathophysiology of bladder dysfunction. Nat Clin Pract Urol 3:206–215
Ryan LM, Rachow JW, McCarty DJ (1991) Synovial fluid ATP: a potential substrate for the production of inorganic pyrophosphate. J Rheumatol 18:716–720
Salas NA, Smith CP, Kiss S, Boone TB, Somogyi GT (2005) Intravesical cholinergic receptor activated spinal ATP release in normal and chronic spinal cord injured rats. J Urol 173:45
Salas NA, Somogyi GT, Gangitano DA, Boone TB, Smith CP (2007) Receptor activated bladder and spinal ATP release in neurally intact and chronic spinal cord injured rats. Neurochem Int 50:345–350
Shabbir M, Burnstock G (2009) Purinergic receptor-mediated effects of adenosine 5′-triphosphate in urological malignant diseases. Int J Urol 16:143–150
Sibley GN (1984) A comparison of spontaneous and nerve-mediated activity in bladder muscle from man, pig and rabbit. J Physiol 354:431–443
Sjogren C, Andersson K-E, Husted S, Mattiasson A, Moller-Madsen B (1982) Atropine resistance of transmurally stimulated isolated human bladder muscle. J Urol 128:1368–1371
Smith CP, Vemulakonda VM, Kiss S, Boone TB, Somogyi GT (2005) Enhanced ATP release from rat bladder urothelium during chronic bladder inflammation: Effect of botulinum toxin A. Neurochem Int 47:291–297
Smith CP, Gangitano DA, Munoz A, Salas NA, Boone TB, Aoki KR, Francis J, Somogyi GT (2008) Botulinum toxin type A normalizes alterations in urothelial ATP and NO release induced by chronic spinal cord injury. Neurochem Int 52:1068–1075
Soto F, Lambrecht G, Nickel P, Stuehmer W, Busch AE (1999) Antagonistic properties of the suramin analogue NF023 at heterologously expressed P2X receptors. Neuropharmacology 38:141–149
Souslova V, Cesare P, Ding Y, Akopian AN, Stanfa L, Suzuki R, Carpenter K, Dickenson A, Boyce S, Hill R, Nebenius-Oosthuizen D, Smith AJH, Kidd EJ, Wood JN (2000) Warm-coding deficits and aberrant inflammatory pain in mice lacking $P2X_3$ receptors. Nature 407:1015–1017
Suadicani SO, Vink MJ, Spray DC (2000) Slow intercellular Ca(2+) signaling in wild-type and Cx43-null neonatal mouse cardiac myocytes. Am J Physiol Heart Circ Physiol 279: H3076–H3088
Sun Y, Chai TC (2002) Effects of dimethyl sulphoxide and heparin on stretch-activated ATP release by bladder urothelial cells from patients with interstitial cystitis. BJU Int 90:381–385
Sun Y, Chai TC (2004) Up-regulation of $P2X_3$ receptor during stretch of bladder urothelial cells from patients with interstitial cystitis. J Urol 171:448–452
Sun Y, Keay S, DeDeyne P, Chai T (2001a) Stretch-activated release of adenosine triphosphate by bladder uroepithelia is augmented in interstitial cystitis. Urology 57:131–135
Sun Y, Keay S, DeDeyne PG, Chai TC (2001b) Augmented stretch activated adenosine triphosphate release from bladder uroepithelial cells in patients with interstitial cystitis. J Urol 166:1951–1956
Sun Y, MaLossi J, Jacobs SC, Chai TC (2002) Effect of doxazosin on stretch-activated adenosine triphosphate release in bladder urothelial cells from patients with benign prostatic hyperplasia. Urology 60:351–356
Sun Y, Keay S, Lehrfeld TJ, Chai TC (2009) Changes in adenosine triphosphate-stimulated ATP release suggest association between cytokine and purinergic signaling in bladder urothelial cells. Urology 74:1163–1168
Thuringer D (2004) The vascular endothelial growth factor-induced disruption of gap junctions is relayed by an autocrine communication via ATP release in coronary capillary endothelium. Ann NY Acad Sci 1030:14–27
Tong YC, Hung YC, Shinozuka K, Kunitomo M, Cheng JT (1997) Evidence of adenosine 5′-triphosphate release from nerve and P2x-purinoceptor mediated contraction during electrical stimulation of rat urinary bladder smooth muscle. J Urol 158:1973–1977

Tornroth-Horsefield S, Neutze R (2008) Opening and closing the metabolite gate. Proc Natl Acad Sci USA 105:19565–19566

Tsuda M, Ueno S, Inoue K (1999a) Evidence for the involvement of spinal endogenous ATP and P2X receptors in nociceptive responses caused by formalin and capsaicin in mice. Br J Pharmacol 128:1497–1504

Tsuda M, Ueno S, Inoue K (1999b) In vivo pathway of thermal hyperalgesia by intrathecal administration of α,β-methylene ATP in mouse spinal cord: Involvement of the glutamate-NMDA receptor system. Br J Pharmacol 127:449–456

Ueno S, Moriyama T, Honda K, Kamiya H, Sakurada T, Katsuragi T (2003) Involvement of $P2X_2$ and $P2X_3$ receptors in neuropathic pain in a mouse model of chronic constriction injury. Drug Dev Res 59:104–111

Ulmann L, Hatcher JP, Hughes JP, Chaumont S, Green PJ, Conquet F, Buell GN, Reeve AJ, Chessell IP, Rassendren F (2008) Up-regulation of P2X4 receptors in spinal microglia after peripheral nerve injury mediates BDNF release and neuropathic pain. J Neurosci 28:11263–11268

Ulmann L, Hirbec H, Rassendren F (2010) P2X4 receptors mediate PGE2 release by tissue-resident macrophages and initiate inflammatory pain. EMBO J 29:2290–2300

Velasco C, Guarneri L, Leonardi A, Testa R (2003) Effects of intravenous and infravesical administration of suramin, terazosin and BMY 7378 on bladder instability in conscious rats with bladder outlet obstruction. BJU Int 92:131–136

Vial C, Evans RJ (2000) P2X receptor expression in mouse urinary bladder and the requirement of $P2X_1$ receptors for functional P2X receptor responses in the mouse urinary bladder smooth muscle. Br J Pharmacol 131:1489–1495

Vial C, Evans RJ (2001) Smooth muscle does not have a common P_{2X} receptor phenotype: expression, ontogeny and function of P_{2X1} receptors in mouse ileum, bladder and reproductive systems. Auton Neurosci 92:56–64

Vlaskovska M, Kasakov L, Rong W, Bodin P, Bardini M, Cockayne DA, Ford AP, Burnstock G (2001) $P2X_3$ knock-out mice reveal a major sensory role for urothelially released ATP. J Neurosci 21:5670–5677

Vulchanova L, Riedl MS, Shuster SJ, Buell G, Surprenant A, North RA, Elde R (1997) Immunohistochemical study of the $P2X_2$ and $P2X_3$ receptor subunits in rat and monkey sensory neurons and their central terminals. Neuropharmacology 36:1229–1242

Vulchanova L, Riedl MS, Shuster SJ, Stone LS, Hargreaves KM, Buell G, Surprenant A, North RA, Elde R (1998) $P2X_3$ is expressed by DRG neurons that terminate in inner lamina II. Eur J Neurosci 10:3470–3478

Wang ECY, Lee JM, Ruiz WG, Balestreire EM, von Bodungen M, Barrick S, Cockayne DA, Birder LA, Apodaca G (2005) ATP and purinergic receptor-dependent membrane traffic in bladder umbrella cells. J Clin Invest 115:2412–2422

Westfall TD, Kennedy C, Sneddon P (1997) The ecto-ATPase inhibitor ARL 67156 enhances parasympathetic neurotransmission in the guinea-pig urinary bladder. Eur J Pharmacol 329:169–173

White TD, Chaudhry A, Vohra MM, Webb D, Leslie RA (1985) Characteristics of P2 (nucleotide) receptors mediating contraction and relaxation of rat aortic strips: possible physiological relevance. Eur J Pharmacol 118:37–44

Windscheif U, Ralevic V, Baumert HG, Mutschler E, Lambrecht G, Burnstock G (1994) Vasoconstrictor and vasodilator responses to various agonists in the rat perfused mesenteric arterial bed – selective inhibition by ppads of contractions mediated via p-2x-purinoceptors. Br J Pharmacol 113:1015–1021

Wink MR, Lenz G, Braganhol E, Tamajusuku AS, Schwartsmann G, Sarkis JJ, Battastini AM (2003) Altered extracellular ATP, ADP and AMP catabolism in glioma cell lines. Cancer Lett 198:211–218

Wu C (1999) A comparison of the mode of action of ATP and carbachol on isolated human detrusor smooth muscle. J Urol 162:1840

Wu G, Whiteside GT, Lee G, Nolan S, Niosi M, Pearson MS, Ilyin VI (2004) A-317491, a selective $P2X_3/P2X_{2/3}$ receptor antagonist, reverses inflammatory mechanical hyperalgesia through action at peripheral receptors in rats. Eur J Pharmacol 504:45–53

Wust M, Averbeck B, Reif S, Brater M, Ravens U (2002) Different responses to drugs against overactive bladder in detrusor muscle of pig, guinea pig and mouse. Eur J Pharmacol 454: 59–69

Yang S, Cheek DJ, Westfall DP, Buxton IL (1994) Purinergic axis in cardiac blood vessels. Agonist-mediated release of ATP from cardiac endothelial cells. Circ Res 74:401–407

Yiangou Y, Facer P, Ford A, Brady C, Wiseman O, Fowler CJ, Anand P (2001) Capsaicin receptor VR1 and ATP-gated ion channel $P2X_3$ in human urinary bladder. BJU Int 87:774–779

Yoshida M, Homma Y, Inadome A, Yono M, Seshita H, Miyamoto Y, Murakami S, Kawabe K, Ueda S (2001) Age-related changes in cholinergic and purinergic neurotransmission in human isolated bladder smooth muscle. Exp Gerontol 36:99–109

Yoshida M, Miyamae K, Iwashita H, Otani M, Inadome A (2004) Management of detrusor dysfunction in the elderly: changes in acetylcholine and adenosine triphosphate release during aging. Urology 63:17–23

Yoshimura N, de Groat WC (1999) Increased excitability of afferent neurons innervating rat urinary bladder after chronic bladder inflammation. J Neurosci 19:4644–4653

Yoshimura N, Seki S, Chancellor MB, de Groat WC, Ueda T (2002) Targeting afferent hyperexcitability for therapy of the painful bladder syndrome. Urology 59:61–67

Yoshimura N, Seki S, Erickson KA, Erickson VL, Chancellor MB, de Groat WC (2003) Histological and electrical properties of rat dorsal root ganglion neurons innervating the lower urinary tract. J Neurosci 23:4355–4361

Zhong Y, Banning AS, Cockayne DA, Ford APDW, Burnstock G, McMahon SB (2003) Bladder and cutaneous sensory neurons of the rat express different functional P2X receptors. Neuroscience 120:667–675

Ziganshin AU, Hoyle CH, Bo X, Lambrecht G, Mutschler E, Baumert HG, Burnstock G (1993) PPADS selectively antagonizes P_{2X}-purinoceptor-mediated responses in the rabbit urinary bladder. Br J Pharmacol 110:1491–1495

Cyclic Nucleotide Metabolism Including Nitric Oxide and Phosphodiesterase-Related Targets in the Lower Urinary Tract

Stefan Ückert and Markus A. Kuczyk

Contents

Abstract The clinical data on the use of the orally active phosphodiesterase (PDE) type 5 inhibitors sildenafil (VIAGRA™), vardenafil (LEVITRA™), and tadalafil (CIALIS™) for the treatment of male erectile dysfunction have boosted research activities on the physiology and pharmacology of the organs of the lower urinary tract (LUT). This includes both intracellular signal transduction in the prostate, urinary bladder (detrusor), and urethra, as well as central brain and spinal cord pathways controlling the function of the LUT. Such efforts provided the basis for the development of new therapeutic modalities into the management of dysfunctions/

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syndromes of the LUT, some of which are already offered to the patients. The pharmacological treatment of the overactive bladder and the so-called benign prostatic syndrome, including LUT symptomatology and bladder outlet obstruction secondary to benign prostatic enlargement, has primarily focused on selective, orally available drugs acting by influencing intracellular regulatory mechanisms. These agents are regarded efficacious, have a fast onset of drug action in the target tissue and an improved effect-to-side-effect ratio. Better understanding of the functional significance of proteins related to cyclic nucleotide-dependent pathways, such as nitric oxide synthase, cytosolic and membrane-bound guanylyl cyclases, PDE isoenzymes and cyclic AMP- and cyclic GMP-binding protein kinases, the relative distribution in tissues of the LUT, and the consequences for urogenital function, seems to be of particular interest in order to identify new or more selective pharmacological approaches to manage disorders of the LUT. The present review focuses on cyclic nucleotide-related targets involved in the control of the function of the bladder, prostate, and urethra and the significance of those proteins in the process of evolving new pharmacological options for the treatment of LUT symptoms secondary to benign prostatic hyperplasia as well as dysfunctions of the storage and voiding capability of the urinary bladder.

Keywords Cyclic adenosine monophosphate (cyclic AMP) · Cyclic guanosine monophosphate (cyclic GMP) · Lower urinary tract · Nitric oxide (NO) · Pharmacotherapy

1 Introduction

The control of the smooth musculature of the lower urinary tract (LUT) is a complex mechanism involving the interaction between the central nervous system and the local release and degradation of various adrenergic, cholinergic, and NANC endogenous factors, known to exert either facilitating or inhibitory activity, deriving from neuronal, endothelial, or epithelial sources. The cyclic nucleotide monophosphates cyclic AMP and cyclic GMP are important intracellular regulators of several physiological processes, including smooth muscle function (Antoni 2000; Lucas et al. 2000). Nitric oxide (NO) has been shown a crucial mediator of the relaxation of smooth muscle in the out-flow region of the urinary tract and is also involved in the modification of afferent neurotransmission from parts of the urogenital region to spinal cord and brain centers (Andersson 1993; Hedlund 2005). NO is synthesized from the amino acid L-arginine by two main classes of nitric oxide synthases (NOS): the NOS occurring in the cytosol of peripheral nerves (nNOS) is a Ca^{2+}/calmodulin-dependent enzyme that releases picomoles of NO in response to receptor stimulation. The endothelial NOS (eNOS) is located in endothelial cells and glandular epithelium where it is mainly associated with the membranes of the mitochondria, endoplasmatic reticulum (ER), and Golgi apparatus. The eNOS is one of the factors accounting for the endothelium-mediated relaxation of vascular and nonvascular smooth muscle of

the urogenital tract (Andersson and Persson 1993). NO interacts with the cytosolic (soluble) guanylyl cyclase (sGC) in the cytoplasm and increases the rate of conversion of guanosine triphosphate (GTP) into cyclic GMP. The structurally related particulate GC (pGC) extends in an extracellular domain to which natriuretic peptides, such as atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and C-type natriuretic peptide (CNP), bind to and subsequently induce accumulation of intracellular cyclic GMP (Lucas et al. 2000). Stimulation of specific G-protein-coupled receptors on the outer cell surface by the binding of β-adrenergic agonists, prostaglandin E_1 (PGE_1), or VIP results in the activation of adenylyl cyclase (AC) activity and subsequent elevation of cyclic AMP. The increase in cyclic AMP or cyclic GMP triggers a signal transduction cascade that encompasses the activation of cyclic nucleotide-dependent protein kinases (cAK, cGK), phosphorylation of the actin-myosin system, as well as Ca^{2+} channels and ATP-driven Ca^{2+} pumps located in the outer cell membrane or the membrane of the sarcoplasmatic reticulum (SR). This cascade leads to a reduction in cytosolic Ca^{2+} and, finally, to smooth muscle relaxation. The present review focuses on the control of smooth muscle function mediated by cyclic AMP/cyclic GMP in the bladder, prostate and urethra, potential disease-related alterations in said pathways in the out-flow region, and possibilities for the pharmacological modification of the signaling pathways.

2 Soluble Guanylyl Cyclase

The main intracellular receptor for NO is the soluble form of the enzyme guanylyl cyclase (sGC). sGC is a heterodimeric protein that consists of an α- and β-subunit, both presenting a homologous domain constituting the catalytic center known to generate cyclic GMP. The enzyme also contains a heme that is attached to a histidine residue of the β-subunit, which is essentially required for the activation of the enzyme by NO. Although the binding of NO occurs in the β-subunit, both subunits are required for the stimulation of enzyme activity (Denninger and Marletta 1999; Lee et al. 2000). Since the pharmacological activation of sGC has been recognized to have great potential for the treatment of a wide variety of diseases, several agents have been identified that have the ability to stimulate the enzyme. While classical NO donors (i.e., organic nitrates) may exert serious drawbacks, compounds that can activate sGC in a manner independent from the release of NO offer a significant advance (Hobbs 2002). Such compounds have been developed recently by several pharmaceutical companies and are characterized as non NO-based sGC activators due to a mechanism of action that involves the binding of the entire drug molecule to the heme component. The prototype of non NO-based sGC activators is YC-1, originally identified as a potent inhibitor of platelet aggregation (Friebe et al. 1998). The stimulating effects of YC-1 cannot be blocked by NO scavengers, indicating that the action of the drug is independent of NO. A group of non NO-based sGC activators, designated as A 344905, A 350619,

BAY 41-2272, BAY 41-8543, and BAY 58-2667, are considerably more potent than YC-1 (Bischoff et al. 2003; Straub et al. 2001; Stasch et al. 2002; Moreland et al. 2002; Miller et al. 2003). While the effects of BAY 41-2272 and BAY 41-8543 are dependent on the interaction with the heme moity of sGC, BAY 58-2667 is independent of heme. BAY 41-2272 and BAY 41-8543 stimulate the sGC in a synergistic manner with NO and, thus, require the presence of the heme group. Therefore, they are sensitive to blockade by sGC inhibitors, such as the quinoxaline derivative ODQ. Recently, another novel class of compounds has gained scientific interest among clinical researchers as these drugs are assumed to have emerging biological and medical significance. These agents are exemplified by NO-releasing derivatives of phosphodiesterase type 5 (PDE5) inhibitors. NicOx S.A., France, has developed NCX 911, a NO-releasing derivative of sildenafil citrate. NCX 911 releases NO spontaneously and increases intracellular cyclic GMP concentrations by activating sGC in the absence of endogenous NO and by inhibiting PDE5 (Riffaud et al. 2001).

3 Phosphodiesterase Isoenzymes

Cyclic nucleotides are degraded by PDE isoenzymes, a heterogenous group of hydrolytic enzymes. It is because of their central role in smooth muscle tone regulation that PDEs have become an attractive target for drug development. PDEs are classified according to their preference or affinity for cyclic AMP or/and cyclic GMP, kinetic parameters of cyclic AMP/cyclic GMP hydrolysis, relative sensitivity to inhibition by various compounds, and allosteric regulation by other molecules. This particular group of enzymes is represented by at least 11 families: Ca^{2+}/calmodulin-stimulated PDE (PDE1), cGMP-stimulated PDE (PDE2), cGMP-inhibited PDE (PDE3), cAMP-specific PDE (PDE4), cGMP-specific PDE (PDE5), and the cGMP-binding, cGMP-specific PDE of mammalian rods and cones (PDE6). While PDE7 (cAMP-high affinity) and PDE8 (IBMX-insensitive) have preferred selectivity for cyclic AMP, PDE9 exclusively degrades cyclic GMP, and the PDE10 and PDE11 can inactivate both cyclic AMP and cyclic GMP. Some of these isoenzyme families contain more than one gene (isogenes), and some genes are alternatively spliced so that, to date, more than 50 isoenzymes or variants have been described (Conti and Jin 1999; Essayan 2001). Subtypes of PDEs with distinct substrate preferences, kinetics, and regulatory properties are variably distributed in tissues throughout the body, and the expression of PDE isoenzymes may also vary in cellular and subcellular compartments. Isoforms or variant-transcripts of most PDE families have been shown to be expressed in tissues or cellular structures of the human urinary and genital tract, but, up until today, the exact role of only a few of these PDE subtypes for urogenital functioning has been established. For example, expression of the PDE5 in the corpus cavernosum penis and the relaxation mediated by cyclic GMP of the penile erectile tissue during sexual stimulation have made inhibition of this enzyme a clinical benefit in the management of erectile

dysfunction (Kalsi and Kell 2004). PDE7 is present in human skeletal muscle, kidney, brain, and pancreas. Although expressed in other tissues, high levels of PDE8, PDE10, and PDE11 are found in the testis, and PDE9 is expressed in intestinal smooth muscle, skeletal muscle, and brain (Han et al. 1997; Fisher et al. 1998a, b; Loughney et al. 1999; Fawcett et al. 2000) (see Table 1). PDE

Table 1 Expression of phosphodiesterase (PDE) isoenzymes in different organs/tissues of the human body, including the male and female urogenital/reproductive tract

Organ/tissue of human body	PDE isoenzymes expressed[a]
Cardiovascular system	
Aorta	PDE1, PDE2^{fs}, PDE3^{fs}, PDE4, PDE5
Cardiac ventricle	PDE1
Heart	PDE1, PDE2^{fs}, PDE3^{fs}, PDE4
Veins	PDE1, PDE4, PDE5
Arterial/venous endothelial cells	PDE2^{fs}, PDE5^{fs}
Central nervous system/visual perception	
Brain	PDE1^{fs}, PDE2, PDE4^{fs}, PDE5, PDE9, PDE10
Retina	PDE6^{fs}
Gastrointestinal tract	
Pancreas	PDE3, PDE4
Glandular system	
Adrenal glands	PDE2
Thyroid gland	PDE1, PDE4, PDE8
Respiratory tract	
Lung	PDE1, PDE2, PDE3, PDE4, PDE5
Trachea	PDE1, PDE2, PDE3, PDE4^{fs}, PDE5
Urogenital tract	
Detrusor	PDE1^{fs}, PDE2, PDE3, PDE4^{fs}, PDE5^{fs}
Kidney	PDE1, PDE2, PDE3, PDE4, PDE5, PDE9^{fs}
Penis (*Corpus cavernosum*)	PDE2, PDE3, PDE4^{fs}, PDE5^{fs}
Clitoris	PDE1, PDE2, PDE4, PDE5
Vagina	PDE4, PDE5, PDE10
Prostate	PDE1(?), PDE4^{fs}, PDE5^{fs}, PDE11^{fs}
Seminal vesicles	PDE1, PDE2, PDE3, PDE4^{fs}, PDE5^{fs}, PDE10, PDE11
Testis	PDE1, PDE3, PDE4, PDE5, PDE8, PDE10, PDE11
Urethra	PDE1, PDE2, PDE4^{fs}, PDE5^{fs}, PDE11

Data were adopted in part from a file kindly provided by Professor Christian G. Stief, Ludwig-Maximilians-University, Faculty of Medicine, Department of Urology, Munich, Germany

PDE1 Ca^{2+}/calmodulin-stimulated PDE, inactivates both cAMP and cGMP; *PDE2* Stimulated by cGMP; *PDE3* Inhibited by cGMP; *PDE4* cAMP-specific PDE; *PDE5* cGMP-specific PDE; *PDE6* cGMP-binding, cGMP-specific PDE of mammalian rods and cones; *PDE7* cAMP-high affinity PDE; *PDE8* cAMP-specific, IBMX-insensitive; *PDE9* cGMP-specific PDE; *PDE10*/*PDE11* Inactivate both cAMP and cGMP

[a]Expression was evaluated by means of molecular biology (RT-PCR analysis), Western blot/dot blot analysis, or chromatographical methods (anion exchange chromatography)

Listing does not discriminate between different isoforms of a PDE family (e.g., PDE4A, PDE4B, PDE4D) or splice variants of distinct isoforms (e.g., PDE11A1, PDE11A2, PDE11A3)

fs indicates that the isoenzyme has been suggested/proven to be of functional significance in the control of the respective tissue

? The presence of the PDE1 in the prostate is questionable

isoenzymes hold a central role to control the intracellular levels of cyclic AMP and cyclic GMP. To date, 6 out of these 11 isoenzymes (PDE1, PDE2, PDE3, PDE4, PDE5, and PDE11) have been proven pharmacological important. Since the distribution and functional significance of PDE isoenzymes varies in different tissues, isoenzyme-selective inhibitors have the potential to exert specific effects on the target tissue. Although human tissues express several members of the PDE families or more than one variant of an individual family, there are numerous examples where an individual PDE is predominantly found in a specific localization.

4 Cyclic Nucleotide-Binding Protein Kinases

It is assumed that the binding to and activation of the cyclic GMP-dependent protein kinase (cGKI) is one crucial step in the mechanism mediating the biological actions of cyclic GMP in the LUT. Cyclic GMP/cGKI-mediated mechanisms have been shown to phosphorylate the inositol-1,4,5-triphosphate receptor and reduce the Ca^{2+} sensitization mediated by protein kinase C (PKC) or Rho-kinase (ROK) (Komalavilas and Lincoln 1994; Sawada et al. 2001). Two isoforms of cGKI (cGKIα, MW = 76 kDa and cGKIβ, MW = 78 kDa) have been identified and there is evidence that both isoforms differ considerably with regard to their functional properties. For example, the cGKIα is activated by a significantly lower concentration of cyclic GMP than is the cGKIβ. It has been demonstrated that disturbances in Ca^{2+} regulation in vascular smooth muscle cells deficient in cGKI can be alleviated by transfection of the cells with the isoform cGKIα but not cGKIβ (Lincoln et al. 1988; Feil et al. 2002). It has also been assumed that the relaxation of smooth muscle mediated by cGKIα involves not only a decrease in cytosolic Ca^{2+} but also an inhibition of Ca^{2+} sensitization and a modulation of the activity of the enzyme myosin phosphatase, known to counteract smooth muscle contraction via the dephosphorylation of the light chain of the protein myosin (Surks et al. 1999). Based on the results from experimental and clinical studies, alterations in the expression and activity of cGKIα and cGKIβ have been related to erectile dysfunction and cardiovascular diseases, such as hypertension, atherosclerosis, and thrombus formation (Elesber et al. 2006; Hedlund et al. 2000; Ganz 2005). As of today, the clinical significance of the cGKI in the control of the function of LUT smooth muscle remains to be elucidated. To date, studies evaluating the expression and potential functional significance of cGKI in the human LUT have been limited to the prostate: Haynes et al. (2006), using molecular biology techniques, detected mRNA transcripts encoding for both isoforms in human prostate tissue (Haynes and Cook 2006). Waldkirch et al. (2007) were the first who described by means of immunohistochemistry the distribution of both cGKIα and cGKIβ in stromal areas of the transition zone, where the enzymes were found colocalized with cyclic GMP (Waldkirch et al. 2007).

5 Targets for the Pharmacological Modulation of the Cyclic AMP/Cyclic GMP Signaling in the LUT

5.1 Prostate

The so-called benign prostatic syndrome (BPS) represents a major health care problem in westernized countries. BPS comprises obstructive and irritative symptoms [*lower urinary tract symptomatology* (LUTS)], as well as benign prostatic enlargement (BPE) with variable degrees of bladder outlet obstruction (Guess 1995; Chiricos and Sanford 1996). Major symptoms include urinary frequency, nocturia, and slow stream. It is estimated that approximately 50% of men older than 50 years have moderate to severe symptoms arising from LUTS (Jacobsen et al. 1993). The current pharmacological management of LUTS and BPE involves α_1-adrenergic blockers, such as alfusozin, doxazosin, and tamsulosin, in order to diminish outflow resistance by reducing the tension of smooth muscle fibers located in the transition zone and periurethral region of the prostate (Hieble and Ruffolo jr 1996). Intervention into the hormonal control of prostate growth by using inhibitors of 5-α-reductase activity is another approach to ease symptoms (Andersen et al. 1995; Roehrborn et al. 2004).

The expression of several cyclic AMP and cyclic GMP PDE isoenzymes (PDE1, PDE2, PDE4, PDE5, PDE7, PDE8, PDE9, PDE10) in the human prostate was shown by means of molecular biology and protein chemistry. Later, the distribution of the PDE4 and PDE5 in stromal and glandular areas of the transition zone was shown using an immunohistochemical approach (Ückert et al. 2001; Ückert et al. 2006). In organ bath studies, the tension of prostate strip preparations mediated via the activation of α_1-adrenergic receptors was dose-dependently reversed by the PDE4 inhibitor rolipram and PDE5 inhibitor sildenafil (Ückert et al. 2001).

The role of PDE5 inhibitors in the treatment of symptoms of LUTS/BPH has been addressed by some open-label studies and, more recently, placebo-controlled clinical trials. Sairam et al. (2002) were the first who examined the effects of sildenafil citrate in patients presenting with ED and LUTS. From the 112 patients enrolled in the open-label study, 20 subjects complained of LUTS, from these, 32% had moderate to severe symptoms (IPSS $>$ 7). After 12 weeks of treatment with sildenafil, there was an overall improvement in the international prostate symptom score (IPSS) and LUTS-specific quality of life (QoL) score. All patients who had severe LUTS showed a moderate improvement of the disease, 60% of those who initially presented with moderate LUTS showed a mild symptomatology after treatment. The authors concluded that treatment with sildenafil appears to improve urinary symptom scores (Sairam et al. 2002) McVary et al. (2007a) assessed in a randomized, double-blind, placebo-controlled trial the effects of sildenafil given for 12 weeks in 366 men suffering from ED and LUTS secondary to BPH (IPSS $\geq$ 12). Patients received 50 mg sildenafil at bedtime or up to 1 h before sexual activity for at least 2 weeks, followed by 100 mg

sildenafil once daily for 10 weeks. Primary outcome measures were changes in the erectile function domain of the international index of erectile function (IIEF), secondary outcome measures were changes in all other domains of the IIEF and IPSS, including QoL, BPH impact index (BPH II), and Q_{max}. Sildenafil significantly improved LUTS, mean IPSS decreased by 6.3 points vs. 1.93 in the placebo group. Interestingly, patients with severe LUTS (-8.6 ± -2.4) experienced greater improvement in IPSS than those with moderate LUTS (-3.6 ± -1.7). A significant reduction of bladder storage symptoms and an increase in the BPH II and QoL were also noted in the treatment arm while no significant changes in Q_{max} were registered. From the later findings, the authors concluded that extraprostatic pathophysiological mechanisms related to an impairment of the activity of the NO-system might be involved in the etiology of LUTS (McVary et al. 2007a).

The efficacy and safety of tadalafil (CIALIS™) were also investigated in a randomized, double-blind, placebo-controlled study in men with moderate to severe LUTS secondary to BPH. Following a 4-week placebo run-in phase, 281 men were randomized to 5 mg tadalafil for 6 weeks, followed by dose escalation to 20 mg for another 6 weeks or placebo for 12 weeks. Primary end point was the change in IPSS after 6 and 12 weeks of treatment. Secondary efficacy endpoints included patient IPSS, QoL, BPH II, and the LUTS Global Assessment Questionnaire (GAQ), as well as parameters from uroflowmetric measurements (Q_{max}). After 6 and 12 weeks, tadalafil showed significant improvement in the patients, the respective mean change from baseline in IPSS was -2.8 in the tadalafil group vs. -1.2 in the placebo group (6 weeks) and -3.8 (verum) vs. -1.7 (placebo) (12 weeks). Mean subscores related to irritative and obstructive symptoms also significantly improved in patients who had received tadalafil. Except for BPH II after 6 weeks, all assessments of the disease-related QoL had significantly improved after treatment with tadalafil. In contrast, no significant changes in uroflowmetric values were observed (McVary et al. 2007b). These findings were later confirmed by means of post hoc analysis of the data from a dose-finding study on the effects of tadalafil in comparison to placebo on Q_{max}, bladder capacity, voiding efficiency, and obstructive symptoms in men with LUTS secondary to BPH. One thousand and fifty eight men with BPH/LUTS had been randomly allocated to receive once-daily treatment with tadalafil (2.5, 5, 10, or 20 mg) or placebo for 12 weeks. Tadalafil treatment significantly improved the IPSS obstructive subscores. The increases in Q_{max} were numerically greater for tadalafil vs. placebo, but did not reach statistical significance. No significant changes in PVR were noted. The most pronounced effects on bladder capacity and voiding efficiency were registered in men with a $Q_{max} < 10$ ml/s at baseline; however, these changes were also not significantly different from the placebo responses. Despite the marginal changes in uroflowmetric measurements, the authors emphasized that treating the patients with tadalafil was associated with clinically meaningful and statistically significant alleviation of the obstructive symptoms of BPH (Roehrborn et al. 2009; Broderick et al. 2010).

In an 8 week randomized, double-blind, placebo-controlled, multicenter study, Stief et al. (2008) examined the efficacy of vardenafil (LEVITRA™) in a cohort of 222 men (aged 45–64 years) presenting with LUTS/BPH (IPSS $\geq$ 12). Patients were randomized either to vardenafil (10 mg twice daily) or placebo. Efficacy outcome included changes in peak urinary flow rate, as well as scores from the IPSS and a nine-item BPH-specific QoL questionnaire (UROLIFE QoL 9). After treatment, a decrease of 5.9 points in the IPSS was observed in the vardenafil group vs. 3.6 points in the placebo group. Significant changes in the IPSS subscores for storage and voiding symptoms and an improvement in the UROLIFE QoL were also noted in the vardenafil group. Since baseline values were already close to normal, Q_{max} and postvoiding residual urine volume did not change significantly with treatment (Stief et al. 2008).

It was shown more recently that sildenafil also improves Q_{max} and Q_{ave} (mean average flow rate) rates in men with LUTS suggestive of BPH. A single dose of the PDE5 inhibitor (50 or 100 mg) resulted in an improvement in Q_{max} in the patients. Q_{ave} and the mean voided volumes of the patients also increased while no significant differences were registered in the Q_{max}, Q_{ave}, and voided volumes in the control group before and after placebo (Gülcer et al. 2008; Guven et al. 2009). These results are in support of the use of inhibitors of PDE5 for treating LUTS and urinary obstruction secondary to BPH.

5.2 Urinary Bladder

Anticholinergic drugs are currently the therapy of choice to treat urgency and urge incontinence (Andersson et al. 2001). Nevertheless, up till now, muscarinic receptor blockers acting exclusively on detrusor smooth muscle are not available. Moreover, the unstable detrusor seems to be regulated in part by noncholinergic mechanisms. These factors may explain the common side effects and the limited clinical efficacy of anticholinergics. The specific modulation of intracellular second messenger pathways may offer a promising possibility to achieve selective modulation of tissue function, especially with regard to the contraction and relaxation of human urinary bladder smooth musculature. Using chromatographic methods, Truss et al. (1996a) were the first who reported the presence of the PDE1 (cAMP/cGMP-PDE, Ca^{2+}/calmodulin-dependent), PDE2 (cAMP-PDE, cGMP-dependent), PDE3 (cAMP-PDE, cGMP-inhibited), PDE4 (cAMP-PDE), and PDE5 (cGMP-PDE) in the human detrusor (Truss et al. 1996a). They also demonstrated relaxant responses of isolated human detrusor strip preparations contracted by the muscarinic agonist carbachol to the nonspecific PDE inhibitor papaverine and the PDE1 inhibitor vinpocetine. The relaxing effects of the drugs were paralleled by an increase in tissue levels of cyclic AMP and cyclic GMP (Truss et al. 1996b). They concluded from their findings that the cyclic AMP-pathway and the PDE1 might be of functional significance in the control of detrusor smooth muscle. The predominant expression of the PDE1 in the human detrusor was later confirmed by

RT-PCR analysis (Ückert et al. 2009). Results from a randomized, double-blind, placebo-controlled study to assess the effects of the PDE1 inhibitor vinpocetine in patients with urgency and urge incontinence, who had failed standard pharmacological therapy, demonstrated that vinpocetine was superior to placebo with regard to the clinical outcome parameters micturition frequency, bladder volume at first sensation, bladder volume at voiding desire, maximum detrusor pressure, and voided volume (Truss et al. 2001). The efficacy of vardenafil was also assessed in a single center, randomized, double-blind, placebo-controlled trial in a group of 25 spinal cord injured male patients with micturition disorders who were on oxybutynin treatment. Following a baseline urodynamic testing, a second test was performed 1–3 h after the administration of 20 mg vardenafil or placebo. Primary endpoints were changes in maximum detrusor pressure during voiding, maximum cystometric capacity, and detrusor volume at first (overactivity) sensation. Although NO does not appear to have direct smooth muscle regulatory functions in the detrusor, vardenafil administration significantly decreased maximum detrusor pressure, considerably improved cystometric capacity, and increased volume at first sensation (Gacci et al. 2007). Hence, modulating the activity of PDE isoenzymes might represent a promising approach to treat patients with LUT dysfunction. Future studies will delineate as to whether PDE inhibitors, such as the PDE1 inhibitor vinpocetine or selective inhibitors of the PDE5, may have significance in the treatment of detrusor instabilities and urge incontinence.

5.3 Ureter

Very much in contrast to the urinary bladder, prostrate or urethra, little is known on the peripheral neurotransmission responsible for relaxation of ureteral smooth muscle. Research on ureteral neurotransmitters focused on afferent innervation; studies on the efferent limb of the autonomic innervation of the ureter were done over two decades ago when the concept of nonadrenergic/noncholinergic innervation was not yet established. In the 1990s, reports on the localization of VIP and NOS in nerves supplying the ureter were published but no in vitro studies done to further corroborate the findings (Smet et al. 1994). Later, it was demonstrated that the tension of isolated human ureteral tissue was dose-dependently reversed by the NO donor drug molsidomine (SIN-1), preincubation with the sGC-inhibitor methylene blue significantly reduced the relaxation response. However, no effects of the NOS-inhibitor L-NORAG were observed on the relaxation induced by means of electrical field stimulation. Immunhistochemistry revealed NOS-containing neuronal axons as well as nerve endings in the muscular layers of the ureter (Stief et al. 1996). Taher et al. (1994) reported the presence of the cGMP PDE5, cAMP/cGMP PDE1, and cAMP PDE2 and PDE4 in cytosolic supernatants prepared from human ureteral tissue. Using the organ bath technique, they demonstrated the potency of the PDE4 inhibitor rolipram and dual PDE5/PDE1 inhibitor zaprinast to reverse the tension induced by KCl of circular ureteral segments (Taher et al. 1994). Kühn et al.

(2000) later confirmed the relaxing properties of inhibitors of PDE4 (rolipram) and PDE5 (E 4021, MSPP) on isolated human ureteral smooth musculature and showed that these effects were paralleled by an elevation in intracellular levels of cyclic AMP or cyclic GMP (Kühn et al. 2000). Based on the results from experiments on the effects of the NO donor drugs sodium nitroprusside (SNP) and SIN-1, and the PDE5 inhibitor zaprinast on the tension induced by KCl of proximal segments of the human ureter, Saighi et al. (2000) concluded that cyclic GMP is an important second messenger in the signaling pathway leading to the relaxation of ureteral smooth muscle (Saighi et al. 2000). In contrast, Gratzke et al. (2007) registered marginal effects of the PDE5 inhibitors vardenafil, sildenafil, and tadalafil on the tension induced by KCl of human ureteral tissue. Although the responses were paralleled by a threefold to fourfold increase in tissue levels of cyclic GMP, the maximum relaxation (R_{max}) induced by the drugs ranged from only 23 to 6% (Gratzke et al. 2007). In an in vivo rabbit model, Becker et al. (1998) examined the potential of the PDE4 inhibitor rolipram in comparison to the nonselective PDE inhibitors papaverine, theophylline, and the muscarinic antagonist scopolamine to induce ureteral relaxation. They found that only rolipram induced pronounced decreases in intraluminal (intraureteral) pressure as well as in the amplitude and frequency of the phasic (peristaltic) contractions of the ureter. No considerable effects on the systemic circulation were observed, whereas the application of scopolamine, papaverine, or theophylline exerted no or only short-lasting effects on the ureter, but significantly affected systemic blood pressure of the animals (Becker et al. 1998). It was concluded from these findings that the application of PDE inhibitors, especially those of the cyclic AMP PDE4, seems promising in order to facilitate effectively and with minimal side effects the spontaneous passage of distal ureteral stones and relieve ureteral colic pain.

5.4 Urethra

It is without doubt that there is a pivotal functional role of the urethra in maintaining continence and enabling coordinated micturition in both genders. While the contraction of urethral smooth muscle mediated by the activation of α-adrenoceptors has been attributed to the continence mechanism, the relaxation of the longitudinal and/or circular muscle layer during micturition has been assumed to be mediated by NO and the cyclic GMP pathway. In the human urethra, large amounts of NOS-containing nerves have been demonstrated in the muscular wall, around blood vessels, close to the urothelium, as well as in the sarcolemma of intramural striated muscle fibers of the membranous urethra (Alm et al. 1993; Ho et al. 1998). Significant enzymatic activity of NOS, measured as the formation of citrulline in response to transmural electrical stimulation of nerves, has also been demonstrated (Ehren et al. 1994). In the anesthetized rat, systemic application of the NOS inhibitor L-NOARG decreased the amplitude and duration of urethral relaxation; this effect was in part reversed by administration of L-arginine (Bennett et al. 1995).

In humans, sublingual administration of the NO donor drug isosorbide dinitrate to healthy male subjects resulted in a reduction of the resting pressure of the external urethral sphincter (Reitz et al. 2004). Immunohistochemical studies performed on sections of the human female urethra demonstrated the expression of the cyclic GMP-specific PDE5 within urethral vascular and nonvascular smooth muscle cells and in the vascular endothelium. In the vascular endothelial cells, PDE5 was found colocalized with its substrate cyclic GMP (Werkström et al. 2006). More recently, the predominant expression of mRNA transcripts specifically encoding for PDE1B and 1C (Ca^{2+}/Calmodulin-dependent PDE), PDE4A and 4D (cAMP-PDE), PDE5A (cGMP-PDE) and PDE11A2 (Dual Substrate PDE) was shown by means of RT-PCR analysis (Kedia et al. 2009). In preliminary organ bath experiments, the contraction induced by noradrenaline of isolated human female human urethra was almost completely reversed in response to 10 μM sildenafil, the reversion of the tension brought about by 30 μM vardenafil and tadalafil was 85% and 47%, respectively (Werkström et al. 2006). The significance of the cGKI (see Sect. 4) in the control of normal urethral function has also been evaluated. In sections of the human urethra, strong immunoreactivity for cGKI and cyclic GMP was seen in smooth muscle cells. Animal studies revealed that mice lacking the gene encoding for cGKI do not exhibit NO/cyclic GMP-mediated relaxations of urethral smooth muscle in response to the activation of nerves or application of NO donor drugs (Persson et al. 2000). Isolated urethral tissue of diabetic rabbits demonstrated reduced NO-dependent relaxation responses and also an impaired ability to produce cyclic GMP when challenged with SNP (Mumtaz et al. 1999). These results have been interpreted in terms that modulation of the cyclic GMP pathway in urethral smooth muscle, e.g., by inhibition of the PDE5 or activation of the cGKI, might represent an interesting option to facilitate relaxation of the outflow region.

6 Conclusion

Based on the knowledge of the physiological mechanisms regulating the male and female urogenital tract, the modulation of key enzymes of the cyclic GMP and/or cyclic AMP-pathway has been suggested a straightforward approach for the treatment of various diseases of the LUT. The increased scientific awareness in this field and the unending charge to conceive first-line treatments demonstrating advanced and superior efficacy than the previous options offer a promising future for the therapy of such dysfunctions. While some approaches should involve the NO/cyclic GMP cascade, other strategies should also take into account the modulation of the cyclic AMP signaling, as well as the combination of various mechanisms of drug action (e.g., a drug combining PDE5 inhibition with the release of NO) in order to affect multiple peripheral intracellular protein targets. It is assumed that new treatment options will be efficacious in terms of promoting normal organ function and exert limited systemic adverse events.

References

Alm P, Larsson B, Ekblad E, Sundler F, Andersson KE (1993) Immunohistochemical localization of peripheral nitric oxide synthase-containing nerves using antibodies raised against synthesized C- and N-terminal fragments of a cloned enzyme from rat brain. Acta Physiol Scand 148:421–429

Andersen JT, Ekman P, Wolf H, Beisland HO, Johansson JE, Kontturi M, Lehtonen T, Tveter K (1995) Can finasteride reverse the progress of benign prostatic hyperplasia? A two-year placebo-controlled study. Urology 46:631–637

Andersson KE (1993) Pharmacology of lower urinary tract smooth muscle and penile erectile tissues. Pharmacol Rev 45:253–308

Andersson KE, Persson K (1993) The L-arginine/nitric oxide pathway and nonadrenergic-noncholinergic relaxation of the lower urinary tract. Gen Pharmacol 24:833–839

Andersson KE, Chapple C, Wein A (2001) The basis for drug treament of the overactive bladder. World J Urol 19:294–298

Antoni FA (2000) Molecular diversity of cAMP signaling. Front Neuroendocrinol 21:103–132

Becker AJ, Stief CG, Meyer M, Truss MC, Forssmann WG, Jonas U (1998) The effect of the specific phosphodiesterase 4 inhibitor rolipram on the ureteral peristalsis in vitro and in vivo. J Urol 160:920–925

Bennett BC, Kruse MN, Roppolo JR, Flood HD, Fraser M, de Groat WC (1995) Neural control of urethral outlet activity in vivo: role of nitric oxide. J Urol 153:2004–2009

Bischoff E, Schramm M, Straub A, Feurer A, Stasch JP (2003) BAY 41-2272: a stimulator of soluble guanylyl cyclase induces nitric oxide dependent penile erection. Urology 61: 464–467

Broderick GA, Brock GB, Roehrborn CG, Watts SD, Elion-Mboussa A, Viktrup L (2010) Effects of tadalafil on lower urinary tract symptoms secondary to benign prostatic hyperplasia in men with or without erectile dysfunction. Urology 75:1452–1458

Chiricos TN, Sanford E (1996) Cost consequences of surveillance, medical management or surgery for benign prostatic hyperplasia. J Urol 155:1311–1316

Conti M, Jin SL (1999) The molecular biology of cyclic nucleotide phosphodiesterases. Prog Nucleic Acid Res Mol Biol 63:1–38

Denninger JW, Marletta MA (1999) Guanylate cyclase and the NO/cGMP signalling pathway. Biochim Biophys Acta 1411:334–350

Ehren I, Iversen H, Jansson O, Adolfsson J, Wiklund NP (1994) Localization of nitric oxide synthase activity in the human lower urinary tract and its correlation with neuroeffector responses. Urology 44:683–687

Elesber AA, Solomon H, Lennon RJ, Mathew V, Prasad A, Pumper G, Nelson RE, McConnell JP, Lerman LO, Lerman A (2006) Coronary endothelial dysfunction is associated with erectile dysfunction and elevated asymmetric dimethylarginine in patients with early atherosclerosis. Eur Heart J 27:824–831

Essayan DM (2001) Cyclic nucleotide phosphodiesterases. J Allerg Clin Immunol 108:671–680

Fawcett L, Baxendale R, Stacey P, McGrouther C, Harrow I, Soderling S, Hetman J, Beavo JA, Philips SC (2000) Molecular cloning and characterization of a distinct human phosphodiesterase gene family: PDE11A. Proc Natl Acad Sci USA 97:3702–3707

Feil R, Gappa N, Rutz M, Schlossmann J, Rose CR, Konnerth A, Brummer S, Kuhbandner S, Hofman F (2002) Functional reconstitution of vascular smooth muscle cells with cGMP-dependent protein kinase I isoforms. Circ Res 90:1080–1086

Fisher AD, Smith JF, Pillar JS, St Denis S, Cheng JB (1998a) Isolation and characterization of PDE8A, a novel cAMP-specific phosphodiesterase. Biochem Biophys Res Commun 246:570–577

Fisher AD, Smith JF, Pillar JS, St Denis S, Cheng JB (1998b) Isolation and characterization of PDE9A, a novel cGMP-specific phosphodiesterase. J Biol Chem 273:15559–15564

Friebe A, Müllershausen F, Smolenski A, Walter U, Schultz G, Koesling D (1998) YC-1 potentiates nitric oxide- and carbon monoxide-induced cyclic GMP effects in human platelets. Mol Pharmacol 54:962–967

Gacci M, del Popolo G, Macchiarella A, Celso M, Vittori G, Lapini A, Serni S, Sandner P, Maggi M, Carini M (2007) Vardenafil improves urodynamic parameters in men with spinal cord injury: results from a single dose, pilot study. J Urol 178:2040–2043

Ganz P (2005) Erectile dysfunction: pathophysiologic mechanisms pointing to underlying cardiovascular disease. Am J Cardiol 96:8M–12M

Gratzke C, Ückert S, Kedia G, Reich O, Schlenker B, Seitz M, Becker AJ, Stief CG (2007) In vitro effects of PDE5 inhibitors sildenafil, vardenafil and tadalafil on isolated human ureteral smooth muscle: a basic research approach. Urol Res 35:49–54

Guess HA (1995) Epidemiology and natural history of benign prostatic hyperplasia. Urol Clin North Am 22:247–261

Gülcer C, Tüzel E, Dogantekin E, Kiziltepe G (2008) Does sildenafil affect uroflowmetry values in men with lower urinary tract symptoms suggestive of benign prostatic enlargement? Urol Int 80:181–185

Guven EO, Balbay MD, Mete K, Serefoglu EC (2009) Uroflowmetric assessment of acute effects of sildenafil on the voiding of men with erectile dysfunction and symptomatic benign prostatic hyperplasia. Int Urol Nephrol 41:287–92

Han P, Zhu X, Michaeli T (1997) Alternative splicing of the high affinity cAMP-specific phosphodiesterase (PDE7A) mRNA in human skeletal muscle and heart. J Biol Chem 272: 16152–16157

Haynes JM, Cook ALM (2006) Protein kinase G-induced activation of K_{ATP} channels reduces contractility of human prostate tissue. Prostate 66:377–385

Hedlund P (2005) Nitric oxide/cGMP-mediated effects in the outflow region of the lower urinary tract: is there a basis for pharmacological targeting of cGMP? World J Urol 23:362–367

Hedlund P, Aszodi A, Pfeifer A, Alm P, Hofman F, Ahmad M, Fassler R, Andersson KE (2000) Erectile dysfunction in cyclic GMP-dependent kinase I-deficient mice. Proc Natl Acad Sci U S A 97:2349–2354

Hieble JR, Ruffolojr RR (1996) The use of alpha-adrenoceptor antagonists in the pharmacological management of benign prostatic hyperplasia: an overview. Pharmacol Res 33:145–160

Ho KM, McMurray G, Brading AF, Noble JG, Ny L, Andersson KE (1998) Nitric oxide synthase in the heterogeneous population of intramural striated muscle fibres of the human membranous urethral sphincter. J Urol 159:1091–1096

Hobbs AJ (2002) Soluble guanylate cyclase: an old therapeutic target re-visited. Br J Pharmacol 136:637–640

Jacobsen SJ, Guess HA, Panser L, Girman CJ, Chute CG, Oesterling JE, Lieber MM (1993) A population-based study of health care seeking behaviour for treatment of urinary symptoms. Arch Fam Med 2:729–735

Kalsi JS, Kell PD (2004) Update on the oral treatments for male erectile dysfunction. J Eur Acad Dermatol Venereol 18:267–274

Kedia GT, Ückert S, Steffenhagen V, Sonnenberg JE, Kuczyk MA (2009) Expression von Phosphodiesterase (PDE)-Isoenzymen in der humanen Urethra – Eine molekularbiologische Studie. Abstract presented at the 3. Nordkongress Urologie, Braunschweig, Germany, 14–16 May 2009

Komalavilas P, Lincoln TM (1994) Phosphorylation of the inositol-1, 4, 5-trisphosphate receptor by cyclic GMP-dependent protein kinase. J Biol Chem 269:8701–8107

Kühn R, Ückert S, Stief CG, Truss MC, Lietz B, Bischoff E, Schramm M, Jonas U (2000) Relaxation of human ureteral smooth muscle in vitro by modulation of cyclic nucleotide dependent pathways. Urol Res 28:110–115

Lee YC, Martin E, Murad F (2000) Human recombinant soluble guanylyl cyclase: expression, purification, and regulation. Proc Natl Acad Sci U S A 97:10763–10768

Lincoln TM, Thompson M, Cornwell TL (1988) Purification and characterization of two forms of cyclic GMP-dependent protein kinase from bovine aorta. J Biol Chem 263:17632–17637

Loughney K, Snyder PB, Uher L, Rosman GJ, Ferguson K, Florio VA (1999) Isolation and characterization of PDE10A, a novel human 3'.5'-cyclic nucleotide phosphodiesterase. Gene 234:109–117

Lucas KA, Pitari GM, Kazerounian S, Ruiz-Stewart I, Park J, Schulz S, Chepenik KP, Waldman SA (2000) Guanylyl cyclases and signaling by cyclic GMP. Pharmacol Rev 52:375–414

McVary KT, Monnig W, Camps JL, Young JM, Tseng LJ, van den Ende G (2007a) Sildenafil citrate improves erectile function and urinary symptoms in men with erectile dysfunction and lower urinary tract symptoms associated with benign prostatic hyperplasia: a randomized, double-blind trial. J Urol 177:1071–1077

McVary KT, Roehrborn CG, Kaminetsky JC, Auerbach SM, Wachs B, Young JM, Esler A, Sides GD, Denes BS (2007b) Tadalafil relieves lower urinary tract symptoms secondary to benign prostatic hyperplasia. J Urol 177:1401–1407

Miller LN, Nakane M, Hsieh GC, Chang R, Kolasa T, Moreland RB, Brioni JD (2003) A-350619: a novel activator of soluble guanylyl cyclase. Life Sci 72:1015–1025

Moreland RB, Hsieh GC, Nakane M, Miller LN, Chang R, Kolasa T, Sullivan J, Brioni JD (2002) Novel soluble guanylate cyclase activators A-344905 and A-305619 activate sGC in vitro and facilitate penile erection in rats. J Urol 167(suppl 4):938 (Abstract)

Mumtaz FH, Sullivan ME, Thompson CS, Dashwood MR, Naseem KM, Bruckdorfer KR, Mikhailidis DP, Morgan RJ (1999) Alterations in the nitric oxide synthase binding sites and non-adrenergic, non-cholinergic mediated smooth muscle relaxation in the diabetic rabbit bladder outlet: possible relevance to the pathogenesis of diabetic cystopathy. J Urol 162: 558–566

Persson K, Pandita RK, Aszodi A, Ahmad M, Pfeifer A, Fassler R, Andersson KE (2000) Functional characteristics of urinary tract smooth muscles in mice lacking cGMP protein kinase type I. Am J Physiol Regul Integr Comp Physiol 279:R1112–R1120

Reitz A, Bretscher S, Knapp PA, Muntener M, Wefer B, Schurch B (2004) The effect of nitric oxide on the resting tone and the contractile behaviour of the external urethral sphincter: a functional urodynamic study in healthy humans. Eur Urol 45:367–373

Riffaud JP, Bernarbe J, Giuliano F, Jones R, Jeremy J (2001) Pharmacological profile of sildenafil nitrate (NCX 911) in various models of penile erection. Inflamm Res 50(suppl 3):84 (Abstract)

Roehrborn CG, Marks LS, Fenter T, Freedman S, Tuttle J, Gittleman M, Morril B, Wolford ET (2004) Efficacy and safety of dutasteride in the four-year treatment of men with benign prostatic hyperplasia. Urology 63:709–715

Roehrborn CG, Kaminetsky JC, Auerbach SM, Montelongo RM, Elion-Mboussa A, Viktrup L (2009) Changes in peak urinary flow and voiding efficiency in men with signs and symptoms of benign prostatic hyperplasia during once daily tadalafil treatment. BJU Int 105:502–507

Saighi D, Zerbib M, Thiounn N, Flam T, Conquy S, Jacob L, dall'Ava-Santucci J, Debré B, Dinh-Xuan AT (2000) In vitro study of the modulation of human ureteral tonus by nitric oxide and zaprinast, a phosphodiesterase inhibitor. Prog Urol 10:1161–1168

Sairam K, Kulinskaya E, McNicholas TA, Boustead GB, Hanbury DC (2002) Sildenafil influences lower urinary tract symptoms. BJU Int 90:836–839

Sawada N, Itoh H, Yamashita J, Doi K, Inoue M, Masatsugu K, Fukunaga Y, Sakaguchi S, Sone M, Yamahara K, Yurugi T, Nakao K (2001) cGMP-dependent protein kinase phosphorylates and inactivates RhoA. Biochem Biophys Res Commun 280:798–805

Smet PJ, Edyvane KA, Jonasvicius J, Marshall VR (1994) Colocalisation of nitric oxide synthase with vasoactive intestinal peptide, neuropeptide Y and thyrosine hydroxylase in nerves supplying the human ureter. J Urol 152:1292–1296

Stasch JP, Alonso-Alija C, Apeler H, Dembowsky K, Feurer A, Minuth T, Perzborn E, Schramm M, Straub A (2002) Pharmacological actions of a novel NO-independent guanylyl cyclase stimulator, BAY 41-8543: in vitro studies. Br J Pharmacol 135:333–343

Stief CG, Ückert S, Truss MC, Becker AJ, Machtens S, Jonas U (1996) A possible role for nitric oxide in the regulation of human ureteral smooth muscle tone in vitro. Urol Res 24:333–337

Stief CG, Porst H, Neuser D, Beneke M, Ulbrich E (2008) A randomised, placebo-controlled study to assess the efficacy of twice-daily vardenafil in the treatment of lower urinary tract symptoms secondary to benign prostatic hyperplasia. Eur Urol 53:1236–1244

Straub A, Stasch JP, Alonso-Alija C, Benet-Buchholz I, Ducke B, Feurer A, Minuth T, Perzborn E, Schramm M, Straub A (2001) NO-independent stimulators of soluble guanylate cyclase. Bioorg Med Chem Lett 11:781–784

Surks HK, Mochizuki N, Kasai Y, Georgescu SP, Tang KM, Ito M, Lincoln TM, Mendelsohn ME (1999) Regulation of myosin phosphatase by a specific interaction with cGMP-dependent protein kinase Iα. Science 286:1583–1587

Taher A, Schulz-Knappe P, Meyer M, Truss MC, Forssmann WG, Stief CG, Jonas U (1994) Characterization of cyclic nucleotide phosphodiesterase isoenzymes in the human ureter and their functional role in vitro. World J Urol 12:286–291

Truss MC, Ückert S, Stief CG, Kuczyk MA, Jonas U (1996a) Cyclic nucleotide phosphodiesterase (PDE) isoenzymes in the human detrusor smooth muscle: I. Identification and characterization. Urol Res 24:123–128

Truss MC, Ückert S, Stief CG, Kuczyk MA, Jonas U (1996b) Cyclic nucleotide phosphodiesterase (PDE) isoenzymes in the human detrusor smooth muscle: II. Effect of various PDE-inhibitors on smooth muscle tone and cyclic nucleotide levels in vitro. Urol Res 24:129–134

Truss MC, Stief CG, Ückert S, Becker AJ, Wefer J, Schultheiss D, Jonas U (2001) Phosphodiesterase 1 inhibition in the treatment of lower urinary tract dysfunction: from bench to bedside. World J Urol 19:344–350

Ückert S, Küthe A, Jonas U, Stief CG (2001) Characterization and functional relevance of cyclic nucleotide phosphodiesterase isoenzymes of the human prostate. J Urol 166:2484–2490

Ückert S, Oelke M, Stief CG, Andersson KE, Jonas U, Hedlund P (2006) Immunohistochemical distribution of cAMP- and cGMP-phosphodiesterase (PDE) isoenzymes in the human prostate. Eur Urol 49:740–745

Ückert S, Sandner P, Sigl K, Ulbrich E, Stief CG, Kuczyk MA (2009) Is there a role of the phosphodiesterase type 5 (PDE5) in the control of detrusor smooth muscle? A functional and molecular biology study. J Urol 181(suppl 4):152 (Abstract)

Waldkirch ES, Ückert S, Langnäse K, Richter K, Jonas U, Wolf G, Andersson KE, Stief CG, Hedlund P (2007) Immunohistochemical distribution of cyclic GMP-dependent protein kinase 1 in human prostate tissue. Eur Urol 52:495–502

Werkström V, Svensson A, Andersson KE, Hedlund P (2006) Phosphodiesterase 5 in the female pig and human urethra: morphological and functional aspects. BJU Int 98:414–423

Rho-Kinase, a Common Final Path of Various Contractile Bladder and Ureter Stimuli

Xinhua Zhang and Michael E. DiSanto

Contents

Abstract Normal urinary bladder function is based on the proper contraction and relaxation of smooth muscle (SM), which constitutes the majority of the bladder wall. The contraction and relaxation of all SM involves a phosphorylation–dephosphorylation pathway involving the enzymes smooth muscle myosin light chain kinase (SMMLCK) and smooth muscle myosin light chain phosphatase (SMMLCP), respectively. Although originally thought to function just as a passive opposition to SMMLCK-driven SM contraction, it is now clear that SMMLCP activity is under an extremely complex molecular regulation via which SMMLCP inhibition can induce "calcium sensitization." This review provides a thorough

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summary of the literature regarding the molecular regulation of the SMMLCP with a focus on one of its major inhibitory pathways that is RhoA/Rho-kinase (ROK) including its activation pathways, effector molecules, and its roles in various pathological conditions associated with bladder dysfunction. Newly emerging roles of ROK outside of SM contractility are also discussed. It is concluded that the RhoA/ROK pathway is critical for the maintenance of basal SM tone of the urinary bladder and serves as a common final pathway of various contractile stimuli in rabbits, rats, mice, and pigs as well as humans. In addition, this pathway is upregulated in response to a number of pathological conditions associated with bladder SM dysfunction. Similarly, RhoA/Rho-kinase signaling is essential for normal ureteral function and development and is upregulated in response to ureteral outlet obstruction. In addition to its critical role in bladder SM function, a role of ROK in the urothelium is also beginning to emerge as well as roles for ROK in bladder infection and invasion and metastasis of bladder cancer.

Keywords Bladder dysfunction · Calcium sensitization · RhoA/Rho-kinase · Smooth muscle · Smooth muscle myosin phosphatase

1 Introduction

Normal urinary bladder function is based on the proper contraction and relaxation of smooth muscle (SM), which constitutes the majority of the bladder wall. Unlike skeletal and cardiac muscle, which are regulated by calcium through a tropomyosin–troponin complex, the contraction and relaxation of all SM involves a phosphorylation–dephosphorylation pathway mediated by the enzymes SM myosin light chain kinase (SMMLCK) and SM myosin light chain phosphatase (SMMLCP), respectively. Although SM contraction was originally thought to be activated only by an elevation of cytosolic calcium $[Ca^{2+}]_I$, it is now clear that SM contraction can also occur even in the absence of changes in $[Ca^{2+}]_I$ by inhibiting SMMLCP via a guanine nucleotide-binding protein (G protein)-coupled mechanism of which an enzyme called Rho-kinase (ROK) has been established to play a major role. This process has been termed "calcium sensitization." The focus of this review article is to thoroughly explore the roles of ROK in SM contraction, the mechanisms which regulate its activity, and the effectors which execute its various functions. In addition, we review the role of ROK in numerous pathological conditions that lead to urinary dysfunction.

2 Smooth Muscle Contraction

As early as 1946, it was thought that calcium was the intracellular trigger for muscle contraction (Heilbrunn and Wiercinski 1946). Then in 1954, Huxley and Hanson demonstrated that there were changes in the cross-striations of muscle during contraction and stretch implicating cross-bridge cycling in the generation of force

by a muscle (Huxley and Hanson 1954). Almost 10 years later, Ebashi and Ebashi discovered that the removal of calcium caused relaxation in actomyosin systems (Ebashi and Ebashi 1962), and it was later determined that troponin was the "calcium sensing" protein (Ebashi et al. 1967). However, unlike in skeletal and cardiac muscle, the troponin system was not operative in SM, and it was subsequently determined that an enzyme known as smooth muscle myosin light chain kinase (SMMLCK) was the putative intracellular calcium sensor for SM contraction (Barron et al. 1979) and that it was dependent upon a protein known as calmodulin (CaM) (Dabrowska et al. 1978)

It is now well established that SM contraction is triggered by an increase in cytosolic Ca^{2+} concentration ($[Ca^{2+}]_c$), which can result either through calcium influx via voltage-dependent channels or stretch-activated channels, and release of intracellular calcium ($[Ca^{2+}]_i$) stored in the sarcoplasmic reticulum (SR) through channels in the SR membrane including calcium-induced calcium release (CICR) (Itoh et al. 1981) and inositol 1,4,5-trisphosphate (IP_3) receptor-mediated release of calcium from the SR (Suematsu et al. 1985; Endo et al. 1990). In response to an initial three to tenfold increase in the free intracellular Ca^{2+} binding to calmodulin (CaM), Ca^{2+}/CaM then activates SMMLCK, which phosphorylates the 20 kDa regulatory myosin light chain (MLC_{20}) located near the head region of the myosin crossbridge that binds to actin (Adelstein and Eisenberg 1980). The result is a conformational change that allows actin to stimulate myosin ATPase activity, crossbridges to cycle, and actin to slide past myosin resulting in myocyte contraction (Okamoto and Sekine 1981). It has been demonstrated that there is a linear relationship between the level of SM MLC_{20} phosphorylation and actin-activated ATPase activity necessary for contraction (Chacko 1981). Ca^{2+} levels then subsequently decline and may return to near baseline levels within a few minutes of cellular activation via a mechanism that involves Ca^{2+} buffering by the SR and Ca^{2+} extrusion by Ca^{2+} pumps serving to lower the $[Ca^{2+}]_I$ (Khalil et al. 1987). A decrease in $[Ca^{2+}]_i$ inactivates SMMLCK and permits dephosphorylation of MLC_{20} by SMMLCP, thus causing relaxation. These pathways are summarized in Fig. 1.

3 Calcium Sensitization

The ability of SM cells to sustain a contractile response in the presence of submaximal intracellular Ca^{2+} levels is an important hallmark of "calcium sensitization" (Somlyo et al. 1989; Kitazawa et al. 1991; Himpens et al. 1988; Morgan and Morgan 1984). Ca^{2+} sensitization can even cause contraction of SM without necessarily changing $[Ca^{2+}]_I$ (Litten et al. 1987). Thus, over the last 20 years, evidence has been accumulating that "calcium sensitization" can play as important a role as changes in intracellular calcium levels in the fine tuning of SM tone in a vast array of different tissues (for review see (Hirano 2007; Christ and Wingard 2005; Ratz et al. 2005). During this time, it has been determined that the key player in "calcium

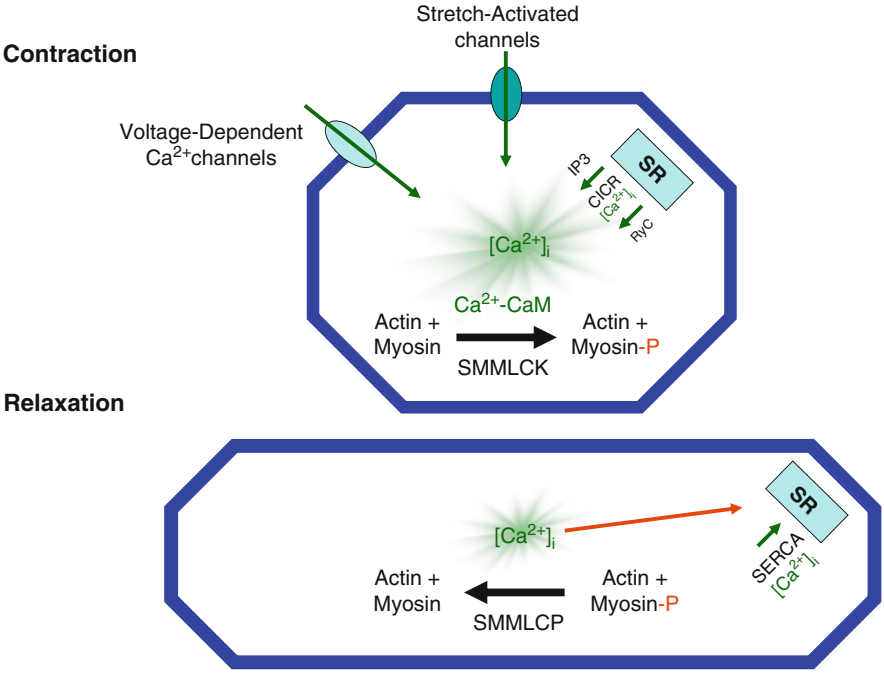

Fig. 1 *Regulation of Bladder Smooth Muscle Contraction*. *Upper Panel*: During contraction of SM, calcium enters the cell via various membrane channels including voltage-dependent Ca^{2+} and stretch-activated channels and is also released from the sarcoplasmic reticulum (SR) via inositol 1,4,5-triphosphate (IP3)-dependent calcium induced calcium release (CCIR) and ryanodine channel (RyC) calcium release. The Ca^{2+} then binds with calmodulin (CaM), and together, they activate the enzyme smooth muscle myosin light chain kinase (SMMLCK), which phosphorylates SM myosin on its 20 kDa regulatory light chain and drives crossbridge cycling and SM contraction. Lower Panel: During relaxation of SM, calcium is buffered and sequestered into the SR via an enzyme known as sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA)

sensitization" appears to be the MLC_{20} phosphorylation-dependent mechanism. Thus the balance between pathways leading to an increase in MLC_{20} phosphorylation and those leading to a decrease in MLC_{20} phosphorylation determines the extent of the "calcium sensitization" (Hirano 2007). Although changes in either of these pathways can influence "calcium sensitization," the focus of this review will be on the regulation of the MLC_{20} phosphatase as this is a primary target for ROK.

3.1 Rho/Rho-kinase

A major mechanism of "calcium sensitization" recently identified involves an enzyme known as ROK. ROK activity is regulated through a complex molecular pathway. A brief summary of the known major participants in this

pathway and a schematic demonstrating how these pathways interact is given in Fig. 2.

One of the most important regulators of ROK activity is RhoA, a small GTP-binding protein (Ishizaki et al. 1996). ROK binds GTP-RhoA at its centrally located Rho-binding domain. This binding of RhoA causes ROK to migrate to the cell membrane where it is maximally active (Leung et al. 1995). One study has shown that ROK can increase the phosphorylation level of SM MLC_{20} by directly phosphorylating the myosin (shown only in vitro) (Amano et al. 1996). However, the currently more accepted mechanism by which ROK increases SM MLC_{20} phosphorylation (which does not require calcium) is indirectly by inhibiting the phosphatase (SMMLCP) responsible for dephosphorylating SM MLC_{20} (Kimura et al. 1996) (Fig. 2).

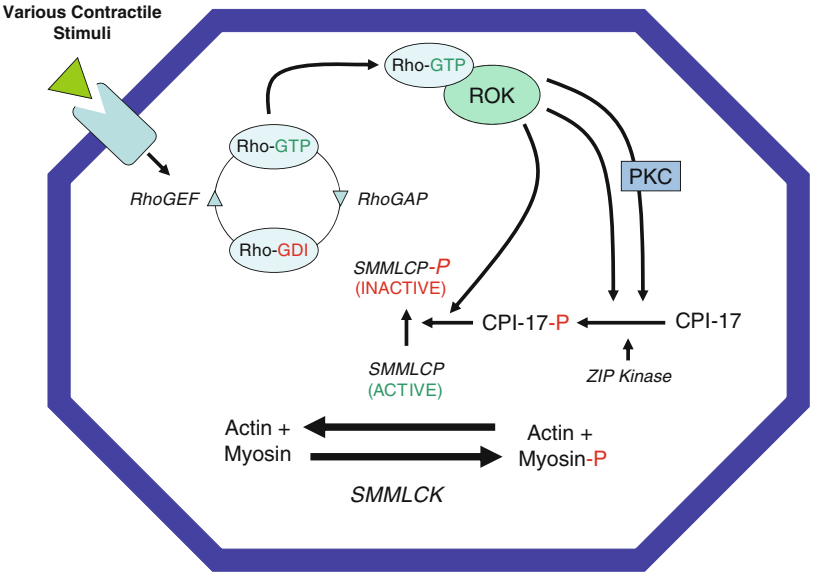

Fig. 2 *Agonist-induced SM Contractile Stimulation Mediated via the RhoA/Rho-kinase Pathway*. In response to the various contractile stimuli outlined in this review the RhoA/Rho-kinase pathway is activated. RhoA exists in an inactive form bound to the inhibitory GDP/GTP exchange protein GDI. Activation of Rho can be accomplished via a Rho guanine nucleotide exchange factor (RhoGEF) to produce Rho-GTP. However, Rho GTPase-activating protein (RhoGAP) can convert Rho-GTP back to RhoGDI. Activated Rho-GTP associates with ROK at the cell membrane wherein ROK is activated. Activated ROK then can directly phosphorylate SMMLCP, which inactivates it and allows the SMMLCK to work with less opposition favoring SM contraction. ROK can also inhibit SMMLCP indirectly by phosphorylating CPI-17, which causes it to become 1,000 fold more active and to bind directly to the SMMLCP inactivating it. Alternatively, ROK can activate PKC to cause phosphorylation of CPI-17

3.2 Rho

The Rho family includes Rho, Rac, and Cdc42, and these small G proteins have been best known for their roles in cytoskeletal reorganization (Olenik et al. 1997) and gene expression (Hill et al. 1995). The Rho subfamily includes RhoA, RhoB, and RhoC, and these proteins share the same amino acid sequence in their effector domains and thus, as expected, seem to have similar intracellular targets (Zong et al. 1999). However, the RhoA GTPase has been the most thoroughly studied, especially in the bladder. Rho acts as a molecular switch in that it cycles between a guanosine-5′-triphosphate (GTP)-bound active state and a guanosine 5′-diphosphate (GDP)-bound inactive state. There are several molecular mechanisms involved in the cycling between the active and inactive states. Rho guanine nucleotide exchange factors (GEFS) catalyze the exchange of GDP for GTP thus activating Rho, GTPase activating proteins (GAPS) inactivate small G proteins by accelerating their intrinsic GTPase activity, while Rho guanine nucleotide dissociation inhibitors (GDIs) bind to Rho and inhibit guanine nucleotide exchange and activation of the Rho GTPases (Zong et al. 1999; Van and D'Souza-Schorey 1997).

3.3 Rho-Kinase

As described above, the small GTPase Rho plays pivotal roles in the Ca^{2+} sensitization of SM. However, the GTP-bound active form of Rho failed to exert Ca^{2+}-sensitizing effects in extensively Triton X-100-permeabilized SM preparations, which was assumed to be due to the loss of an important diffusible cofactor (Gong et al. 1996). Kureishi et al. then demonstrated that a previously identified serine/threonine kinase termed ROKα (Leung et al. 1995) was this missing factor and that ROKα could induce contraction with proportional increases in the level of MLC_{20} phosphorylation both in the presence and absence of calcium (Kureishi et al. 1997). Subsequently, two isoforms of Rho-kinase have been identified: ROKα and ROKβ, also referred to as ROCK-II and ROCK-I, respectively (Nakagawa et al. 1996). These isoforms are relatively large proteins (150–160 kDa) consisting of an amino-terminal kinase domain, a central coiled-coil domain that includes a Rho-binding domain, and a carboxyl-terminal putative pleckstrin homology domain. Although the kinase domain is more than 90% conserved between ROKα and ROKβ in the mouse, the rest of the molecules are only around 55–70% conserved, suggesting that the two enzymes may have somewhat different cellular functions. In the mouse, the ROKβ isoform was expressed abundantly in the heart, lung, liver, stomach, spleen, kidney, testis, placenta, and fetus, but much less in the brain and skeletal muscle, while ROKα was highly expressed in the brain, heart, lung, muscle, placenta, and fetus, and was relatively low in the liver, stomach, spleen, kidney, and testis (Nakagawa et al. 1996). It was later demonstrated by Western blotting that both the ROKα and ROKβ isoenzymes were present in the rat urinary bladder

(Wibberley et al. 2003a), rabbit bladder (Bing et al. 2003), and in human bladder (Takahashi et al. 2004). At present, the complete functional differences between the ROKα and ROKβ isoforms have not been elucidated.

3.4 Activation of the Rho/Rho-kinase Pathway

The RhoA/Rho-kinase pathway can be activated by a number of different molecules and physiological stimuli. The main pathways relevant to the bladder include muscarinic acetylcholine receptors, endothelin, angiotensin, protein kinase C, nitric oxide, reactive oxygen species, sphingosine-1-phosphate (S1P) and sphingosylphosphorylcholine (SPC) pathways, P2-type purinoceptors, lysophosphatidic acid, and prostaglandin F2α.

Jezior et al. initially reported that bethanechol-induced contraction of rabbit detrusor was due in part to a ROK-induced increase in calcium sensitivity (Jezior et al. 2001). This was subsequently followed by a study by Ratz et al. who found that carbachol (CCh)-induced contraction of rabbit bladder SM was inhibited by the ROK inhibitors Y-27632 and HA-1077 (Ratz et al. 2002). Schneider et al. then demonstrated that the selective ROK inhibitor Y-27632 concentration dependently and effectively attenuated the responses to the muscarinic agonist CCh in human urinary bladder (Schneider et al. 2004), while Takahashi confirmed the ROK dependence of CCh-induced contraction of human bladder SM (Takahashi et al. 2004). In a similar manner, Y-27632 reduced the CCh-induced maximal contraction along with CCh potency in normal rat bladder SM strips (Braverman et al. 2006; Fleichman et al. 2004) and inhibited CCh-induced contractions in neonatal and adult mice (Ekman et al. 2006). However, ROK has yet to be shown to play a major role in muscarinic-mediated contraction of the guinea pig bladder (Durlu-Kandilci and Brading 2006; Roosen et al. 2008).

It has been shown that endothelin 1 (ET-1) stimulates the small GTPase, "RhoA," in vascular SM (Sakurada et al. 2001), suggesting that the molecular mechanism by which ET-1 causes SM contraction involves the ROK pathway. This hypothesis is strongly supported by the facts that ET-1-induced contraction of stellate cells and portal vein constriction can be almost completely inhibited by the ROK-selective inhibitor Y-27632 (Kawada et al. 1999). In prior studies, it was reported that this is also true for corpus cavernosum (CC) SM of the penis as it was shown that ET-1 induced CCSM contraction was almost completely abolished by Y-27632 (Chang et al. 2003), and subsequently, it was reported that ET-1 induced contraction of rabbit clitoral cavernous SM was also relaxed by the ROK inhibitor Y-27632(Myung et al. 2008). Recently, it has been shown that although both the ETA and ETB receptors mediate a $G\alpha_q$ receptor-dependent activation of SMMLCK that results in a transient increase in MLC_{20} phosphorylation levels, only the ETA receptor mediates a $G\alpha_{13}$ receptor-dependent activation of RhoA, and the ETA-$G\alpha_{13}$ pathway was found to selectively modulate the sustained phase of SM contraction (Hersch et al. 2004). Concentration–response curves to ET-1

(0.01–100 nM)-induced bladder contraction were significantly attenuated following incubation with the Rho-kinase inhibitors H-1152 (0.1–1 μM), Y-27632 (1–10 μM), or HA-1077 (10 μM) (Teixeira et al. 2007).

Although canine detrusor did not respond well to either angiotensin I (Ang 1) or angiotensin II (Ang 2) (Steidle et al. 1990), both Ang 1 and 2 induced potent contraction of human detrusor muscle (Saito et al. 1992) and rat bladder (Rouissi et al. 1990; Tanabe et al. 1993). However, canine bladder base and urethra did contract to Ang (Ye and Jin 1997). Even though it does not seem to have been demonstrated yet in the bladder, Ang-induced contraction is largely mediated via the ROK pathway in other SMs (Hisaoka et al. 2001), and thus, Ang is likely to be an activator of the ROK pathway in the bladder as well as other urogenital SM.

Kandabashi et al. provided evidence for protein kinase C (PKC)-mediated activation of ROK in a porcine model of coronary artery spasm by showing that PDBu (a direct activator of PKC-induced contraction) was significantly inhibited by the ROK inhibitor hydroxyfasudil, identifying PKC to be located upstream of ROK and that among the PKC isoforms, only PKCδ appeared to be involved (Kandabashi et al. 2003). More recently, Nobe et al. demonstrated that glucose-dependent enhancement of diabetic bladder contraction is associated with an ROK-regulated PKC pathway (Nobe et al. 2009).

Soliman et al. reported that cardiomyocytes from rats cultured in the presence of the NO donor sodium nitroprusside (SNP) increased RhoA expression, while cardiomyocytes from diabetic rats showed elevation of RhoA expression that could be normalized by the iNOS inhibitor L-NIL (Soliman et al. 2008). Furthermore, eNOS knockout mice as well as eNOS/nNOS double knockout mice exhibited decreased ROK activity but with no significant change in RhoA or ROK isoforms expression (Bivalacqua et al. 2007).

Reactive oxygen species (ROS) has been shown to activate the ROK pathway as Jin et al. demonstrated that the ROK signaling pathway is activated by ROS in rat aorta (Jin et al. 2004). Similarly, calcium sensitization by ROK involved in the regulation of airway SM tone was shown to be induced by H_2O_2 in guinea pigs (Kojima et al. 2007). Since the overdistension-induced oxidative stress in rat bladders is associated with ROS production (Yu et al. 2004), ROS-induced ROK activation may be intimately involved in bladder SM function and dysfunction.

S1P is a sphingolipid that acts on five types of G-protein-coupled receptors termed $S1P_1$–$S1P_5$ [originally termed EDG receptors] (Lockman et al. 2004; Ishii et al. 2004; Chun et al. 2002). S1P, via the $S1P_2$ and $S1P_3$ receptors, has been shown to activate constriction of SM via the RhoA/ROK pathways ($G_{12/13}$) (Salomone et al. 2003; Ishii et al. 2002) including human SM (Hudson et al. 2007). Using the ROK inhibitor H-1152, it was demonstrated that S1P-induced bladder SM contraction in the rat is largely mediated via the RhoA/Rho-kinase pathway (Aydin et al. 2010).

Extracellular nucleotides can bind to P2-type purinoceptors, which can be ion channels (P2X receptors) or molecules that couple to trimeric G proteins (P2Y receptors) and have been shown to induce contraction and Y-27632-sensitive Ca^{2+} sensitization in aortic rings (Sauzeau et al. 2000). Contraction of the rat urinary

bladder SM with the P2X receptor agonist α,β-methylene ATP was attenuated by Y-27632 (Wibberley et al. 2003b). In contrast, P2Y is associated with bladder SM relaxation (Aronsson et al. 2010; Boland et al. 1993), demonstrating the tissue-specific effects of the P2Y receptor.

Finally, lysophosphatidic acid (LPA), prostaglandin F2a (PGF2a), and SPC have been shown to increase phosphorylation of MYPT1 at Thr695 and enhance Ca^{2+} sensitivity through activation of ROK. Feng et al. developed a site- and phosphorylation-specific antibody to Thr695 and demonstrated that stimulation of serum-starved Swiss 3T3 cells by LPA induced an increase in Thr695 phosphorylation on MYPT1 and that this effect was blocked by an ROK inhibitor, Y-27632 (Feng et al. 1999). Indeed LPA induced significant contraction of both human and rat cultured bladder SM cells (Kropp et al. 1999). In isolated canine basilar arteries, SPC and PGF2a-induced contraction were both dose-dependently inhibited by Y-27632 (Hashiba et al. 2007). PGF2a has been shown to contract guinea pig detrusor strips but interestingly relax strips from the bladder outlet (Finkbeiner and Bissada 1981), while SPC induced a concentration-dependent contraction of SM cells from the rectosigmoid of the rabbit (Bitar and Yamada 1995).

Tachykinins (also referred to in mammalian species as neurokinins) are a family of peptides that share a common C-terminal sequence, Phe-X-Gly-Leu-Met-NH_2. The mammalian tachykinins include substance P, neurokinin A, and neurokinin B. Neurokinin A & B and substance P have been shown to cause contraction of rat urinary bladder SM, and Y-27632 significantly attenuated both the tonic and phasic components of bladder contraction for neurokinin A and substance P (Wibberley et al. 2003b; Quinn et al. 2004; Hall et al. 1992).

3.5 Effectors of the Rho/Rho-kinase Pathway

Numerous molecules have been demonstrated as effectors of the RhoA/ROK pathway. The most important of these is the SMMLCP, which is composed of three subunits: MYPT1 (a 110–130 kDa MLC_{20} targeting subunit); PP1c (a 38 kDa catalytic subunit of protein phosphatase type 1); and a small 20 kDa subunit whose function is not clear (Shimizu et al. 1994; Fujioka et al. 1998; Shirazi et al. 1994; Alessi et al. 1992). Kimura et al. showed that ROK, which is activated by GTP. RhoA, phosphorylated MYPT1, and then consequently inactivated the SMMLCP enzyme (Kimura et al. 1996). The major sites of phosphorylation of MYPT1 by ROK have been identified as T695/T696/697 (chicken/human/rat) and S850/S853/855 (chicken/human/rat) (Ito et al. 2004; Kawano et al. 1999; Haystead 2005), with ROK exhibiting a threefold preference for the serine site (Hirano 2007). Phosphorylation of both T696 and T853 has been now shown to decrease the activity of the SMMLCP (Feng et al. 1999; Muranyi et al. 2005), while T853 was also shown to interfere with the binding of MYPT1 to myosin (Velasco et al. 2002).

Alternatively, ROK may exert its influence on SMMLCP activity via a molecule known as CPI-17, which is a PKC-potentiated SMMLCP-inhibitory protein of

17 kDa that is expressed predominantly in SM (Eto et al. 1997). Similar to ROK, CPI-17 can potentiate contraction at constant $[Ca^{2+}]_I$ (Li et al. 1998). Although significantly active in its unphosphorylated form, phosphorylation at Thr^{38} increases the affinity of CPI-17 for SMMLCP by more than 1,000-fold (Eto et al. 1995). In addition to PKC, it has been revealed that ROK can also phosphorylate CPI-17 at the Thr^{38} site (Koyama et al. 2000). Importantly, this suggests that CPI-17 could be a molecular switch that would allow cross-talk between the ROK and PKC pathways. Most recently, Wang et al. reported that Thr^{38} phosphorylation could be reduced by inhibition of PKC during the entire contraction but by ROK inhibition only during the sustained phase of contraction (Wang et al. 2009).

One of the key questions that have arisen is how the activated RhoA/ROK complex, which should presumably be located at the cell membrane, can activate the contractile apparatus, which is located in the sarcoplasm. A potential explanation that has arisen involves a molecule that has been termed zipper interacting protein kinase (Zip-kinase; also referred to as ZIP-like kinase or DAPK3 or ZIPK) that has been shown to selectively phosphorylate the regulatory subunit (MYPT1) of the SMMLCP and the inhibitory protein CPI-17 inducing Ca^{2+} sensitization in SM (MacDonald et al. 2001a; b). It was subsequently determined that dominant negative RhoA inhibited the ZIPK–SMMLCP interaction, supporting a role for Rho in promoting the ZIPK–SMMLCP interaction and suggesting ZIPK as a bridge between membrane associated activated ROK and the contractile apparatus (Endo et al. 2004). Discrete kinase activities toward Thr-265 and Thr-299 were defined and identified by mass spectrometry as ROKβ, and in vitro, it has been demonstrated that ROK phosphorylated ZIPK at Thr-265 and Thr-299 as well as bringing about its activation (Hagerty et al. 2007). Furthermore, it has been shown that phosphorylation at Thr-299 regulates the intracellular localization of ZIPK (Graves et al. 2005).

4 Rho-kinase in Dysfunctional Bladder Contractility

Some of these above ROK-mediated contractile mechanisms have been shown to be extensively altered in response to various pathological insults. In the following section, we discuss the role of ROK in these common pathologies of the lower urinary tract.

4.1 Partial Bladder Outlet Obstruction

Bladder outlet obstruction (BOO) is a common urologic pathology observed in children with posterior urethral valves (Dinneen and Duffy 1996), in adults with benign prostatic hyperplasia (BPH) (Nordling 1994), or in response to urethral stricture (Mondet et al. 2001). BOO has been associated with numerous and

substantial changes in the phenotype of the urinary bladder SM including an altered SM contractility. In general, there is an increase in the connective tissue-to-muscle ratio (Inui et al. 1999), an increase in detrusor muscle cell hypertrophy (Collado et al. 2006; Elbadawi et al. 1993), and an increase in SM contractility (Bouchelouche et al. 2005).

Experimentally, many pathologic characteristics of human obstructed bladder disease can be recapitulated in animal models (Levin et al. 2000). In rabbits, rats, and mice with BOO, the detrusor SM undergoes hypertrophy to compensate for the increased force required to expel urine against the obstruction. Bing et al. reported that surgically induced BOO male rabbit decompensated bladder exhibited a tonic type SM contraction to KCl depolarization compared to sham-operated rats that exhibited a more phasic-type SM contraction (Bing et al. 2003). In this study, rabbits with BOO first underwent metabolic cage monitoring to determine in vivo bladder function, and only those rabbits that exhibited decompensated bladder function were used as the BOO group for the study. They also determined that the bladder from BOO rabbits exhibited lower SMMLCP activity and showed more Ca^{2+}-independent MLC_{20} phosphorylation, which was partially inhibited by Y-27632, suggesting a role for the RhoA/Rho-kinase pathway in the BOO-induced tonic-type detrusor contraction. Although the expression of SMMLCK or ROKα was not significantly different between bladders from BOO and sham-operated rabbits, the expression of ROKβ was significantly increased in bladders from BOO rabbits at both the mRNA and protein levels, determined by RT-PCR and Western blotting, respectively, further suggesting a role for ROK in the increased tonicity of the bladder SM strips in response to BOO (Bing et al. 2003).

The above findings are in agreement with a subsequent study by Levin and colleagues also in male rabbits that in general found an increase in the expressions of ROKα and ROKβ in the bladder at 2 and 8 weeks post BOO (Lin et al. 2008). In their study, they also found that the increase in ROKα and ROKβ protein correlated with decreases in relaxation of phenylephrine-precontracted detrusor strips by electrical field stimulation (EFS), adenosine triphosphate (ATP), acetylcholine and sodium nitroprusside, and a significant decrease in the SM to collagen ratio. Similarly, the work of Takahashi et al. found an increase in the expression of RhoA, ROKα, and ROKβ after 4 weeks of BOO in the male rat (Takahashi et al. 2009). This study also demonstrated an increase in the sustained component of detrusor contraction like the Bing et al. study above and further showed that the sustained component of CCh-induced contraction for the detrusor from BOO rats was decreased to a greater degree by the ROK inhibitor Y-27632 than the sustained force component from control rats. Most recently, Aydin et al. reported an approximately twofold increase in the expression of both of these ROK isoforms in the male rat after 2 weeks BOO (Aydin et al. 2010) consistent with the above studies.

However, another study by the Levin group found a more varied response to BOO in the male rabbit with an increase in the expression of ROKα at both the mRNA and protein levels from 2–8 weeks but a substantial decrease in the expression of the ROKβ isoform from 1–8 weeks BOO (Guven et al. 2007). In order to determine if the age of the rabbit influenced the animal's response to BOO,

they compared the response of young and mature male rabbits to BOO over 2 weeks. Again, the expression of the ROKα isoform was increased and the expression of the ROKβ isoform was decreased at both the mRNA and protein levels (Guven et al. 2008). The only major difference between the young and old group was that from 7–14 days, the expression of both ROKα and ROKβ recovered to some degree in the young group but did not recover in the old group. It is possible that the expression of the ROK isoforms correlates with the severity of the bladder obstruction and/or the in vivo functional capacity of the urinary bladder, but this awaits future studies with larger cohorts of animals coupled with urodynamic evaluation.

Bladders from 2-week BOO rats also had greater mRNA expression of the $S1P_2$ (fivefold) and $S1P_3$ (twofold) receptors, which as described above mediate their contractile effects at least partly via the RhoA/ROK pathway (Aydin et al. 2010). Moreover, sphingosine kinase-1 (SPHK1), an enzyme that converts sphingosine to S1P, was also increased nearly threefold, suggesting that there would be more endogenous S1P available to activate this pathway. Western blotting and organ-bath contractility studies showed similar changes at the protein and in vitro functional level, with an increased contractility of bladder strips from BOO rats to exogenous S1P. Transfection of SPHK into isolated SM cells increased ROK expression. Thus, for the first time, it was demonstrated that the S1P signaling pathway is significantly upregulated in response to BOO in male rats at both the molecular (mRNA and protein level) and in vitro functional levels (increased force development in response to exogenous S1P stimulation), correlating with an activation of the RhoA/ROK pathway. In addition, using selective in vitro antagonists, it was shown that S1P-mediated contraction in bladder can be completely attenuated by blocking the ROK pathway and that the $S1P_2$ and the $S1P_3$ receptors play a major role in bladder contraction. Further, SPHK overexpression increases ROK expression in SM cells in vitro, suggesting a novel hypothesis of S1P-induced bladder overactivity in the mechanism for BOO-induced bladder dysfunction and the S1P signaling pathway as a possible therapeutic target for bladder overactivity (Aydin et al. 2010).

There is also mounting evidence that ischemia/reperfusion (I/R) is a major etiologic factor in the progression of bladder dysfunction associated with BOO. Juan et al. showed that ischemia alone caused an immediate increase in the expression of ROKα in the bladder smooth muscle and that this expression returned to normal levels after reperfusion (Juan et al. 2009). In this same study, CPI-17 expression was also significantly increased in the muscle layer and then decreased after 2 weeks of reperfusion.

4.2 Diabetic Bladder

One of the most common complications of diabetes mellitus (DM) is bladder dysfunction, which for years has been accepted to be a manifestation of diabetic

neuropathy (Andersen and Bradley 1976; Faerman et al. 1973) with an early loss of bladder sensation (Kebapci et al. 2007). The bladder cannot sense that it is full and overdistends, leading to impaired detrusor contractility and atony of the bladder (Frimodt-Moller 1978). However, it is now thought by many that diabetic cystopathy likely represents end-stage bladder failure. Interestingly, bladder dysfunction in response to DM can also be manifested early on as detrusor overactivity, and this pathology has been reported to be the most frequent urodynamic finding in patients with peripheral neuropathy with 70% of patients exhibiting detrusor overactivity (Kaplan et al. 1995). It has also been determined that diabetes is associated with decreased urethral relaxation in response to NO, again suggestive of an increase in SM contractility or tone (Yang et al. 2007). To date, diabetic animal models of T1D have yielded conflicting data on detrusor contractility with some studies finding decreased force generation (Changolkar et al. 2005; Gupta et al. 1996; Longhurst and Belis 1986; Su et al. 2004) and other studies finding increased force generation (Kodama and Takimoto 2000; Tammela et al. 1994; Waring and Wendt 2000).

Increased Rho/ROK signaling occurs in several nonbladder tissues of diabetic animals (Kizub et al. 2010; Didion et al. 2005; Xie et al. 2010; Soliman et al. 2008). Concerning the bladder, Chang et al., using two-dimensional gel electrophoresis, showed that the basal MLC_{20} phosphorylation level was significantly higher in detrusor from alloxan-induced Type 1 diabetic rabbits than detrusor from normal or diuretic controls, and that the ROK inhibitor, Y-27632, decreased the MLC phosphorylation level (Chang et al. 2006). Furthermore, in this same study, adding Y-27632 to bethanechol-precontracted detrusor SM strips induced muscle relaxation, but it occurred much more slowly in diabetic samples compared with controls, and this correlated with tenfold, and twofold increased expression of the ROKβ isoform at the mRNA and protein levels, respectively. Chang et al. also found a similar twelvefold and 2.5 fold higher mRNA and protein expression, respectively, of the ROK effector protein CPI-17 in diabetic rabbits compared to their nondiabetic control counterparts (Chang et al. 2006).

Using a mouse model of type 2 diabetes known as the ob/ob mouse, Nobe et al. found that CCh induced time- and dose-dependent contractions in ob/ob and C57BL mice; however, maximal responses differed significantly (14.34 ± 0.32 and 12.69 ± 0.22 mN/mm in ob/ob and C57BL mice, respectively, after 30 μM CCh treatment), which correlated with an approximate twofold increase in blood glucose levels in the ob/ob mice (Nobe et al. 2009). Additionally, pretreatment of bladder SM strips under high glucose conditions led to the enhancement of CCh-induced contraction solely in the diabetic mice, and the correlation between intracellular calcium concentration and contraction was enhanced only in the ob/ob mouse, suggesting a diabetes-induced "calcium sensitization." This enhancement of contraction was inhibited by pretreatment with the ROK inhibitor, fasudil, while this inhibitor also suppressed the differences between ob/ob and C57BL mouse bladder contractions under high glucose conditions.

Since cooling of urinary bladder SM normally is a potent stimulus to micturition due to increase in muscle tone, Ismael et al. studied the effects of cooling on normal

and diabetic bladder specimens (Ismael et al. 2010). Using urinary bladder SM strips from rats, they showed that stepwise cooling from 37 to 5°C induced a rapid and reproducible increase in basal tone, proportional to the cooling temperature and that this response was more pronounced in diabetic specimens. The ROK inhibitor Y-27632 (1 μM) inhibited cooling (20°C)-induced contraction by 52.1 ± 10.0% in control and by 70.0 ± 12.0% in diabetic rats, which was a statistically significant difference.

4.3 Hypertension-Associated Bladder Dysfunction

Spontaneously hypertensive rats (SHR) exhibit hyperactive voiding (Spitsbergen et al. 1998) with lower bladder capacity, decreased micturition volume, and an increased amplitude of nonvoiding bladder contractions consistent with the overactive bladder syndrome (OAB) (Persson et al. 1998). Rajasekaran et al. confirmed and extended these findings by demonstrating that SHR rats had a higher voiding rate and rate of nonvoiding contractions compared to control Wistar Kyoto (WKY) rats (Rajasekaran et al. 2005). In addition, they showed that the ROK inhibitor Y-27632 could significantly reduce these rates in the SHR to near control levels. Additionally, using immunohistochemistry, they reported stronger reactivity of antibody to RhoA in the bladders of the SHR compared to the control WKY rats.

Schneider et al. provided contrasting data suggesting that ROK does not contribute to bladder dysfunction in SHR rats (Schneider et al. 2005). In this study, they determined that the mean total number of muscarinic receptors, the relative roles of their muscarinic receptor subtypes, contractile response to CCh, and the effect of the ROK inhibitor Y-27632 on CCh-induced contraction were quantitatively similar for SHRs and WKY control rats. However, subsequently, Nobe et al. provided further data supporting a role for ROK in the spontaneously hypertensive and hyperlipidemic rat (SHHR rat) (Nobe et al. 2008). They found that CCh induced dose-dependent contractions in Sprague-Dawley (age-matched control) rats and SHHRs with an approximately 2.5-fold lesser force in the SHHR rats. Moreover, this difference, which was maintained in calcium-replaced physiological salt solution, was suppressed by pretreatment with the ROK inhibitor Y-27632. Thus, unlike the increase in CCh-induced maximum contraction observed by Rajesekaran et al. in the SHR, this study found a much lower maximum force in the SHHR rat. Yet in both of these studies, an ROK inhibitor attenuated these changes. Although the decreased SHHR contraction to CCh occurred prior to any significant change in blood glucose level (ruling out a hyperglycemia-induced effect), the SHHR rat did have two to threefold higher levels of total cholesterol and trigylcerides, which may have influenced SM phenotype (Nobe et al. 2008).

Most recently, Morelli et al. reported that the intercontraction interval and bladder capacity were decreased in SHRs (similar to the Rajasekaran et al. study) and restored by the phosphodiesterase V (PDE5) inhibitor vardenafil (Morelli et al. 2009). However, nonvoiding contractions were not significantly different

between the two groups. Also, they noted that the in vitro relaxant effect of the ROK inhibitor Y-27632 was higher in bladder strips from SHR than from WKY control rats and also restored toward normal levels by vardenafil. Interestingly, vardenafil prevented RhoA membrane translocation/activation and decreased ROK activity both in vivo in the rat and in vitro in human bladder cells. Thus the effect of vardenfil on OAB could be determined at least partly by RhoA/ROK signaling.

4.4 Hormonal Regulation of Rho-kinase

Although this issue of a menopausal influence on voiding function in women remains quite a controversial topic, evidence is mounting that alterations in circulating estrogen levels can influence lower urinary tract (LUT) functions (Robinson and Cardozo 2003; Parekh et al. 2004). Hong et al. showed that incubation with 10 μM Y-27632 resulted in the largest decrease in baseline tension of strips from a bilateral ovariectomy-only group compared to the sham-operated and bilateral ovariectomy + estrogen replacement groups, but this was not statistically significant (Hong et al. 2006). For CCh-induced tonic contractions, however, strips from the bilateral ovariectomy-only group were attenuated the most among the three groups after adding Y-27632, and this change was found to be significant. However, at the protein level, there were no significant expression differences in the levels of RhoA and the two ROK isoforms in bladder tissues from the three groups. Their data suggest that estrogen might inhibit the function of ROK in bladder smooth muscle, while having no significant effect on its expression. This work is partially supported by a study from Li et al. that revealed ovariectomized rabbits exhibited a deceased contractile function that correlated with a significantly increased expression of ROKα but rather with a significantly decreased expression of the ROKβ isoform (Li et al. 2009).

But in a more detailed study, Chavalmane et al. showed that aromatase and sex steroid receptors, including GPR30, were expressed in human male bladder and they mediate several biological functions (Chavalmane et al. 2010). Both 17beta-estradiol and the orphan estrogen receptor GPR30/GPER1 agonist G1 activated calcium transients and induced RhoA/ROK signaling (cell migration, cytoskeleton remodeling, and smooth muscle gene expression) in human bladder cells, while RhoA/ROK inhibitors blunted these effects. Estrogen- but not androgen-supplementation to male castrated rats increased the sensitivity to the ROK inhibitor Y-27632 in isolated bladder strips. In cultured human bladder SM cells, testosterone elicited effects similar to estrogen, which were attenuated by blocking its aromatization through letrozole. These data thus indicated for the first time that estrogen more than androgen receptors upregulate RhoA/ROK signaling. These authors speculated that since an altered estrogen/androgen ratio characterizes conditions, such as aging, obesity, and metabolic syndrome, often associated to LUTS, it is speculated that a relative hyperestrogenism may induce bladder overactivity

through the upregulation of RhoA/ROK pathway. However, this speculation is in contrast to the above study by Hong et al. that suggested that estrogen may inhibit rather than activate ROK activity in the bladder. Clearly, more detailed studies in this area are needed to address this issue.

4.5 The Aging Bladder

In general, aging impairs the contractile response of detrusor strips. Gomez-Pinilla et al. found that agonist-induced contractions in adult guinea pig bladder strips were sensitive to blockade with Y27362, an ROK inhibitor, and GF109203X, an inhibitor of PKC, but that these inhibitors had negligible effects on aged strips, suggesting an alteration in ROK expression and/or activity (Gomez-Pinilla et al. 2008). The decreased response to ROK and PKC inhibitors in aged bladders correlated well with lower levels of RhoA, ROK, PKC, and the two Rho-kinase effectors CPI-17 and MYPT1, and with the absence of CPI-17 and MYPT1 phosphorylation in response to agonist stimulation.

5 Rho-kinase in Ureteral Contractility

In normal rat, but not guinea-pig ureter, three of the major ROK inhibitors, Y-27632, HA-1077, and H-1152, were shown to significantly decrease phasic contractions and Ca^2+ transients (Shabir et al. 2004). The fact that these inhibitors could modulate phasic contractions in the absence of changes in calcium suggests a role for ROK in calcium sensitization of ureteral SM. Levent et al. extended these studies to sheep showing that both the ROKα and ROKβ isoforms were detected in sheep ureter and that it mediated agonist- and electrical field stimulation (EFS)-induced contractions as well as spontaneous contractile activity of isolated sheep ureter (Levent and Buyukafsar 2004). Next, Hong et al. extended these studies to human ureteral SM demonstrating via immunohistochemistry and immunoblotting that the ROKα isoform is present in human ureteral SM (Hong et al. 2005). In functional analysis, they showed that Y-27632 could decrease not only baseline tension but also spontaneous and EFS-induced contractile responses of human ureteral strips in a concentration-dependent manner.

Turna et al. later demonstrated that, similar to bladder BOO, unilateral ureteric obstruction (UUO) increased the expression of both the ROKα and ROKβ isoforms in rabbits (Turna et al. 2007). In addition, functional analysis revealed that the contractions induced by EFS, KCl, phenylephrine, and carbachol in the ureteric strips from rabbits with UUO were significantly greater than those from the control rabbits and that Y-27632 considerably suppressed the ureter contractile responses in both UUO and control rabbits while normalizing UUO contractions to control levels.

6 Non SM Contractility Effects of Rho-kinase in the Bladder and Ureter

Both the normal bladder and, to a much greater degree, the BOO bladder undergo cyclical stretch. The cysteine-rich protein 61 (Cyr61) is a signaling molecule with functions in cell migration, adhesion, and proliferation. Tamura et al. demonstrated that Cyr61 mRNA levels increase sharply in response to cyclic mechanical stretch applied to cultured bladder SM cells and that selective inhibition of the ROK pathway altered this stretch effect, suggesting a role for ROK in the proliferative/hypertrophic response of the bladder to BOO (Tamura et al. 2001). Stress has also been recently shown to increase expression of ROK. Yoon et al. reported that rats exposed to scheduled stress environments for 14 days exhibited an increased expression of ROKα that correlated with significantly decreased levels of estrogen but increased levels of testosterone, dopamine, and norepinephrine (Yoon et al. 2010)

ROK has also been shown to exist in the urothelium layer of the bladder. In fact, Nakanishi et al. demonstrated in the pig a higher level of RhoA mRNA and activated RhoA enzyme (two- and fourfold, respectively) in the urothelium compared to bladder SM (Nakanishi et al. 2009). Furthermore, the ROK inhibitor Y-27632 caused a greater tension decrease in CCh-precontracted bladder strips that had an intact urothelium compared to those that had the urothelium removed. In a similar study, Tatsumiya et al. showed that in bladder strips with and without urothelium, low concentrations of the ROK inhibitor fasudil (1 or 10 mmol/L) inhibited CCh-induced contraction almost identically, but at a higher concentration (30 mmol/L) of fasudil, E_{max} was twofold lower in tissues with urothelium compared to those without urothelium, suggesting that the urothelium may be involved in regulating CCh-induced contraction in urinary bladder via the RhoA/ROK pathway (Tatsumiya et al. 2009). Related to the dysfunctional bladder, in the ischemia/reperfusion study by Juan et al. described above, they found that in contrast to what occurred in the SM layer, the urothelium showed no change in the expression of ROKα in response to ischemia alone but then an increase in ROKα expression in response to reperfusion (Juan et al. 2009).

The RhoA/ROK pathway has also been implicated in the molecular mechanism for bladder infections caused by uropathogenic *Escherichia coli* (UPEC) (Martinez and Hultgren 2002), with invasion and metastasis of bladder cancer (Kamai et al. 2003; Chang et al. 2010) and with microfilament-based contraction in branching morphogenesis of the ureteric bud (Michael et al. 2005).

7 Conclusions

The data summarized in this review show that the RhoA/ROK pathway is critical for the maintenance of basal SM tone of the urinary bladder and serves as a common final pathway of various contractile stimuli in rabbits, rats, mice, and pigs

as well as humans. In addition, this pathway is upregulated in response to a number of pathological conditions associated with bladder SM dysfunction. Similarly, RhoA/ROK signaling is essential for normal ureteral function and development and is upregulated in response to ureteral outlet obstruction. ROK induces "calcium sensitization" of SM predominantly via the inhibition of SMMLCP either directly or via activation of the 17 kDa SMMLCP inhibitory protein CPI-17 by directly phosphorylating it or activating PKC-mediated CPI-17 phosphorylation. In addition to its critical role in bladder SM function, a role of ROK in the urothelium is also beginning to emerge as well as roles in bladder infection and invasion and metastasis of bladder cancer.

However, much still remains to be elucidated about the RhoA/ROK pathway including whether true functional differences exist between ROKα and ROKβ, which may be extremely important in generating some type of isoform-selective inhibitors, which may indeed be necessary to target such a universally active enzyme. In addition, there are still many discrepancies between different species with regard to ROK function, and these must be unified and translated to the human condition. Finally, clinical data on the use of ROK inhibitors should begin to emerge in the near future and will need to be critically evaluated.

References

Adelstein RS, Eisenberg E (1980) Regulation and kinetics of the actin-myosin-ATP interaction. [review]. Annu Rev Biochem 49:921–956

Alessi D, MacDougall LK, Sola MM, Ikebe M, Cohen P (1992) The control of protein phosphatase-1 by targetting subunits. The major myosin phosphatase in avian smooth muscle Is a novel form of protein phosphatase-1. Eur J Biochem 210:1023–1035

Amano M, Ito M, Kimura K, Fukata Y, Chihara K, Nakano T, Matsuura Y, Kaibuchi K (1996) Phosphorylation and activation of myosin by rho-associated kinase (rho-kinase). J Biol Chem 271:20246–20249

Andersen JT, Bradley WE (1976) Abnormalities of bladder innervation in diabetes mellitus. Urology 7:442–448

Aronsson P, Andersson M, Ericsson T, Giglio D (2010) Assessment and characterization of Purinergic contractions and relaxations in the rat urinary bladder. Basic Clin Pharmacol Toxicol 107:603–613

Aydin M, Downing K, Villegas G, Zhang X, Chua R, Melman A, DiSanto ME (2010) The sphingosine-1-phosphate pathway is upregulated in response to partial urethral obstruction in male rats and activates RhoA/Rho-kinase signalling. BJU Int 106:562–571

Barron JT, Barany M, Barany K (1979) Phosphorylation of the 20, 000-Dalton light chain of myosin of intact arterial smooth muscle in rest and in contraction. J Biol Chem 254: 4954–4956

Bing W, Chang S, Hypolite JA, DiSanto ME, Zderic SA, Rolf L, Wein AJ, Chacko S (2003) Obstruction-induced changes in urinary bladder smooth muscle contractility: a role for Rho kinase. Am J Physiol Renal Physiol 285:F990–F997

Bitar KN, Yamada H (1995) Modulation of smooth muscle contraction by Sphingosylphosphorylcholine. Am J Physiol 269:G370–G377

Bivalacqua TJ, Liu T, Musicki B, Champion HC, Burnett AL (2007) Endothelial nitric oxide synthase keeps erection regulatory function balance in the penis. Eur Urol 51:1732–1740

Boland B, Himpens B, Paques C, Casteels R, Gillis JM (1993) ATP induced-relaxation in the mouse bladder smooth muscle. Br J Pharmacol 108:749–753

Bouchelouche K, Andersen L, Alvarez S, Nordling J, Bouchelouche P (2005) Increased contractile response to phenylephrine in detrusor of patients with bladder outlet obstruction: effect of the alpha1a and alpha1d-adrenergic receptor antagonist tamsulosin. J Urol 173:657–661

Braverman AS, Tibb AS, Ruggieri MR Sr (2006) M2 and M3 muscarinic receptor activation of urinary bladder contractile signal transduction. I. Normal rat bladder. J Pharmacol Exp Ther 316:869–874

Chacko S (1981) Effects of phosphorylation, calcium ion, and tropomyosin on actin-activated adenosine 5′-triphosphatase activity of mammalian smooth muscle myosin. Biochemistry 20:702–707

Chang HR, Huang HP, Kao YL, Chen SL, Wu SW, Hung TW, Lian JD, Wang CJ (2010) The suppressive effect of Rho kinase inhibitor, Y-27632, on oncogenic Ras/RhoA induced invasion/migration of human bladder cancer TSGH cells. Chem Biol Interact 183:172–180

Chang S, Hypolite JA, Changolkar A, Wein AJ, Chacko S, DiSanto ME (2003) Increased contractility of diabetic rabbit corpora smooth muscle in response to endothelin is mediated via Rho-kinase beta. Int J Impot Res 15:53–62

Chang S, Hypolite JA, DiSanto ME, Changolkar A, Wein AJ, Chacko S (2006) Increased basal phosphorylation of detrusor smooth muscle myosin in alloxan-induced diabetic rabbit is mediated by up-regulation of Rho-kinase Beta and CPI-17. Am J Physiol Renal Physiol 290: F650–F656

Changolkar AK, Hypolite JA, DiSanto M, Oates PJ, Wein AJ, Chacko S (2005) Diabetes induced decrease in detusor smooth muscle force associated with oxidative stress and overactivity of aldose reductase. J Urol 173:309–313

Chavalmane AK, Comeglio P, Morelli A, Filippi S, Fibbi B, Vignozzi L, Sarchielli E, Marchetta M, Failli P, Sandner P, Saad F, Gacci M, Vannelli GB, Maggi M (2010) Sex steroid receptors in male human bladder: expression and biological function. J Sex Med 7:2698–2713

Christ G, Wingard C (2005) Calcium sensitization as a pharmacological target in vascular smooth-muscle regulation. Curr Opin Investig Drugs 6:920–933

Chun J, Goetzl EJ, Hla T, Igarashi Y, Lynch KR, Moolenaar W, Pyne S, Tigyi G (2002) International union of pharmacology. XXXIV. Lysophospholipid receptor nomenclature. Pharmacol Rev 54:265–269

Collado A, Batista E, Gelabert-Mas A, Corominas JM, Arano P, Villavicencio H (2006) Detrusor quantitative morphometry in obstructed males and controls. J Urol 176:2722–2728

Dabrowska R, Sherry JM, Aromatorio DK, Hartshorne DJ (1978) Modulator protein as a component of the myosin light chain kinase from chicken gizzard. Biochemistry 17:253–258

Didion SP, Lynch CM, Baumbach GL, Faraci FM (2005) Impaired endothelium-dependent responses and enhanced influence of Rho-Kinase in cerebral arterioles in type II diabetes. Stroke 36:342–347

Dinneen MD, Duffy PG (1996) Posterior urethral valves. Br J Urol 78:275–281

Durlu-Kandilci NT, Brading AF (2006) Involvement of Rho kinase and protein kinase C in carbachol-induced calcium sensitization in beta-escin skinned rat and guinea-pig bladders. Br J Pharmacol 148:376–384

Ebashi F, Ebashi S (1962) Removal of calcium and relaxation in actomyosin systems. Nature 194:378–379

Ebashi S, Ebashi F, Kodama A (1967) Troponin as the Ca++-receptive protein in the contractile system. J Biochem 62:137–138

Ekman M, Andersson KE, Arner A (2006) Developmental regulation of nerve and receptor mediated contractions of mammalian urinary bladder smooth muscle. Eur J Pharmacol 532:99–106

Elbadawi A, Yalla SV, Resnick NM (1993) Structural basis of geriatric voiding dysfunction. IV. Bladder outlet obstruction. J Urol 150:1681–1695

Endo A, Surks HK, Mochizuki S, Mochizuki N, Mendelsohn ME (2004) Identification and characterization of zipper-interacting protein kinase as the unique vascular smooth muscle myosin phosphatase-associated kinase. J Biol Chem 279:42055–42061

Endo M, Iino M, Kobayashi T, Yamamoto T (1990) Control of calcium release in smooth muscle cells. Prog Clin Biol Res 327:193–204

Eto M, Ohmori T, Suzuki M, Furuya K, Morita F (1995) A novel protein phosphatase-1 inhibitory protein potentiated by protein kinase C. Isolation from porcine aorta media and characterization. J Biochem 118:1104–1107

Eto M, Senba S, Morita F, Yazawa M (1997) Molecular cloning of a novel phosphorylation-dependent inhibitory protein of protein phosphatase-1 (CPI17) in smooth muscle: its specific localization in smooth muscle. FEBS Lett 410:356–360

Faerman I, Glocer L, Celener D, Jadzinsky M, Fox D, Maler M, Alvarez E (1973) Autonomic nervous system and diabetes. Histological and histochemical study of the autonomic nerve fibers of the urinary bladder in diabetic patients. Diabetes 22:225–237

Feng J, Ito M, Ichikawa K, Isaka N, Nishikawa M, Hartshorne DJ, Nakano T (1999) Inhibitory phosphorylation site for Rho-associated kinase on smooth muscle myosin phosphatase. J Biol Chem 274:37385–37390

Finkbeiner AE, Bissada NK (1981) In vitro effects of prostaglandins on the guinea pig detrusor and bladder outlet. Urol Res 9:281–288

Fleichman M, Schneider T, Fetscher C, Michel MC (2004) Signal transduction underlying carbachol-induced contraction of rat urinary bladder. II. Protein kinases. J Pharmacol Exp Ther 308:54–58

Frimodt-Moller C (1978) Diabetic cystopathy. A review of the urodynamic and clinical features of neurogenic bladder dysfunction in diabetes mellitus. Dan Med Bull 25:49–60

Fujioka M, Takahashi N, Odai H, Araki S, Ichikawa K, Feng J, Nakamura M, Kaibuchi K, Hartshorne DJ, Nakano T, Ito M (1998) A new isoform of human myosin phosphatase targeting/regulatory subunit (MYPT2): CDNA cloning, tissue expression, and chromosomal mapping. Genomics 49:59–68

Gomez-Pinilla PJ, Gomez MF, Sward K, Hedlund P, Hellstrand P, Camello PJ, Andersson KE, Pozo MJ (2008) Melatonin restores impaired contractility in aged guinea pig urinary bladder. J Pineal Res 44:416–425

Gong MC, Iizuka K, Nixon G, Browne JP, Hall A, Eccleston JF, Sugai M, Kobayashi S, Somlyo AV, Somlyo AP (1996) Role of guanine nucleotide-binding proteins–ras-family or trimeric proteins or both–in ca2+ sensitization of smooth muscle. Proc Natl Acad Sci USA 93:1340–1345

Graves PR, Winkfield KM, Haystead TA (2005) Regulation of zipper-interacting protein kinase activity in vitro and in vivo by multisite phosphorylation. J Biol Chem 280:9363–9374

Gupta S, Yang S, Cohen RA, Krane RJ, Saenz de Tejada I (1996) Altered contractility of urinary bladder in diabetic rabbits: relationship to reduced Na+ pump activity. Am J Physiol Cell Physiol 271:C2045–C2052

Guven A, Onal B, Kalorin C, Whitbeck C, Chichester P, Kogan B, Levin R, Mannikarottu A (2007) Long term partial bladder outlet obstruction induced contractile dysfunction in male rabbits: a role for rho-kinase. Neurourol Urodyn 26:1043–1049

Guven A, Lin WY, Neuman P, Kogan BA, Levin R, Mannikarottu A (2008) Effect of age on the role of rho-kinase in short-term partial bladder outlet obstruction. Urology 71:541–545

Hagerty L, Weitzel DH, Chambers J, Fortner CN, Brush MH, Loiselle D, Hosoya H, Haystead TA (2007) ROCK1 phosphorylates and activates zipper-interacting protein kinase. J Biol Chem 282:4884–4893

Hall JM, Flowers JM, Morton IK (1992) A pharmacological study of NK1 and NK2 tachykinin receptor characteristics in the rat isolated urinary bladder. Br J Pharmacol 107(7): 77–784

Hashiba Y, Tosaka M, Saito N, Imai H, Shimizu T, Sasaki T (2007) Vasorelaxing effect of the rho-kinase inhibitor, Y-27632, in isolated canine basilar arteries. Neurol Res 29:485–489

Haystead TA (2005) ZIP kinase, a key regulator of myosin protein phosphatase 1. Cell Signal 17:1313–1322

Heilbrunn LV, Wiercinski FJ (1946) The action of various cations of muscle protoplasm. Biol Bull 91:237

Hersch E, Huang J, Grider JR, Murthy KS (2004) Gq/G13 Signaling by ET-1 in smooth muscle: MYPT1 phosphorylation via ETA and CPI-17 dephosphorylation via ETB. Am J Physiol Cell Physiol 287:C1209–C1218

Hill CS, Wynne J, Treisman R (1995) The Rho family GTPases RhoA, Rac1, and CDC42Hs regulate transcriptional activation by SRF. Cell 81:1159–1170

Himpens B, Matthijs G, Somlyo AV, Butler TM, Somlyo AP (1988) Cytoplasmic free calcium, myosin light chain phosphorylation, and force in phasic and tonic smooth muscle. J Gen Physiol 92:713–729

Hirano K (2007) Current topics in the regulatory mechanism underlying the Ca2+ sensitization of the contractile apparatus in vascular smooth muscle. J Pharmacol Sci 104:109–115

Hisaoka T, Yano M, Ohkusa T, Suetsugu M, Ono K, Kohno M, Yamada J, Kobayashi S, Kohno M, Matsuzaki M (2001) Enhancement of Rho/Rho-kinase system in regulation of vascular smooth muscle contraction in tachycardia-induced heart failure. Cardiovasc Res 49:319–329

Hong SK, Kwak C, Chang JB, Kim BS, Kim HH (2005) Involvement of Rho-kinase in the contractile mechanism of human ureteral smooth muscle. Neurourol Urodyn 24:136–141

Hong SK, Yang JH, Kim TB, Kim SW, Paick JS (2006) Effects of ovariectomy and oestrogen replacement on the function and expression of Rho-kinase in rat bladder smooth muscle. BJU Int 98:1114–1117

Hudson NK, O'Hara M, Lacey HA, Corcoran J, Hemmings DG, Wareing M, Baker P, Taggart MJ (2007) Modulation of human arterial tone during pregnancy: the effect of the bioactive metabolite sphingosine-1-phosphate. Biol Reprod 77:45–52

Huxley H, Hanson J (1954) Changes in the cross-striations of muscle during contraction and stretch and their structural interpretation. Nature 173:973–976

Inui E, Ochiai A, Naya Y, Ukimura O, Kojima M (1999) Comparative morphometric study of bladder detrusor between patients with benign prostatic hyperplasia and controls. J Urol 161:827–830

Ishii I, Ye X, Friedman B, Kawamura S, Contos JJ, Kingsbury MA, Yang AH, Zhang G, Brown JH, Chun J (2002) Marked perinatal lethality and cellular signaling deficits in mice null for the two Sphingosine 1-Phosphate (S1P) receptors, S1P(2)/LP(B2)/EDG-5 and S1P(3)/LP(B3)/EDG-3. J Biol Chem 277:25152–25159

Ishii I, Fukushima N, Ye X, Chun J (2004) Lysophospholipid receptors: signaling and biology. Annu Rev Biochem 73:321–354

Ishizaki T, Maekawa M, Fujisawa K, Okawa K, Iwamatsu A, Fujita A, Watanabe N, Saito Y, Kakizuka A, Morii N, Narumiya S (1996) The small GTP-binding protein Rho binds to and activates a 160 KDa ser/thr protein kinase homologous to myotonic dystrophy kinase. EMBO J 15:1885–1893

Ismael HN, Mustafa S, Thulesius O (2010) Effect of diabetes on cooling-induced detrusor muscle contraction: mediation via Rho-kinase activation. Urology 75:891–895

Ito M, Nakano T, Erdodi F, Hartshorne DJ (2004) Myosin phosphatase: structure, regulation and function. Mol Cell Biochem 259:197–209

Itoh T, Kuriyama H, Suzuki H (1981) Excitation–contraction coupling in smooth muscle cells of the guinea-pig mesenteric artery. J Physiol 321:513–535

Jezior JR, Brady JD, Rosenstein DI, McCammon KA, Miner AS, Ratz PH (2001) Dependency of detrusor contractions on calcium sensitization and calcium entry through LOE-908-sensitive channels. Br J Pharmacol 134:78–87

Jin L, Ying Z, Webb RC (2004) Activation of Rho/Rho kinase signaling pathway by reactive oxygen species in rat aorta. Am J Physiol Heart Circ Physiol 287:H1495–H1500

Juan YS, Li S, Levin RM, Kogan BA, Schuler C, Leggett RE, Huang CH, Mannikarottu A (2009) The effect of ischemia/reperfusion on rabbit bladder–role of Rho-kinase and smooth muscle regulatory proteins. Urology 73:1126–1130

Kamai T, Tsujii T, Arai K, Takagi K, Asami H, Ito Y, Oshima H (2003) Significant association of Rho/ROCK pathway with invasion and metastasis of bladder. Cancer Clin Cancer Res 9:2632–2641

Kandabashi T, Shimokawa H, Miyata K, Kunihiro I, Eto Y, Morishige K, Matsumoto Y, Obara K, Nakayama K, Takahashi S, Takeshita A (2003) Evidence for protein kinase C-mediated activation of Rho-kinase in a porcine model of coronary artery spasm. Arterioscler Thromb Vasc Biol 23:2209–2214

Kaplan SA, Te AE, Blaivas JG (1995) Urodynamic findings in patients with diabetic cystopathy. J Urol 153:342–344

Kawada N, Seki S, Kuroki T, Kaneda K (1999) ROCK Inhibitor Y-27632 attenuates stellate cell contraction and portal pressure increase induced by endothelin-1. Biochem Bioph Res Co 266:296–300

Kawano Y, Fukata Y, Oshiro N, Amano M, Nakamura T, Ito M, Matsumura F, Inagaki M, Kaibuchi K (1999) Phosphorylation of myosin-binding subunit (MBS) of myosin phosphatase by Rho-kinase in vivo. J Cell Biol 147:1023–1038

Kebapci N, Yenilmez A, Efe B, Entok E, Demirustu C (2007) Bladder dysfunction in type 2 diabetic patients. Neurourol Urodyn 26:814–819

Khalil R, Lodge N, Saida K, van Breeman C (1987) Mechanism of calcium activation in vascular smooth muscle. J Hypertens Suppl 5:S5–15

Kimura K, Ito M, Amano M, Chihara K, Fukata Y, Nakafuku M, Yamamori B, Feng J, Nakano T, Okawa K, Iwamatsu A, Kaibuchi K (1996) Regulation of myosin phosphatase by Rho and Rho-associated kinase (Rho-kinase). [See comments]. Science 273:245–248

Kitazawa T, Masuo M, Somlyo AP (1991) G Protein-mediated inhibition of myosin light-chain phosphatase in vascular smooth muscle. Proc Natl Acad Sci USA 88:9307–9310

Kizub IV, Pavlova OO, Johnson CD, Soloviev AI, Zholos AV (2010) Rho kinase and protein kinase C involvement in vascular smooth muscle myofilament calcium sensitization in arteries from diabetic rats. Br J Pharmacol 159:1724–1731

Kodama M, Takimoto Y (2000) Influence of 5-Hydroxytryptamine and the effect of a new serotonin receptor antagonist (Sarpogrelate) on detrusor smooth muscle of streptozotocin-induced diabetes mellitus in the rat. Int J Urol 7:231–235

Kojima K, Kume H, Ito S, Oguma T, Shiraki A, Kondo M, Ito Y, Shimokata K (2007) Direct effects of hydrogen peroxide on airway smooth muscle tone: roles of Ca2+ influx and Rho-kinase. Eur J Pharmacol 556:151–156

Koyama M, Ito M, Feng J, Seko T, Shiraki K, Takase K, Hartshorne DJ, Nakano T (2000) Phosphorylation of CPI-17, an inhibitory phosphoprotein of smooth muscle myosin phosphatase, by Rho-Kinase. FEBS Lett 475:197–200

Kropp BP, Zhang Y, Tomasek JJ, Cowan R, Furness PD, Vaughan MB, Parizi M, Cheng EY (1999) Characterization of cultured bladder smooth muscle cells: assessment of in vitro contractility. J Urol 162:1779–1784

Kureishi Y, Kobayashi S, Amano M, Kimura K, Kanaide H, Nakano T, Kaibuchi K, Ito M (1997) Rho-associated kinase directly induces smooth muscle contraction through myosin light chain phosphorylation. J Biol Chem 272:12257–12260

Leung T, Manser E, Tan L, Lim L (1995) A novel serine/threonine kinase binding the Ras-related RhoA GTPase which translocates the kinase to peripheral membranes. J Biol Chem 270:29051–29054

Levent A, Buyukafsar K (2004) Expression of Rho-kinase (ROCK-1 and ROCK-2) and its substantial role in the contractile activity of the sheep ureter. Br J Pharmacol 143:431–437

Levin RM, Brading AF, Mills IW, Longhurst PA (2000) Experimental models of bladder outlet obstruction. In: Lepor H (ed) Prostatic diseases. WB Saunders, Philadelphia, PA, p 169

Li L, Eto M, Lee MR, Morita F, Yazawa M, Kitazawa T (1998) Possible involvement of the novel CPI-17 protein in protein kinase C signal transduction of rabbit arterial smooth muscle. J Physiol 508:871–881

Li S, Hydery T, Juan Y, Lin WY, Kogan B, Mannikarottu A, Leggett RE, Schuler C, Levin RM (2009) The effect of 2- and 4-week ovariectomy on female rabbit urinary bladder function. Urology 74:691–696

Lin WY, Mannikarottu A, Chichester P, Neuman P, Johnson A, Perez-Martinez FC, Levin RM (2008) The effect of chronic partial bladder outlet obstruction on corpus cavernosum smooth muscle and Rho-kinase in rabbits. Neurourol Urodyn 27:826–831

Litten RZ, Suba EA, Roth BL (1987) Effects of a phorbol ester on rat aortic contraction and calcium influx in the presence and absence of BAY k 8644. Eur J Pharmacol 144:185–191

Lockman K, Hinson JS, Medlin MD, Morris D, Taylor JM, Mack CP (2004) Sphingosine 1-phosphate stimulates smooth muscle cell differentiation and proliferation by activating separate serum response factor co-factors. J Biol Chem 279:42422–42430

Longhurst PA, Belis JA (1986) Abnormalities of rat bladder contractility in streptozotocin-induced diabetes mellitus. J Pharmacol Exp Ther 238:773–777

MacDonald JA, Borman MA, Muranyi A, Somlyo AV, Hartshorne DJ, Haystead TA (2001a) Identification of the endogenous smooth muscle myosin phosphatase-associated kinase. Proc Natl Acad Sci USA 98:2419–2424

MacDonald JA, Eto M, Borman MA, Brautigan DL, Haystead TA (2001b) Dual ser and thr phosphorylation of CPI-17, an inhibitor of myosin phosphatase, by MYPT-associated kinase. FEBS Lett 493:91–94

Martinez JJ, Hultgren SJ (2002) Requirement of Rho-family GTPases in the invasion of type 1-piliated uropathogenic *Escherichia coli*. Cell Microbiol 4:19–28

Michael L, Sweeney DE, Davies JA (2005) A role for microfilament-based contraction in branching morphogenesis of the ureteric bud. Kidney Int 68:2010–2018

Mondet F, Chartier-Kastler EJ, Conort P, Bitker MO, Chatelain C, Richard F (2001) Anatomic and functional results of transperitoneal-transvesical vesicovaginal fistula repair. Urology 58:882–886

Morelli A, Filippi S, Sandner P, Fibbi B, Chavalmane AK, Silvestrini E, Sarchielli E, Vignozzi L, Gacci M, Carini M, Vannelli GB, Maggi M (2009) Vardenafil modulates bladder contractility through CGMP-mediated inhibition of RhoA/Rho Kinase signaling pathway in spontaneously hypertensive rats. J Sex Med 6:1594–1608

Morgan JP, Morgan KG (1984) Stimulus-specific patterns of intracellular calcium levels in smooth muscle of ferret portal vein. J Physiol 351:155–167

Muranyi A, Derkach D, Erdodi F, Kiss A, Ito M, Hartshorne DJ (2005) Phosphorylation of Thr695 and Thr850 on the myosin phosphatase target subunit: inhibitory effects and occurrence in A7r5 cells. FEBS Lett 579:6611–6615

Myung SC, Lee MY, Lee SY, Yum SH, Park SH, Kim SC (2008) Contractile changes of the clitoral cavernous smooth muscle in female rabbits with experimentally induced overactive bladder. J Sex Med 5:1088–1096

Nakagawa O, Fujisawa K, Ishizaki T, Saito Y, Nakao K, Narumiya S (1996) ROCK-I and ROCK-II, two isoforms of Rho-associated coiled-coil forming protein serine/threonine kinase in mice. FEBS Lett 392:189–193

Nakanishi K, Kamai T, Mizuno T, Arai K, Yamanishi T (2009) Expression of RhoA MRNA and activated RhoA in urothelium and smooth muscle, and effects of a Rho-kinase inhibitor on contraction of the porcine urinary bladder. Neurourol Urodyn 28:521–528

Nobe K, Yamazaki T, Kumai T, Okazaki M, Iwai S, Hashimoto T, Kobayashi S, Oguchi K, Honda K (2008) Alterations of glucose-dependent and -independent bladder smooth muscle contraction in spontaneously hypertensive and hyperlipidemic rat. J Pharmacol Exp Ther 324:631–642

Nobe K, Yamazaki T, Tsumita N, Hashimoto T, Honda K (2009) Glucose-dependent enhancement of diabetic bladder contraction is associated with a Rho kinase-regulated protein kinase C pathway. J Pharmacol Exp Ther 328:940–950

Nordling J (1994) Definition of prostatic urethral obstruction. Urol Res 22:267–271
Okamoto Y, Sekine T (1981) Light chain phosphorylation alters the N terminal structure of gizzard myosin heavy chain in an ATP dependent manner. J Biochem 89:697–700
Olenik C, Barth H, Just I, Aktories K, Meyer DK (1997) Gene expression of the small GTP-binding proteins RhoA, RhoB, Rac1, and Cdc42 in adult rat brain. Brain Res Mol Brain Res 52:263–269
Parekh MH, Chichester P, Lobel RW, Aikawa K, Levin RM (2004) Effects of castration on female rabbit bladder physiology and morphology. Urology 64:1048–1051
Persson K, Pandita RK, Spitsbergen JM, Steers WD, Tuttle JB, Andersson KE (1998) Spinal and peripheral mechanisms contributing to hyperactive voiding in spontaneously hypertensive rats. Am J Physiol 275:R1366–R1373
Quinn T, Collins C, Baird AW (2004) Mechanisms of neurokinin A- and substance P-induced contractions in rat detrusor smooth muscle in vitro. BJU Int 94:651–657
Rajasekaran M, Wilkes N, Kuntz S, Albo E (2005) Rho-kinase inhibition suppresses bladder hyperactivity in spontaneously hypertensive rats. Neurourol Urodyn 24:295–300
Ratz PH, Berg KM, Urban NH, Miner AS (2005) Regulation of smooth muscle calcium sensitivity: KCl as a calcium-sensitizing stimulus. Am J Physiol Cell Physiol 288:C769–C783
Ratz PH, Meehl JT, Eddinger TJ (2002) RhoA kinase and protein kinase C participate in regulation of rabbit stomach fundus smooth muscle contraction. Br J Pharmacol 137:983–992
Robinson D, Cardozo LD (2003) The role of estrogens in female lower urinary tract dysfunction. Urology 62:45–51
Roosen A, Wu C, Sui GP, Fry CH (2008) Synergistic effects in neuromuscular activation and calcium-sensitization in the bladder trigone. BJU Int 101:610–614
Rouissi N, Nantel F, Drapeau G, Rhaleb NE, Dion S, Regoli D (1990) Inhibitors of peptidases: how they influence the biological activities of substance P neurokinins, bradykinin and angiotensin in guinea pig, hamster and rat urinary bladders. Pharmacology 40: 196–204
Saito M, Kato T, Kondo A, Miyake K (1992) Response of human urinary bladders to angiotensins: comparison between neurogenic and control bladders. Hinyokika Kiyo 38:525–530
Sakurada S, Okamoto H, Takuwa N, Sugimoto N, Takuwa Y (2001) Rho activation in excitatory agonist-stimulated vascular smooth muscle. Am J Physiol Cell Physiol 281:C571–C578
Salomone S, Yoshimura S, Reuter U, Foley M, Thomas SS, Moskowitz MA, Waeber C (2003) S1P3 Receptors mediate the potent constriction of cerebral arteries by sphingosine-1-phosphate. Eur J Pharmacol 469:125–134
Sauzeau V, Le JH, Cario-Toumaniantz C, Vaillant N, Gadeau AP, Desgranges C, Scalbert E, Chardin P, Pacaud P, Loirand G (2000) P2Y(1), P2Y(2), P2Y(4), and P2Y(6) receptors are coupled to Rho and Rho kinase activation in vascular myocytes. Am J Physiol Heart Circ Physiol 278:H1751–H1761
Schneider T, Fetscher C, Krege S, Michel MC (2004) Signal transduction underlying carbachol-induced contraction of human urinary bladder. J Pharmacol Exp Ther 309:1148–1153
Schneider T, Hein P, Bai J, Michel MC (2005) A role for muscarinic receptors or Rho-kinase in hypertension associated rat bladder dysfunction? J Urol 173:2178–2181
Shabir S, Borisova L, Wray S, Burdyga T (2004) Rho-kinase inhibition and electromechanical coupling in rat and guinea-pig ureter smooth muscle: Ca2+-dependent and -independent mechanisms. J Physiol 560:839–855
Shimizu H, Ito M, Miyahara M, Ichikawa K, Okubo S, Konishi T, Naka M, Tanaka T, Hirano K, Hartshorne DJ (1994) Characterization of the myosin-binding subunit of smooth muscle myosin phosphatase. J Biol Chem 269:30407–30411
Shirazi A, Iizuka K, Fadden P, Mosse C, Somlyo AP, Somlyo AV, Haystead TA (1994) Purification and characterization of the mammalian myosin light chain phosphatase holoenzyme. The differential effects of the holoenzyme and its subunits on smooth muscle. J Biol Chem 269:31598–31606

Soliman H, Craig GP, Nagareddy P, Yuen VG, Lin G, Kumar U, McNeill JH, MacLeod KM (2008) Role of inducible nitric oxide synthase in induction of RhoA expression in hearts from diabetic rats 1. Cardiovasc Res 79:322–330

Somlyo AP, Kitazawa T, Himpens B, Matthijs G, Horiuti K, Kobayashi H, Goldman YE, Somlyo AV (1989) Modulation of Ca2+-sensitivity and of the time course of contraction in smooth muscle: a major role of protein phosphatases? Adv Protein Phosphatases 5:181–195

Spitsbergen JM, Clemow DB, McCarty R, Steers WD, Tuttle JB (1998) Neurally mediated hyperactive voiding in spontaneously hypertensive rats. Brain Res 790:151–159

Steidle CP, Cohen ML, Neubauer BL (1990) Bradykinin-induced contractions of canine prostate and bladder: effect of angiotensin-converting enzyme inhibition. J Urol 144:390–392

Su X, Changolkar A, Chacko S, Moreland RS (2004) Diabetes decreases rabbit bladder smooth muscle contraction while increasing levels of myosin light chain phosphorylation. Am J Physiol Renal Physiol 287:F690–F699

Suematsu E, Hirata M, Sasaguri T, Hashimoto T, Kuriyama H (1985) Roles of Ca2+ on the inositol 1, 4, 5-trisphosphate-induced release of Ca2+ from saponin-permeabilized single cells of the porcine coronary artery. Comp Biochem Physiol A Comp Physiol 82:645–649

Takahashi N, Shiomi H, Kushida N, Liu F, Ishibashi K, Yanagida T, Shishido K, Aikawa K, Yamaguchi O (2009) Obstruction alters muscarinic receptor-coupled RhoA/Rho-kinase pathway in the urinary bladder of the rat. Neurourol Urodyn 28:257–262

Takahashi R, Nishimura J, Hirano K, Seki N, Naito S, Kanaide H (2004) Ca2+ sensitization in contraction of human bladder smooth muscle. J Urol 172:748–752

Takakashi R, Nishimura J, Hirano K, Seki N, Naito S, Kanaide H (2004) Ca2+ Sensitization in contraction of human bladder smooth muscle. J Urol 172:748–752

Tammela TL, Briscoe JA, Levin RM, Longhurst PA (1994) Factors underlying the increased sensitivity to field stimulation of urinary bladder strips From streptozotocin-induced diabetic rats. Br J Pharmacol 113:195–203

Tamura I, Rosenbloom J, Macarak E, Chaqour B (2001) Regulation of Cyr61 gene expression by mechanical stretch through multiple signaling pathways. Am J Physiol Cell Physiol 281: C1524–C1532

Tanabe N, Ueno A, Tsujimoto G (1993) Angiotensin II receptors in the rat urinary bladder smooth muscle: type 1 subtype receptors mediate contractile responses. J Urol 150:1056–1059

Tatsumiya K, Yamanishi T, Watanabe M, Masuda A, Mizuno T, Kamai T, Yoshida K (2009) Effects of fasudil, a Rho-kinase inhibitor, on contraction of pig bladder tissues with or without urothelium. Int J Urol 16:959–966

Teixeira CE, Jin L, Priviero FB, Ying Z, Webb RC (2007) Comparative pharmacological analysis of Rho-kinase inhibitors and identification of molecular components of Ca2+ sensitization in the rat lower urinary tract. Biochem Pharmacol 74:647–658

Turna B, Cinar MG, Canda AE, Orhan EC, Tiftik NR, Nazli O, Buyukafsar K (2007) Role of Rho-kinase in contractions of ureters from rabbits with unilateral ureteric obstruction. BJU Int 100:1166–1171

Van AL, D'Souza-Schorey C (1997) Rho GTPases and signaling networks. Genes Dev 11:2295–2322

Velasco G, Armstrong C, Morrice N, Frame S, Cohen P (2002) Phosphorylation of the regulatory subunit of smooth muscle protein phosphatase 1 M at Thr850 induces its dissociation from myosin. FEBS Lett 527:101–104

Wang T, Kendig DM, Smolock EM, Moreland RS (2009) Carbachol-induced rabbit bladder smooth muscle contraction: roles of protein kinase C and Rho kinase. Am J Physiol Renal Physiol 297:F1534–F1542

Waring JV, Wendt IR (2000) Effects of streptozotocin-induced diabetes mellitus on intracellular calcium and contracttion of longitudinal smooth muscle from rat urinary bladder. J Urol 163:323–330

Wibberley A, Chen Z, Hu E, Hieble JP, Westfall TD (2003a) Expression and functional role of Rho-kinase in rat urinary bladder smooth muscle. Br J Pharmacol 138:757–766

Wibberley A, Chen Z, Hu E, Hieble JP, Westfall TD (2003b) Expression and functional role of Rho-kinase in rat urinary bladder smooth muscle. Br J Pharmacol 138:757–766

Xie Z, Gong MC, Su W, Xie D, Turk J, Guo Z (2010) Role of calcium-independent phospholipase A2beta in high glucose-induced activation of RhoA, Rho kinase, and CPI-17 in cultured vascular smooth muscle cells and vascular smooth muscle hypercontractility in diabetic animals. J Biol Chem 285:8628–8638

Yang Z, Dolber PC, Fraser MO (2007) Diabetic urethropathy compounds the effects of diabetic cystopathy. J Urol 178:2213–2219

Ye G, Jin X (1997) Action of autonomic drugs on the in vivo bladder base and proximal urethra in dog. Chin Med J (Engl) 110:173–176

Yoon H, Lee D, Chun K, Yoon H, Yoo J (2010) Effect of stress on the expression of Rho-kinase and collagen in rat bladder tissue. Korean J Urol 51:132–138

Yu HJ, Chien CT, Lai YJ, Lai MK, Chen CF, Levin RM, Hsu SM (2004) Hypoxia preconditioning attenuates bladder overdistension-induced oxidative injury by up-regulation of Bcl-2 in the rat. J Physiol 554:815–828

Zong H, Raman N, Mickelson-Young LA, Atkinson SJ, Quilliam LA (1999) Loop 6 of RhoA confers specificity for effector binding, stress fiber formation, and cellular transformation. J Biol Chem 274:4551–4560

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