Urheberrechtlich geschütztes Material

Advances in Anatomy
Embryology and Cell Biology
185

W. Neuhuber · M. Raab
H.-R. Berthoud · J. Wörl

Innervation of the Mammalian Esophagus

Urheberrechtlich geschütztes Material

185
Advances in Anatomy Embryology and Cell Biology

Editors

F. F. Beck, Melbourne · B. Christ, Freiburg
F. Clascá, Madrid · D. E. Haines, Jackson
H.-W. Korf, Frankfurt · W. Kummer, Giessen
E. Marani, Leiden · R. Putz, München
Y. Sano, Kyoto · T. H. Schiebler, Würzburg
K. Zilles, Düsseldorf

W. L. Neuhuber · M. Raab ·
H.-R. Berthoud · J. Wörl

Innervation of the Mammalian Esophagus

With 14 Figures

Prof. Dr. Winfried L. Neuhuber
Dr. Marion Raab
Dr. Jürgen Wörl

Institute of Anatomy
University of Erlangen-Nuremberg
Krankenhausstr. 9
91054 Erlangen
Germany

e-mail: winfried.neuhuber@anatomie1.med.uni-erlangen.de

Prof. Dr. Hans-Rudolf Berthoud

Pennington Biomedical Research Center
Louisiana State University System
6400 Perkins Road
Baton Rouge, LA 70808
USA

ISSN 0301-5556
ISBN-10 3-540-29205-5 Springer Berlin Heidelberg New York
ISBN-13 978-3-540-29205-0 Springer Berlin Heidelberg New York

This work is subject to copyright. All rights reserved, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilm or in any other way, and storage in data banks. Duplication of this publication or parts thereof is permitted only under the provisions of the German Copyright Law of September, 9, 1965, in its current version, and permission for use must always be obtained from Springer-Verlag. Violations are liable for prosecution under the German Copyright Law.

Springer is a part of Springer Science+Business Media
springeronline.com
© Springer-Verlag Berlin Heidelberg 2006
Printed in Germany

The use of general descriptive names, registered names, trademarks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.
Product liability: The publisher cannot guarantee the accuracy of any information about dosage and application contained in this book. In every individual case the user must check such information by consulting the relevant literature.

Editor: Simon Rallison, Heidelberg
Desk editor: Anne Clauss, Heidelberg
Production editor: Nadja Kroke, Leipzig
Cover design: design & production GmbH, Heidelberg
Typesetting: LE-TEX Jelonek, Schmidt & Vöckler GbR, Leipzig
Printed on acid-free paper SPIN 11533467 27/3150/YL – 5 4 3 2 1 0

Acknowledgements

Work in the authors' laboratory was supported by Deutsche Forschungsgemeinschaft SFB 353 B9 and B15 (to W.L.N.), Swiss National Foundation (to W.L.N.), Johannes and Frieda Marohn-Stiftung, Erlangen (to M.R. and J.W.) and NIH grants DK 47348 and 57242 (to H.R.B.). Special thanks to Sabine Richter (Zurich) and Andrea Hilpert and Anita Hecht (Erlangen) for excellent technical assistance.

List of Contents

1	**Introduction**	1
1.1	Muscle Layers of the Esophagus	2
2	**Materials and Methods**	3
2.1	Anterograde WGA-HRP Tracing from the Nodose Ganglion	3
2.2	Anterograde DiI Tracing from Thoracic Dorsal Root Ganglia	5
3	**Extrinsic Innervation**	5
3.1	Vagal Innervation	5
3.1.1	Efferent Vagal Innervation	5
3.1.1.1	Nucleus Ambiguus	5
3.1.1.2	Dorsal Motor Nucleus	7
3.1.2	Afferent Vagal Innervation	8
3.1.2.1	Vagal Afferent Structures in the Tunica Muscularis	8
3.1.2.2	Vagal Afferent Innervation of Mucosa and Submucosa	31
3.1.2.3	Central Projections of Vagal Afferents from the Esophagus	34
3.1.2.4	Vagal Nociceptors	36
3.2	Spinal Innervation	36
3.2.1	Efferent Spinal Innervation	36
3.2.2	Afferent Spinal Innervation	37
3.2.2.1	Functional Aspects	39
3.3	Innervation of Esophageal Sphincters	41
3.3.1	Upper Esophageal Sphincter	41
3.3.2	Lower Esophageal Sphincter	41
3.4	Swallowing Central Pattern Generator	43
3.5	Cortical Representation of the Esophagus in Human	44
4	**Intrinsic Innervation**	45
4.1	General Organization	45
4.2	Enteric Coinnervation	46
4.2.1	Spatial Relationships of Vagal and Enteric Terminals and Neurochemical Coding	47
4.2.2	Comparative Anatomy of Enteric Coinnervation	48
4.2.3	Ontogeny of the Esophageal Neuromuscular Junction	49
5	**Functional Considerations**	51
5.1	General Remarks	51
5.2	Cooperation Between Extrinsic and Intrinsic Systems	51

6 Concluding Remarks 55

References 56

Subject Index 75

Abbreviations

(The abbreviations apply to all figures.)

ACh	Acetylcholine
AChE	Acetylcholine esterase
AMB	Nucleus ambiguus
AMBc	Nucleus ambiguus, compact formation
ASIC	Acid-sensitive ion channels
CGRP	Calcitonin gene-related peptide
ChAT	Choline acetyl transferase
Co-GOD	Cobalt glucose oxidase
DAB	Diamino benzidine
DEA-NO	Diethylamine-NO
DiA	4-(4-Dihexadecyl-aminostyryl)-*N*-methylpyridinium iodide
DiI	1,1′-Dioleyl-3,3,3′,3′-tetramethylindocarbocyanine methanosulfonate
DMPG	Deglutitive motor pattern generator
DMX	Dorsal motor nucleus of vagus nerve
E	Embryonic day
GABA	γ-Aminobutyric acid
GAL	Galanin
GERD	Gastroesophageal reflux disease
GFAP	Glial fibrillary acidic protein
ICC	Interstitial cells of Cajal
IGLE	Intraganglionic laminar ending
IMAs	Intramuscular arrays
LES	Lower esophageal sphincter
L-NAME	N^G-nitro-L-arginine methyl ester
L-NNA	Nω-Nitro-L-arginine
mAChR	Muscarinic acetylcholine receptor
mGluR	Metabotropic glutamate receptor
M-ENK	Met-enkephalin
NADPH-d	Nicotinamide adenine dinucleotide phosphate diaphorase
NKA	Neurokinin A
NK-1R	Neurokinin-1 receptor
NO	Nitric oxide

nNOS	Neuronal nitric oxide synthase
NONOate	[Z]-1-[2-aminoethyl]-*N*-[2-aminoethyl]diazen-1-ium-1,2-diolate
NPY	Neuropeptide Y
NT	Neurotrophin
NTS	Nucleus tractus solitarii
NTSce	NTS subnucleus centralis
NTSim	NTS subnucleus intermedius
NTSis	NTS subnucleus interstitialis
P	Postnatal day
RLN	Recurrent laryngeal nerve
SLN	Superior laryngeal nerve
SP	Substance P
TMB	Tetramethyl benzidine
Trk	Tyrosine receptor kinase
TRP	Transient receptor potential
UES	Upper esophageal sphincter
VAChT	Vesicular acetylcholine transporter
VGLUT	Vesicular glutamate transporter
VIP	Vasoactive intestinal peptide
VMAT	Vesicular monoamine transporter
WGA-HRP	Wheat germ agglutinin-horseradish peroxidase conjugate

1 Introduction

The esophagus is a relatively simple though vital organ. It consists of a two-layered muscular tube whose lumen is lined by squamous stratified epithelium. Beyond its role of propelling food from the pharynx to the stomach by a propulsive contraction wave representing the esophageal phase of deglutition (Conklin and Christensen 1994; Jean 2001), it is more and more recognized as a sensory organ from which a variety of respiratory and cardiovascular reflexes can be triggered, thus cooperating with the larynx in protecting the lower airways from aspiration (Barthélémy et al. 1996; Lang et al. 2002; Lang et al. 2001; Loomis et al. 1997; Medda et al. 2003). In ruminants, there is additional antiperistalsis for regurgitation. During emesis, the esophagus is a merely passive conduit except for some antiperistalsis in its upper part. In the interval between swallows, both oral and aboral ends of the esophagus are tonically closed by the upper and lower esophageal sphincters, UES and LES respectively, while the tubular esophagus is flaccid and partly filled with air. Despite this apparent simplicity, neuronal control of esophageal functions is quite complex.

Esophageal swallowing requires the well-coordinated opening of the UES, oral-to-aboral peristalsis, and opening of the LES. These events are organized by a central pattern generator in the brainstem and controlled by vago–vagal reflexes making the esophagus highly dependent on extrinsic vagal innervation (Bieger 1993; Chang et al. 2003; Conklin and Christensen 1994; Jean 2001; Miller 1986). In addition, the esophagus contains, as all other organs of the gastrointestinal tract, enteric ganglia providing a local neuronal network for motility control (Conklin and Christensen 1994). Although the esophagus harbors some mucous glands, and its blood vessels receive both intrinsic and extrinsic innervation, the major task of the esophageal nerve tissue is motility control. Understanding the innervation of the esophagus is a prerequisite for successful treatment of a variety of disorders, e.g., dysphagia, achalasia, gastroesophageal reflux disease (GERD), and non-cardiac chest pain (Castell et al. 2004; Clouse et al. 1999; Orlando 2003; Orlando 2004; Qualman et al. 1984; Storr and Allescher 1999). In particular, GERD with its high prevalence of more than 10% represents a significant health problem. This review aims at summarizing current knowledge of anatomy of esophageal innervation and will focus on peculiarities of motor innervation of striated esophageal muscle, i.e., enteric coinnervation, and possible involvement of vagal afferent neurons in myenteric ganglionic circuitry. For a more extensive coverage, in particular of the older literature and of functional and clinical data, the reader may consult the classical handbook article by Stöhr (1957) or recent reviews (Chang et al. 2003; Conklin and Christensen 1994; Orlando 2003; Orlando 2004; Sengupta 2000).

The vagus nerve and branches from the sympathetic trunk and the celiac ganglion provide motor, preganglionic parasympathetic, and postganglionic sympathetic input, and these elements also carry numerous afferent fibers projecting

to the brainstem and spinal cord (Aharinejad and Firbas 1989; Collman et al. 1992; Collman et al. 1993; Fryscak et al. 1984; Hudson and Cummings 1985; Khurana and Petras 1991). A well developed ganglionated myenteric plexus is present in both smooth and striated muscle portions of the esophagus (Christensen and Robison 1982; Greving 1931; Gruber 1968). In the submucous plexus, ganglia are rare or even absent, especially in small mammals. Extrinsic motor innervation has to match the peculiar anatomy of the esophageal tunica muscularis which consists either entirely of striated muscle fibers or a mixture of striated and smooth muscle depending on the species. In contrast to the pharynx, the striated part of the esophagus also harbors a smooth muscle lamina muscularis mucosae which typically separates the mucosa from the submucosa all along the gastrointestinal tract.

1.1 Muscle Layers of the Esophagus

The tunica muscularis of the tubular esophagus in mammals consists of varying amounts of striated muscle fibers mixed with smooth muscle. This differs markedly from the esophagus of birds, which is typically built up of smooth muscle only (Shiina et al. 2005). In rodents, dogs, and sheep, striated muscle accounts for almost 100% of esophageal length, while in the cat, opossum, dolphin, macaque, and human the striated portion is more or less confined to the cervical esophagus and blends with smooth muscle in its thoracic portion (Shiina et al. 2005; Wörl and Neuhuber 2005a). Insertion of striated fibers with elastic brush-like tendons into the connective tissue layers of the smooth muscle portion was supposed to dampen the fast contraction twitches, thus smoothening the transition of contraction waves from the striated to the smooth muscle part of the esophagus (Rohen 1955). Rather than separable into an outer longitudinal and inner circular muscle layer, the tunica muscularis of the esophagus consists of spiraling chains of fiber bundles of various steepness (Kaufmann et al. 1968). Outer and inner spirals are twisted in opposite directions and at places blend into each other. Striated esophageal muscle fibers are of the twitch type, although fiber-type composition varies among species (Wörl and Neuhuber 2005a).

In late embryonic and fetal life, the tunica muscularis becomes gradually transformed from smooth into striated muscle from oral to aboral. This transformation process extends to various levels in different species and is completed in rodents in the second postnatal week. The origin of striated esophageal muscle is a matter of debate. In contrast to striated muscle of the pharynx, it is neither branchiomeric nor of somitic origin. During the past decade arguments for both transdifferentiation of smooth into striated muscle fibers and myogenesis from separate striated muscle precursors have been put forward (Wörl and Neuhuber 2005b). However, a recent ultrastructural study demonstrated apoptosis of smooth muscle cells and also presence of mesenchymal putative striated muscle precursor cells, thus lending support to the hypothesis of replacement of smooth by newly generated striated muscle (Wörl and Neuhuber 2005b).

The UES is composed of the caudal portion of the inferior pharyngeal constrictor, in particular the cricopharyngeus muscle and also the uppermost esophageal muscle, the cricopharyngeus being the most important component (Lang and Shaker 2000). All these muscles are striated. In contrast, the LES consists of smooth muscle in all species, even in those with a striated tubular esophagus. In most species it is not a typical ring of thickened inner circular muscle layer but consists of two opposing forceps-like fiber bundles, which were described in the human LES as semicircular clasps at the lesser curve and gastric sling fibers at the greater curve, respectively (Liebermann-Meffert et al. 1979; Stein et al. 1995).

The lamina muscularis mucosae with its smooth muscle fibers extends from the pharyngo-esophageal junction throughout the whole esophagus and further the entire gastrointestinal tract. It becomes particularly thick in the abdominal esophagus (Clerc 1983b; Fryscak et al. 1984; Nagai et al. 2003).

In the paragraphs to follow, extrinsic and intrinsic innervation systems will be described as well as possible cooperation between the two. A brief summary of current knowledge about the central pattern generator controlling the esophageal phase of deglutition and of cortical representation of both efferent and afferent innervation of the esophagus will be included in the section on extrinsic innervation.

2 Materials and Methods

The methods used to gather the results presented in this review have been described in detail in the quoted publications, and some data are included from unpublished work performed at the Institute of Anatomy, University of Zürich on the tracing of ultrastructural wheat germ agglutinin-horseradish peroxidase (WGA-HRP) and 1,1′-dioleyl-3,3,3′,3′-tetramethylindocarbocyanine methanosulfonate (DiI). The methodological modification in ultrastructural WGA-HRP tracing relates to the protocol for identification of anterogradely transported WGA-HRP in IGLEs. In contrast to the tetramethylbenzidine (TMB) method as utilized in the original study (Neuhuber 1987), a combination of the cobalt-glucoseoxidase method (Co-GOD; Itoh et al. 1979) and a silver-gold intensification procedure (Ag-Au; Liposits et al. 1982) was adopted in these experiments. The advantage over the TMB method is a significantly better ultrastructural preservation, thus facilitating identification of specialized contacts of IGLEs with other neuronal and glial elements of enteric ganglia.

2.1 Anterograde WGA-HRP Tracing from the Nodose Ganglion

Pressure injections of 0.5 to 1.0 μl of 2% WGA-HRP (Sigma, Buchs, Switzerland) in saline through a glass micropipette into the right nodose ganglion or bilat-

erally were performed in Wistar rats (body weights 200–250 g; combined fluanisone/fentanyl/diazepam IM anesthesia; 2 rats with right nodose, 6 rats with bilateral injections; 3 rats without injection served as controls). All procedures were approved by the local governmental veterinary authorities of Zürich, Switzerland. In the two rats with right nodose injections, supranodose vagotomy was performed 14 days before ganglion injections. After 18 to 48 hours, rats were euthanized with pentobarbital IP and perfusion fixed with 800 ml of 1.25% glutaraldehyde and 1% paraformaldehyde in 0.1 M phosphate buffer after a prewash with saline. Perfusion was completed by a flush of 500 ml of phosphate buffer. Subdiaphragmatic esophagi and segments of the stomach were sliced on a vibratome at 80–100 μm and processed for combined Co-GOD/Ag-Au tracer detection. After osmication, specimens were dehydrated and flat embedded in epoxy resin according to standard protocols. Myenteric ganglia were identified in semithin sections stained with methylene blue, and ultrathin sections through ganglia stained with uranyl acetate were examined in Philips 300 (Philips, Eindhoven, Netherlands) or Zeiss EM 6 (Zeiss, Oberkochen, Germany) electron microscopes. Altogether, serial ultrathin sections from 12 myenteric ganglia of the subdiaphragmatic esophagus and two ganglia of the gastric fundus were examined. After combined Co-GOD/Ag-Au procedure, anterogradely transported WGA-HRP appeared as electron-dense, sharply demarcated profiles that were not observed in controls. Conventional prints of electron micrographs were scanned at a resolution of 600 dpi, and images were optimized for contrast and brightness using Adobe Photoshop CS, version 8.0.1 (Adobe, Unterschleissheim, Germany). To apply text and scale bars, and organize the final layouts for printing both Photoshop and CorelDraw (Corel, Dublin) software, version 11, were used.

In order to assess the overall regeneration speed of both efferent and afferent vagal axons in the rat, the cervical vagus nerve was sectioned about 5 mm distal to the nodose ganglion and immediately reanastomosed in another three rats anesthetized as described above, and 14 days were allowed for regeneration. Then, WGA-HRP was injected into nodose ganglia and the anterograde transport front was determined light microscopically after 2 days of survival by TMB histochemistry on cryostat sections of the vagus nerve along its course. Most of the labeled regenerating axons had regrown for a distance of about 10–15 mm, and only very few labeled growth cones were seen at the branching point of the left recurrent laryngeal nerve, i.e., about 30 mm distal to the point of vagus nerve reanastomosis. Thus, regenerating vagal nerve fibers grow at maximum for about 30 mm within 14 days, i.e., less than half the distance from the nodose ganglion to the subdiaphragmatic esophagus. Even if efferent vagal axons regenerating upon supranodose vagotomy in the rats that were used for ultrastructural studies would have had taken up and anterogradely transported WGA-HRP injected into the nodose ganglion, this would not have led to terminal labeling in the subdiaphragmatic esophagus. This, and previous evidence of negligible WGA-HRP uptake and anterograde transport by efferent fibers en passant (Neuhuber 1987), allowed for ascribing WGA-HRP terminal labeling in myenteric ganglia exclusively to IGLEs

connected to afferent axons originating from sensory neurons in the nodose ganglion. Moreover, recent studies indicated that afferent but not efferent sectioned vagal axons regenerate into their original terminal fields (Phillips et al. 2003; Powley et al. 2005).

2.2 Anterograde DiI Tracing from Thoracic Dorsal Root Ganglia

In four Wistar rats (body weights 350–450 g), lower thoracic dorsal root ganglia (T 10–12) were exposed through a dorsal midline incision and laminectomy under general fluanisone/fentanyl/diazepam anesthesia. Small amounts of DiI (Molecular Probes, Eugene, OR, USA) dissolved in ethanol (0.3–0.5 μl) were pressure injected through a glass micropipette uni- or bilaterally over several minutes. After wound closure, animals were allowed to recover and survived for 6 weeks. After IP introduction of a lethal dose of pentobarbital, rats were perfusion fixed with 500 ml of 4%-phosphate-buffered formaldehyde, and the esophagus and stomach were excised and stored in the same fixative until examined. Vibratome slices of segments of the esophagus and cardia 80–100 μm thick were coverslipped in glycerol-propylgallate and examined in a Biorad MRC 1000 (Bio-Rad, Hemel Hempstead, UK) confocal laser scanning unit attached to a Nikon Diaphot 300 inverted microscope (Nikon, Tokyo). The yellow line (568 nm) of the krypton-argon laser (ILC, Salt Lake City, UT, USA) was used to excite DiI whereas the blue line (488 nm) was used to generate green background fluorescence of tissues as a sort of counterstain. Merged two-channel images were converted to TIFF format using confocal assistant software and adjusted for contrast and brightness using Adobe Photoshop CS 8.01.

3 Extrinsic Innervation

3.1 Vagal Innervation

3.1.1 Efferent Vagal Innervation

3.1.1.1 Nucleus Ambiguus

Striated muscle fibers of the esophagus are innervated by neurons located in the nucleus ambiguus (AMB) in the ventrolateral medulla oblongata (Bieger and Hopkins 1987; Collman et al. 1993; Fryscak et al. 1984; Sang and Young 1998). Within this nucleus, they are clustered in the compact formation located at its rostral end (AMBc). There are some species differences as to the cytoarchitectonic partitioning of AMB and the detailed somatotopic arrangement of motor neurons

for esophagus, pharynx, and larynx among rat, rabbit, and cat, and presumably other species also (Lang et al. 2004). This may correlate with species-different subnuclear organization of premotor neurons in the nucleus tractus solitarii (NTS) and the wiring of the central pattern generator for swallowing (Gai et al. 1995; Jean 2001; Lang et al. 2004). Axons exiting the AMB typically travel first dorsomedially before turning ventrolaterally to their exit through the rootlets of the vagus nerve, thus forming a "genu" similar to axons of facial motoneurons (Bieger and Hopkins 1987; Kalia and Mesulam 1980). Axons reach the esophagus through the vagus nerve and its recurrent laryngeal branch (RLN; Gruber 1968) and, to a minor extent, superior laryngeal (SLN) nerves (Andrew 1956) and establish motor endplates on striated muscle fibers in the esophagus (Neuhuber et al. 1998; Ottaviani 1937/38; Stefanelli 1938). Although these motor endplates look at first glance like typical motor endplates of striated muscle, they are on average smaller and shallower than endplates in the pharynx or skeletal muscle and the unmyelinated portion of preterminal motor axons is longer than in skeletal muscle (Gruber 1968; Whitmore 1983; Whitmore and Notman 1987). This, among other peculiarities, was taken as indication of the unique nature of esophageal striated muscle. Acetylcholine is the prime transmitter as indicated by both functional (Bartlet 1968; Kantrowitz et al. 1970; Storr et al. 2001) and immunohistochemical evidence (Sang and Young 1997; Wörl et al. 2002). In addition, vagal motor terminals contain CGRP (Neuhuber et al. 1994; Sang and Young 1998) and VGLUT1 (Kraus et al. 2004). The latter suggests that glutamate acts as a co-transmitter in striated esophagus. There is no indication that AMB contributes to innervation of myenteric ganglia (Neuhuber et al. 1998; Sang and Young 1998).

Motor endplates in the rat esophagus from the nucleus ambiguus, as demonstrated by anterograde tracing with DiI, were distributed largely to the ipsilateral half of the organ (Neuhuber et al. 1998). However, at upper and lower thoracic levels, some motor axons crossed the midline to establish endplates contralaterally, too. In a study using electrical stimulation of the rat vagus nerve and the glycogen-depletion method for demonstrating activated muscle fibers, unilateral right or left stimulation were each shown to excite 50% of muscle fibers distributed around the circumference of the esophagus, thus indicating extensive right/left overlap of motor innervation (Gruber 1978).

Esophageal motor neurons in the AMBc are smaller than pharyngeal motorneurons in the semicompact formation of AMB. Their dendrites are bundled rostrocaudally, thus presumably favoring synchronous activity (Hayakawa et al. 1996; Hopkins 1995). Synchronization of neuronal activity during swallowing may also be facilitated by gap junctions, which are found in about 30% of motor neurons in the compact formation (Lewis 1994). Ultrastructural analysis of neurons partly identified by retrograde tracing from pharynx, cervical, and abdominal esophagus revealed that those innervating the abdominal esophagus were contacted by a majority of Gray's type I (presumptive excitatory) synapses, while motor neurons for both the cervical esophagus and pharynx received Gray's type I and type II

(presumptive inhibitory) synapses to about an equal extent (Hayakawa et al. 1996; Hopkins 1995; Saxon et al. 1996). Although the significance of this finding is unclear at present, it may help in interpreting the increasing rate of enteric coinnervation in lower parts of the esophagus (see below).

3.1.1.2 Dorsal Motor Nucleus

Smooth muscle fibers of both the tunica muscularis, in species with a "mixed" esophagus muscle coat, and the lamina muscularis mucosae are innervated by enteric motor neurons commanded by preganglionic neurons of the dorsal motor nucleus of the vagus (DMX). In all species, smooth muscle of the LES is controlled by DMX neurons via an enteric relay. As the lamina muscularis mucosae is also present in the striated muscle portion of the esophagus, DMX projections to this part were likewise to be expected. Retrograde tracing studies in rat and dog (both with an entirely striated tunica muscularis) and cat (with a mixed tunica muscularis) resulted in labeled cell bodies in the DMX, although tracer injections into the cervical esophagus were usually less effective than into its abdominal part (Collman et al. 1993; Fryscak et al. 1984; Hudson and Cummings 1985; Sang and Young 1998). Esophageal preganglionic neurons are concentrated in the rostral and caudal portions of the DMX. There is evidence that the rostral cluster provides excitatory, the caudal one inhibitory input to the tubular esophagus and also the LES (Chang et al. 2003; Hornby and Abrahams 2000; Hyland et al. 2001; Rossiter et al. 1990). Excitation and inhibition apparently results from preganglionic cholinergic projections respectively onto cholinergic and nitrergic enteric neurons. Vagally induced contraction of smooth muscle is mediated through enteric cholinergic neurons and muscarinic ACh receptors on the postganglionic side and develops more slowly than the fast contraction of striated muscularis propria, which is typically mediated by nicotinic ACh receptors (Bieger and Triggle 1985; Storr et al. 2001; Watson et al. 1995).

Anterograde tracing with DiA from the DMX of the rat labeled varicose fibers in less than 10% of myenteric ganglia in the esophagus, although almost 100% of myenteric ganglia in the stomach were endowed with labeled fibers in the same animals (Neuhuber et al. 1998). This suggested a sparse vagal preganglionic supply to the esophagus in the rat. However, electrical stimulation of the vagus nerve resulted in prompt activation of the immediate/early gene c-fos in myenteric neurons of the rat esophagus (Zheng et al. 1997). This discrepancy may be partly explained by underestimation of preganglionic innervation in the anterograde tracing study due to inadequate access of tracer to esophageal DMX neurons. Intriguingly, anterogradely labeled preganglionic terminals were more often found in myenteric ganglia of the cervical than more caudal parts of the esophagus (Neuhuber et al. 1998). This contrasts with data from retrograde tracing experiments that indicated weak or even no projections from the DMX to the cervical esophagus of the rat and significant input only to the abdominal por-

tions (Collman et al. 1993; Fryscak et al. 1984). This was interpreted as reflecting greater thickness of the lamina muscularis mucosae in the abdominal esophagus or preganglionic innervation mainly devoted to the LES. Nevertheless, since both retrograde and anterograde tracing methods have their quantitative limitations, the complementary data from all these experiments should be taken as evidence for preganglionic innervation from the DMX to both striated and smooth muscle esophagus portions and significant preganglionic vagal innervation from DMX even in species with entirely striated tunica muscularis. In particular, enteric coinnervation of striated esophageal muscle fibers that originates in myenteric ganglia (Neuhuber et al. 1994) allows for interpreting DMX projections to striated esophagus not only destined to control of smooth lamina muscularis mucosae but also of striated tunica muscularis. Data from anterograde tracing studies lacking, preganglionic vagal innervation of enteric ganglia in species with mixed striated/smooth muscle esophagus, e.g., cat, opossum or human, is presumably even more pronounced.

3.1.2
Afferent Vagal Innervation

Afferent vagal neurons innervate all layers of the esophageal wall. Their perikarya reside in the nodose-jugular-petrosal complex and their axons reach the esophagus via esophageal rami of the vagus nerve and the superior and recurrent laryngeal nerves. Although vagal afferents were hitherto considered to represent mainly low threshold sensors serving as the afferent limb in reflexes controlling the "physiological" functions of the esophagus (Andrews and Lang 1982; Clarke and Davison 1975; Sengupta et al. 1989), there are recent indications of a significant population of vagal nociceptors innervating the esophagus (Yu et al. 2005). The most prominent vagal afferent structures in the muscle coat of the esophagus (and also the rest of the digestive tract) are the so called intraganglionic laminar endings (IGLEs; Berthoud et al. 1997a; Wang and Powley 2000) that were recently identified as sites of low threshold mechanosensory transduction (Zagorodnyuk and Brookes 2000; Zagorodnyuk et al. 2001). In contrast to earlier reports (Asaad et al. 1983; Slawik 1942), muscle spindles could not be found either in the esophageal body or the UES (Bonington et al. 1988; Neuhuber 1987). Also of particular interest is the rather dense vagal innervation of the mucosa in the upper esophagus displaying a variety of distinct terminal structures (Dütsch et al. 1998; Wank and Neuhuber 2001).

3.1.2.1
Vagal Afferent Structures in the Tunica Muscularis

Intraganglionic Laminar Endings

The term intraganglionic laminar ending (IGLE) was coined by José Rodrigo (Rodrigo et al. 1975b) in order to describe a bizarre leafy structure equipped with

numerous delicate spinous extensions in myenteric ganglia of the feline esophagus impregnated with the zinc-iodide-osmium technique (Champy-Maillet stain). These leaflets originated from relatively coarse myelinated branching nerve fibers and more or less extensively covered enteric ganglia sandwiched between outer and inner layers of the tunica muscularis (Rodrigo et al. 1975b). Some of the processes even penetrated deeply between ganglionic neurons. Similar neuronal structures had been described with silver or gold impregnation techniques decades before by Lawrentjew and Nonidez in the esophagus of the dog (Lawrentjew 1929; Nonidez 1946) and by Ottaviani (his Figs. 1, 3) and Stefanelli (his Fig. 1) in rat and mouse esophagus (Ottaviani 1937, 1938; Stefanelli 1938). Ottaviani even described these structures as "dilatazioni laminari", and Stefanelli emphasized that the parent axons of these terminals were less regularly myelinated than thick axons leading to motor endplates of striated esophageal muscle. Although less strikingly, nerve fiber arborizations around myenteric ganglia described in other parts of the gastrointestinal tract also resemble IGLEs (Kolossow and Milochin 1963). These studies remained largely neglected in the international literature until more recently when IGLEs were re-discovered using anterograde tracing from the nodose ganglion with tritiated leucine, HRP, lectin coupled HRP, DiI (Fig. 1A, B) or dextranes [rat esophagus: (Neuhuber 1987); guinea pig esophagus: (Lindh et al. 1989); rat stomach: (Berthoud and Powley 1992); mouse stomach: (Fox et al. 2000)]. It has been firmly established by control experiments including tracer injections into the cervical vagus nerve and supranodose vagotomy before nodose tracer injections (in order to induce degeneration of efferent vagal fibers originating in the brainstem) that tracer injections into the nodose ganglion specifically label only afferent fibers originating in this ganglion. Efferent fibers of passage were labeled only over a few millimeters and could not account for IGLE labeling in the periphery (Neuhuber 1987; see also Materials and Methods). However, if tracer, e.g., biotinamide, was applied to cut vagal branches close to the esophagus, efferent fibers were also labeled in addition to IGLEs (Zagorodnyuk and Brookes 2000). Using the classical zinc-iodide-osmium method, IGLEs were also found in the esophagus of the opossum (Christensen et al. 1987). In the ruminant stomach, IGLEs were described using neurofilament immunostaining (Yamamoto et al. 1994).

A vagal origin of IGLEs had been already shown by (infranodose) vagotomy experiments (Lawrentjew 1929). However, most of the classical authors regarded IGLEs as preganglionic efferent endings. Back then the concept of a two-neuron chain consisting of a preganglionic neuron transmitting its activity via a postganglionic neuron to the effector was relatively novel (Langley 1921). This concept was based primarily on experiments in the sympathetic nervous system, and researchers were eager to find paradigms where a pre-/postganglionic arrangement could be demonstrated also in the vagal realm. Visceral afferents, although recognized by early workers (Langley 1921; Müller 1924), were ill-defined, particularly in anatomical terms, and not yet described within the scope of intramural ganglionic circuitry. After half a century of uncertainty, the vagal afferent nature of IGLEs was

definitely elucidated by selective nodose ganglionectomy in cat (Rodrigo et al. 1982) and by anterograde neuronal tracing from the nodose ganglion in rat (Neuhuber 1987). In particular, the anterograde tracing technique revealed that IGLEs are the most prominent vagal afferent terminal structures in the tunica muscularis of the gastrointestinal tract from the esophagus down to the distal colon, although their density drops markedly anal to the mid duodenum (Berthoud et al. 1997a; Wang and Powley 2000). In the esophagus, IGLEs supply virtually every myenteric ganglion (Neuhuber et al. 1998), and about one half of gastric myenteric ganglia are innervated by IGLEs (Berthoud et al. 1997a). Thus, the distribution of IGLEs parallels that of vagal preganglionic efferents (Berthoud et al. 1991).

Remarkably, IGLEs are more evenly scattered along the digestive tract than the other type of intramuscular vagal afferents, the so-called intramuscular arrays (IMAs; Fig. 1C, D), which are almost confined to the gastric fundus and sphincter regions, in particular to the lower esophageal and pyloric sphincters (Berthoud and Powley 1992; Kressel et al. 1994; Neuhuber et al. 1998; Phillips and Powley 2000; Wang and Powley 2000). In the esophagus, IGLEs are most numerous in its cervical part and at the level of the esophageal hiatus (Wang and Powley 2000).

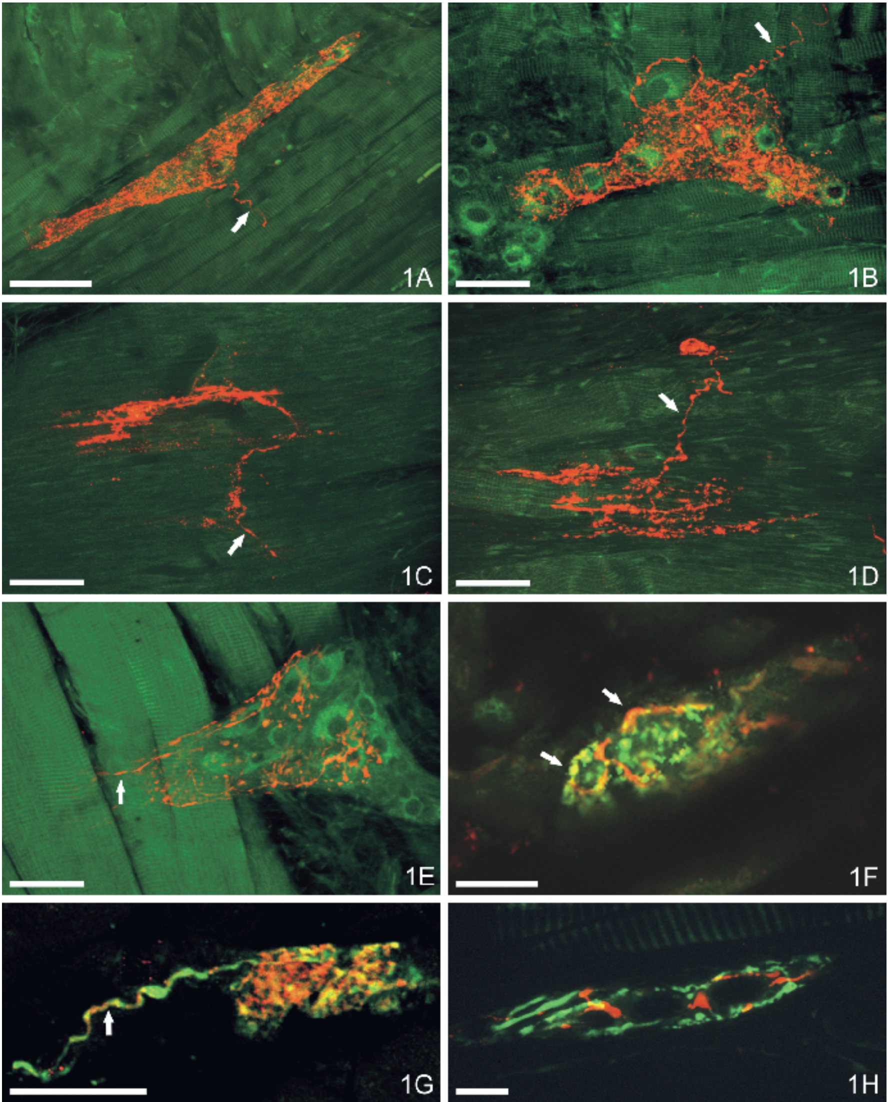
Recently, anterograde tracing, though not from sensory ganglia but from peripheral nerve trunks, revealed IGLE-like structures in the rectum of the guinea pig that apparently originate in lower lumbar and sacral dorsal root ganglia, thus representing "parasympathetic" afferents ("rIGLEs"; Lynn et al. 2003, 2005). Until now, anterograde tracing from thoracolumbar dorsal root ganglia and colonic nerves that carry "sympathetic" afferents from the distal gut have never labeled IGLEs

Fig. 1 A–H Vagal muscular afferents in the rat esophagus as demonstrated by anterograde DiI tracing from the nodose ganglion (**A**–**D**) and immunocytochemistry for calretinin, a specific marker for vagal afferents in the rat esophagus (**E**–**H**). **A, B** Vagal afferent axons (*arrows*) supplying profuse coarse and fine terminals, so-called IGLEs (*red*), to myenteric ganglia situated between the two layers of striated muscle in the thoracic esophagus. Myenteric neurons and muscle fibers appear green due to their autofluorescence. **C, D** IMAs (*red*) in the lower esophageal sphincter. Afferent axons (*arrows*) branching into numerous varicose fibers arranged in parallel between smooth muscle fibers (*green*). Confocal all-in-focus images taken from vibratome slices. **E** IGLEs in the thoracic esophagus immunostained for calretinin (*red*). Leafy endings originating from a parent axon (*arrow*) cover a group of myenteric neurons (*green autofluorescence*). **F** Calretinin immunopositive IGLEs (*red*) costaining for VGLUT2 (*green*) resulting in the mixed color yellow (examples indicated by *arrows*). **G** IGLEs originating from a wavy parent axon costaining for calretinin (*green*) and purinergic P2X2 receptor (*red*) resulting in the mixed color yellow. **H** Calretinin-positive IGLEs (*green*) and CGRP-immunoreactive spinal afferent fibers (*red*) in close apposition (*yellow*), providing a basis for possible peripheral interaction of vagal and spinal afferents. **E**–**G** All-in-focus images of stacks of optical sections; **H** single optical section. *Bars* are 50 µm in **A**–**E**, 25 µm **G**, and 20 µm in **F** and **H**. (**E, H** From Dütsch et al. 1998, **F** from Raab and Neuhuber 2003, **G** from Wang and Neuhuber 2003, with permission)

(Aldskogius et al. 1986; Lynn et al. 2003; Nance et al. 1988). Thus, IGLEs appear to represent typical "parasympathetic," i.e., vagal and sacral, afferent structures in the gastrointestinal tract.

Chemical Neuroanatomy of IGLEs

The first specific immunohistochemical stains for IGLEs, at least in the esophagus of the rat, were the calcium-binding proteins calbindin and calretinin (Figs. 1E, 2; Dütsch et al. 1998; Kressel 1998; Kuramoto and Kuwano 1994; Kuramoto and



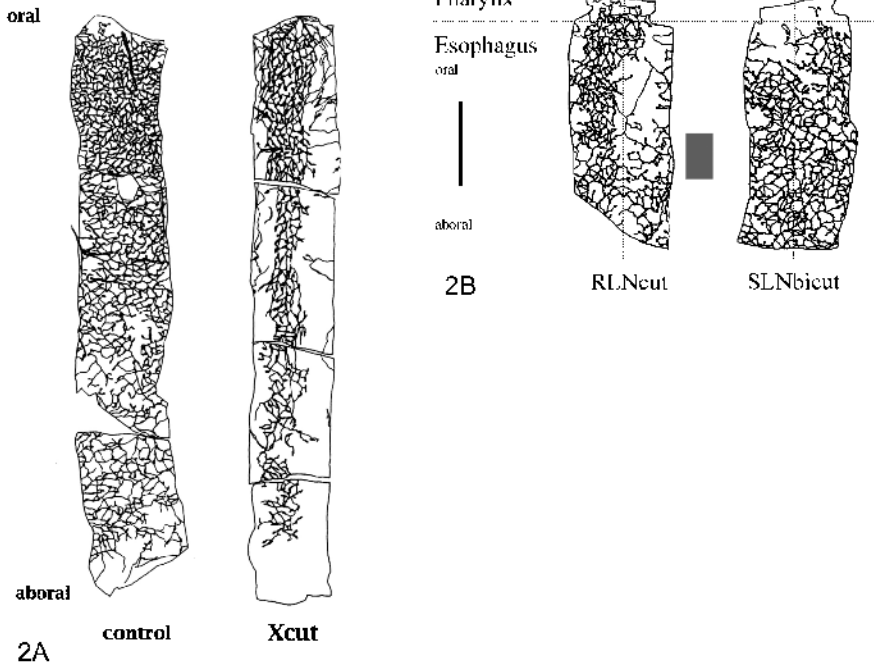


Fig. 2 A,B *Camera lucida* drawings of wholemounts of the rat esophageal tunica muscularis for demonstration of calretinin-positive IGLEs. **A** IGLEs are most dense in the cervical esophagus and relatively sparse at the LES level. Unilateral cervical vagotomy (*Xcut*) markedly depleted IGLE staining ipsilaterally. **B** In the cervical esophagus, IGLEs were significantly depleted ipsilateral to transection of the recurrent laryngeal nerve (*RLNcut*) while transection of the superior laryngeal nerve (*SLNcut*) had almost no effect. *Gray rectangle* indicates level of *RLN cut*. *Bar* in **B** is 1 cm. (From Wank and Neuhuber 2001, with permission)

Kuwano 1995). Thus, IGLEs share a chemical coding with low-threshold muscular and cutaneous mechanosensors (Duc et al. 1994). Another calcium binding protein, neurocalcin, was also detected in IGLEs but additionally in efferent nerve endings in the esophagus and thus could not be regarded as a specific marker (Iino et al. 1998). More recently, purinergic receptors P2X2 (Fig. 1G) and P2X3 (Castelucci et al. 2003; Wang and Neuhuber 2003; Xiang and Burnstock 2004) and vesicular glutamate transporter 2 (VGLUT2, Fig. 1F; Raab and Neuhuber 2003) were added to the repertoire of specific markers for IGLEs. This is noteworthy since these latter compounds can be used as selective immunohistochemical markers in mice also, thus rendering studies on IGLEs in genetically manipulated animals more feasible than with the time consuming and more laborious tracing technique (Fox et al. 2001a; Raab et al. 2003). However, these compounds differ in their suitability as quantitative markers for IGLEs. A recent

study in C57Bl/6 mice demonstrated that P2X2 purinergic receptor immunoreactivity revealed only about half the total IGLE population in the esophagus as calculated from anterograde tracing experiments. In contrast, VGLUT2 immunocytochemistry visualized twice as much IGLEs, apparently the entire population (Raab and Neuhuber 2005). Remarkably, P2X2 immunoreactive IGLEs were concentrated in the abdominal esophagus and were rare in its cervical and thoracic portions. It remains to be determined if this neurochemical bias represents a special feature of C57Bl/6 mice and what its significance might be. The observation that calretinin immunoreactivity was more intense in IGLEs of the cervical than abdominal esophagus (Kressel and Radespiel-Tröger 1999) already indicated topographical differences of IGLEs regarding equipment with various functionally relevant molecules, and it is tempting to speculate about relationships between intracellular levels of certain calcium-binding proteins and expression of purinergic receptors.

IGLEs in rat, mouse, and guinea pig do not stain for CGRP (Fig. 1H; Dütsch et al. 1998; Lindh et al. 1989; Raab and Neuhuber 2003; Wang and Neuhuber 2003) although some CGRP immunopositive IGLEs were detected in the ferret (Costa et al. 2004). Specific immunohistochemistry corroborated earlier data on the morphology of IGLEs and opened new possibilities of multilabel immunostaining for detecting functionally relevant molecules within IGLEs and for more thoroughly investigating the relationships of IGLEs with other neuronal and non-neuronal components of enteric ganglia (Castelucci et al. 2003; Raab and Neuhuber 2004).

The recent availability of specific antibodies against vesicular glutamate transporters (VGLUTs; Fujiyama and Furuta 2001; Tong et al. 2001) and earlier findings of synapse-like contacts between IGLEs and myenteric neurons (Neuhuber 1987) prompted us to test the hypothesis that VGLUT2 is contained in IGLEs. Anterograde WGA-HRP tracing from the nodose ganglion combined with calretinin immunostaining had demonstrated the colocalization of both tracer and calretinin in esophageal IGLEs of rats (Kressel 1998; Kressel and Radespiel-Tröger 1999). Thus, the rat esophagus, in particular its oral portions, was seen to represent an ideal model for investigating the existence of VGLUT2 in identified calretinin positive IGLEs. VGLUT2 labeling in myenteric ganglia almost perfectly matched calretinin immunostaining of the profusely arborizing laminar structures that enveloped myenteric ganglia indicating presence of VGLUT2 in IGLEs (Raab and Neuhuber 2003; arrows in Fig. 1F). Triple-labeling of calretinin, VGLUT2, and synaptophysin additionally showed extensive colocalization of VGLUT2 immunoreactivity (ir) and synaptophysin, demonstrating the location of VGLUT2 in synaptic vesicles (Raab and Neuhuber 2003). Colocalization of synaptophysin and VGLUT2 in IGLEs supports the suggestion that glutamate may be released via fast synaptic mechanisms from IGLEs. About one third of synaptophysin-ir spots in calretinin-immunopositive IGLEs were found without VGLUT2-ir, probably indicating that glutamate may not be the only transmitter in esophageal IGLEs stored in vesicles.

Since calretinin immunohistochemistry does not stain IGLEs in the mouse esophagus (Castelucci et al. 2003; Raab and Neuhuber 2003), a specific immunohistochemical marker for IGLEs in the mouse was not available until recently. Therefore, we utilized anterograde tracing from nodose ganglion combined with VGLUT2 immunohistochemistry in order to test this concept in the mouse. In sections processed for combined WGA-HRP demonstration and VGLUT2 immunohistochemistry, the pattern of VGLUT2 labeling was similar to that described in rat esophagus (Raab and Neuhuber 2003). Yellow spots resulting from the colocalization of green-stained VGLUT2 and red-stained tracer were scattered throughout the ganglion. Thus, IGLEs in the mouse esophagus also contain VGLUT2 and may use glutamate as transmitter. There was no evidence for VGLUT2-immunopositive enteric neurons or other cell types either in rat or mouse (Raab and Neuhuber 2003).

In the guinea pig esophagus, anterogradely biotinamide-labeled IGLEs were strongly immunoreactive for VGLUT1 and only weakly for VGLUT2 (Zagorodnyuk et al. 2003). Thus, species differences appear to exist with respect to the type of VGLUT utilized in a given neuronal structure. However, recent data from our laboratory indicate that rat esophageal IGLEs contain VGLUT1 as well as VGLUT2 (Ewald et al., submitted). When a cocktail of antibodies against both VGLUT1 and 2 was combined in multilabeling experiments with a calretinin antibody, colocalization rates of the IGLE marker calretinin and VGLUT1/2 amounted to 100%. This indicates that, at least in rat, every IGLE contains VGLUTs, either VGLUT1 or VGLUT2 or both. Myenteric neurons were immunonegative for VGLUT2 (Raab and Neuhuber 2003) and only few of them contained VGLUT1 immunoreactivity (Ewald et al., submitted). Since IGLEs innervate every myenteric ganglion in the esophagus (Neuhuber et al. 1998), they probably represent a major source for neuronally released glutamate.

This evidence from immunohistochemical studies suggests that IGLEs are glutamatergic. The targets of glutamate released from IGLEs have still to be determined. A recent study provided evidence for expression of metabotropic glutamate receptors (mGluRs) in vagal afferents and their transport to peripheral afferent axons in various species including humans (Page et al. 2005b). Vagal mechanosensory afferents from esophagus and stomach of mouse and ferret were inhibited by glutamate via mGluRs. Thus, glutamate released from IGLEs may act on autoreceptors, reducing their mechanosensitivity. Whether glutamate from IGLEs also affects enteric neurons or glia has to be determined in forthcoming experiments.

IGLEs in the Context of Myenteric Ganglia

In this section, myenteric ganglia of the esophagus will only be discussed as targets for vagal afferent neurons.

Relationships of IGLEs to Enteric Nitrergic/Peptidergic Neurons In both mouse and rat esophagus, we saw close relationships of VGLUT2-ir IGLEs with neuronal

nitric oxide synthase (nNOS)-, vasoactive intestinal peptide (VIP)-, galanin (GAL)-, and neuropeptide Y (NPY)-immunopositive varicosities and enteric neuronal cell bodies, in particular with their dendrites (Raab and Neuhuber 2004). The extensive coexistence of these peptide transmitters with nNOS in myenteric neurons has already been described in guinea pig small intestine (Costa et al. 1992) and rat esophagus (Wörl et al. 1997). Intense homogeneous cytoplasmatic nNOS-ir and NADPH-diaphorase staining was found in about 80% of myenteric neuronal cell bodies of rat and mouse esophagus (Grozdanovic et al. 1992; Neuhuber et al. 1994; Raab and Neuhuber 2004), proximal dendrites, and varicose fibers. Double-channel confocal analysis revealed that VGLUT2-ir and nNOS-ir were closely apposed to each other, and even formed key–lock associations. However, we never found colocalization within the same profile (Raab and Neuhuber 2004). These close appositions are highly suggestive though not diagnostic of direct synaptic contacts. For analysis of presumptive direct contacts, i.e., without intervening glial processes, between varicosities and between varicosities and neuronal cell bodies, we additionally used electron microscopy. In a preembedding procedure we performed NADPH-diaphorase histochemistry for staining nitrergic neuronal components followed by VGLUT2-immunostaining using diaminobenzidine (DAB) for detection of antibody binding sites. At higher magnification asymmetric synapses between dendrites of a NADPH-diaphorase-positive enteric neuron and VGLUT2-DAB-positive IGLE were evident (M.R., unpublished observation). Close relationships of anterogradely labeled IGLEs with nitrergic neurons were previously described in the stomach using confocal laser scanning microscopy (Berthoud 1995). About one third of NADPH-diaphorase-positive myenteric neurons were closely associated with DiI-labeled IGLEs. Our data concur well with these findings and extend them to the esophagus.

Synaptic contacts of IGLEs, i.e., peripheral endings of glutamatergic vagal primary afferents, with nitrergic enteric neurons apparently mirror the connectivity of central vagal afferent terminals in the NTS, in particular its central subnucleus (NTSce; Atkinson et al. 2003; Hayakawa et al. 2003). Here, terminals of vagal primary afferents, which are devoid of nitrergic markers, frequently synapse with nitrergic second order neurons. It has been suggested that glutamatergic signals from vagal afferent terminals may trigger NO release from second order neurons, which modulates glutamate release from afferent terminals in turn. It is tempting to speculate that similar processes take place in the periphery. Likewise, modulation of gastroesophageal afferents by galanin, as recently demonstrated experimentally (Page et al. 2005a), may also occur under physiological conditions in vivo, based on the intimate relationship between IGLEs and GAL-containing enteric neurons (Raab & Neuhuber 2004).

Relationships to Enteric Cholinergic Neurons The vesicular acetylcholine transporter (VAChT) gene is transcribed, along with the acetylcholine biosynthetic enzyme choline acetyltransferase (ChAT), from a genomic location called the choliner-

gic gene locus (Eiden 1998). All cholinergic neurons of the mammalian nervous system, both central and peripheral, therefore contain VAChT and ChAT.

Myenteric cholinergic neurons are known as both excitatory motor neurons to the intestinal longitudinal and circular muscle and excitatory interneurons (Furness and Costa 1987). In the rat esophagus, enteric ChAT-positive neurons may serve as motor neurons to the muscularis mucosae or as ascending interneurons between ganglia in the esophagus (Kuramoto and Brookes 2000). It is not known if enteric coinnervation of striated muscle fibers from cholinergic myenteric neurons also exists. Although ChAT-immunopositive enteric neuronal cell bodies in mouse and rat esophagus never showed VGLUT2-ir, VGLUT2-immunopositive varicosities were often found in close apposition to ChAT-immunopositive cell bodies and dendrites (Raab and Neuhuber 2004). Only 4% of mouse esophageal myenteric ganglia also showed colocalization of VAChT-ir and VGLUT2-ir in IGLEs, probably representing a minor cholinergic subtype (Raab and Neuhuber 2004). In the remaining 96% of these mouse ganglia and in all myenteric ganglia of the rat esophagus we found close appositions of VAChT-ir and VGLUT2-ir, sometimes in a key–lock manner, but no colocalization within the same profiles. This is illustrated by double immunostaining for VAChT and VGLUT2 of the rat esophagus, where these intermingling fibers appear as yellow and partly overlaps (Raab and Neuhuber 2004). Ultrastructural analysis of these close contacts will be necessary for proper appreciation.

Thus, VGLUT2 containing IGLEs are in close contact with both major enteric neuron classes, i.e., cholinergic and nitrergic, which suggests glutamatergic interaction of IGLEs with both excitatory and inhibitory enteric neurons. These enteric neurons themselves, at least in the esophagus, do not contain VGLUT2. However, they may use other vesicular glutamate transporters, e.g., VGLUT1 in the rat (Ewald et al., submitted), thus tending to confute IGLEs as the sole source of glutamate in myenteric ganglia.

Relationships to Catecholaminergic Neurons Catecholaminergic neurons were detected using immunohistochemistry for tyrosine hydroxylase (TH) and dopamine-beta hydroxylase (DBH, for distinguishing noradrenergic neurons). VGLUT-ir IGLEs were often closely apposed to TH- and DBH-positive fibers (Raab and Neuhuber 2004). Interestingly, also some neuronal perikarya were immunoreactive for TH or DBH. They may represent dopaminergic (Anlauf et al. 2003) or even noradrenergic myenteric neurons.

IGLEs and Substance P Besides substance P (SP)-immunoreactivity in spinal primary afferents, most SP-immunopositive varicosities in the enteric nervous system are of intrinsic origin and SP is often contained in cholinergic neurons (Brookes 2001a; Furness and Costa 1987; Li and Furness 1998). However, in a previous study WGA-HRP transported anterogradely from the nodose ganglion could be found colocalized with both calretinin-ir and SP-ir in 27% of rat esophageal IGLEs (Kressel and Radespiel-Tröger 1999). Therefore, it was not sur-

prising that SP-ir varicosities were found colocalized with VGLUT2-ir in about one third of mouse and about 80% of rat esophageal myenteric ganglia (Raab and Neuhuber 2004). Thus, IGLEs not only contain VGLUT2, indicating a glutamatergic phenotype, but a subset of them also contains SP. The major reason why SP was heretofore never considered as a marker for IGLEs may be on one hand because of a masking effect in single immunohistochemical SP preparations by intrinsic or/and spinal primary afferent SP-positive fibers, and on the other hand due to the fact that not all IGLEs contain SP. Close contacts of VGLUT2- or VGLUT2/SP colocalized spots to SP-immunopositive myenteric neurons which are probably also cholinergic, were found in numerous ganglia, in agreement with results of IGLEs closely contacting cholinergic enteric neurons.

Relationship of IGLEs to Spinal Afferent Neurons Spinal primary afferents use glutamate as transmitter at their central terminals (Alvarez et al. 2004; Keast and Stephensen 2000), and up to 90% of these neurons innervating the esophagus contain CGRP (Dütsch et al. 1998). CGRP additionally colocalizes with SP in large granular vesicles of many primary spinal afferents. However, SP is additionally found in intrinsic neurons and in vagal IGLEs, as described above, and therefore not useful as a marker for spinal primary afferents. In contrast, CGRP can be considered a fairly specific marker for spinal afferents at least in the thoracic and abdominal esophagus of rat, mouse, and guinea pig. In the cervical esophagus, CGRP is also contained in numerous fine vagal afferent fibers (Wank and Neuhuber 2001).

Immunostaining of myenteric ganglia of rat and mouse for CGRP showed fine varicose fibers with sparse ramifications similar to previous descriptions (Dütsch et al. 1998). Although VGLUT2-ir and CGRP-ir were found closely apposing each other, sometimes in a key–lock manner, no colocalization within the same profile was observed within the ganglionic neuropil (Raab and Neuhuber 2003). Similar close appositions were found between calretinin-positive IGLEs and CGRP-positive spinal afferents in myenteric ganglia of the rat esophagus (Fig. 1H; Dütsch et al. 1998). This close relationship between vagal and spinal afferent terminals suggests peripheral interactions between them. This idea is not far-fetched as vagal–spinal afferent modulation is common at the spinal cord level (Chandler et al. 1991; Randich and Gebhart 1992).

Relationships of IGLEs to Enteric Glia The phenotype of enteric glia cells closely resembles that of astrocytes (Jessen and Mirsky 1983). Astrocytes have been shown to modulate synaptic neurotransmission by releasing glutamate in a Ca^{2+}-dependent manner (Araque et al. 1998; Kang et al. 1998). In our recent study we investigated colocalization of the glial markers S100 and glial acidic fibrillary protein (GFAP) with VGLUT2 in mouse and rat esophagus (Raab and Neuhuber 2004). In both species, S100-ir and GFAP-ir showed compact staining of enteric glial cell bodies and their processes while sparing myenteric perikarya.

However, we never found colocalization of S100-ir and GFAP-ir with VGLUT2-ir in the same profile, indicating that enteric glia in the esophagus lacks VGLUT2-ir. It remains to be determined if enteric glia contains other VGLUTs, for example VGLUT1 or VGLUT3. Nevertheless, several S100- and GFAP-immunopositive glial processes were in close contact, or even in key–lock apposition to VGLUT2 immunopositive endings. Single consecutive optical section analysis of these areas revealed interdigitations of glial processes and VGLUT2-immunopositive endings, indicating close proximity. Electron microscopy also demonstrated direct apposition of IGLEs and glial processes (see below). Thus, enteric glia may interact with IGLEs in a way similar to that of astrocytes with central neurons.

Ultrastructure of IGLEs

Ultrastructural analysis of tracer-identified IGLEs in the rat esophagus (Neuhuber 1987; Neuhuber and Clerc 1990) and of IGLEs of the rat esophagus immunostained for neurocalcin (Iino et al. 1998) revealed their superficial location immediately beneath the basal lamina of myenteric ganglia, thus facing the periganglionic extracellular matrix (Figs. 3–7, 9, 10). This typical superficial location was already noticed at the light microscopic level (Nonidez 1946). Parent axons of IGLEs measured typically 2–3 μm in diameter but were unmyelinated at the level of the myenteric ganglion (Fig. 4A), losing their myelin sheaths at various distances away from the ganglia (Stefanelli 1938). The large leafy endings which often measured 5 to 10 μm in length (Figs. 3A, 6B) were connected by thin axonal segments (Figs. 5A, 6B) and stacked with mitochondria, a feature considered typical for afferent nerve endings (Figs. 3, 5, 6A, 7, 9A, 10A) (Iggo and Andres 1982). Other parts of IGLEs were filled with small clear vesicles which, however, were not related to synaptic contacts in most cases (Figs. 5B, 6B). The cytoplasm beneath the plasma membrane facing the extracellular matrix contained a fine filamentous material, the so-called receptor matrix (Figs. 3B, 6A; Iggo and Andres 1982; von Düring and Andres 1990). At places, finger-, hook- or mushroom-like extensions of IGLEs even protruded into the periganglionic extracellular matrix (Figs. 3B, 4B, 9B). Collagen fibrils often appeared to attach to the basal lamina covering IGLEs, and membrane thickenings reminiscent of hemidesmosomes or focal adhesion plaques were evident (Fig. 6A). Thus, IGLEs share ultrastructural features of established mechanosensors. The either more smooth or more dentate surface of myenteric ganglia probably depended on the contraction stage of the esophagus and hence its myenteric ganglia at the time of fixation (Gabella 1990; Gabella and Trigg 1984). IGLEs and their delicate processes interdigitated with glial processes that also reached the surface of the ganglion (Figs. 4B, 7, 9). IGLEs also extensively intermingled with other neuronal and glial profiles in deeper regions of the ganglia (Fig. 8; Iino et al. 1998). Sometimes, IGLEs were connected to neuronal perikarya or dendrites by symmetric contacts of the adherens type (Fig. 7A; Neuhuber 1987). Of particular interest were specialized contacts of IGLEs with enteric neuronal cell bodies and, more often, dendrites displaying all the ultrastructural features

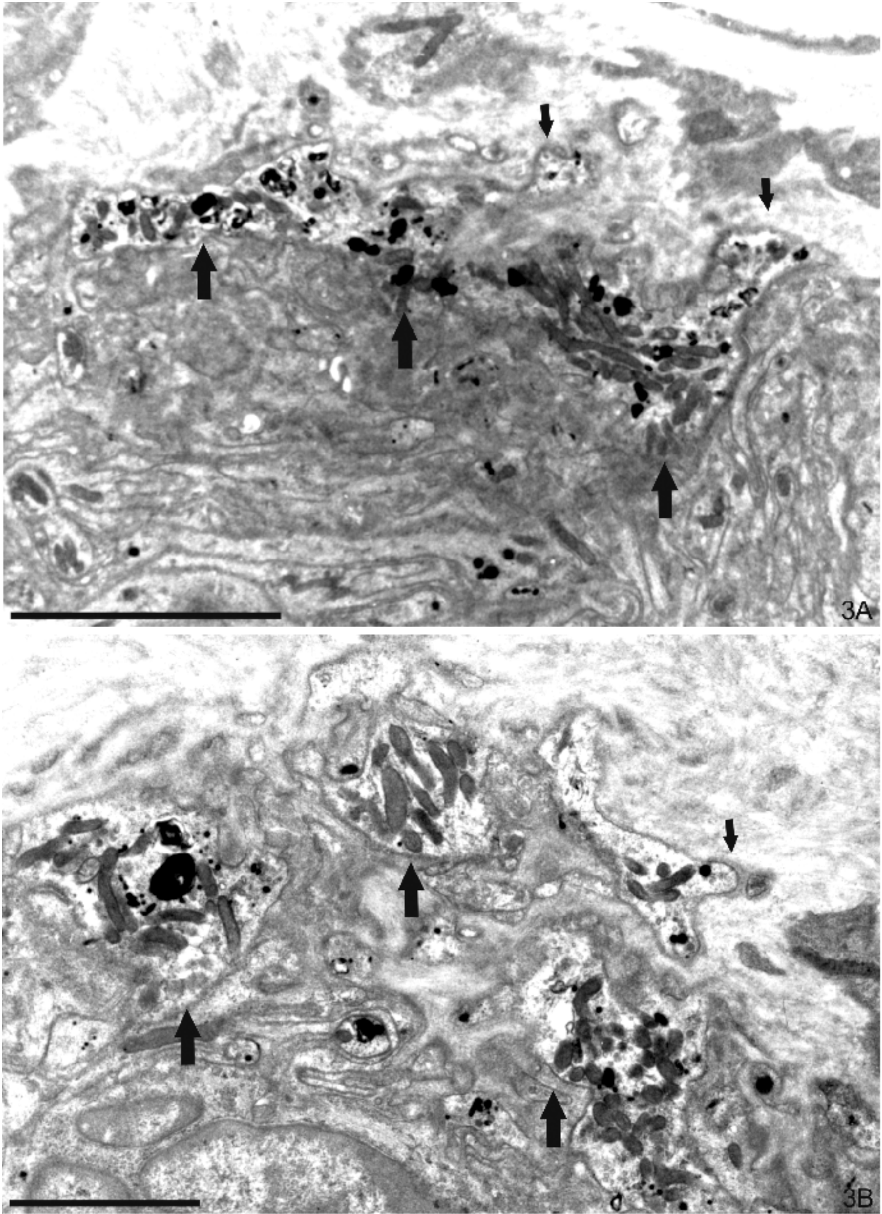


Fig. 3 A,B Electron micrographs of superficial areas of myenteric ganglia in the abdominal rat esophagus. Several anterogradely labeled IGLE profiles containing sharply demarcated electron dense HRP reaction product (*black*) are indicated by *large arrows*. *Small arrows* denote IGLEs facing the periganglionic extracellular matrix. In **B**, *small arrow* points to finger-like IGLE process covered by basal lamina. Note fine filamentous material underlying the plasma membrane, typical for receptor matrix. *Bars* are 5 μm in **A** and 2 μm in **B**

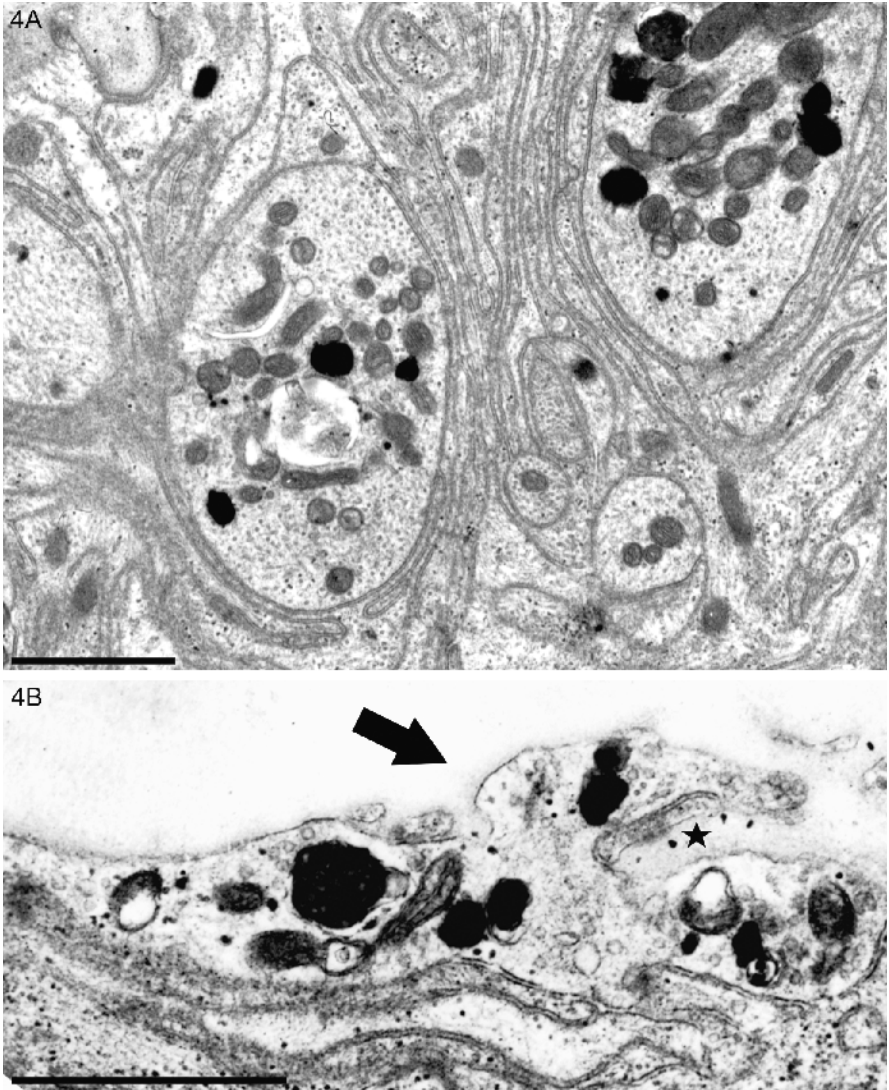


Fig. 4 A,B IGLE profiles in myenteric ganglia of the rat abdominal esophagus anterogradely labeled with WGA-HRP (*black*) from the nodose ganglion. In **A**, two large unmyelinated axonal profiles are labeled which represent parent axons of IGLEs. They contain neurofilaments, microtubules and many mitochondria, and are enwrapped by several layers of glial lamellae. In **B**, a hook-like IGLE process accompanied by a thin glia leaflet (*asterisk*) projects to the periganglionic extracellular matrix. *Arrow* indicates the "free" surface of the IGLE separated from the extracellular matrix by a basal lamina. *Bars* are 1 μm

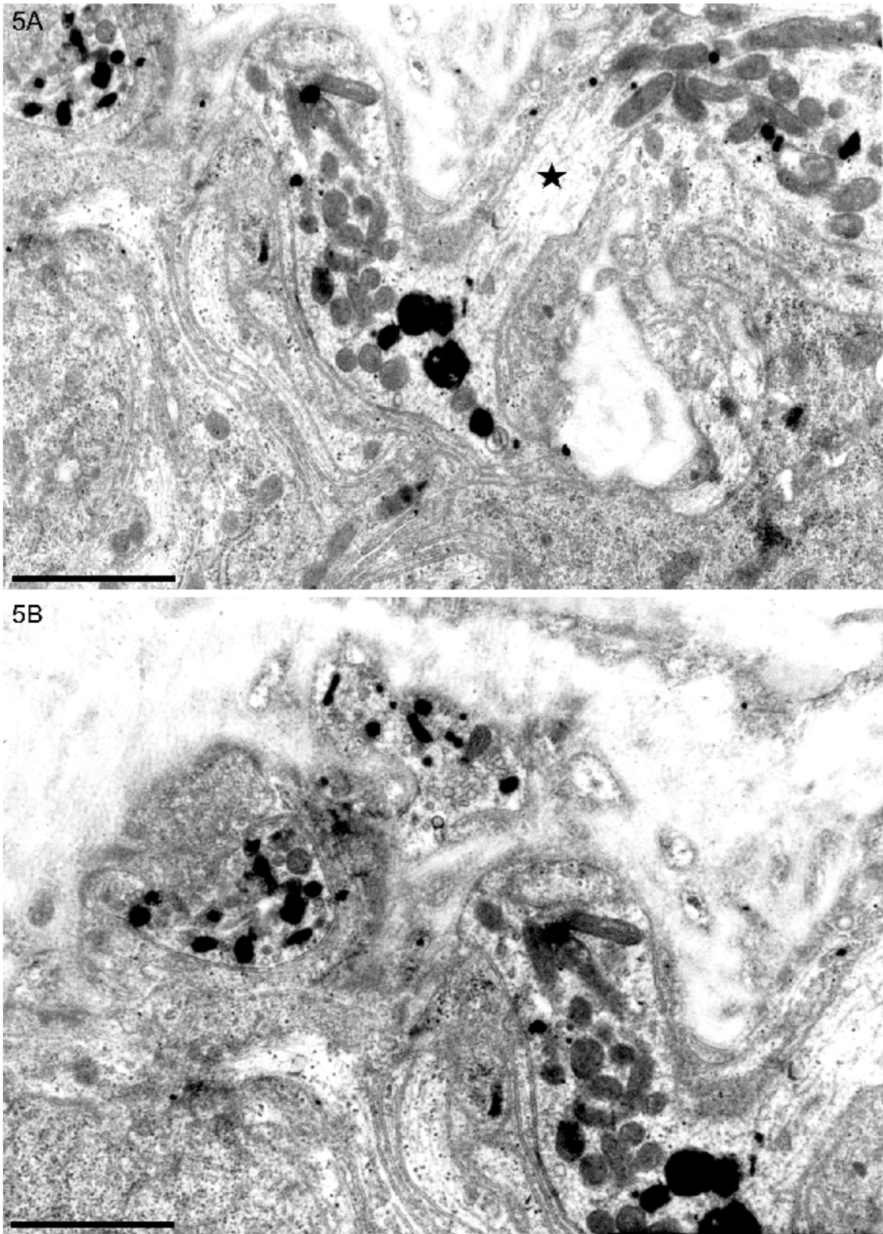


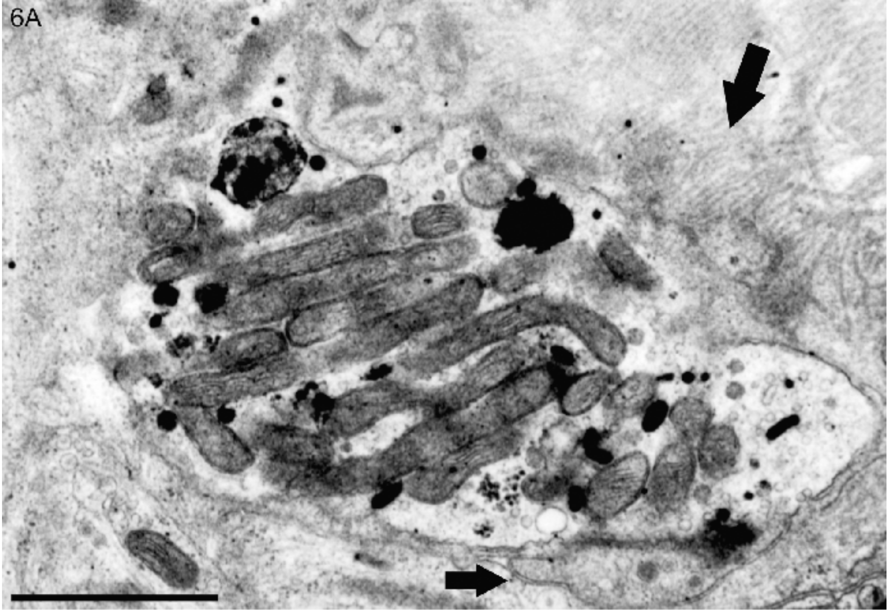
Fig. 5 A,B Two overlapping electron micrographs of the same ganglionic area in the abdominal esophagus. **A** Two WGA-HRP (*black*) labeled IGLE profiles stacked with mitochondria are connected by a thin axonal segment (*asterisk*). In **B**, two additional labeled IGLE profiles are seen, one of them almost completely surrounded by extracellular matrix and filled with small clear vesicles. *Bars* are 1 μm

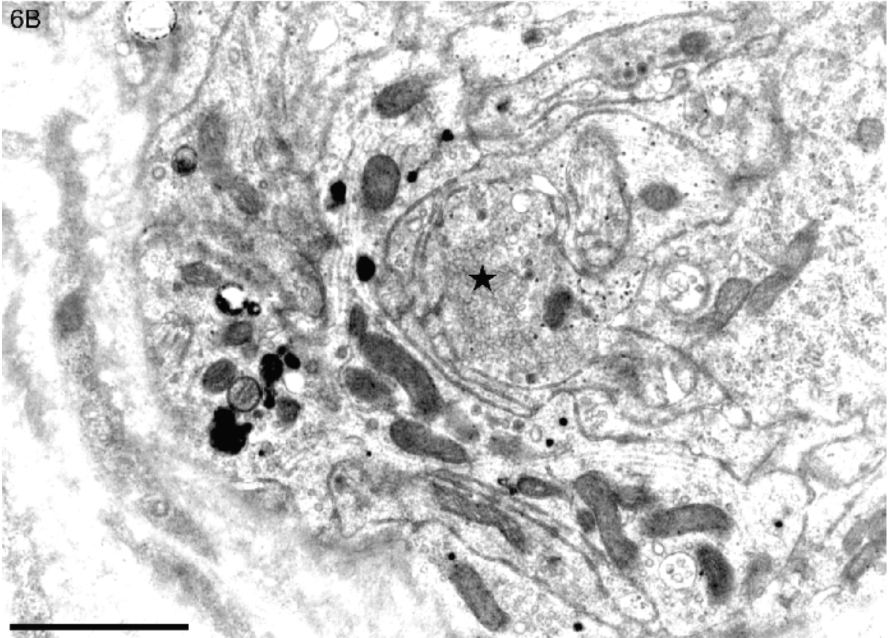
of asymmetric chemical synapses (Figs. 6A, 7B, 8, 9; Neuhuber 1987; Neuhuber and Clerc 1990). These synapses were typically located opposite the "free" surface of IGLEs, on which latter surface the contacts with the extracellular matrix were established. Several of these contacts with enteric neurons were found in every ganglion investigated. Although neurocalcin is not a specific marker for IGLEs, the neurocalcin-positive profile depicted in Fig. 4D of the Iino et al. paper (1998) may indeed represent a synaptic contact formed by an IGLE process. Dendrites of enteric neurons often contained large granular vesicles indicative of storage of peptides (Figs. 6A, 8B, 9). Similar dendritic structures had been described in the rat pelvic ganglion (Yokota and Burnstock 1983). Small round clear vesicles and an occasional large granular vesicle were clustered within IGLEs at these specialized contacts, thus suggesting IGLEs as presynaptic structures impinging onto myenteric neurons (Neuhuber 1987; Neuhuber and Clerc 1990). The presence within IGLEs of VGLUT2 colocalized with synaptophysin (Raab and Neuhuber 2003) supports the idea that these contacts represent true chemical synapses with glutamate contained in the small vesicles. The large granular vesicles may contain substance P as suggested by data on colocalization of substance P-ir with calretinin (Kressel and Radespiel-Tröger 1999) and VGLUT2 (Raab and Neuhuber 2004) in IGLEs. Some synaptic contacts formed by IGLEs were capping spiny dendrites of enteric neurons resulting in ring-like profiles if the section plane was perpendicular to the axis of the spine (Fig. 8B). Pre- and postsynaptic densities were often not as pronounced as in central nervous system synapses, which is not unusual in the autonomic nervous system (Smolen 1988). Synaptic zones occupied only a small fraction of total IGLE membrane area directly apposed to enteric neurons. However, this is common also in large synapses of vagal afferents in the NTS (Hayakawa et al. 2003) and in synapses in other parts of the central nervous system (Sätzler et al. 2002). IGLEs in the rat stomach revealed basically the same ultrastructural features. In particular, they extensively contacted extracellular matrix and established close non-synaptic and also some synaptic contacts with enteric neurons (Fig. 10).

From an anatomical point of view IGLEs resemble specialized encapsulated afferent terminals, e.g., Meissner, Merkel, Ruffini, or Pacinian corpuscles (Halata 1975; Iggo and Andres 1982; Kannari et al. 1991; Munger et al. 1988), as they are specifically related to other cellular elements, i.e., enteric neuronal and glial

Fig. 6 A,B Two WGA-HRP (*black*) labeled IGLEs in the abdominal esophagus. The IGLE in **A** contains densely packed mitochondria and establishes a direct specific contact with a small enteric dendritic profile containing two large granular vesicles (*small arrow*). This contact is located opposite the "free" surface of the IGLE. Note membrane thickenings on the IGLE's "free" surface and underlying receptor matrix where bundles of collagen fibrils (*large arrow*) abut onto the basal lamina covering the IGLE. In **B**, a superficially located IGLE profile emanates from a thin-caliber axon coursing perpendicularly from the *top*. *Asterisk* indicates unlabeled bouton filled with small clear vesicles. *Bars* are 1 µm

cells. They also share basic morphological traits with vagal and glossopharyngeal chemosensory endings in paraganglia, i.e., large size, content of small clear vesicles and synaptic contacts with associated cells, and, in case of chemosensory endings,





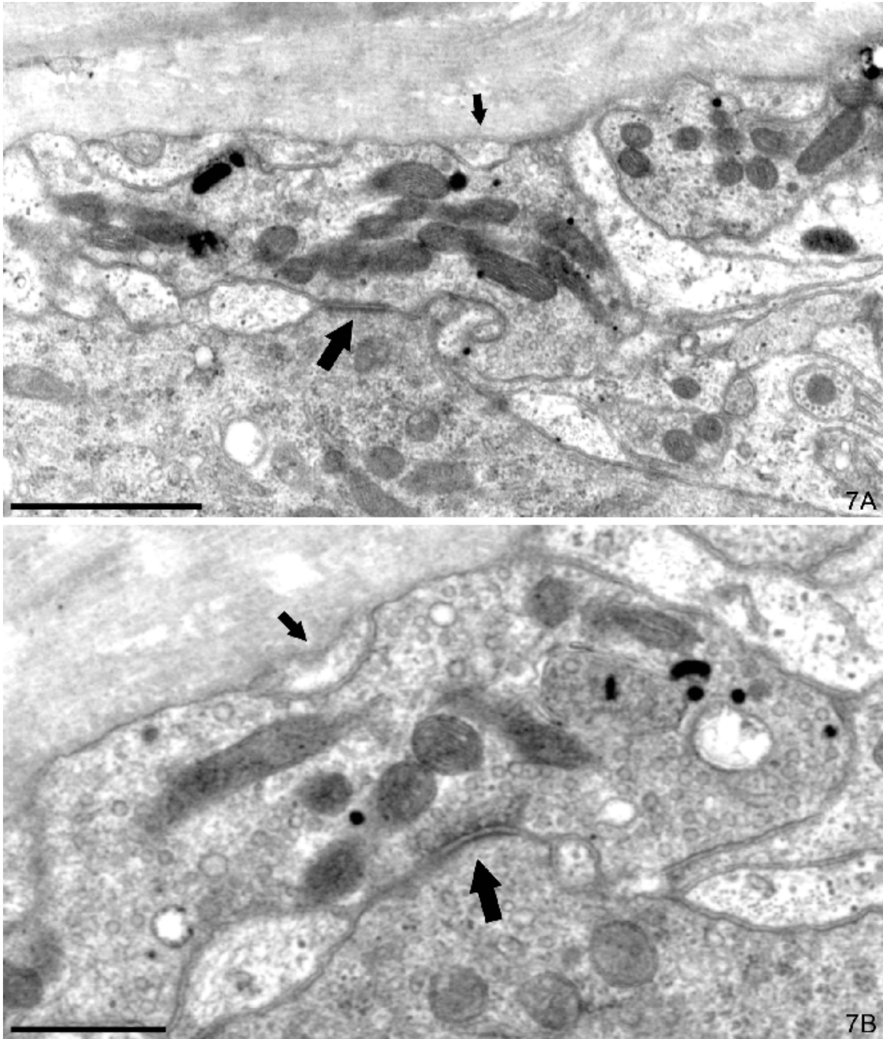


Fig. 7 A,B Two consecutive ultrathin sections through a labeled (*black*) IGLE profile located on the surface of a myenteric ganglion in the abdominal esophagus immediately beneath the basal lamina. In **A**, *large arrow* points to a contact of the adherens type with the underlying myenteric neuronal cell body. In **B**, *large arrow* indicates a synaptic contact with the same neuron with pre- and postsynaptic densities, presynaptic dense projections, and an accumulation of small round clear vesicles on the side of the IGLE. Note that the synapse is located opposite the "free" surface of the IGLE. *Small arrows* point to thin glial leaflets interdigitating with the IGLE on the ganglionic surface. *Bars* are 1 μm in **A** and 0.5 μm in **B**

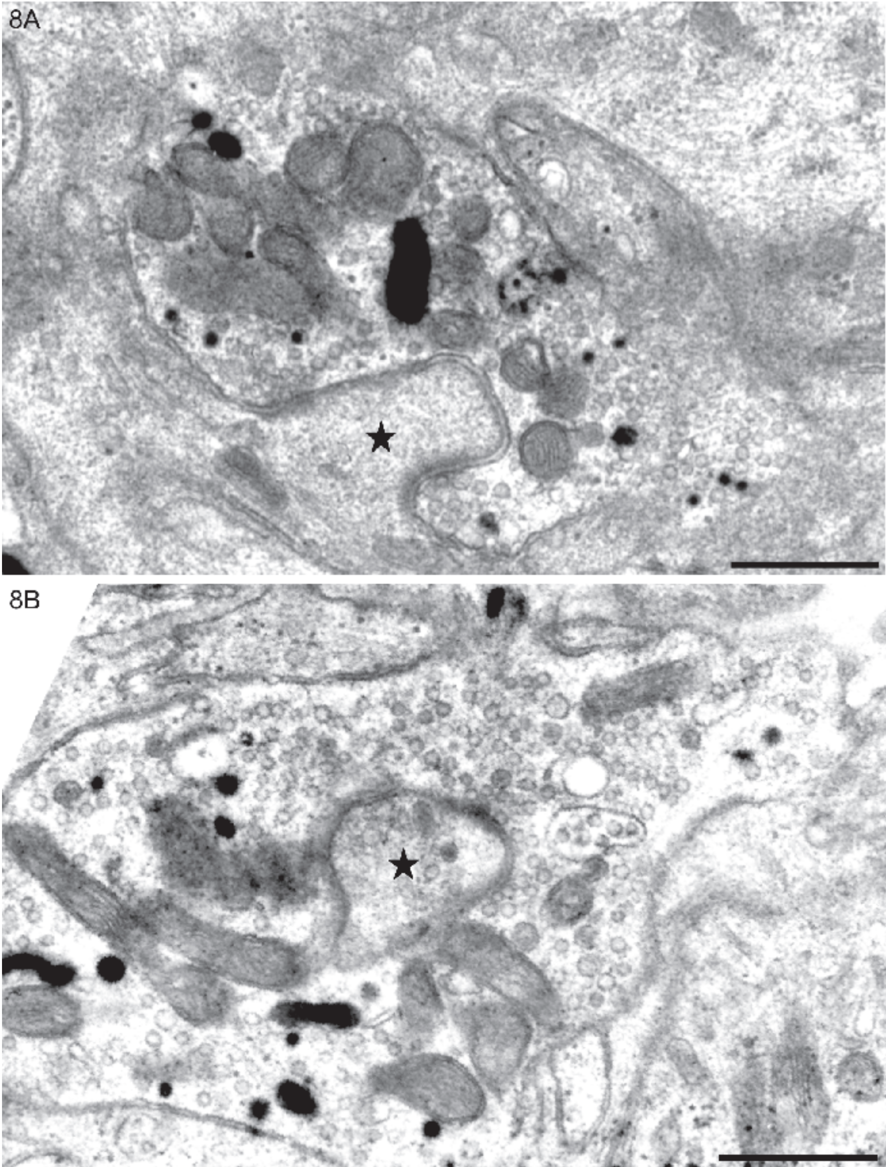


Fig. 8 A,B Two labeled IGLE profiles in deeper regions of myenteric ganglia synaptically contacting small enteric dendrites (*asterisks*). Note pre- and postsynaptic densities and accumulation of small round clear vesicles in IGLEs. The small dendrite in **B** contains two granular and several clear pleomorphic vesicles. *Bars* are 0.5 μm

the glomus cells (Dahlqvist et al. 1994; Fidone et al. 1975; Kummer and Neuhuber 1989). Thus, IGLEs together with associated enteric ganglia may be considered complex sensor structures. This is in striking contrast to the classical concept of free nerve endings as the structural substratum of visceroafferent terminals (Grundy 1988; Iggo 1957; Leek 1972).

Development of IGLEs

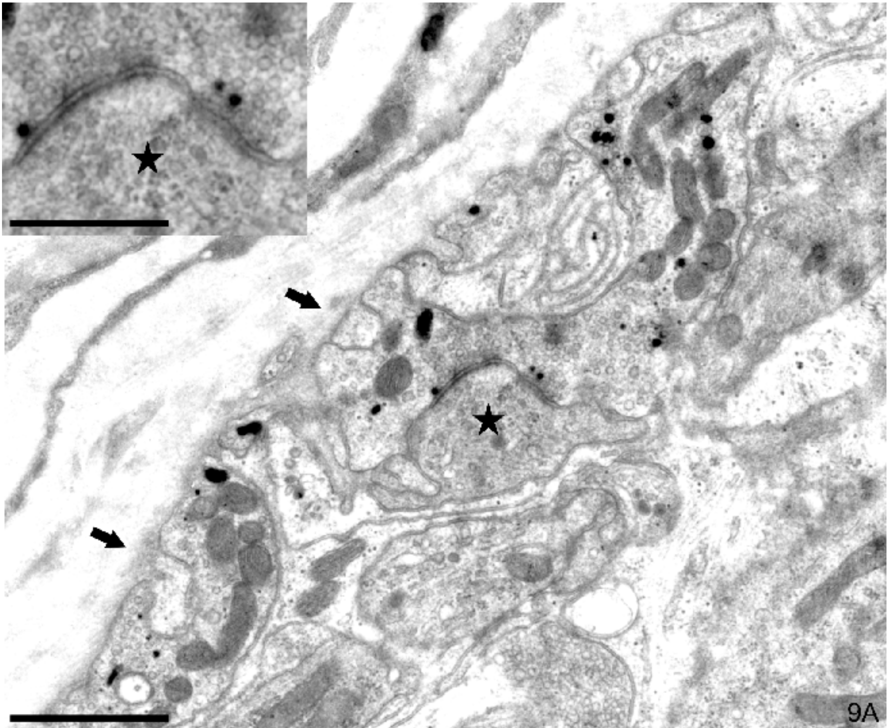
Postmortem anterograde DiI tracing in fixed fetal mouse specimens revealed putative IGLEs in the esophagus as early as E15 (Sang and Young 1998). Immunohistochemistry for calretinin demonstrated IGLEs in the rat esophagus somewhat later and showed their increasing complexity over the first postnatal week progressing from cranial to caudal (Suft et al. 1997). This discrepancy may indicate that synthesis, axonal transport and accumulation of calretinin in peripheral vagal afferent terminals to immunohistochemically detectable amounts required several days beyond structural establishment of IGLEs had taken place. The cranio-caudal development of IGLEs parallels the development of esophageal muscle and its innervation (see below) and is even slightly ahead of the transition from smooth to striated muscle. IGLEs in the stomach become detectable several days later than in the esophagus and displayed a mature configuration at P10 (Swithers et al. 2002). This indicates oral-to-aboral ingrowth of vagal afferents along the gastrointestinal tract. However, different types of afferents follow different time schedules as indicated by the later appearance of IMAs than IGLEs in the gastric fundus.

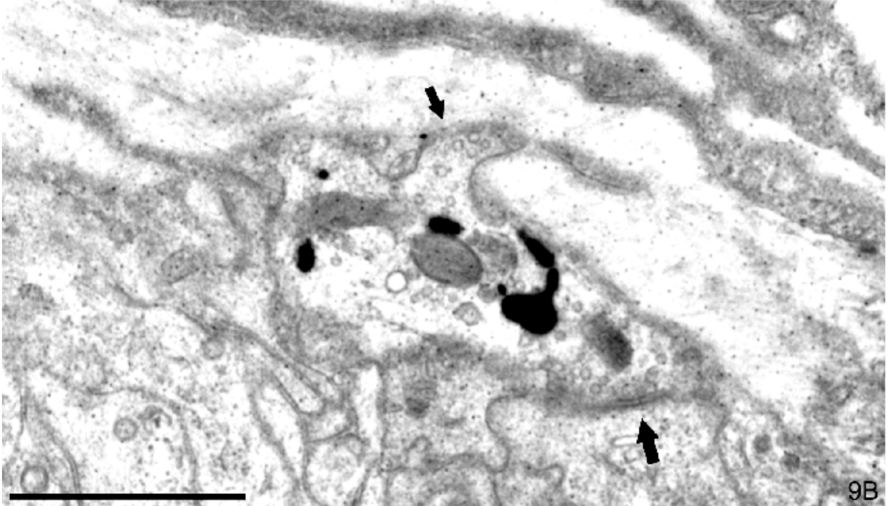
Neurotrophin Dependence and Plasticity of IGLEs

Development of IGLEs appears to depend on neurotrophins. Anterograde tracing from the nodose ganglion in NT-$4^{-/-}$ knockout mice revealed that IGLEs were almost lacking in the duodenum while gastric IGLEs were not altered (Fox et al. 2001a). Anterograde WGA-HRP labeling of IGLEs in the esophagus of mutant mice revealed that they depend in a similar way on NT-3 and its high affinity receptor TrkC (Raab et al. 2003). In NT-$3^{+/-}$- mice IGLEs were reduced by about 50% while $TrkC^{+/-}$ mice showed a less pronounced reduction. In particular, NT-3/TrkC dependence points to similarities of IGLEs with low threshold mechanosensors in

Fig. 9 A,B Two labeled IGLEs establishing synaptic contacts with enteric dendrites. **A** IGLE profiles separated from the periganglionic extracellular matrix only by basal lamina, partly covered by delicate glial processes (*arrows*). IGLEs are filled with mitochondria and small round clear vesicles clustered at synaptic contacts (see also *inset*). The dendrite labeled by *asterisk* is contacted by two active zones. Note large granular vesicle in the dendrite. **B** Labeled IGLE profile on the surface of a myenteric ganglion projecting a hook-like process (*small arrow*) into the extracellular matrix. The IGLE is covered by basal lamina and partly by delicate glial processes. *Large arrow* points to a synaptic contact with a dendrite. Note accumulation of small clear vesicles inside the IGLE. Again, the synapses are located opposite the “outer” surface of IGLEs. *Bars* are 1 μm in **A** and **B**, and 0.5 μm in the *inset*

skeletal muscles, which are typically depleted in NT-$3^{-/-}$ mice (Ernfors et al. 1994; Kucera et al. 1995). This is in line with both morphological and functional data indicating a mechanosensor function of IGLEs.





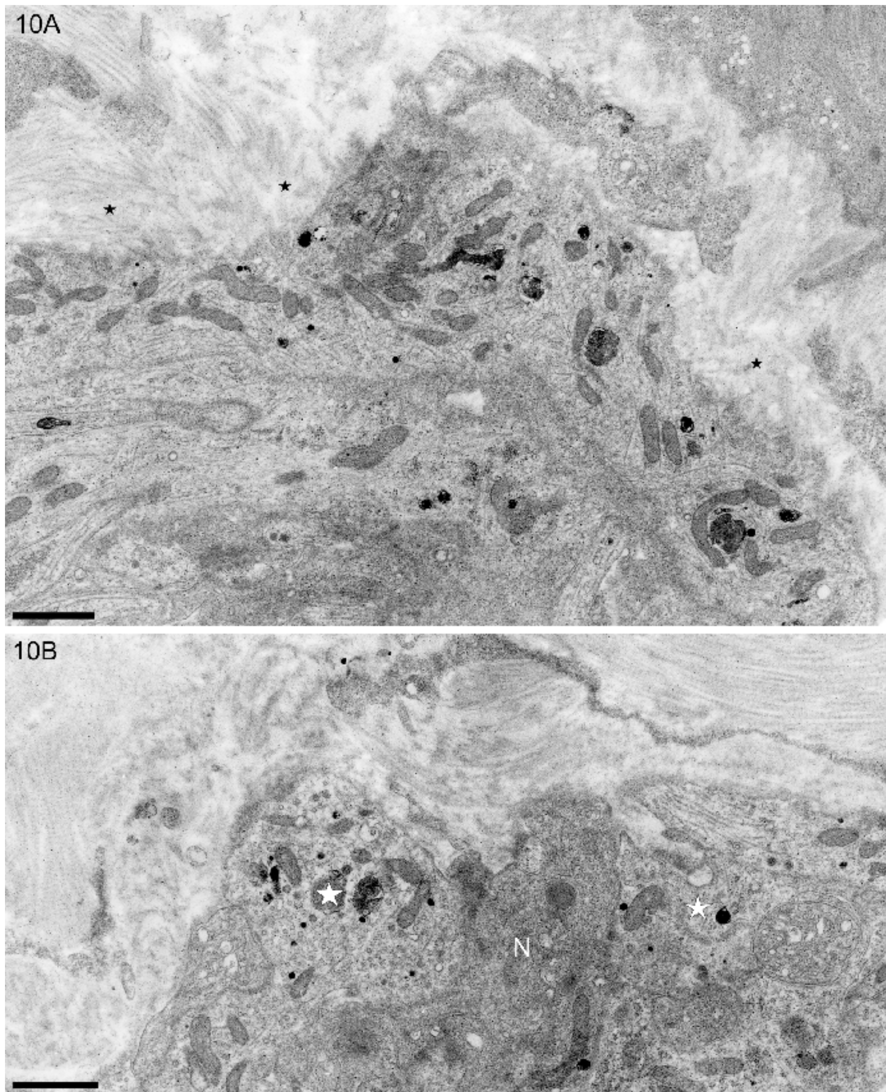


Fig. 10 A,B IGLEs in a myenteric ganglion of the stomach. **A** Large labeled profile containing numerous mitochondria and facing the periganglionic extracellular matrix (*asterisks*). **B** Two labeled profiles (*asterisks*), presumably belonging to the same IGLE complex, flanking a large dendrite of a myenteric neuron (*N*). Both IGLE profiles are rich in mostly small clear and some granular vesicles. In this sample, no synaptic contact can be discerned. *Bars* are 2 µm

Most IGLEs in the esophagus and stomach are resistant to capsaicin (Berthoud et al. 1997b). This corresponds to a lack of the immunohistochemically detectable vanilloid receptor VR1/TRPV1 in some of these terminals (Patterson et al. 2003).

However, chemical or inflammatory challenge may enhance the expression of TRPV1 or acid-sensitive ion channels (ASIC; Holzer 2003; Holzer 2004) that could be relevant for the pathogenesis of mechanical hypersensitivity.

The regenerating capacity of vagal neurons shows significant differences. Studies in the esophagus, stomach, and small intestine of vagotomized rats demonstrated that IGLEs regenerated while vagal efferents did not (Phillips et al. 2003). Nevertheless, regeneration of IGLEs and IMAs was incomplete even after 45 weeks, and regenerating fibers often established abnormal patterns.

Functional Considerations

Mechanosensory Function The first to ascribe a mechanosensor function to IGLEs, based on morphological observations, was Nonidez (1946). Other classical authors, who correctly recognized the vagal origin of IGLEs in the esophagus, considered them as terminals of preganglionic efferent vagal neurons and used these observations in support of Langley's preganglionic/postganglionic concept for the vagus nerve also (Lawrentjew 1929; Ottaviani 1937/38; Stefanelli 1938). Ironically, the observation of synaptic contacts of IGLEs with myenteric neurons (Neuhuber 1987) and the recent finding of VGLUT2 colocalized with synaptophysin in IGLEs (Raab and Neuhuber 2003) favor an efferent function of these well established afferent terminal structures. Although the afferent vagal nature of IGLEs had been determined by Rodrigo and colleagues, these authors entertained the idea of a tension receptor function of IGLEs with some reservation (Rodrigo et al. 1982). However, based on the observation that tracer labeled IGLEs were the only vagal afferent structures in the tunica muscularis of the rat esophagus, and on ultrastructural findings demonstrating similarities with established somatic mechanosensors, IGLEs were proposed as the anatomical equivalent to muscular mechanosensors detecting shearing forces between outer and inner layers of the tunica muscularis and deformation of myenteric ganglia during passive distension or peristalsis (Neuhuber 1987; Neuhuber and Clerc 1990). This idea was further elaborated in the extensive systematic studies of Berthoud, Powley and colleagues who on the basis of careful morphological observation and thoughtful functional reasoning attempted to ascribe various aspects of muscular mechanosensation in the gastrointestinal tract to the two different types of proposed vagal mechanosensors, IGLEs and IMAs (Berthoud and Powley 1992; Phillips and Powley 2000; Wang and Powley 2000). Furthermore, endowment with calretinin and calbindin (Dütsch et al. 1998; Kuramoto and Kuwano 1994) and dependence of IGLEs on NT-3 (Raab et al. 2003) has been established as compatible with a mechanosensory function.

These inferences from anatomy received strong and definite support from elegant studies combining anterograde tracing and single fiber recording in ex vivo preparations of the guinea pig esophagus and stomach (Zagorodnyuk and Brookes 2000; Zagorodnyuk et al. 2001; Zagorodnyuk et al. 2003). IGLEs identified by anterograde biotinamide tracing from branches of the vagus nerve attached

to a wholemount of the esophagus or stomach could be convincingly correlated to hot spots of mechanosensory transduction as localized by stimulation with von Frey hairs and electrophysiological recording from vagal filaments with the wholemount under tension. Thus, IGLEs can be considered the structures subserving low-threshold mechanosensation in the digestive tract in the sense of slowly adapting tension receptors (Clarke and Davison 1975; Iggo 1957; Leek 1972; Mei 1983). Although their sensitivity can be modulated by ATP, the sensory transduction process itself appears to be independent of chemical transmission and most likely involves stretch sensitive membrane channels (Zagorodnyuk et al. 2003). Other molecules also may modulate mechanosensory properties of IGLEs, in particular GABA(B) agonists, which were shown to inhibit vagal mechanosensors in the ferret esophagus (Page and Blackshaw 1999). However, species differences appear to exist with respect to GABA(B) receptor expression on peripheral mechanosensory terminals. Although GABA(B) receptors could be immunohistochemically identified in nodose ganglion neurons, they were not detectable in IGLEs of guinea pig (Zagorodnyuk et al. 2002). Recently, glutamate was shown to inhibit mechanosensation in the esophagus via metabotropic glutamate receptors (Page et al. 2005b). Since IGLEs contain both VGLUT2 (Raab and Neuhuber 2003) and VGLUT1 (Ewald et al., submitted) and likely also release glutamate, they may regulate their own mechanosensory properties in an autocrine manner.

Remarkably, IMAs, which were also anterogradely labeled in vagus nerve–stomach in vitro preparations, could not be correlated with mechanosensory transduction sites as yet (Zagorodnyuk et al. 2001). This is surprising as IMAs represent long trails of branching varicose vagal afferent fibers embedded within smooth muscle and arranged parallel to the muscle fibers (Berthoud and Powley 1992; Phillips and Powley 2000). Thus, their anatomy seemed at first glance much more compatible with predictions from physiological studies as to what tension receptors might look like, than were IGLEs.

Low-threshold tension receptors are vital to proper functioning of the tubular esophagus. Chronic deafferentation may lead to severe motility disorders. Vagal deafferentation in the sheep resulted in the inability to swallow a solid bolus while saliva was still swallowed (Falempin et al. 1986). In dogs treated with acrylamide, the development of megaesophagus could be linked to neuropathic dysfunction of vagal low-threshold, fast-conducting tension receptors in the esophagus (Satchell and McLeod 1984). Congenital idiopathic megaesophagus in dogs (Holland et al. 2002) and similar conditions in human (Longstreth and Walker 1994) may result from similar defects in vagal mechanosensors. This may be explained partly by the intricate connection of esophageal afferents to core structures of the swallowing central pattern generator (Jean 2001). Studies using electrical stimulation of the superior laryngeal nerve or natural bolus stimuli for elicitation of deglutition highlighted the activation (as shown by enhanced c-fos expression) of sensory relay nuclei which also harbor premotor neurons, i.e., the NTSce in case of the esophageal phase (Lang et al. 2004; Sang and Goyal 2001). Disruption of afferent feedback to

this central pattern generator contributes to deficient peristalsis control, which may even alter the structure of the esophagus.

Local Effector Function The ultrastructural finding of synaptic contacts between IGLEs and myenteric neurons with accumulation of predominantly small clear vesicles near the membrane suggested an efferent influence of vagal mechanosensors on local neurons. Alluding to the paradigm of local effector function of peptidergic thin caliber primary afferent fibers (Holzer 1988), a similar local effector function was also proposed for IGLEs, thus suggesting that IGLEs may be considered to be complex vagal sensor–effector structures (Neuhuber 1987). Earlier results indicating that axon reflexes in the gastrointestinal tract are mediated by vagal afferents were taken as support for this proposal (Delbro 1985). In a similar vein, the fact that IGLEs and IMAs in the fundic stomach were sometimes seen to originate from the same parent axon suggested an axon reflex arrangement, with IMAs as sensors and IGLEs as the efferent branch (Berthoud and Powley 1992). Likewise, gastric relaxation upon distension of the distal esophagus was partly ascribed to an axon reflex in vagal afferents supplying both the distal esophagus and myenteric ganglia in the stomach with collaterals (Wei et al. 1997). Disappointingly, a functional study using antidromic electrical stimulation of vagal afferents was unable to provide significant evidence for c-fos induction in myenteric neurons of the rat esophagus and stomach, although stimulation of preganglionic vagal efferents was effective (Zheng et al. 1997). However, the recent finding that IGLEs contain VGLUT2 colocalized with synaptophysin again fuels the old idea of a local effector role of IGLEs (Raab and Neuhuber 2003). Given the intricate relationships of IGLEs with nitrergic enteric neurons (Berthoud 1995; Raab and Neuhuber 2004), a local feedback regulation by glutamate release from IGLEs stimulating NO release from enteric neurons, which in turn modulates glutamate release from IGLEs similar to mechanisms described in the NTS (Atkinson et al. 2003), can be suggested.

3.1.2.2 Vagal Afferent Innervation of Mucosa and Submucosa

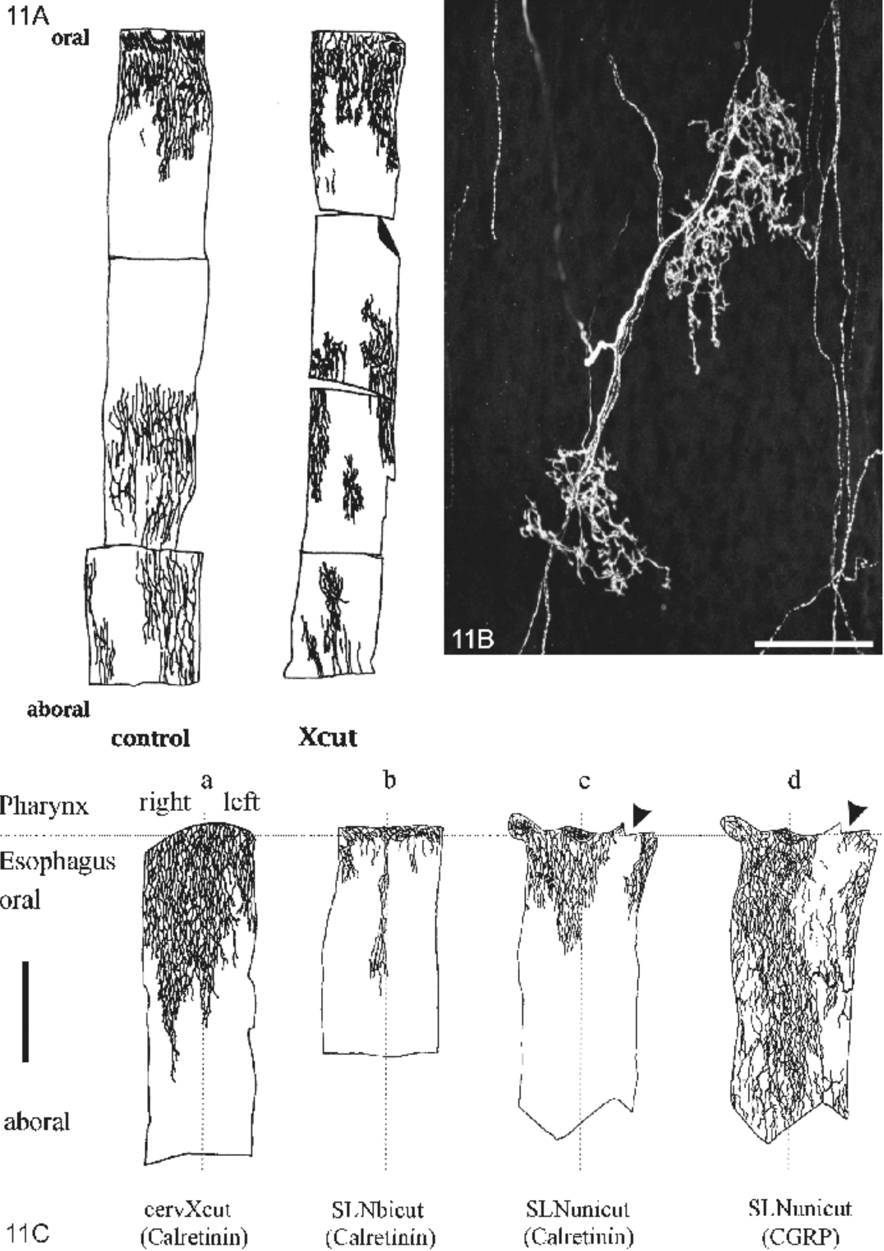
Vagal afferent innervation of the esophageal mucosa and submucosa had been investigated for quite a while with electrophysiological (Andrew 1957; Clerc 1984; Page et al. 2002; Sengupta and Gebhart 1994) and also morphological methods (Cecio and Califano 1967; Robles-Chillida et al. 1981; Rodrigo et al. 1975a; Rodrigo et al. 1980a; Rodrigo et al. 1980b). In more recent years, neuronal tracing and immunohistochemistry have added valuable new data (Clerc and Condamin 1987; Dütsch et al. 1998). Against a background of increasing incidence of gastroesophageal reflux disease and related complications, ranging from reflux induced asthma to Barrett's carcinoma, understanding of the functional anatomy and pathophysiology of esophageal mucosal afferents is particularly significant.

Both anterograde tracing from vagal sensory ganglia and immunohistochemistry for calcium binding proteins in the rodent esophagus indicated the greatest innervation density to be in the upper cervival esophagus, corresponding to the oralmost 10 mm in the rat (Fig. 11; Dütsch et al. 1998; Neuhuber 1987; Wank and Neuhuber 2001). Innervation density steeply decreased in the lower cervical and thoracic esophagus, then slightly increased again in its abdominal portion. In the upper cervical esophagus, numerous thin varicose calretinin-positive fibers formed a dense net stretched in the long axis of the tube. Many of these fibers had short finger-like branches directed to the epithelium. Others were simple unbranched varicose fibers, many costaining for CGRP. In addition, there were thin varicose fibers staining for CGRP only. Transsection of the superior laryngeal nerve ablated almost all of the thin calretinin-positive fibers and the majority of CGRP-positive axons, while cutting the recurrent laryngeal nerve had little such effect (Fig. 11C; Wank and Neuhuber 2001). Retrograde tracing combined with immunocytochemistry indicated that the cell bodies of origin displaying a compatible chemical phenotype reside in the jugular-petrosal ganglia (Wank and Neuhuber 2001).

In addition to these thin caliber afferent axons, specialized afferent terminals immunoreactive for calretinin and calbindin deriving from thick caliber axons and exhibiting complex arborizations were present in the uppermost 5 mm segment of the esophagus (Fig. 11B; Dütsch et al. 1998; Wank and Neuhuber 2001). They accounted for about 20 terminal complexes in each rat, most of them abutting and even penetrating the epithelium with fine branches. Some of these endings were associated with submucosal blood vessels or were located in the submucosal connective tissue without obvious relationships to other structures. The axons

Fig. 11 A–C Vagal afferent innervation of the esophageal mucosa in the rat. **A** *Camera lucida* overview of mucosal wholemounts immunostained for calretinin. Nerve fibers are most dense in the upper cervical portion and in the aboral third of the organ. The middle third is almost free of vagal afferent mucosal innervation. Unilateral transection of the cervical vagus nerve (*Xcut*) resulted in some patchy loss of calretinin stained fibers in the lower third, whereas fiber density in the upper portion was unaffected. **B** Confocal image of two calretinin-positive complex Ruffini endings in the mucosa of the uppermost cervical esophagus, emanating from a bifurcating thick parent axon. Note thin varicose fibers partly accompanying the thick axon, partly running alone longitudinally. **C** Calretinin-positive nerve fibers in the cervical esophageal mucosa were almost completely depleted by bilateral SLN transection (*SLNbicut*, *b*) and depleted on the side of unilateral SLN transection (*SLNunicut, c*), while transection of the left cervical vagus (which also carries afferent axons of the RLN) was ineffective (*cervXcut, a*). Unilateral transection of SLN also markedly reduced the density of CGRP-positive nerve fibers in the cervical esophagus (*d*), indicating a vagal afferent contribution in addition to the fiber population originating in dorsal root ganglia. *Arrowheads* indicate the side ipsilateral to nerve transection. *Bars* are 100 μm in **B** and 1 cm in **C**. (**A, B** From Dütsch et al. 1998, **C** from Wank and Neuhuber 2001, with permission)

leading to these complex terminals traveled exclusively in the superior laryngeal nerve as evidenced by their disappearance upon SLN cut. At first glance, because of their leafy appearance these complex endings resembled IGLEs, a resemblance



underlined by their chemical coding for calretinin and calbindin and immunonegativity for CGRP (Dütsch et al. 1998; Kuramoto and Kuwano 1994; Wank and Neuhuber 2001). Similar terminals of vagal afferent fibers, classified by some authors as Ruffini endings, were demonstrated in the esophagus of both cat and monkey using the zinc-iodide-osmium stain (Rodrigo et al. 1980a; Rodrigo et al. 1980b), and in rabbit using gold impregnation (Cecio and Califano 1967). The laryngeal mucosa of rat and dog has also been found to harbor this type of sensor (Yamamoto et al. 1998; Yamamoto et al. 1997). They are also reminiscent of Ruffini endings in periodontal ligaments (Kannari 1990) and Golgi tendon organs (Schoultz and Swett 1974). Again, complex mucosal terminals in the esophagus share calretinin immunoreactivity with periodontal Ruffini endings (Ichikawa et al. 1997).

It is tempting to ascribe distinct functions to these various types of vagal mucosal afferents. Both caliber and chemical coding of thin fibers reaching the esophagus via the superior laryngeal nerve suggest a chemosensory or even nociceptive role. This has to be elucidated by direct functional testing. The complex endings in the mucosa and submucosa of the uppermost esophagus connected to thick caliber axons most likely represent low-threshold mechanosensors. Their intraepithelial extensions suggest also thermo- or chemosensory function. Interestingly, functional studies have indicated greater sensitivity of the upper versus lower esophagus to both mechanical and chemical stimulation (Hummel et al. 2003). The most parsimonious explanation for this is greater afferent innervation density of the upper part of the organ. Dense innervation of the uppermost esophagus makes sense if one considers the importance of protective reflexes triggered in this area.

3.1.2.3
Central Projections of Vagal Afferents from the Esophagus

Transganglionic tracing studies combined with nerve transections have demonstrated the central termination sites of vagal afferents from the esophagus. Afferents from all portions of the organ project to the central subnucleus of the nucleus tractus solitarii (NTSce) in a somatotopic manner, oral portions of the organ being represented rostral to more caudal portions (Fryscak et al. 1984; Altschuler et al. 1989). However, afferents from the cervical esophagus, at least in the rat, follow two different pathways and project to two distinct subnuclear regions in the NTS. Most of muscular afferents, largely equivalent to IGLEs, travel in the recurrent laryngeal nerve (Fig. 2B) and project to the NTSce. Mucosal afferents with both thin and thick caliber axons, including presumptive chemo- and nociceptors, coursing through the superior laryngeal nerve project heavily to the interstitial and intermediate subnuclei of the NTS (NTSis and NTSim, respectively; Fig. 12; Wank and Neuhuber 2001). This is noteworthy because the NTSce is the established premotor neuron pool for the compact formation of the nucleus ambiguus, i.e., the esophageal motor neurons (Cunningham and Sawchenko 1989; Hayakawa

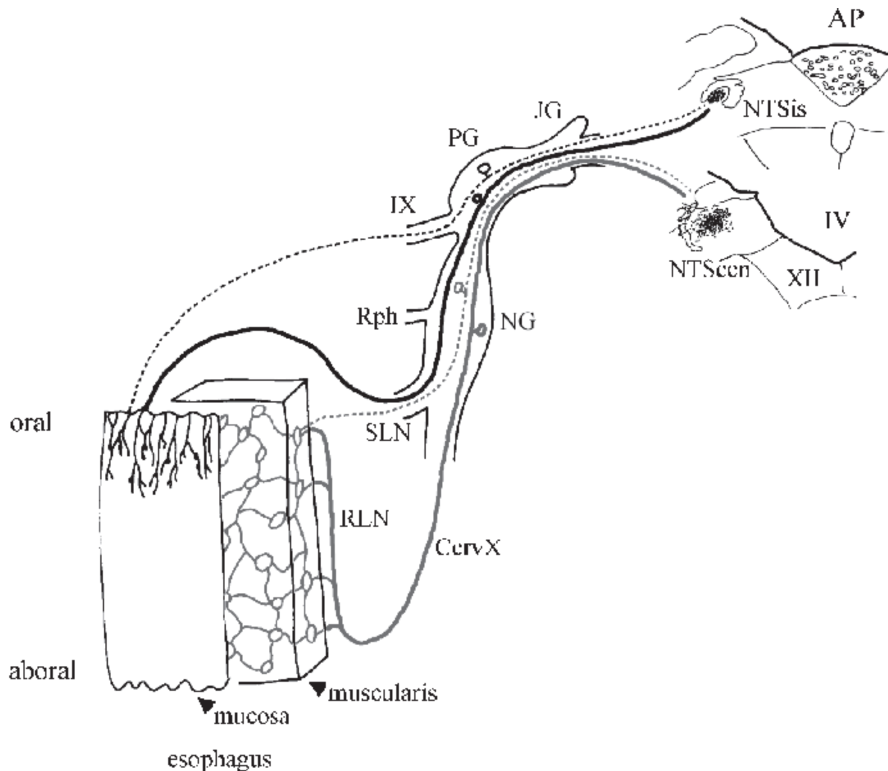


Fig. 12 Summary diagram of vagal afferent innervation of the cervical esophagus in the rat. Muscular afferents, largely representing IGLEs, are carried by the RLN and cervical vagus (*CervX*), with minor contributions by the SLN to their cell bodies in the nodose ganglion (*NG*), and terminate in the central subnucleus of the NTS (*NTScen*). Mucosal afferents travel through the SLN, and only to minor extent via the glossopharyngeal nerve (*IX*), to their perikarya in petrosal-jugular ganglia (*PG, JG*) and project to the NTSis. *AP,* area postrema; *IV,* fourth ventricle; *Rph,* ramus pharyngeus n. vagi; *XII,* hypoglossal nucleus. (From Wank and Neuhuber 2001, with permission)

et al. 1997), while the NTSis and NTSim receive convergent afferents from pharynx, larynx, and tongue (Altschuler et al. 1989; Hamilton and Norgren 1984; Nazruddin et al. 1989; Neuhuber and Fryscak-Benes 1987) and harbor premotor neurons for pharyngeal and laryngeal motor neurons (Barrett et al. 1994; Broussard et al. 1998). In addition, the NTSis projects to the NTSce (Broussard and Altschuler 2000a; Broussard and Altschuler 2000b). Thus, the NTSis is in a strategic position for coordinating protective and aversive reflexes in the upper aerodigestive tract, and mucosal afferents from the upper esophagus appear to directly feed into this network.

Transganglionic tracing from both thoracic and abdominal esophagus labeled afferent terminals in the NTSce (Altschuler et al. 1989; Wank and Neuhuber 2001).

This may indicate that vagal mucosal afferents from these parts of the esophagus also project to the NTSce. However, since vagal mucosal innervation of the thoracic and abdominal esophagus is much less dense than of its cervical part (Dütsch et al. 1998), a projection of mucosal afferents to the NTSis and NTSim was probably not adequately demonstrated by transganglionic tracing.

3.1.2.4 Vagal Nociceptors

Although classically considered a non-nociceptive pathway (Sengupta and Gebhart 1994), some recent studies on afferents from esophagus and also other vagally innervated organs suggest presence of nociceptive or nociceptive-like afferents in the vagus nerve and its branches. Esophageal balloon distension in the rat with presumptive nociceptive volumes resulted in greater pseudoaffective responses (as determined by neck muscle electromyography) when the cervical as compared to the lower thoracic esophagus was stimulated (Hummel et al. 2003). These differences were even more pronounced with HCl stimulation of the mucosa. Transsection of the superior laryngeal nerve reduced this response. Other experiments on gastric afferents using various chemical stimuli (Danzer et al. 2004; Holzer 2004; Holzer et al. 2004; Lamb et al. 2003) also indicated a significant role of vagal afferents in chemonociception while mechanonociception was still ascribed to spinal afferents (Jou et al. 2002; Ozaki and Gebhart 2001; Ozaki et al. 1999). A recent report showed slowly conducting vagal afferents from all portions of the guinea pig esophagus displaying graded responses to increasing distension pressures (Yu et al. 2005). Most of these fibers were also sensitive to capsaicin and were interpreted as vagal nociceptors. Even if these afferents do not lead to conscious pain experience in a classical sense, they may contribute to the emotional aspect of pain and may trigger respiratory and cardiovascular reflexes and homeostatic reactions which are of pathophysiological significance. Another factor which has to be considered in this context is sensitization of non-nociceptive vagal afferent neurons by, e.g., acid challenge, inflammatory cytokines or nerve growth factor (Dinh et al. 2004; Holzer 2003; Holzer 2004). These substances can lead to increased expression of TRPV1 (Matthews et al. 2004) and possibly also other receptors, e.g., ASIC, that are of paramount importance in chemonociception by previously non-nociceptive afferents. These recent data may stimulate reconsideration of current models for understanding mechanisms of non-cardiac chest pain arising in the esophagus.

3.2 Spinal Innervation

3.2.1 Efferent Spinal Innervation

Spinal efferent innervation of the esophagus is accomplished by the sympathetic nervous system. Preganglionic sympathetic neurons presumably reside in the tho-

racic spinal intermediolateral nucleus and in more medially located areas, as can be inferred from studies on preganglionics of the greater splanchnic nerve (Cervero and Connell 1984; Neuhuber et al. 1986). Postganglionic axons from neurons in superior cervical, stellate, thoracic, and celiac ganglia reach the esophagus via delicate branches (Aharinejad and Firbas 1989; Hudson and Cummings 1985; Uddman et al. 1995). Data obtained with catecholamine fluorescence histochemistry comply well with more recent immunohistochemical results showing that the main targets of sympathetic postganglionic axons are myenteric ganglia and blood vessels (Baumgarten and Lange 1969; Raab and Neuhuber 2004). Although not directly investigated, noradrenergic sympathetic innervation may have the same inhibitory effect on esophageal enteric neuronal functions as it has in the intestines (Furness and Costa 1987).

3.2.2
Afferent Spinal Innervation

Axons of dorsal root ganglion neurons at cervical and thoracic levels travel through fine branches from the sympathetic trunk and, to the abdominal portion, also through the greater splanchnic nerve and the celiac ganglion. Retrograde tracing studies in rat, mouse, cat, and dog demonstrated a broad distribution of spinal esophageal afferents from upper cervical to upper lumbar dorsal root ganglia (Collman et al. 1992; Dütsch et al. 1998; Green and Dockray 1987; Hudson and Cummings 1985; Khurana and Petras 1991; Sang and Young 1998). For example, dorsal root ganglia C1-L2 contributed afferents to the dog esophagus (Khurana and Petras 1991), and results in the other species were quite similar. As indicated by retrograde tracing combined with immunohistochemistry, up to 90% of spinal afferent neurons contain CGRP and about 40% costain for substance P, nNOS, and/or calbindin (Dütsch et al. 1998; Green and Dockray 1987; Kuramoto and Kuwano 1995; Sang and Young 1998; Uddman et al. 1995). Since CGRP is lacking in most nodose ganglion neurons and in IGLES (Dütsch et al. 1998; Lindh et al. 1989), this peptide can be used as a fairly specific marker for spinal afferent fibers at least in the thoracic and abdominal esophagus. In wholemount specimens of the mucosa, a delicate net of fine varicose fibers staining for CGRP was revealed (Dütsch et al. 1998). In contrast to the marked differences in density of calretinin immunoreactive vagal afferents, CGRP positive spinal afferents are distributed rather evenly from oral to aboral. It is only in the upper cervical esophagus where, in addition to spinal afferents, a majority of CGRP fibers is contributed by the superior laryngeal nerve (Fig. 11C; Wank and Neuhuber 2001). Upon entering into the esophagus, CGRP positive spinal afferents are distributed through the myenteric plexus, either terminating in myenteric ganglia (Fig. 13C) or en route to the mucosa. Anterograde tracing from dorsal root ganglia directly demonstrated labeled axons in myenteric ganglia of the esophagus (Fig. 13A, B; Clerc and Mazzia 1994; Mazzia and Clerc 1997). In striking contrast to vagal IGLEs, these fibers and branches are far less nu-

merous and complex. Intraganglionic spinal afferent terminals were seen as fine varicosities rather than coarse lamellar expansions as typical for IGLEs. However, spinal afferents also establish direct contacts to myenteric ganglion neurons as evidenced by electron microscopy (Mazzia and Clerc 1997). Intraepithelial innervation in thoracic and abdominal esophagus by spinal afferents is sparse (Robles-Chillida et al. 1981; Rodrigo et al. 1975a; Rodrigo et al. 1985). This contrasts to vagal mucosal afferents in the upper cervical esophagus and particularly in the pharynx where intraepithelial innervation is fairly dense (Dütsch et al. 1998; Terenghi et al. 1986; Wang and Neuhuber 2003; Wank and Neuhuber 2001).

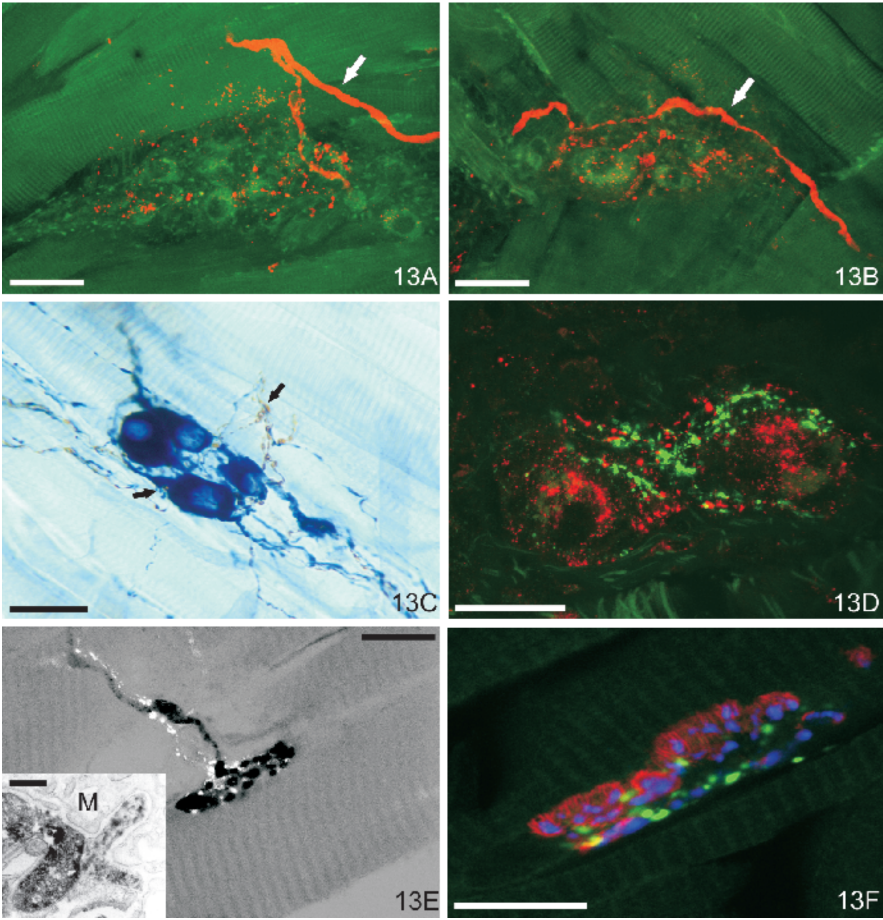
Spinal afferent fibers from the cervical esophagus enter the spinal cord via both cervical and, upon descending in the sympathetic trunk, upper thoracic spinal nerves, while thoracic esophageal afferents reach the spinal cord through thoracic spinal nerves. Thus, second order neurons in the thoracic dorsal horn may receive convergent input from widely distributed portions of the esophagus (Qin et al. 2003; Qin et al. 2004).

Although central projections of spinal afferents from the esophagus to date have not been studied with transganglionic tracing, it can be assumed that their termination pattern in the spinal cord is similar to those of splanchnic and hypogastric nerve afferents, i.e., laminae I, outer II, V, and X (Cervero and Connell 1984; Morgan et al. 1986; Neuhuber 1982; Neuhuber et al. 1986). Second order spinal neurons activated upon esophageal distension were located largely within these laminae (Euchner-Wamser et al. 1993; Qin et al. 2004).

Fig. 13 A,B Confocal images of spinal afferent axons labeled by anterograde tracing with DiI (*red*) from lower thoracic dorsal root ganglia. Fine varicosities originating from relatively thick parent axons are dispersed over myenteric ganglia of the rat thoracic esophagus. Autofluorescence of ganglionic neurons and striated muscle fibers is coded *green*. **C** NADPH-d-positive myenteric neurons (*blue*) in the rat esophagus intermingling with CGRP-immunoreactive spinal afferent fibers (*brown*). Some CGRP-positive varicosities closely contact myenteric neurons as indicated by *left arrow*. **D** VIP-immunoreactive neuronal cell bodies (*red*) surrounded by VAChT-positive cholinergic varicosities (*green*) in a myenteric ganglion of the human esophagus. Confocal all-in-focus image. **E**, **F** Enteric coinnervation. **E** CGRP-positive vagal motor axon (*black*) forming a motor endplate on a striated muscle fiber in the rat esophagus. A thin varicose GAL-positive enteric nerve fiber (*white*) accompanies the vagal motor axon and intermingles with vagal motor terminals in the endplate area. As shown in the *inset*, enteric terminals, as labeled with coarse electron-dense NADPH-d reaction product, are in direct contact to both a vagal CGRP motor terminal, as demonstrated with fine electron-dense DAB reaction product, and the striated muscle fiber (*M*). **F** Motor endplate in the rat esophagus labeled with α-bungarotoxin (*red*). Coarse vagal cholinergic boutons (*blue*) intermingle with fine enteric terminals immunostained for nNOS (*green*). *Bars* are 40 μm in **C**, 20 μm in **A**, **B**, **D**, and **E**, 10 μm in **F**, and 0.5 μm in the *inset* in **E**. (**E** From Wörl et al. 1998, inset in **E** from Wörl et al. 1997, **F** from Wörl et al. 2002, with permission)

3.2.2.1
Functional Aspects

Electrophysiological studies of spinal afferents from the esophagus in various species demonstrated that they include muscular, mucosal, and serosal sensors (Clerc 1984; Clerc and Mei 1983a). They typically exhibited features compatible with a nociceptive function since they conducted at a slow rate, responded to graded distension either with increasing spike frequency or at high threshold, and displayed sensitivity to bradykinin (Sengupta et al. 1990; Sengupta et al. 1992). Their sensitivity to H^+ via the capsaicin receptor TRPV1 can be inferred from their chemical coding, i.e., substance P and CGRP content, and from functional studies. Intriguingly, the recently described vagal afferents from the guinea pig esophagus with cell bodies in the jugular and nodose ganglia displayed similar properties, in particular graded responses to distension and sensitivity to cap-



saicin (Yu et al. 2005). Although experimentally demonstrated in gastric and not strictly esophageal spinal afferents, upregulation of TRPV1 by acid challenge (Schicho et al. 2004) can also be expected to occur in the latter. Thus, the increased nerve fiber immunostaining for TRPV1 as reported in the inflamed human esophagus (Matthews et al. 2004) could well be attributed to spinal afferents. In the periphery, i.e., the various layers of the esophageal wall and its enteric ganglia, substance P and CGRP containing spinal primary afferents may be engaged in local reflexes resulting in vasodilatation and increased vascular permeability, the so-called neurogenic inflammatory response (Holzer 1988a; Sandler et al. 1993), or in effects on motility. Capsaicin application to the whole guinea pig esophagus in vitro resulted in muscular contraction (Barthó et al. 1999) while it reduced the force of vagally induced striated muscle contraction in the hamster (Izumi et al. 2003). In both situations, however, capsaicin may have led to release of substance P and CGRP from spinal and probably also vagal capsaicin-sensitive primary afferents with subsequent stimulation of, respectively, excitatory and inhibitory enteric motor neurons. Recently, neurokinin-1 receptor (NK-1R) was immunohistochemically demonstrated on myenteric neurons of the rat esophagus (Kuramoto et al. 2004). About 75% of NK-1R positive neurons were ChAT immunoreactive, i.e. presumptive excitatory cholinergic, while 25% were presumptive inhibitory, and about 50% of NK-1R positive were closely approached by primary spinal afferents costaining for substance P and CGRP. These results provide a link for the proposed local reflex. Since the tunica muscularis of all three species, i.e., guinea pig, hamster, and rat, consists of striated muscle, enteric motor neurons may exert their contractile or relaxing effects via coinnervation of motor endplates, or by innervating the smooth muscularis mucosae.

Spinal afferents from the esophagus converge with cutaneous afferents onto the same second order spinal neurons, thus providing a substratum for referral of pain from the esophagus to skin areas (Euchner-Wamser et al. 1993). Second order spinal neurons also receive convergent input from various other viscera, in addition to the esophagus (Jou et al. 2002). A prediction of this multiplicity might have been made from the extensive overlap of spinal primary afferent projections from esophagus, stomach, and duodenum (Khurana and Petras 1991) as well as from heart and bronchpulmonary area (Kummer et al. 1992; Kuo et al. 1984). Viscero-visceral convergence provides a pathophysiological basis for development of esophageal hypersensitivity upon duodenal acidification (Hobson et al. 2004).

Vagal afferent modulation of second order spinal neuronal activity via descending projections also occurs in case of the esophagus, as has been shown for afferents from other chest organs (Chandler et al. 1991; Hummel et al. 1997; Randich and Gebhart 1992). Spinal and vagal afferents may interact at spinal cord and probably also higher levels, thus determining the intensity of painful sensation from the esophagus. In a recent study using balloon distension or hydrochloric acid stimulation of upper or lower esophagus in the rat, pseudoaffective responses (neck

muscle EMG activity, taken as equivalent to pain reactions) were significantly higher upon upper versus lower stimulation (Hummel et al. 2003). The responses to upper stimulation were gradually reduced by consecutive sectioning of the cervical vagus nerve (which carries esophageal afferents of the recurrent laryngeal nerve) and the SLN, thus eliminating the dense vagal afferent innervation of the cervical esophagus. However, the same neurectomy procedures gradually increased the responses upon stimulation of the lower esophagus. This may be explained by release of spinal second order neurons from tonic descending inhibition mediated by vagal afferents.

3.3 Innervation of Esophageal Sphincters

3.3.1 Upper Esophageal Sphincter

The various components of the upper esophageal sphincter (UES) are innervated by neurons of the nucleus ambiguus that send axons through both the superior (Kobler et al. 1994; Sanders and Mu 1998) and recurrent (Brok et al. 1999; Mu and Sanders 1996) laryngeal nerves and the pharyngeal branch of the vagus (Fukushima et al. 2003). Tonically contracted at rest, its relaxation at the transition from the pharyngeal to the esophageal phase of swallowing is coordinated by the deglutition central pattern generator (Lang and Shaker 2000). Supposedly, the dense mucosal afferent innervation of the pharynx (Terenghi et al. 1986; Wang and Neuhuber 2003) and upper esophagus (Dütsch et al. 1998; Wank and Neuhuber 2001) is intimately related to UES motor control (Ali et al. 1994).

3.3.2 Lower Esophageal Sphincter

The LES is also of crucial importance for proper swallowing as its relaxation allows for transport of the bolus into the stomach. However, ill-coordinated LES relaxation contributes to gastroesophageal reflux disease (GERD). Thus, understanding the neuronal regulation of LES is mandatory for effective treatment of this condition; for proper appreciation of the enormous body of literature on LES control, the reader is referred to recent reviews (Chang et al. 2003; Goyal et al. 2001; Orlando 2001). Relaxation of LES is mediated by vagal preganglionic projections onto inhibitory nitrergic local neurons (see below). Not surprisingly, inhibitory nitrergic myenteric motor neurons are relatively more numerous than excitatory cholinergic neurons at the level of the LES as compared to stomach and small intestine (Brookes 1996). The perikarya of most motor neurons, both cholinergic and nitrergic, are located in the area of the sphincter itself indicating short axonal projections. Some reside in the abdominal esophagus, nitrergic neurons here located more oral than cholinergic ones. There is no significant ascending projection to the sphincter from myenteric neurons in the stomach. The important

role of inhibitory local neurons is emphasized by the lack of VIP- and NADPH-diaphorase-positive neuronal perikarya and nerve fibers in the LES of patients suffering from achalasia (Aggestrup et al. 1983; Mearin et al. 1993; Vanderwinden 1994). In contrast, excitatory cholinergic innervation is not affected in this condition (Holloway et al. 1986).

Afferent innervation of the LES is provided by both vagal and dorsal root ganglion neurons (Clerc 1983a). In electrophysiological studies, slowly adapting muscular mechansosensors from both sources were found, while mucosal afferents were exclusively vagal (Clerc 1984; Clerc and Mei 1983b). Anterograde tracing from vagal and dorsal root ganglia in cat revealed afferent fibers in both muscularis and mucosa of the LES (Clerc and Condamin 1987; Clerc and Mazzia 1994; Mazzia and Clerc 1997; Mazzia and Clerc 2000). Anterograde tracing in the rat demonstrated that IGLEs were few in the sphincter area proper whereas IMAs were present particularly in the inner zone of the circular muscle (Neuhuber et al. 1998; Wang and Powley 2000).

LES pressure control is achieved by a balanced interplay of both vagal and splanchnic reflex mechanisms involving cholinergic, adrenergic, nitrergic, and peptidergic signals, and many studies have been devoted to the neuromuscular mechanisms of LES relaxation (Aggestrup et al. 1986; Allescher et al. 1988; Behar et al. 1982; Beyak et al. 2003; Gilbert and Dodds 1987; Gonella et al. 1977; Gonella et al. 1979; Gonella et al. 1980; Niel 1986a; Niel 1986b; Smid and Blackshaw 2000b). LES relaxation during deglutition results from activation of intrinsic nitrergic inhibitory neurons by preganglionic vagal efferents with cell bodies in the caudal DMX (Chang et al. 2003; Hornby and Abrahams 2000; Hyland et al. 2001). Activation of the proper set of preganglionic vagal efferents is managed by a central pattern generator located in the medulla oblongata (see next section). Transient LES relaxation, which may lead to gastroesophageal reflux, is triggered by stimulation of gastric vagal afferents and mediated via vago-vagal reflexes (Mittal et al. 1995; Orlando 2001; Orlando 2003; Sifrim and Holloway 2001). The pattern of brainstem neuronal activation by gastric-afferent-induced LES relaxation as detected in the mouse by c-fos expression differs significantly from the deglutitive pattern induced by SLN stimulation (Sang and Goyal 2000). In particular, while deglutitive activity is seen in NTSis, NTSim, NTSce, and AMBc, gastric-afferent LES relaxation is paralleled by c-fos expression in commissural and dorsomedial NTS subnuclei and the caudal ("inhibitory") DMX, but not in the aforementioned nuclei. Data from experiments in ferrets suggested involvement also of tachykininergic spinal afferents in LES relaxation, which may activate enteric inhibitory neurons via axon reflexes (Blackshaw et al. 1997). However, studies on isolated muscle strips from the human LES argue against this mechanism (Smid and Blackshaw 2000a). Vagal reflex mechanisms leading to LES relaxation can be inhibited by GABA(B) agonists binding to receptors expressed on both vagal efferent and afferent neurons (Blackshaw et al. 2000; Page and Blackshaw 1999), a finding that may be of considerable therapeutic significance in GERD (Cange et al. 2002).

3.4 Swallowing Central Pattern Generator

Although beyond the scope of this review, some aspects of the swallowing central pattern generator (deglutitive motor pattern generator, DMPG; Bieger 1993; Jean 2001) may help in understanding peculiarities of extrinsic and intrinsic innervation of the esophagus.

As shown by elaborate studies in several species, in particular rat and sheep, both of which possess a striated esophageal muscle coat, the DMPG consists of a neuronal network in the medulla oblongata harboring second order neurons receiving primary afferent input, interneurons, premotor neurons, and motor neurons innervating muscles engaged in both oropharyngeal and esophageal stages of deglutition. For esophageal deglutition, the key neuronal pool resides in the NTSce, which receives vagal afferents from the esophagus (in particular its muscle coat) and projects monosynaptically to esophageal motor neurons in the compact formation of the AMB. Interneurons in the immediate vicinity of the NTSce are likely also to be part of the so-called dorsal swallowing group. Interneurons around the AMB, so-called periambigual neurons in the ventrolateral medulla. are also involved as part of the ventral swallowing group. Glutamate, acetylcholine, GABA, nitric oxide, and somatostatin appear to be the main transmitters involved in excitatory and inhibitory control of esophageal peristalsis (Bieger 2001; Cunningham and Sawchenko 1990; Jean 2001; Wiedner et al. 1995). Elicitation of esophageal deglutition by either electrical SLN stimulation or quasi-natural bolus stimuli resulted in enhanced c-fos expression in the NTSce and AMBc in mouse and the homologous NTS and AMB subnuclei in cat, and also in both the rostral and caudal DMX (Lang et al. 2004; Sang and Goyal 2001). Synaptic activation of the NTSce and the AMBc was expected from previous anatomical and functional studies. Activation of rostral (excitatory) and caudal (inhibitory) DMX neurons in cat most likely indicates preganglionic vagal control of both peristalsis in the tubular esophagus and relaxation of the LES. In mouse with an entirely striated tubular esophagus and a smooth LES, the same c-fos activation pattern was seen. This indicates DMX-induced relaxation of LES plus a DMX contribution to peristalsis control in the tubular esophagus. DMX activity during peristalsis in tubular striated esophagus may be related to control of the smooth lamina muscularis mucosae. However, it may also point to DMX influence on myenteric neurons coinnervating striated muscle fibers (see below). This suggests cooperation of the DMX and the AMB in regulation of motility of striated tunica muscularis. This may apply not only to species with an entirely striated but also to those with a mixed esophagus, e.g., cat and human. Coordinated activation of DMX neurons is mediated most likely by NTSce interneurons projecting to vagal preganglionics. This circuit probably also orchestrates gastric adaptive relaxation at the completion of the swallowing act (Rogers et al. 1999, 2003).

A notable difference between oropharyngeal and esophageal stages of deglutition is that the latter is modulated by peripheral afferent input to a much greater extent (Jean 2001; Miller 1986). Thus, the propagation of esophageal peristalsis and the amplitude of pressure propelling the bolus can be finely tuned to accommodate its size and consistency. As noted above, disruption of this afferent feedback abolishes this accommodative function and leads to motility disorders and even megaesophagus (Falempin et al. 1986; Satchell and McLeod 1984).

Although swallowing occurs in utero, the DMPG appears not to be fully developed at birth (Jean 2001; Miller 1986). This may help in understanding why enteric coinnervation of striated esophageal muscle in rodents is much more pronounced in early postnatal stages than at a later time (Breuer et al. 2004; Wörl et al. 2002). As will be outlined below, peripheral mechanisms of peristalsis control may be more important postnatally than in adulthood.

3.5 Cortical Representation of the Esophagus in Human

Transcranial magnetic stimulation was used in order to elucidate the cortical motor representation of various muscles involved in swallowing (Hamdy et al. 1996). A discrete somatotopic order in both primary motor and premotor cortices was found for mylohyoid, pharynx, and esophagus muscles. Interestingly, although this representation was bilateral, asymmetry existed independent of handedness. Presumably, these cortical areas project to the central pattern generator in a lateralized manner. This lateralization may be compensated for by commissural projections at brainstem level (Jean 2001) and by overlapping innervation of esophageal muscle by both vagi (Gruber 1978).

Several studies were devoted to localize the cortical representation of afferents from the esophagus. Electrical and mechanical stimulation were applied, and electro- and magnetoencephalography, positron emission tomography, and functional magnetic resonance imaging were used to detect cortical activation. Electrical stimulation activated primarily A delta vagal afferents, presumably both mucosal and muscular. Although results differed in some detail, presumably due to technical reasons, e.g., stimulation modalities or sites within the esophagus, both primary and secondary somatosensory cortices (SI and SII, respectively) were consistently activated by non-painful stimulation. Painful stimulation led to more pronounced responses in these areas and additionally resulted in activity in insular and anterior cingulate cortices (Aziz et al. 1997; Furlong et al. 1998; Hecht et al. 1999; Loose et al. 1999; Schnitzler et al. 1999). It is reasonable to assume that vagal afferent pathways are primarily involved in mediating non-painful cortical events, whereas discomfort and pain may be mediated primarily by spinal afferents (Söllenböhmer et al. 1996). However, recent indications for vagal nociceptors in the esophagus (Yu et al. 2005) and ample evidence for modulation of spinal nociceptive second order neurons by vagal afferents (Chandler et al. 1991; Hummel et al. 2003; Hummel et al. 1997; Ren et al.

1989) suggest that sensation from the esophagus is the result of a complex interplay of both vagal and spinal afferent pathways. In particular, chemical nociceptive stimulation may primarily activate vagal afferents (Holzer et al. 2004; Lamb et al. 2003).

4 Intrinsic Innervation

4.1 General Organization

The esophagus harbors a well developed intrinsic nervous system consisting of a ganglionated myenteric plexus and an aganglionic (in small mammals) or sparsely ganglionated (mostly in larger mammals) submucosal plexus (Cecio 1976; Christensen and Robison 1982; Greving 1931; Gruber 1968; Teixeira et al. 2001). It is well established that these local ganglia are essential for peristalsis regulation in the smooth muscle esophagus and for both excitatory and inhibitory innervation of the lamina muscularis mucosae (Bieger and Triggle 1985; Christensen et al. 1995; Conklin and Christensen 1994). Curiously though, these myenteric ganglia are also present in the striated muscle portion of the esophagus, a finding that has been enigmatic for a long time (Gruber 1968; Weisbrodt 1976). Density and sizes of myenteric ganglia vary along the esophagus and among species. For example, ganglia in the cervical esophagus of the rat are larger and contain more neurons than in the abdominal part of the organ (Neuhuber et al. 1998) while the reverse is true for the mouse (Raab et al. 2003). In the area of the LES proper, myenteric ganglia are sparse in most species (Christensen and Robison 1982). Although myenteric ganglia in the esophagus are significantly smaller than their counterparts in other gastrointestinal organs (Christensen et al. 1983), their intrinsic organization is similar. In particular, they contain basically the same set of excitatory (cholinergic; Kuramoto and Brookes 2000; Sang and Young 1998) and inhibitory (nitrergic; Fig. 13C; Fang and Christensen 1994; Grozdanovic et al. 1992; Morikawa and Komuro 1998; Ny et al. 1994; Ny et al. 1995; Rodrigo et al. 1998; Singaram et al. 1994; Wörl et al. 1994; Wu et al. 2003b) neurons, the former projecting mainly ascending and the latter projecting mainly descending. Likewise, several peptides known from enteric neurons elsewhere were described in esophageal ganglia, and their coexistence patterns were similar (Aggestrup et al. 1985; Leander et al. 1982; Melander et al. 1985; Uddman et al. 1978; Uddman et al. 1980; Wattchow et al. 1987; Wienbeck 1987). In particular, VIP (Fig. 13D), galanin, and NPY are typically colocalized with nitrergic markers in presumptive inhibitory neurons (Reichel et al. 1995; Wörl et al. 1998; Wörl et al. 1997), while substance P may be contained in cholinergic neurons. On the other hand, significant morphological and functional differences between esophageal and intestinal enteric neurons were revealed upon closer scrutiny. Differences in the proportions of Dogiel type I and type II neurons between esophagus and intestines were

already noted by classical investigators (Lawrentjew 1929). Electrophysiological studies demonstrated some peculiarities of myenteric neurons of the rat esophagus (De Laet et al. 2002) that preclude functional phenotyping along the lines established for the guinea pig intestine (Furness and Costa 1987). In the guinea pig some myenteric esophageal neurons also project their axons out of the organ to innervate the trachealis muscle (Fischer et al. 1998). This connection reflects the close developmental relationship between the two structures and mediates nonadrenergic, noncholinergic relaxation of the trachealis muscle upon vagal stimulation.

As with other organs of the gastrointestinal tract, smooth muscle of the esophagus harbors interstitial cells of Cajal (ICC) intercalated between nerve fibers and smooth muscle cells (Berezin et al. 1994; Daniel and Posey-Daniel 1984; Faussone-Pellegrini and Cortesini 1985). However, ICC as identified by the Kit receptor were also found in the striated tunica muscularis of the mouse (Rumessen et al. 2001) and the pig esophagus (Wu et al. 2003a). Here, they were contacted by nitrergic nerve fibers of presumably intrinsic origin and may function as specialized mechanosensors. A link between ICC and mechanosensory function is also suggested by their association with IMAs in the stomach (Fox et al. 2002; Fox et al. 2000; Fox et al. 2001b).

Thus, the intrinsic nervous system of the esophagus is as specialized as intrinsic neural networks in other digestive organs in depending on functional demands. Still, the most striking feature of myenteric neurons in the esophagus is their affinity to striated muscle fibers. In the following, anatomy and possible functional significance of this so-called enteric coinnervation of striated esophageal muscle is briefly summarized. For a more detailed account the reader is referred to the recent review of Wörl and Neuhuber (2005a).

4.2 Enteric Coinnervation

There is no doubt that smooth muscle of both the tunica muscularis and muscularis mucosae are innervated by myenteric neurons that are in turn controlled by the DMX. However, the classical view that striated esophageal muscle is exclusively innervated by cholinergic vagal input from the brainstem via motor endplates has been repeatedly challenged by findings and suggestions in favor of a contribution of the esophageal myenteric plexus to such innervation (Gruber 1968; Gruber 1978; Jurica 1926; Toyama et al. 1975; Weisbrodt 1976). This issue was found to be definitely resolvable by using NADPH-d histochemistry and nNOS immunocytochemistry in combination with markers for vagal motor fibers, e.g., CGRP, VAChT, or anterograde tracing from the nucleus ambiguus. Thus, we were able to demonstrate that a majority of motor endplates in the rat esophagus are associated with fine varicose NADPH-d/nNOS-positive nerve fibers (Neuhuber et al. 1994; Wörl et al. 1994). In these and later studies two morphologically and chemically distinct types of nerve endings on motor endplates could be demonstrated: coarse

boutons immunoreactive for CGRP and VAChT arising from thick axons, and smaller NADPH-d/nNOS-positive boutons arising from thin fibers (Figs. 13E, F). Fibers leading to coarse boutons could be demonstrated to originate in the nucleus ambiguus while the origin of the small boutons turned out to be myenteric ganglia. This unorthodox double supply of striated muscle fibers by both vagal and enteric neurons was termed enteric coinnervation. Shortly before this, nitric oxide (NO), which is synthesized by a multienzyme complex including NOS and NADPH-d, had been proposed as the main inhibitory mediator of nonadrenergic, noncholinergic (NANC) neurons in the gastrointestinal tract (Bult et al. 1990; Gibson et al. 1990; Tottrup et al. 1991). Nitrergic coinnervation was restricted to the esophagus and could be detected in neither branchiomeric nor myotomal musculature (Neuhuber et al. 1994; see, however, Hisa et al. 1996 for the rare coinnervated motor endplate in the larynx). Since the pattern of activity of the UES, whose major tone-generating muscle is the cricopharyngeus, is different from that of the other pharyngeal constrictors (Kobler et al. 1994; Lang and Shaker 2000; Sivarao and Goyal 2000), the question arose whether enteric nerve fibers also contribute to its motor innervation. Therefore we investigated the pharyngo-esophageal junction of the rat with histochemistry for NADPH-d and AChE. The first enteric coinnervated motor endplates were detected immediately caudal to the semicircular compartment of the rat thyropharyngeus muscle (Beyer et al. 1996), which is considered homologous to the human cricopharyngeus muscle (Kobler et al. 1994). Thus, enteric coinnervation is restricted to the tubular esophagus caudal to the UES. This new evidence for an enteric coinnervation of esophageal motor endplates draws the attention to a component of esophagus innervation that was hitherto neglected in concepts of peristalsis control in the striated muscle part. In retrospect, coinnervating thin caliber nerve fibers coursing parallel to thick motor axons can be recognized in early work of Ottaviani (his Figs. 5, 8; Ottaviani 1937/38). This author considered them either vagal "satellite" fibers or sympathetic.

4.2.1
Spatial Relationships of Vagal and Enteric Terminals and Neurochemical Coding

Confocal laser scanning and electron microscopy revealed intimate relationships between enteric and vagal terminals on motor endplates on the one hand, and enteric terminals and the sarcolemma on the other, suggesting both pre- and postsynaptic modulatory effects of enteric neurons on vagal neuromuscular transmission in striated esophageal muscle (Fig. 13E, F; Wörl et al. 1997). In further studies it was demonstrated that VIP, NPY, GAL (Fig. 13E), and M-ENK were also present in enteric neurons and nerve terminals on motor endplates in the esophagus of different species, and that in most cases these peptides were co-localized (Wörl and Neuhuber 2005a). It is noteworthy that these peptides are typical for inhibitory neurons in the enteric nervous system of the intestine (for review see Brookes 2001; Furness 2000). Nerve terminals coded similar to enteric

inhibitory neurons were completely separated from vagal cholinergic endings (Fig. 13E, F). Although the peptides and transmitters localized in enteric nerve terminals were described several years ago, knowledge about receptors of these ligands on esophageal motor endplates remains scarce. Until now it could be demonstrated only by immunocytochemistry, that: (1) soluble guanylyl cyclase (sGC), the most important receptor for the signaling molecule NO (Friebe and Koesling 2003; Koesling et al. 2004; Kuhn 2003), can be localized to a zone immediately beneath the motor endplate, indicating a postjunctional mechanism of NO signaling; (2) muscarinic acetylcholine receptors (mAChRs) are located on coinnervating enteric terminals in close proximity to cholinergic vagal terminals, indicating a function as a sort of sensor for acetylcholine release; and (3) mu-opioid receptor antisera stain the entire sarcolemma including the endplate area of almost all muscle fibers (Neuhuber et al. 2001). Based on the reduction of vagally induced contractions of striated esophageal-muscle by mu-opioid receptor agonists, as shown in a recent in vitro study, a role of mu-opioid receptors in the peripheral modulation of esophageal contractions was suggested (Neuhuber et al. 2001; Storr et al. 2000).

4.2.2 Comparative Anatomy of Enteric Coinnervation

Originally described in the rat (Neuhuber et al. 1994; Wörl et al. 1994), enteric coinnervation has meanwhile been found also in the esophagus of other mammals, including mouse, hamster, guinea pig, rabbit, cat, pig, sheep, and monkey (Wörl and Neuhuber 2005a). In addition to the results in lower animals, we likewise found enteric nerve fibers on motor endplates in the human esophagus by means of enzyme histochemistry for NADPH-d and AChE (Sörensen et al. 1995). Taken together, the wide distribution of enteric coinnervation among mammalian species, including human, possessing either mixed or entirely striated esophagus strongly suggests a significant functional role.

The percentage of vagally innervated motor endplates additionally receiving enteric nerve fibers, the so-called coinnervation rate (Neuhuber et al. 1994; Wörl et al. 1994), varied depending on both species and markers used for enteric neurons. For example, in NMRI mice nitrergic coinnervation rates were as low as 10%, while in BALB/c and C57Bl/6 mice these were significantly higher at 32% and 37%, respectively (Wörl and Neuhuber 2005a). Average coinnervation rate in rats even reached values between 55% and 88% as determined with nNOS and galanin immunohistochemistry, respectively. In C57Bl/6 mice, nNOS immunohistochemistry revealed coinnervation at a rate of 37% whereas galanin showed a value of 15% (Breuer et al. 2004). This may reflect species-specific variability of expression levels for the respective markers or simply be due to technical factors. Determination of coinnervation rates in cervical, thoracic, and abdominal portions of the esophagus revealed in most species increasing values from oral to aboral, the highest values being found in the abdominal esophagus. In our original study

in rat using NADPH-d as a marker, coinnervation rates of 42, 59, and 72% were found for the cervical, thoracic, and abdominal esophagus, respectively (Neuhuber et al. 1994).

As low coinnervation rates were not related to gross swallowing disorders, these differences raised doubts concerning the significance of enteric coinnervation for normal functioning of the adult esophagus. Investigation of enteric and vagal innervation during development of the esophagus revealed average coinnervation rates of more than 80% in early postnatal stages in rats and in NMRI and BALB/c mice (Vietze et al. 1995; Wörl et al. 2002). These high coinnervation rates declined over several weeks until a stable adult situation was reached. Hence, the question arose whether enteric coinnervation may be functionally important mainly during development of esophageal striated muscle.

4.2.3 Ontogeny of the Esophageal Neuromuscular Junction

In order to elucidate the precise developmental timing of vagal and enteric innervation of striated muscle, esophagi from mice from age E 17 to 3 months were triple stained for VAChT, nNOS, and α-bungarotoxin and investigated at 1 mm intervals along their entire length using confocal laser scanning microscopy (Breuer et al. 2004). At closer scrutiny, enteric nerve fibers appeared to grow toward newly developed endplates once they had become innervated by vagal motor fibers. There was even a certain stage when all endplates were contacted by enteric fibers. While vagal innervation remained stable through adulthood, enteric fibers retracted from many endplates (Breuer et al. 2004; Wörl et al. 2002). As outlined above, the proportion of coinnervated endplates in adult individuals varied among species and even mouse strains. The factors determining this species-specific coinnervation rate remain to be elucidated. However, although variable in adult animals, coinnervation rates were at almost the same level in perinatal rats and mice. The time course of this well-ordered sequential development of enteric coinnervation is in perfect register with the cranio-caudal transition from smooth to striated tunica muscularis that takes place from late fetal to early postnatal life in rodents. At the end of this process, the tunica muscularis of the tubular esophagus is entirely striated. This does not necessarily mean that the coinnervation of enteric neurons is causally related to smooth-to-striated transformation. Indeed, striated muscle fibers develop apparently normally in the esophagus of Mash $1^{-/-}$ knockout mice, which almost completely lack enteric neurons, hence coinnervation, in the esophagus (Sang et al. 1999). However, these mice die shortly after birth when smooth-to-striated muscle transformation is not yet complete (Wörl and Neuhuber 2000), an untimely demise that precludes detailed studies on the full developmental sequence of the esophageal tunica muscularis in these animals. Transition from smooth to striated muscle also occurs in the esophagus of other species, notably the human (Jit 1974). However, this change apparently dies out in the upper thoracic area and does not reach the esophago-gastric junc-

tion. As younger stages of striated myogenesis are found more caudal than older ones, development of motor endplates and their vagal and enteric innervation is also caudo-cranially aligned. Careful analysis of the spatio-temporal organization of these processes led us to conclude that enteric nerve fibers are attracted by and maintained on all maturing motor endplates, only to be retracted from the majority thereof when their maturity is accomplished, all in a well-ordered sequence (Breuer et al. 2004). This process suggests an important role for enteric coinnervation in the development of neuromuscular contacts and probably also in that of striated muscle fibers themselves. For a more detailed review of neuromuscular development of the rodent esophagus see Wörl and Neuhuber (2005a, b).

It has been suggested that the presence of enteric terminals at motor endplates of adult animals represents a non-specific residual from early developmental stages when the tunica muscularis still consisted of smooth muscle fibers embedded in a meshwork of enteric nerve fibers (Sang and Young 1997). However, the observations that enteric nerve fibers contacted striated esophageal muscle fibers at specialized sites, i.e. motor endplates, and that these contacts appeared in an ordered cranio-caudal and temporal sequence after vagal neuromuscular junctions had been established (Breuer et al. 2004; Wörl et al. 2002) argue against this suggestion. Rather, the transient presence of nNOS-positive enteric nerve terminals on every developing motor endplate during maturation of vagal myoneuronal contacts suggests an import role for enteric coinnervation in the ontogeny of esophageal striated muscle (Breuer et al. 2004). Remarkably, in rat and in three mice strains with significantly different adult coinnervation rates, the perinatal innervation patterns were more or less identical (Breuer et al. 2004; Vietze et al. 1995; Wörl et al. 2002). It is possible that enteric coinnervating nerve fibers are involved in maturation of the neuromuscular junction itself (Breuer et al. 2004). Mash1$^{-/-}$ mice, which have almost no enteric neurons in the esophagus, have significantly more vagal motor terminals in developing endplates, suggesting disturbed development of vagal myoneural contacts (Sang et al. 1999). In addition, the prominent enteric coinnervation in the early postnatal period may indicate functional compensation for a presumably immature central pattern generator (Jean 2001; Miller 1986) by local modulation of striated muscle contractions. Local neural circuits could play a more important role in the regulation of peristalsis in developing than in adult esophagus (Breuer et al. 2004). Possible components of these local circuits are IGLEs as primary afferent elements and their synaptic contacts to myenteric motor neurons. Depending on the advancement of smooth-to-striated transformation of the tunica muscularis at the respective postnatal stages, these myenteric neurons may innervate smooth muscle or coinnervate striated muscle. In addition, IGLEs also appear to contact excitatory and inhibitory interneurons (Raab and Neuhuber 2004). Clearly, our understanding of development of intrinsic neuronal circuits in the esophagus is still too fragmentary as to corroborate this hypothesis.

5 Functional Considerations

5.1 General Remarks

In addition to the complexity of the DMPG, innervation of the esophagus is more complex than that of other muscles involved in deglutition. The most striking differences are (1) vagal efferent innervation originating from two different brainstem nuclei, i.e., the AMB and DMX, and (2) vagal afferent neurons intermingling with enteric circuitry via IGLEs. This peculiarity is further emphasized by the notion that even in species with an entirely striated esophagus, e.g., rat and mouse, myenteric neurons, and probably also the DMX supplying them with preganglionic fibers, are engaged in innervation of the tunica muscularis through coinnervation of striated muscle fibers. This innervation pattern is summarized in Fig. 14. In the following section, some ideas taking into account these peculiarities are proposed.

5.2 Cooperation Between Extrinsic and Intrinsic Systems

The chemical coding of enteric coinnervation and spatial relationships of vagal and enteric nerve terminals in motor endplates point to an inhibitory effect of enteric neurons on esophageal motility at the motor endplate level. Thus, efforts were undertaken to prove this hypothesis by functional experiments. In a first approach using a vagus nerve–esophagus preparation from the rat, the influence of VIP, GAL, the NOS inhibitor L-NNA, and the NO-donor DEA-NO on vagally induced contraction of the striated esophageal muscle was tested, but no significant effect could be ascertained (Storr et al. 2001). However, a similar vagus nerve–esophagus preparation from the hamster yielded results supporting an inhibitory effect on vagally mediated contractions of esophageal striated muscle (Izumi et al. 2003). Both vagally induced contraction force and ACh release were significantly diminished by mimicking or activating the intrinsic nitrergic neuronal system. Different inhibitors of NOS and NO donors L-NAME and NONOate were used, which might partly explain results directly contrary to those of Storr et al. (2001). Interestingly, this effect could also be elicited by applying capsaicin and piperine to the organ bath. This is the first study to experimentally demonstrate an inhibitory effect of nitrergic coinnervating nerve terminals on esophageal striated muscle contractions in vitro in support of the idea of a physiological relevance for this novel innervation component. Remarkably, the inhibition of muscle contraction was accomplished not through direct stimulation of nitrergic neurons, but via capsaicin-sensitive afferent neurons, which in turn supposedly supply the direct stimulation to nitrergic myenteric neurons. Thus, Izumi and colleagues (2003) probably interfered with a local reflex arc involving capsaicin-sensitive neurons

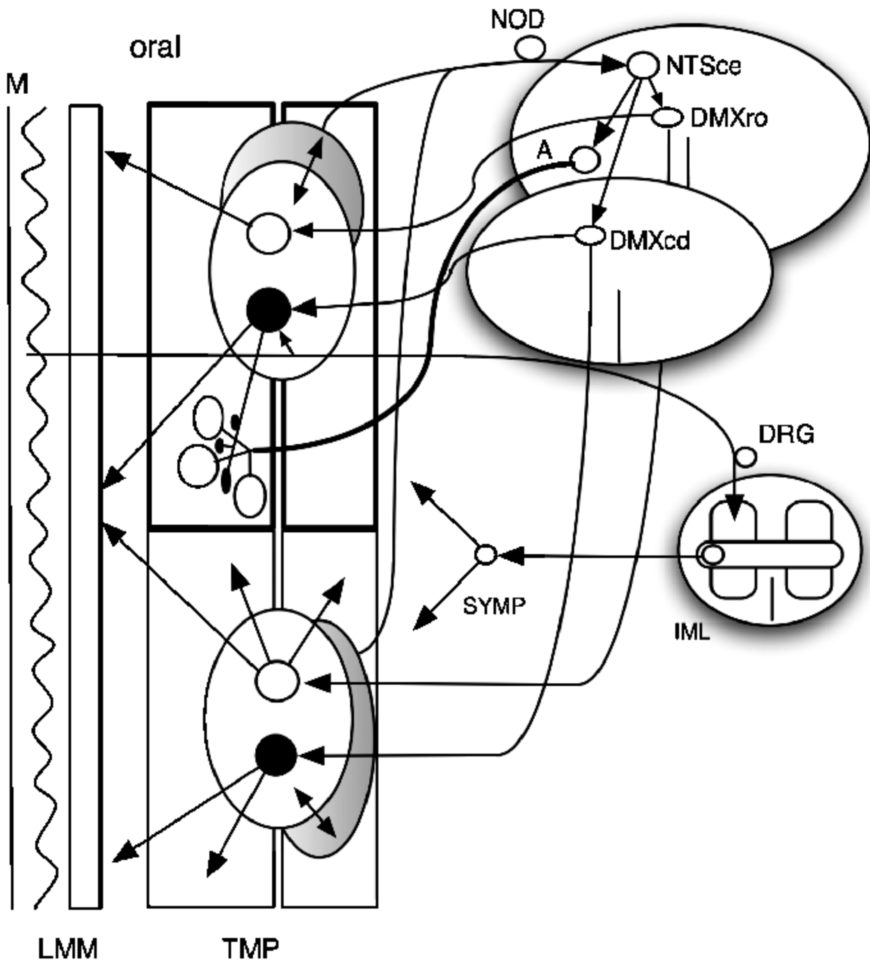
that activated inhibitory nitrergic neurons to decrease the striated muscle contraction force (Izumi et al. 2003). Since most vagal primary afferent IGLEs (Berthoud et al. 1997b) and enteric neurons in the rat esophagus (Neuhuber et al. 1994) are capsaicin resistant, the most likely source for the capsaicin-sensitive nerve fibers are spinal ganglia (Holzer 1988). Spinal primary afferents from the esophagus and gastrointestinal tract typically co-express SP, NKA, and CGRP (Dütsch et al. 1998; Holzer 1988; Holzer and Holzer-Petsche 1997a; Holzer and Holzer-Petsche 1997b; Kuramoto et al. 2004; Rodrigo et al. 1985). Tachykinins and CGRP released from primary afferents mediate the "local effector" function of their source (Holzer 1988). Spinal afferent nerve fibers were shown to contact myenteric neurons in the esophagus (Mazzia and Clerc 1997), and these neurons express NKA receptors (Kuramoto et al. 2004). Thus, key elements of a local reflex arc from capsaicin-sensitive afferents via nitrergic myenteric neurons to motor endplates are present in the esophagus. Modulation of gastrointestinal motility by capsaicin- and piperine-sensitive primary afferent neurons has been reported previously (Holzer et al. 1987; Takaki et al. 1990). In addition, IGLEs may locally influence myenteric neurons through release of glutamate and SP (Kressel and Radespiel-Tröger 1999; Raab and Neuhuber 2003; Raab and Neuhuber 2004). Thus, both capsaicin-sensitive (spinal) and capsaicin-resistant (IGLEs) afferents may feed into a local reflex arc for modulation of vagally induced muscle contraction in the esophagus.

AMBc neurons innervating the abdominal esophagus in the rat receive significantly fewer inhibitory Gray type II synapses than ambiguus motor neurons

Fig. 14 Summary diagram of vagal and spinal innervation of the esophagus. The compact formation of the nucleus ambiguus (*A*) provides innervation of striated muscle (*thick contoured*) via motor endplates. Inhibitory myenteric neurons (*filled circle*) coinnervate these motor endplates. Preganglionic neurons in the rostral dorsal motor nucleus (*DMXro*) innervate excitatory cholinergic neurons (*open circles*), while neurons in the caudal dorsal motor nucleus (*DMXcd*) innervate inhibitory nitrergic myenteric neurons (*filled circles*), thus providing excitatory (ascending) and inhibitory (descending) innervation, respectively, to smooth muscle (*thin contoured*) of both the tunica muscularis (*TMP*) and lamina muscularis mucosae (*LMM*). Preganglionic innervation from DMXcd to coinnervating myenteric nitrergic neurons is hypothetical. For the sake of clarity, excitatory and inhibitory neurons to TMP, LMM and motor endplates are summarized in one *open circle* and one *filled circle*, respectively. Low threshold muscular mechanosensory information from IGLEs (*shaded*) connected to the nodose ganglion (*NOD*) afferent axons is relayed through the central subnucleus of the solitary complex (*NTSce*) to esophageal motor neurons in the nucleus ambiguus and probably also to the DMX. Reciprocal connections of IGLEs to myenteric neurons are suggested by *double arrows*. Preganglionic sympathetic innervation from the intermediolateral nucleus (*IML*) in the spinal cord is relayed through sympathetic ganglia (*SYMP*). Dorsal root ganglion (*DRG*) afferents make *en passant* contacts with myenteric neurons. Innervation of the LES is similar to that of smooth muscle of the tubular esophagus except for a paucity of IGLEs, which are probably substituted for by IMAs. *M*, mucosa

supplying the cervical esophagus and pharynx (Hayakawa et al. 1996; Hopkins 1995; Saxon et al. 1996). The significance of this finding is unclear at present. However, paucity of inhibitory synapses on esophageal motor neurons may result in greater demand for peripheral inhibitory modulation of striated muscle contraction in the abdominal esophagus, thus requiring the higher coinnervation rates that are typically found in the abdominal esophagus (Neuhuber et al. 1994; Wörl and Neuhuber 2005a).

Both vagal and enteric nerve terminals on motor endplates in the rat esophagus have been shown to be immunoreactive for the M2 subtype of mAChRs (Neuhuber et al. 2001). This may indicate multiple modulatory roles for ACh. First, ACh released from vagal motor terminals may modulate its own release via presynaptic autoreceptors. Such autoreceptors involved in feedback regulation of ACh release



have been demonstrated in neuromuscular junctions of both mouse (Minic et al. 2002) and rat (Santafe et al. 2003). Second, ACh from vagal terminals may modulate via mAChRs' release of, e.g., NO from enteric coinnervating terminals, similar to what has been shown in various situations (Hebeiss and Kilbinger 1999; Lanzafame et al. 2003; Sterin-Borda et al. 2003). Since NO and possibly other transmitters also present in enteric terminals, e.g., VIP and NPY, can in turn modulate ACh release (Prast and Philippu 1992), mutual regulation of vagal and enteric terminals could take place in esophageal motor endplates. As a third possibility, ACh released from vagal terminals during peristalsis could trigger a retrograde signal in coinnervating enteric neurons that may be picked up by IGLEs. Thus, IGLEs may detect motility-related events in the esophagus via retrograde signals from enteric neurons. Communication between enteric neurons and IGLEs, e.g., via NO release, may easily occur given the intricate structural relationship between them.

However, at present only scarce experimental evidence exists for vagal–enteric interactions at the endplate level (Izumi et al. 2003), and none for involvement of enteric coinnervation in inhibitory modulation of striated esophageal muscle contraction in vivo. Rather, distal and deglutitive inhibition as observed in the striated rat esophagus appear to be entirely mediated by medullary mechanisms (Dong et al. 2000). Nevertheless, a further possibility exists for inhibitory modulation of vagally mediated contraction of striated esophageal muscle. Interestingly, the proximal striated muscle of the human esophagus behaves manometrically more like the distal smooth muscle rather than like the striated muscle of the pharynx, i.e., striated muscle of the proximal esophagus contracts more slowly than expected (Peghini et al. 1998). The mechanism of this contraction delay is unclear. It is tempting to speculate that slowing down the fast-contracting striated muscle for better coordination with the slow contracting smooth muscle is an important function of enteric coinnervation. This accommodation may contribute to uniformity in progression of peristalsis through the esophagus. This slowing down of striated muscle contraction is present also in species with an entirely striated muscle coat (Jean 2001). Since striated esophageal muscle fibers, e.g., in rat, are of the fast-twitch type, this reduction in velocity cannot by fully explained by factors intrinsic to the muscle fibers. In this context it would be interesting if high or low coinnervation rates in different species or mouse strains correlate with predominance of fast- and slow-twitch muscle fibers, respectively. It remains to be elucidated if the role of preganglionic innervation from the DMX to myenteric ganglia in the striated esophagus is to activate inhibitory enteric neurons that slow down striated muscle contraction during peristalsis. Interestingly, recent c-fos studies in the mouse demonstrated activity in both rostral and caudal areas of the DMX during the esophageal phase of swallowing (Sang and Goyal 2001). If electrical stimulation of the SLN was set at 10 Hz, which was able to elicit the whole swallowing sequence, enhanced c-fos expression was seen over more extended areas of the DMX than with 5 Hz stimulation, which elicited the buccopharyngeal sequence and LES relaxation only. This sug-

gests involvement of DMX in control of peristalsis in the entirely striated tubular esophagus of the mouse. Enteric esophageal neurons coinnervating striated muscle may represent a relay in this pathway. DMX activation during tubular esophageal peristalsis may also simply relate to control of the smooth muscularis mucosae.

6 Concluding Remarks

Studies over the past two decades have significantly broadened our knowledge of the complexity of esophageal innervation. Although many of the experimental data, in particular on enteric coinnervation of striated muscle fibers and possible involvement of terminals of vagal and spinal primary afferent neurons in synaptic circuitry of myenteric ganglia, have yet to be evaluated as to clinical impact, some results have already opened new therapeutic options, e.g., treatment of GERD with GABA(B) agonists based on experimental insights on GABA(B)-receptor-mediated inhibition of gastroesophageal mechanosensors. It can be expected that the recent discovery of key molecules of the glutamatergic system in vagal mechanosensors, i.e., VGLUT1 and VGLUT2 and metabotropic glutamate receptors, will have similar consequences for the treatment of esophageal motility disorders. Likewise, the detailed knowledge of the structure of, and of functionally relevant molecules within, both motor and sensory terminals in the esophagus provides a reasonably sound basis for future studies on the pathogenesis of various motility-related diseases of the esophagus. For example, previous experimental observations on acrylamide-induced afferent neuropathy associated with megaesophagus may be re-evaluated and expanded toward molecular analysis of changes in IGLEs that had not yet been identified as tension receptors when these studies were performed 20 years ago. The possibility that expression of TRPV1 or ASIC is upregulated in IGLEs under inflammatory conditions, thus leading to mechanical hypersensitivity, i.e., visceral allodynia, remains to be explored. Recent insight into development of esophageal muscle and its innervation will probably help in understanding developmental malformations such as atresia of the esophagus. Thus, combination of fine-grained anatomical, molecular, and functional approaches will hopefully further improve the currently rather unsatisfactory therapeutic options for esophageal neuromuscular disorders.

References

Aggestrup S, Uddman R, Jensen SL, Hakanson R, Sundler F, Schaffalitzky de Muckadell O, Emson P (1986) Regulatory peptides in lower esophageal sphincter of pig and man. Dig Dis Sci 31:1370–1375

Aggestrup S, Uddman R, Jensen SL, Sundler F, Schaffalitzky de Muckadell O, Holst JJ, Hakanson R, Ekman R, Sorensen HR (1985) Regulatory peptides in the lower esophageal sphincter of man. Regul Pept 10:167–178

Aggestrup S, Uddman R, Sundler F, Fahrenkrug J, Hakanson R, Sorensen HR, Hambraeus G (1983) Lack of vasoactive intestinal polypeptide nerves in esophageal achalasia. Gastroenterology 84:924–927

Aharinejad S, Firbas W (1989) Die Innervation des menschlichen Ösophagus. Acta Anat (Basel) 136:715–720

Aldskogius H, Elfvin LG, Forsman CA (1986) Primary sensory afferents in the inferior mesenteric ganglion and related nerves of the guinea pig. An experimental study with anterogradely transported wheat germ agglutinin-horseradish peroxidase conjugate. J Auton Nerv Syst 15:179–190

Ali GN, Laundl TM, Wallace KL, Shaw DW, Decarle DJ, Cook IJ (1994) Influence of mucosal receptors on deglutitive regulation of pharyngeal and upper esophageal sphincter function. Am J Physiol 267:G644–649

Allescher HD, Berezin I, Jury J, Daniel EE (1988) Characteristics of canine lower esophageal sphincter: a new electrophysiological tool. Am J Physiol 255:G441–453

Altschuler SM, Bao X, Bieger D, Hopkins DA, Miselis RR (1989) Viscerotopic representation of the upper alimentary tract in the rat: sensory ganglia and nuclei of the solitary and spinal trigeminal tracts. J Comp Neurol 283:248–268

Alvarez FJ, Villalba RM, Zerda R, Schneider SP (2004) Vesicular glutamate transporters in the spinal cord, with special reference to sensory primary afferent synapses. J Comp Neurol 472:257–280

Andrew BL (1956) The nervous control of the cervical oesophagus of the rat during swallowing. J Physiol 134:729–740

Andrew BL (1957) Activity in afferent nerve fibres from the cervical oesophagus. J Physiol 135:54–55P

Andrews PLR, Lang KM (1982) Vagal afferent discharge from mechanoreceptors in the lower oesophagus of the ferret. J Physiol 332:29P

Anlauf M, Schäfer MK, Eiden L, Weihe E (2003) Chemical coding of the human gastrointestinal nervous system: cholinergic, VIPergic, and catecholaminergic phenotypes. J Comp Neurol 459:90–111

Araque A, Parpura V, Sanzgiri RP, Haydon PG (1998) Glutamate-dependent astrocyte modulation of synaptic transmission between cultured hippocampal neurons. Eur J Neurosci 10:2129–2142

Asaad K, Abd-El Rahman S, Nawar NN, Mikhail Y (1983) Intrinsic innervation of the oesophagus in dogs with special reference to the presence of muscle spindles. Acta Anat (Basel) 115:91–96

Atkinson L, Batten TF, Corbett EK, Sinfield JK, Deuchars J (2003) Subcellular localization of neuronal nitric oxide synthase in the rat nucleus of the solitary tract in relation to vagal afferent inputs. Neuroscience 118:115–122

Aziz Q, Andersson JL, Valind S, Sundin A, Hamdy S, Jones AK, Foster ER, Langstrom B, Thompson DG (1997) Identification of human brain loci processing esophageal sensation using positron emission tomography. Gastroenterology 113:50–59

Barrett RT, Bao X, Miselis RR, Altschuler SM (1994) Brain stem localization of rodent esophageal premotor neurons revealed by transneuronal passage of pseudorabies virus. Gastroenterology 107:728–737

Barthélémy P, Sabeur G, Jammes Y (1996) Reflex relationships between the cervical esophagus and the respiratory system in cats. Neurosci Lett 217:81–84

Barthó L, Lenard L, Jr., Patacchini R, Halmai V, Wilhelm M, Holzer P, Maggi CA (1999) Tachykinin receptors are involved in the "local efferent" motor response to capsaicin in the guinea-pig small intestine and oesophagus. Neuroscience 90:221–228

Bartlet AL (1968) The effect of vagal stimulation and eserine on isolated guinea-pig oesophagus. Q J Exp Physiol Cogn Med Sci 53:170–174

Baumgarten HG, Lange W (1969) Adrenergic innervation of the oesophagus in the cat (Felis domestica) and Rhesus monkey (Macacus rhesus). Z Zellforsch Mikrosk Anat 95:529–545

Behar J, Kerstein M, Biancani P (1982) Neural control of the lower esophageal sphincter in the cat: studies on the excitatory pathways to the lower esophageal sphincter. Gastroenterology 82:680–688

Berezin I, Daniel EE, Huizinga JD (1994) Ultrastructure of interstitial cells of Cajal in the canine distal esophagus. Can J Physiol Pharmacol 72:1049–1059

Berthoud HR (1995) Anatomical demonstration of vagal input to nicotinamide acetamide dinucleotide phosphate diaphorase-positive (nitrergic) neurons in rat fundic stomach. J Comp Neurol 358:428–439

Berthoud HR, Carlson NR, Powley TL (1991) Topography of efferent vagal innervation of the rat gastrointestinal tract. Am J Physiol 260:R200–207

Berthoud HR, Patterson LM, Neumann F, Neuhuber WL (1997a) Distribution and structure of vagal afferent intraganglionic laminar endings (IGLEs) in the rat gastrointestinal tract. Anat Embryol (Berl) 195:183–191

Berthoud HR, Patterson LM, Willing AE, Mueller K, Neuhuber WL (1997b) Capsaicin-resistant vagal afferent fibers in the rat gastrointestinal tract: anatomical identification and functional integrity. Brain Res 746:195–206

Berthoud HR, Powley TL (1992) Vagal afferent innervation of the rat fundic stomach: morphological characterization of the gastric tension receptor. J Comp Neurol 319:261–276

Beyak MJ, Collman PI, Xue S, Valdez DT, Diamant NE (2003) Release of nitric oxide in the central nervous system mediates tonic and phasic contraction of the cat lower oesophageal sphincter. Neurogastroenterol Motil 15:401–407

Beyer S, Wörl J, Neuhuber WL (1996) Nitrerge Co-Innervation von motorischen Endplatten im pharyngo-ösophagealen Übergang bei der Ratte. Ann Anat Suppl 178:344

Bieger D (1993) The brainstem esophagomotor network pattern generator: a rodent model. Dysphagia 8:203–208

Bieger D (2001) Rhombencephalic pathways and neurotransmitters controlling deglutition. Am J Med 111 Suppl 8A:85S–89S

Bieger D, Hopkins DA (1987) Viscerotopic representation of the upper alimentary tract in the medulla oblongata in the rat: the nucleus ambiguus. J Comp Neurol 262:546–562

Bieger D, Triggle C (1985) Pharmacological properties of mechanical responses of the rat oesophageal muscularis mucosae to vagal and field stimulation. Br J Pharmacol 84:93–106

Blackshaw LA, Haupt JA, Omari T, Dent J (1997) Vagal and sympathetic influences on the ferret lower oesophageal sphincter. J Auton Nerv Syst 66:179–188

Blackshaw LA, Smid SD, O'Donnell TA, Dent J (2000) GABA(B) receptor-mediated effects on vagal pathways to the lower oesophageal sphincter and heart. Br J Pharmacol 130:279–288

Bonington A, Mahon M, Whitmore I (1988) A histological and histochemical study of the cricopharyngeus muscle in man. J Anat 156:27–37

Breuer C, Neuhuber WL, Wörl J (2004) Development of neuromuscular junctions in the mouse esophagus: morphology suggests a role for enteric co-innervation during maturation of vagal myoneural contacts. J Comp Neurol 475:47–69

Brok HA, Copper MP, Stroeve RJ, Ongerboer de Visser BW, Venker-van Haagen AJ, Schouwenburg PF (1999) Evidence for recurrent laryngeal nerve contribution in motor innervation of the human cricopharyngeal muscle. Laryngoscope 109:705–708

Brookes SJ (2001) Classes of enteric nerve cells in the guinea-pig small intestine. Anat Rec 262:58–70

Brookes SJH (1996) Characterization of excitatory and inhibitory motor neurons to the guinea pig lower esophageal sphincter. Gastroenterology 111:108–117

Broussard DL, Altschuler SM (2000a) Brainstem viscerotopic organization of afferents and efferents involved in the control of swallowing. Am J Med 108 Suppl 4a:79S–86S

Broussard DL, Altschuler SM (2000b) Central integration of swallow and airway-protective reflexes. Am J Med 108 Suppl 4a:62S–67S

Broussard DL, Lynn RB, Wiedner EB, Altschuler SM (1998) Solitarial premotor neuron projections to the rat esophagus and pharynx: implications for control of swallowing. Gastroenterology 114:1268–1275

Bult H, Boeckxstaens GE, Pelckmans PA, Jordaens FH, Van Maercke YM, Herman AG (1990) Nitric oxide as an inhibitory non-adrenergic non-cholinergic neurotransmitter. Nature 345:346–347

Cange L, Johnsson E, Rydholm H, Lehmann A, Finizia C, Lundell L, Ruth M (2002) Baclofen-mediated gastro-oesophageal acid reflux control in patients with established reflux disease. Aliment Pharmacol Ther 16:869–873

Castell DO, Murray JA, Tutuian R, Orlando RC, Arnold R (2004) Review article: the pathophysiology of gastro-oesophageal reflux disease—oesophageal manifestations. Aliment Pharmacol Ther 20 Suppl 9:14–25

Castelucci P, Robbins HL, Furness JB (2003) P2X(2) purine receptor immunoreactivity of intraganglionic laminar endings in the mouse gastrointestinal tract. Cell Tissue Res 312:167–174

Cecio A (1976) Further histophysiological observations on the lower esophagus of the rabbit. Cell Tissue Res 168:475–488

Cecio A, Califano G (1967) Neurohistological observations on the oesophageal innervation of rabbit. Z Zellforsch Mikrosk Anat 83:30–39

Cervero F, Connell LA (1984) Distribution of somatic and visceral primary afferent fibres within the thoracic spinal cord of the cat. J Comp Neurol 230:88–98

Chandler MJ, Hobbs SF, Bolser DC, Foreman RD (1991) Effects of vagal afferent stimulation on cervical spinothalamic tract neurons in monkeys. Pain 44:81–87

Chang HY, Mashimo H, Goyal RK (2003) Musings on the wanderer: what's new in our understanding of vago-vagal reflex? IV. Current concepts of vagal efferent projections to the gut. Am J Physiol Gastrointest Liver Physiol 284:G357–366

Christensen J, Fang S, Rick GA (1995) NADPH-diaphorase-positive nerve fibers in smooth muscle layers of opossum esophagus: gradients in density. J Auton Nerv Syst 52:99–105

Christensen J, Rick GA, Robison BA, Stiles MJ, Wix MA (1983) Arrangement of the myenteric plexus throughout the gastrointestinal tract of the opossum. Gastroenterology 85:890–899

Christensen J, Rick GA, Soll DJ (1987) Intramural nerves and interstitial cells revealed by the Champy-Maillet stain in the opossum esophagus. J Auton Nerv Syst 19:137–151

Christensen J, Robison BA (1982) Anatomy of the myenteric plexus of the opossum esophagus. Gastroenterology 83:1033–1042

Clarke GD, Davison JS (1975) Tension receptors in the oesophagus and stomach of the rat. J Physiol 244:41P–42P

Clerc N (1983a) Afferent innervation of the lower oesophageal sphincter of the cat. An HRP study. J Auton Nerv Syst 9:623–636

Clerc N (1983b) Histological characteristics of the lower oesophageal sphincter in the cat. Acta Anat (Basel) 117:201–208

Clerc N (1984) Afferent innervation of the lower esophageal sphincter of the cat. Pathways and functional characteristics. J Auton Nerv Syst 10:213–216

Clerc N, Condamin M (1987) Selective labeling of vagal sensory nerve fibers in the lower esophageal sphincter with anterogradely transported WGA-HRP. Brain Res 424:216–224

Clerc N, Mazzia C (1994) Morphological relationships of choleragenoid horseradish peroxidase-labeled spinal primary afferents with myenteric ganglia and mucosal associated lymphoid tissue in the cat esophagogastric junction. J Comp Neurol 347:171–186

Clerc N, Mei N (1983a) Thoracic esophageal mechanoreceptors connected with fibers following sympathetic pathways. Brain Res Bull 10:1–7

Clerc N, Mei N (1983b) Vagal mechanoreceptors located in the lower oesophageal sphincter of the cat. J Physiol 336:487–498

Clouse RE, Richter JE, Heading RC, Janssens J, Wilson JA (1999) Functional esophageal disorders. Gut 45 (Suppl II): II31–II36

Collman PI, Tremblay L, Diamant NE (1992) The distribution of spinal and vagal sensory neurons that innervate the esophagus of the cat. Gastroenterology 103:817–822

Collman PI, Tremblay L, Diamant NE (1993) The central vagal efferent supply to the esophagus and lower esophageal sphincter of the cat. Gastroenterology 104:1430–1438

Conklin JL, Christensen J (1994) Motor function of the pharynx and esophagus. In: Johnson LR (eds) Physiology of the gastrointestinal tract. Raven Press, New York, 903–928

Costa M, Brookes SH, Zagorodnyuk V (2004) How many kinds of visceral afferents? Gut 53 Suppl 2:ii1–4

Costa M, Furness JB, Pompolo S, Brookes SJ, Bornstein JC, Bredt DS, Snyder SH (1992) Projections and chemical coding of neurons with immunoreactivity for nitric oxide synthase in the guinea-pig small intestine. Neurosci Lett 148:121–125

Cunningham ET, Jr., Sawchenko PE (1989) A circumscribed projection from the nucleus of the solitary tract to the nucleus ambiguus in the rat: anatomical evidence for somatostatin-28-immunoreactive interneurons subserving reflex control of esophageal motility. J Neurosci 9:1668–1682

Cunningham ET, Jr., Sawchenko PE (1990) Central neural control of esophageal motility: a review. Dysphagia 5:35–51

Dahlqvist A, Neuhuber WL, Forsgren S (1994) Innervation of laryngeal nerve paraganglia: an anterograde tracing and immunohistochemical study in the rat. J Comp Neurol 345:440–446

Daniel EE, Posey-Daniel V (1984) Neuromuscular structures in opossum esophagus: role of interstitial cells of Cajal. Am J Physiol 246:G305–315

Danzer M, Samberger C, Schicho R, Lippe IT, Holzer P (2004) Immunocytochemical characterization of rat brainstem neurons with vagal afferent input from the stomach challenged by acid or ammonia. Eur J Neurosci 19:85–92

De Laet A, Cornelissen W, Adriaensen D, Van Bogaert PP, Scheuermann DW, Timmermans JP (2002) Ca^{2+} involvement in the action potential generation of myenteric neurones in the rat oesophagus. Neurogastroenterol Motil 14:161–172

Delbro D (1985) The role of substance P in the control of gut motility. In: Hakanson R, Sundler F (eds) Tachykinin Antagonists. Elsevier, Amsterdam, 223–230

Dinh QT, Groneberg DA, Peiser C, Springer J, Joachim RA, Arck PC, Klapp BF, Fischer A (2004) Nerve growth factor-induced substance P in capsaicin-insensitive vagal neurons innervating the lower mouse airway. Clin Exp Allergy 34:1474–1479

Dong H, Loomis CW, Bieger D (2000) Distal and deglutitive inhibition in the rat esophagus: role of inhibitory neurotransmission in the nucleus tractus solitarii. Gastroenterology 118:328–336

Duc C, Barakat-Walter I, Droz B (1994) Innervation of putative rapidly adapting mechanoreceptors by calbindin- and calretinin-immunoreactive primary sensory neurons in the rat. Eur J Neurosci 6:264–271

von Düring M, Andres KH (1990) Topography and ultrastructure of group III and IV nerve terminals of the cat's gastrocnemius-soleus muscle. In: Zenker W, Neuhuber WL (eds) The primary afferent neuron. Plenum Press, New York, 35–41

Dütsch M, Eichhorn U, Worl J, Wank M, Berthoud HR, Neuhuber WL (1998) Vagal and spinal afferent innervation of the rat esophagus: a combined retrograde tracing and immunocytochemical study with special emphasis on calcium-binding proteins. J Comp Neurol 398:289–307

Eiden LE (1998) The cholinergic gene locus. J Neurochem 70:2227–2240

Ernfors P, Lee KF, Kucera J, Jaenisch R (1994) Lack of neurotrophin-3 leads to deficiencies in the peripheral nervous system and loss of limb proprioceptive afferents. Cell 77:503–512

Euchner-Wamser I, Sengupta JN, Gebhart GF, Meller ST (1993) Characterization of responses of T2-T4 spinal cord neurons to esophageal distension in the rat. J Neurophysiol 69:868–883

Falempin M, Madhloum A, Rousseau JP (1986) Effects of vagal deafferentation on oesophageal motility and transit in the sheep. J Physiol 372:425–436

Fang S, Christensen J (1994) Distribution of NADPH diaphorase in intramural plexuses of cat and opossum esophagus. J Auton Nerv Syst 46:123–133

Faussone-Pellegrini MS, Cortesini C (1985) Ultrastructural features and localization of the interstitial cells of Cajal in the smooth muscle coat of human esophagus. J Submicrosc Cytol 17:187–197

Fidone SJ, Stensaas LJ, Zapata P (1975) Sensory nerve endings containing "synaptic" vesicles: an electron-microscope autoradiographic study. J Neurobiol 6:423–427

Fischer A, Canning BJ, Undem BJ, Kummer W (1998) Evidence for an esophageal origin of VIP-IR and NO synthase-IR nerves innervating the guinea pig trachealis: a retrograde neuronal tracing and immunohistochemical analysis. J Comp Neurol 394:326–334

Fox EA, Phillips RJ, Baronowsky EA, Byerly MS, Jones S, Powley TL (2001a) Neurotrophin-4 deficient mice have a loss of vagal intraganglionic mechanoreceptors from the small intestine and a disruption of short-term satiety. J Neurosci 21: 8602–8615

Fox EA, Phillips RJ, Byerly MS, Baronowsky EA, Chi MM, Powley TL (2002) Selective loss of vagal intramuscular mechanoreceptors in mice mutant for steel factor, the c-Kit receptor ligand. Anat Embryol 205:325–342

Fox EA, Phillips RJ, Martinson FA, Baronowsky EA, Powley TL (2000) Vagal afferent innervation of smooth muscle in the stomach and duodenum of the mouse: morphology and topography. J Comp Neurol 428:558–576

Fox EA, Phillips RJ, Martinson FA, Baronowsky EA, Powley TL (2001b) C-Kit mutant mice have a selective loss of vagal intramuscular mechanoreceptors in the forestomach. Anat Embryol 204:11–26

Friebe A, Koesling D (2003) Regulation of nitric oxide-sensitive guanylyl cyclase. Circulation Res 93:96–105

Fryscak T, Zenker W, Kantner D (1984) Afferent and efferent innervation of the rat esophagus. A tracing study with horseradish peroxidase and nuclear yellow. Anat Embryol 170:63–70

Fujiyama F, Furuta T (2001) Immunocytochemical localization of candidates for vesicular glutamate transporters in the rat cerebral cortex. J Comp Neurol 435:379–387

Fukushima S, Shingai T, Kitagawa J, Takahashi Y, Taguchi Y, Noda T, Yamada Y (2003) Role of the pharyngeal branch of the vagus nerve in laryngeal elevation and UES pressure during swallowing in rabbits. Dysphagia 18:58–63

Furlong PL, Aziz Q, Singh KD, Thompson DG, Hobson A, Harding GF (1998) Cortical localisation of magnetic fields evoked by oesophageal distension. Electroencephalogr Clin Neurophysiol 108:234–243

Furness JB (2000) Types of neurons in the enteric nervous system. J Auton Nerv Syst 81:87–96

Furness JB, Costa M (1987) The Enteric Nervous System. Churchill Livingstone, Edinburgh

Gabella G (1990) On the plasticity of form and structure of enteric ganglia. J Auton Nerv Syst 30 Suppl:S59–66

Gabella G, Trigg P (1984) Size of neurons and glial cells in the enteric ganglia of mice, guinea-pigs, rabbits and sheep. J Neurocytol 13:49–71

Gai WP, Messenger JP, Yu YH, Gieroba ZJ, Blessing WW (1995) Nitric oxide-synthesising neurons in the central subnucleus of the nucleus tractus solitarius provide a major innervation of the rostral nucleus ambiguus in the rabbit. J Comp Neurol 357:348–361

Gibson A, Mirzazadeh S, Hobbs AJ, Moore PK (1990) L-NG-monomethyl arginine and L-NG-nitro arginine inhibit non-adrenergic, non-cholinergic relaxation of the mouse anococcygeus muscle. Br J Pharmacol 99:602–606

Gilbert RJ, Dodds WJ (1987) Subtypes of muscarinic receptors in vagal inhibitory pathway to the lower esophageal sphincter of the opossum. Dig Dis Sci 32:1130–1135

Gonella J, Niel JP, Roman C (1977) Vagal control of lower oesophageal sphincter motility in the cat. J Physiol 273:647–664

Gonella J, Niel JP, Roman C (1979) Sympathetic control of lower oesophageal sphincter motility in the cat. J Physiol 287:177–190

Gonella J, Niel JP, Roman C (1980) Mechanism of the noradrenergic motor control on the lower oesophageal sphincter in the cat. J Physiol 306:251–260

Goyal RK, Padmanabhan R, Sang Q (2001) Neural circuits in swallowing and abdominal vagal afferent-mediated lower esophageal sphincter relaxation. Am J Med 111 Suppl 8A:95S–105S

Green T, Dockray GJ (1987) Calcitonin gene-related peptide and substance P in afferents to the upper gastrointestinal tract in the rat. Neurosci Lett 76:151–156

Greving R (1931) Über die motorische und sensible Innervation der Speiseröhre, zugleich ein Beitrag zum Regulationsmechanismus des peripherischen vegetativen Nervensystems. Dtsch Arch klin Med 171:10–26

Grozdanovic Z, Baumgarten HG, Brüning G (1992) Histochemistry of NADPH-diaphorase, a marker for neuronal nitric oxide synthase, in the peripheral autonomic nervous system of the mouse. Neuroscience 48:225–235

Gruber H (1968) Über die Struktur und Innervation der quergestreiften Muskulatur des Ösophagus der Ratte. Z Zellforsch 91:236–247

Gruber H (1978) Motor innervation of the striated oesophagus muscle. Part 1. Intramural distribution of the right and left vagus nerve in the rat esophagus as revealed by the glycogen depletion technique. J Neurol Sci 36:41–53

Grundy D (1988) Speculations on the structure/function relationship for vagal and splanchnic afferent endings supplying the gastrointestinal tract. J Auton Nerv Syst 22:175–180

Halata Z (1975) The mechanoreceptors of the mammalian skin ultrastructure and morphological classification. Adv Anat Embryol Cell Biol 50:3-77

Hamdy S, Aziz Q, Rothwell JC, Singh KD, Barlow J, Hughes DG, Tallis RC, Thompson DG (1996) The cortical topography of human swallowing musculature in health and disease. Nat Med 2:1217–1224

Hamilton RB, Norgren R (1984) Central projections of gustatory nerves in the rat. J Comp Neurol 222:560–577

Hayakawa T, Takanaga A, Tanaka K, Maeda S, Seki M (2003) Ultrastructure of the central subnucleus of the nucleus tractus solitarii and the esophageal afferent terminals in the rat. Anat Embryol 206:273–281

Hayakawa T, Yajima Y, Zyo K (1996) Ultrastructural characterization of pharyngeal and esophageal motoneurons in the nucleus ambiguus of the rat. J Comp Neurol 370:135–146

Hayakawa T, Zheng JQ, Yajima Y (1997) Direct synaptic projections to esophageal motoneurons in the nucleus ambiguus from the nucleus of the solitary tract of the rat. J Comp Neurol 381:18–30

Hebeiss K, Kilbinger H (1999) Cholinergic and GABAergic regulation of nitric oxide synthesis in the guinea pig ileum. Am J Physiol 276:G862–866

Hecht M, Kober H, Claus D, Hilz M, Vieth J, Neundorfer B (1999) The electrical and magnetical cerebral responses evoked by electrical stimulation of the esophagus and the location of their cerebral sources. Clin Neurophysiol 110:1435–1444

Hisa Y, Tadaki N, Uno T, Koike S, Tanaka M, Okamura H, Ibata Y (1996) Nitrergic innervation of the rat larynx measured by nitric oxide synthase immunohistochemistry and NADPH-diaphorase histochemistry. Ann Oto Rhinol Laryngol 105:550–554

Hobson AR, Khan RW, Sarkar S, Furlong PL, Aziz Q (2004) Development of esophageal hypersensitivity following experimental duodenal acidification. Am J Gastroenterol 99:813–820

Holland CT, Satchell PM, Farrow BR (2002) Selective vagal afferent dysfunction in dogs with congenital idiopathic megaoesophagus. Auton Neurosci 99:18–23

Holloway RH, Dodds WJ, Helm JF, Hogan WJ, Dent J, Arndorfer RC (1986) Integrity of cholinergic innervation to the lower esophageal sphincter in achalasia. Gastroenterology 90:924–929

Holzer P (1988) Local effector functions of capsaicin-sensitive sensory nerve endings: involvement of tachykinins, calcitonin gene-related peptide and other neuropeptides. Neuroscience 24:739–768

Holzer P (2003) Acid-sensitive ion channels in gastrointestinal function. Curr Opin Pharmacol 3:618–625

Holzer P (2004) TRPV1 and the gut: from a tasty receptor for a painful vanilloid to a key player in hyperalgesia. Eur J Pharmacol 500:231–241

Holzer P, Danzer M, Schicho R, Samberger C, Painsipp E, Lippe IT (2004) Vagal afferent input from the acid-challenged rat stomach to the brainstem: enhancement by interleukin-1beta. Neuroscience 129:439–445
Holzer P, Holzer-Petsche U (1997a) Tachykinins in the gut. Part I. Expression, release and motor function. Pharmacol Ther 73:173–217
Holzer P, Holzer-Petsche U (1997b) Tachykinins in the gut. Part II. Roles in neural excitation, secretion and inflammation. Pharmacol Ther 73:219–263
Holzer P, Schluet W, Lippe IT, Sametz W (1987) Involvement of capsaicin-sensitive sensory neurons in gastrointestinal function. Acta Physiol Hung 69:403–411
Hopkins DA (1995) Ultrastructure and synaptology of the nucleus ambiguus in the rat: the compact formation. J Comp Neurol 360:705–725
Hornby PJ, Abrahams TP (2000) Central control of lower esophageal sphincter relaxation. Am J Med 108 Suppl 4a:90S–98S
Hudson LC, Cummings JF (1985) The origins of innervation of the esophagus of the dog. Brain Res 326:125–136
Hummel T, Barz S, Holscher T, Neuhuber WL (2003) Differences in responses to nociceptive stimulation of the oral and aboral oesophagus. J Clin Neurosci 10:223–225
Hummel T, Sengupta JN, Meller ST, Gebhart GF (1997) Responses of T2–4 spinal cord neurons to irritation of the lower airways in the rat. Am J Physiol 273:R1147–1157
Hyland NP, Abrahams TP, Fuchs K, Burmeister MA, Hornby PJ (2001) Organization and neurochemistry of vagal preganglionic neurons innervating the lower esophageal sphincter in ferrets. J Comp Neurol 430:222–234
Ichikawa H, Jacobowitz DM, Sugimoto T (1997) Coexpression of calretinin and parvalbumin in Ruffini-like endings in the rat incisor periodontal ligament. Brain Res 770:294–297
Iggo A (1957) Gastro-intestinal tension receptors with unmyelinated afferent fibres in the vagus of the cat. Q J Exp Physiol Cogn Med Sci 42:130–143
Iggo A, Andres KH (1982) Morphology of cutaneous receptors. Annu Rev Neurosci 5:1–31
Iino S, Kato M, Hidaka H, Kobayashi S (1998) Neurocalcin-like immunoreactivity in the rat esophageal nervous system. Cell Tissue Res 294:57–68
Itoh K, Konishi A, Nomura S, Mizuno N, Nakamura Y, Sugimoto T (1979) Application of coupled oxidation reaction to electron microscopic demonstration of horseradish peroxidase: cobalt-glucose oxidase method. Brain Res 175:341–346
Izumi N, Matsuyama H, Ko M, Shimizu Y, Takewaki T (2003) Role of intrinsic nitrergic neurones on vagally mediated striated muscle contractions in the hamster oesophagus. J Physiol 551:287–294
Jean A (2001) Brain stem control of swallowing: neuronal network and cellular mechanisms. Physiol Rev 81:929–969
Jessen KR, Mirsky R (1983) Astrocyte-like glia in the peripheral nervous system: an immunohistochemical study of enteric glia. J Neurosci 3:2206–2218
Jit I (1974) Development of striated muscle fibres in the human oesophagus. Indian J Med Res 62:838–844
Jou CJ, Farber JP, Qin C, Foreman RD (2002) Convergent pathways for cardiac- and esophageal-somatic motor reflexes in rats. Auton Neurosci 99:70–77
Jurica EJ (1926) Studies on the motility of the denervated mammalian esophagus. Am J Physiol 77:371–384
Kalia M, Mesulam MM (1980) Brain stem projections of sensory and motor components of the vagus complex in the cat: I. The cervical vagus and nodose ganglion. J Comp Neurol 193:435–465
Kang J, Jiang L, Goldman SA, Nedergaard M (1998) Astrocyte-mediated potentiation of inhibitory synaptic transmission. Nat Neurosci 1:683–692

Kannari K (1990) Sensory receptors in the periodontal ligament of hamster incisors with special reference to the distribution, ultrastructure and three-dimensional reconstruction of Ruffini endings. Arch Histol Cytol 53:559–573

Kannari K, Sato O, Maeda T, Iwanaga T, Fujita T (1991) A possible mechanism of mechanoreception in Ruffini endings in the periodontal ligament of hamster incisors. J Comp Neurol 313:368–376

Kantrowitz PA, Siegel CI, Strong MJ, Hendrix TR (1970) Response of the human oesophagus to d-tubocurarine and atropine. Gut 11:47–50

Kaufmann P, Lierse W, Stark J, Stelzner F (1968) Die Muskelanordnung in der Speiseröhre. Erg Anat Entw-Gesch 40:5–34

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

Khurana RK, Petras JM (1991) Sensory innervation of the canine esophagus, stomach, and duodenum. Am J Anat 192:293–306

Kobler JB, Datta S, Goyal RK, Benecchi EJ (1994) Innervation of the larynx, pharynx, and upper esophageal sphincter of the rat. J Comp Neurol 349:129–147

Koesling D, Russwurm M, Mergia E, Mullershausen F, Friebe A (2004) Nitric oxide-sensitive guanylyl cyclase: structure and regulation. Neurochem Int 45:813–819

Kolossow NG, Milochin AA (1963) Die afferente Innervation der Ganglien des vegetativen Nervensystems. Z Mikrosk-Anat Forsch 70:426–464

Kraus T, Neuhuber WL, Raab M (2004) Vesicular glutamate transporter 1 immunoreactivity in motor endplates of striated esophageal but not skeletal muscles in the mouse. Neurosci Lett 360:53–56

Kressel M (1998) Tyramide amplification allows anterograde tracing by horseradish peroxidase-conjugated lectins in conjunction with simultaneous immunohistochemistry. J Histochem Cytochem 46:527–533

Kressel M, Berthoud HR, Neuhuber WL (1994) Vagal innervation of the rat pylorus: an anterograde tracing study using carbocyanine dyes and laser scanning confocal microscopy. Cell Tissue Res 275:109–123

Kressel M, Radespiel-Tröger M (1999) Anterograde tracing and immunohistochemical characterization of potentially mechanosensitive vagal afferents in the esophagus. J Comp Neurol 412:161–172

Kucera J, Fan G, Jaenisch R, Linnarsson S, Ernfors P (1995) Dependence of developing group Ia afferents on neurotrophin-3. J Comp Neurol 363:307–320

Kuhn M (2003) Structure, regulation, and function of mammalian membrane guanylyl cyclase receptors, with a focus on guanylyl cyclase-A. Circulation Res 93:700–709

Kummer W, Fischer A, Kurkowski R, Heym C (1992) The sensory and sympathetic innervation of guinea-pig lung and trachea as studied by retrograde neuronal tracing and double-labelling immunohistochemistry. Neuroscience 49:715–737

Kummer W, Neuhuber WL (1989) Vagal paraganglia of the rat. J Electron Microsc Tech 12:343–355

Kuo DC, Oravitz JJ, DeGroat WC (1984) Tracing of afferent and efferent pathways in the left inferior cardiac nerve of the cat using retrograde and transganglionic transport of horseradish peroxidase. Brain Res 321:111–118

Kuramoto H, Brookes SJ (2000) Cholinergic and nitrergic innervation of the rat esophagus. In: Krammer HJ, Singer MV (eds) Neurogastroenterology From the Basics to the Clinics. Kluwer Academic Publishers, Dordrecht, 78–82

Kuramoto H, Kuwano R (1994) Immunohistochemical demonstration of calbindin-containing nerve endings in the rat esophagus. Cell Tissue Res 278:57–64

Kuramoto H, Kuwano R (1995) Location of sensory nerve cells that provide calbindin-containing laminar nerve endings in myenteric ganglia of the rat esophagus. J Auton Nerv Syst 54:126–136

Kuramoto H, Oomori Y, Murabayashi H, Kadowaki M, Karaki S, Kuwahara A (2004) Localization of neurokinin 1 receptor (NK1R) immunoreactivity in rat esophagus. J Comp Neurol 478:11–21

Lamb K, Kang YM, Gebhart GF, Bielefeldt K (2003) Gastric inflammation triggers hypersensitivity to acid in awake rats. Gastroenterology 125:1410–1418

Lang IM, Dana N, Medda BK, Shaker R (2002) Mechanisms of airway protection during retching, vomiting, and swallowing. Am J Physiol Gastrointest Liver Physiol 283:G529–536

Lang IM, Dean C, Medda BK, Aslam M, Shaker R (2004) Differential activation of medullary vagal nuclei during different phases of swallowing in the cat. Brain Res 1014:145–163

Lang IM, Medda BK, Shaker R (2001) Mechanisms of reflexes induced by esophageal distension. Am J Physiol Gastrointest Liver Physiol 281:G1246–1263

Lang IM, Shaker R (2000) An overview of the upper esophageal sphincter. Curr Gastroenterol Reports 2:185–190

Langley JN (1921) The autonomic nervous system. Heffner & Sons, Cambridge

Lanzafame AA, Christopoulos A, Mitchelson F (2003) Cellular signaling mechanisms for muscarinic acetylcholine receptors. Receptor and Channel 9:241–260

Lawrentjew BI (1929) Experimentell-morphologische Studien über den feineren Bau des Autonomen Nervensystems. II. Über den Aufbau der Ganglien der Speiseröhre nebst einigen Bemerkungen über das Vorkommen und die Verteilung zweier Arten von Nervenzellen in dem autonomen Nervensystem. Z Mikrosk-Anat Forsch 18:233–267

Leander S, Brodin E, Hakanson R, Sundler F, Uddman R (1982) Neuronal substance P in the esophagus. Distribution and effects on motor activity. Acta Physiol Scand 115:427–435

Leek BF (1972) Abdominal visceral receptors. In: Neil E (eds) Handbook of Sensory Physiology, Vol 3. Springer, Berlin, 113–160

Lewis DI (1994) Dye-coupling between vagal motoneurones within the compact region of the adult rat nucleus ambiguus, in-vitro. J Auton Nerv Syst 47:53–58

Li ZS, Furness JB (1998) Immunohistochemical localisation of cholinergic markers in putative intrinsic primary afferent neurons of the guinea-pig small intestine. Cell Tissue Res 294:35–43

Liebermann-Meffert D, Allgower M, Schmid P, Blum AL (1979) Muscular equivalent of the lower esophageal sphincter. Gastroenterology 76:31–38

Lindh B, Aldskogius H, Hokfelt T (1989) Simultaneous immunohistochemical demonstration of intra-axonally transported markers and neuropeptides in the peripheral nervous system of the guinea pig. Histochemistry 92:367–376

Liposits Z, Gorcs T, Gallyas F, Kosaras B, Setalo G (1982) Improvement of the electron microscopic detection of peroxidase activity by means of the silver intensification of the diaminobenzidine reaction in the rat nervous system. Neurosci Lett 31:7–11

Longstreth GF, Walker FD (1994) Megaesophagus and hereditary nervous system degeneration. J Clin Gastroenterol 19:125–127

Loomis CW, Yao D, Bieger D (1997) Characterization of an esophagocardiovascular reflex in the rat. Am J Physiol 272: R1783–1791

Loose R, Schnitzler A, Sarkar S, Schmitz F, Volkmann J, Frieling T, Freund HJ, Witte OW, Enck P (1999) Cortical activation during oesophageal stimulation: a neuromagnetic study. Neurogastroenterol Mot 11:163–171

Lynn PA, Olsson C, Zagorodnyuk V, Costa M, Brookes SJ (2003) Rectal intraganglionic laminar endings are transduction sites of extrinsic mechanoreceptors in the guinea pig rectum. Gastroenterology 125:786–794

Lynn P, Zagorodnyuk V, Hennig G, Costa M, Brookes SJ (2005) Mechanical activation of rectal intraganglionic laminar endings in the guinea pig distal gut. J Physiol 564:589–601
Matthews PJ, Aziz Q, Facer P, Davis JB, Thompson DG, Anand P (2004) Increased capsaicin receptor TRPV1 nerve fibres in the inflamed human oesophagus. Eur J Gastroenterol Hepatol 16:897–902
Mazzia C, Clerc N (1997) Ultrastructural relationships of spinal primary afferent fibers with neuronal and non-neuronal cells in the myenteric plexus of the cat esophago-gastric junction. Neuroscience 80:925–937
Mazzia C, Clerc N (2000) Ultrastructural analysis of spinal primary afferent fibers within the circular muscle of the cat lower esophageal sphincter. Histochem Cell Biol 113:235–239
Mearin F, Mourelle M, Guarner F, Salas A, Riveros-Moreno V, Moncada S, Malagelada JR (1993) Patients with achalasia lack nitric oxide synthase in the gastro-oesophageal junction. Eur J Clin Invest 23:724–728
Medda BK, Kern M, Ren J, Xie P, Ulualp SO, Lang IM, Shaker R (2003) Relative contribution of various airway protective mechanisms to prevention of aspiration during swallowing. Am J Physiol Gastrointest Liver Physiol 284:G933–939
Mei N (1983) Recent studies on intestinal vagal afferent innervation. Functional implications. J Auton Nerv Syst 9:199–206
Melander T, Hokfelt T, Rokaeus A, Fahrenkrug J, Tatemoto K, Mutt V (1985) Distribution of galanin-like immunoreactivity in the gastro-intestinal tract of several mammalian species. Cell Tissue Res 239:253–270
Miller AJ (1986) Neurophysiological basis of swallowing. Dysphagia 1:91–100
Minic J, Molgo J, Karlsson E, Krejci E (2002) Regulation of acetylcholine release by muscarinic receptors at the mouse neuromuscular junction depends on the activity of acetylcholinesterase. Eur J Neurosci 15:439–448
Mittal RK, Holloway RH, Penagini R, Blackshaw LA, Dent J (1995) Transient lower esophageal sphincter relaxation. Gastroenterology 109:601–610
Morgan C, deGroat WC, Nadelhaft I (1986) The spinal distribution of sympathetic preganglionic and visceral primary afferent neurons that send axons into the hypogastric nerves of the cat. J Comp Neurol 243:23–40
Morikawa S, Komuro T (1998) Distribution of myenteric NO neurons along the guinea-pig esophagus. J Auton Nerv Syst 74:91–99
Mu L, Sanders I (1996) The innervation of the human upper esophageal sphincter. Dysphagia 11:234–238
Müller LR (1924) Die Lebensnerven. 2. Aufl. Springer, Berlin
Munger BL, Yoshida Y, Hayashi S, Osawa T, Ide C (1988) A re-evaluation of the cytology of cat Pacinian corpuscles. I. The inner core and clefts. Cell Tissue Res 253:83–93
Nagai K, Noguchi T, Hashimoto T, Uchida Y, Shimada T (2003) The organization of the lamina muscularis mucosae in the human esophagus. Arch Histol Cytol 66:281–288
Nance DM, Burns J, Klein CM, Burden HW (1988) Afferent fibers in the reproductive system and pelvic viscera of female rats: anterograde tracing and immunocytochemical studies. Brain Res Bull 21:701–709
Nazruddin, Suemune S, Shirana Y, Yamauchi K, Shigenaga Y (1989) The cells of origin of the hypoglossal afferent nerves and central projections in the cat. Brain Res 490:219–235
Neuhuber W (1982) The central projections of visceral primary afferent neurons of the inferior mesenteric plexus and hypogastric nerve and the location of the related sensory and preganglionic sympathetic cell bodies in the rat. Anat Embryol 164:413–425
Neuhuber WL (1987) Sensory vagal innervation of the rat esophagus and cardia: a light and electron microscopic anterograde tracing study. J Auton Nerv Syst 20:243–255

Neuhuber WL, Clerc N (1990) Afferent innervation of the esophagus in cat and rat. In: Zenker W, Neuhuber WL (eds) The primary afferent neuron. Plenum Press, New York, 93–107

Neuhuber WL, Eichhorn U, Wörl J (2001) Enteric co-innervation of striated muscle fibers in the esophagus: just a "hangover"? Anat Rec 262:41–46

Neuhuber WL, Fryscak-Benes A (1987) Die zentralen Projektionen afferenter Neurone des Nervus hypoglossus bei der Albinoratte. Verh Anat Ges 81:981–983

Neuhuber WL, Kressel M, Stark A, Berthoud HR (1998) Vagal efferent and afferent innervation of the rat esophagus as demonstrated by anterograde DiI and DiA tracing: focus on myenteric ganglia. J Auton Nerv Syst 70:92–102

Neuhuber WL, Sandoz PA, Fryscak T (1986) The central projections of primary afferent neurons of greater splanchnic and intercostal nerves in the rat. A horseradish peroxidase study. Anat Embryol 174:123–144

Neuhuber WL, Wörl J, Berthoud HR, Conte B (1994) NADPH-diaphorase-positive nerve fibers associated with motor endplates in the rat esophagus: new evidence for co-innervation of striated muscle by enteric neurons. Cell Tissue Res 276:23–30

Niel JP (1986a) Reflex activation of the lower oesophageal sphincter in the cat induced by stimulation of the splanchnic afferent fibres. J Auton Nerv Syst 16:211–218

Niel JP (1986b) Reflex excitation and inhibition of the lower oesophageal sphincter induced by gastric distension in the cat. J Auton Nerv Syst 16:205–209

Nonidez JF (1946) Afferent nerves in the intermuscular plexus of the dog's oesophagus. J Comp Neurol 85:177–189

Ny L, Alm P, Ekstrom P, Hannibal J, Larsson B, Andersson KE (1994) Nitric oxide synthase-containing, peptide-containing, and acetylcholinesterase-positive nerves in the cat lower oesophagus. Histochem J 26:721–733

Ny L, Alm P, Larsson B, Ekstrom P, Andersson KE (1995) Nitric oxide pathway in cat esophagus: localization of nitric oxide synthase and functional effects. Am J Physiol 268: G59–70

Orlando RC (2001) Overview of the mechanisms of gastroesophageal reflux. Am J Med 111 Suppl 8A:174S–177S

Orlando RC (2003) Pathogenesis of gastroesophageal reflux disease. Am J Med Sci 326:274–278

Orlando RC (2004) Esophageal perception and noncardiac chest pain. Gastroenterol Clin North Am 33:25–33

Ottaviani G (1937/38) Osservazioni istoanatomiche e sperimentali sulla innervazione dell'esofago di alcuni mammiferi. Z Zellforsch Mikrosk Anat 27:393–429

Ozaki N, Gebhart GF (2001) Characterization of mechanosensitive splanchnic nerve afferent fibers innervating the rat stomach. Am J Physiol Gastrointest Liver Physiol 281:G1449–1459

Ozaki N, Sengupta JN, Gebhart GF (1999) Mechanosensitive properties of gastric vagal afferent fibers in the rat. J Neurophysiol 82:2210–2220

Page AJ, Blackshaw LA (1999) GABA(B) receptors inhibit mechanosensitivity of primary afferent endings. J Neurosci 19:8597–8602

Page AJ, Martin CM, Blackshaw LA (2002) Vagal mechanoreceptors and chemoreceptors in mouse stomach and esophagus. J Neurophysiol 87:2095–2103

Page AJ, Slattery JA, O'Donnell TA, Cooper NJ, Young RL, Blackshaw LA (2005a) Modulation of gastro-oesophageal vagal afferents by galanin in mouse and ferret. J Physiol 563:809–819

Page AJ, Young RL, Martin CM, Umaerus M, O'Donnell TA, Cooper NJ, Coldwell JR, Hulander M, Mattsson JP, Lehmann A, Blackshaw LA (2005b) Metabotropic glutamate receptors inhibit mechanosensitivity in vagal sensory neurons. Gastroenterology 128:402–410

Patterson LM, Zheng H, Ward SM, Berthoud HR (2003) Vanilloid receptor (VR1) expression in vagal afferent neurons innervating the gastrointestinal tract. Cell Tissue Res 311:277–287

Peghini PL, Pursnani KG, Gideon MR, Castell JA, Nierman J, Castell DO (1998) Proximal and distal esophageal contractions have similar manometric features. Am J Physiol 274:G325–G330

Phillips RJ, Baronowsky EA, Powley TL (2003) Long-term regeneration of abdominal vagus: efferents fail while afferents succeed. J Comp Neurol 455:222–237

Phillips RJ, Powley TL (2000) Tension and stretch receptors in gastrointestinal smooth muscle: re- evaluating vagal mechanoreceptor electrophysiology. Brain Res Brain Res Rev 34:1–26

Powley TL, Chi MM, Baronowsky EA, Phillips RJ (2005) Gastrointestinal Tract Innervation of the Mouse: Afferent Regeneration and Meal Patterning after Vagotomy. Am J Physiol Regul Integr Comp Physiol (in press)

Prast H, Philippu A (1992) Nitric oxide releases acetylcholine in the basal forebrain. Eur J Pharmacol 216:139–140

Qin C, Chandler MJ, Foreman RD (2003) Afferent pathways and responses of T3-T4 spinal neurons to cervical and thoracic esophageal distensions in rats. Auton Neurosci 109:10–20

Qin C, Chandler MJ, Foreman RD (2004) Esophagocardiac convergence onto thoracic spinal neurons: comparison of cervical and thoracic esophagus. Brain Res 1008:193–197

Qualman SJ, Haupt HM, Yang P, Hamilton SR (1984) Esophageal Lewy bodies associated with ganglion cell loss in achalasia. Similarity to Parkinson's disease. Gastroenterology 87:848–856

Raab M, Neuhuber WL (2003) Vesicular glutamate transporter 2 immunoreactivity in putative vagal mechanosensor terminals of mouse and rat esophagus: indication of a local effector function? Cell Tissue Res 312:141–148

Raab M, Neuhuber WL (2004) Intraganglionic laminar endings and their relationships with neuronal and glial structures of myenteric ganglia in the esophagus of rat and mouse. Histochem Cell Biol 122:445–459

Raab M, Neuhuber WL (2005) Number and distribution of intraganglionic laminar endings in the mouse esophagus as demonstrated with two different immunohistochemical markers. J Histochem Cytochem 53:1023–1031

Raab M, Wörl J, Brehmer A, Neuhuber WL (2003) Reduction of NT-3 or TrkC results in fewer putative vagal mechanoreceptors in the mouse esophagus. Auton Neurosci 108:22–31

Randich A, Gebhart GF (1992) Vagal afferent modulation of nociception. Brain Res Brain Res Rev 17:77–99

Reichel B, Wörl J, Neuhuber WL (1995) NPY-positive nerve fibers on motor endplates in the rat esophagus stain also for VIP and NADPH-diaphorase. J Anat 187:226

Ren K, Randich A, Gebhart GF (1989) Vagal afferent modulation of spinal nociceptive transmission in the rat. J Neurophysiol 62:401–415

Robles-Chillida EM, Rodrigo J, Mayo I, Arnedo A, Gomez A (1981) Ultrastructure of free-ending nerve fibres in oesophageal epithelium. J Anat 133:227–233

Rodrigo J, de Felipe J, Robles-Chillida EM, Perez Anton JA, Mayo I, Gomez A (1982) Sensory vagal nature and anatomical access paths to esophagus laminar nerve endings in myenteric ganglia. Determination by surgical degeneration methods. Acta Anat 112:47–57

Rodrigo J, Hernandez CJ, Vidal MA, Pedrosa JA (1975a) Vegetative innervation of the esophagus. III. Intraepithelial endings. Acta Anat 92:242–258
Rodrigo J, Hernandez J, Vidal MA, Pedrosa JA (1975b) Vegetative innervation of the esophagus. II. Intraganglionic laminar endings. Acta Anat 92:79–100
Rodrigo J, Polak JM, Fernandez L, Ghatei MA, Mulderry P, Bloom SR (1985) Calcitonin gene-related peptide immunoreactive sensory and motor nerves of the rat, cat, and monkey esophagus. Gastroenterology 88:444–451
Rodrigo J, Robles Chillida EM, De Felipe J, Perez Anton JA, Pedrosa JA, Arnedo A (1980a) Sensorivagal nature of oesophageal submucous layer nerve endings. Determination of surgical degeneration methods. Acta Anat 108:540–550
Rodrigo J, Robles Chillida EM, Espinosa F, De Felipe J, Hernandez CJ, Arnedo A, Mayo I (1980b) New contribution on the oesophageal mucous innervation in certain monkeys (Cercopithecidae). Acta Anat 108:510–520
Rodrigo J, Uttenthal LO, Peinado MA, Esteban FJ, Fernandez AP, Serrano J, Martinez de Velasco J, Santacana M, Bentura ML, Martinez-Murillo R, Pedrosa JA (1998) Distribution of nitric oxide synthase in the esophagus of the cat and monkey. J Auton Nerv Syst 70:164–179
Rogers RC, Hermann GE, Travagli A (1999) Brainstem pathways responsible for oesophageal control of gastric motility and tone in the rat. J Physiol 514:369–383
Rogers RC, Travagli RA, Hermann GE (2003) Noradrenergic neurons in the rat solitary nucleus participate in the esophageal-gastric relaxation reflex. Am J Physiol Integr Comp Physiol 285:R479–R489
Rohen J (1955) Über den funktionellen Zusammenhang zwischen glatter und quergestreifter Muskulatur im menschlichen Oesophagus (Oesophagusstudien I.). Anat Anz 102:210–216
Rossiter CD, Norman WP, Jain M, Hornby PJ, Benjamin S, Gillis RA (1990) Control of lower esophageal sphincter pressure by two sites in dorsal motor nucleus of the vagus. Am J Physiol 259:G899–906
Rumessen JJ, de Kerchove d'Exaerde A, Mignon S, Bernex F, Timmermans JP, Schiffmann SN, Panthier JJ, Vanderwinden JM (2001) Interstitial cells of Cajal in the striated musculature of the mouse esophagus. Cell Tissue Res 306:1–14
Sanders I, Mu L (1998) Anatomy of the human internal superior laryngeal nerve. Anat Rec 252:646–656
Sandler AD, Schmidt C, Richardson K, Murray J, Maher JW (1993) Regulation of distal esophageal mucosal blood flow: the roles of nitric oxide and substance P. Surgery 114:285–293
Sang Q, Ciampoli D, Greferath U, Sommer L, Young HM (1999) Innervation of the esophagus in mice that lack Mash-1. J Comp Neurol 408:1–10
Sang Q, Goyal RK (2000) Lower esophageal sphincter relaxation and activation of medullary neurons by subdiaphragmatic vagal stimulation in the mouse. Gastroenterology 119:1600–1609
Sang Q, Goyal RK (2001) Swallowing reflex and brain stem neurons activated by superior laryngeal nerve stimulation in the mouse. Am J Physiol Gastrointest Liver Physiol 280: G191–200
Sang Q, Young HM (1997) Development of nicotinic receptor clusters and innervation accompanying the change in muscle phenotype in the mouse esophagus. J Comp Neurol 386:119–136
Sang Q, Young HM (1998) The origin and development of the vagal and spinal innervation of the external muscle of the mouse esophagus. Brain Res 809:253–268

Santafe MM, Salon I, Garcia N, Lanuza MA, Uchitel OD, Tomas J (2003) Modulation of ACh release by presynaptic muscarinic autoreceptors in the neuromuscular junction of the newborn and adult rat. Eur J Neurosci 17:119–127

Satchell PM, McLeod JG (1984) Abnormalities of oesophageal mechanoreceptors in canine acrylamide neuropathy. J Neurol Neurosurg Psychiatry 47:692–698

Sätzler K, Söhl LF, Bollmann JH, Borst JG, Frotscher M, Sakmann B, Lübke JH (2002) Three-dimensional reconstruction of a calyx of Held and its postsynaptic principal neuron in the medial nucleus of the trapezoid body. J Neurosci 22:10567–10579

Saxon DW, Robertson GN, Hopkins DA (1996) Ultrastructure and synaptology of the nucleus ambiguus in the rat: the semicompact and loose formations. J Comp Neurol 375:109–127

Schicho R, Florian W, Liebmann I, Holzer P, Lippe IT (2004) Increased expression of TRPV1 receptor in dorsal root ganglia by acid insult of the rat gastric mucosa. Eur J Neurosci 19:1811–1818

Schnitzler A, Volkmann J, Enck P, Frieling T, Witte OW, Freund HJ (1999) Different cortical organization of visceral and somatic sensation in humans. Eur J Neurosci 11:305–315

Schoultz TW, Swett JE (1974) Ultrastructural organization of the sensory fibers innervating the Golgi tendon organ. Anat Rec 179:147–162

Sengupta JN (2000) An overview of esophageal sensory receptors. Am J Med 108 Suppl 4a:87S–89S

Sengupta JN, Gebhart GF (1994) Gastrointestinal afferent fibers and sensation. In: Johnson LR (ed) Physiology of the Gastrointestinal Tract, 3rd ed. Raven Press, New York, 483–519

Sengupta JN, Kauvar D, Goyal RK (1989) Characteristics of vagal esophageal tension-sensitive afferent fibers in the opossum. J Neurophysiol 61:1001–1010

Sengupta JN, Saha JK, Goyal RK (1990) Stimulus-response function studies of esophageal mechanosensitive nociceptors in sympathetic afferents of opossum. J Neurophysiol 64:796–812

Sengupta JN, Saha JK, Goyal RK (1992) Differential sensitivity to bradykinin of esophageal distension-sensitive mechanoreceptors in vagal and sympathetic afferents of the opossum. J Neurophysiol 68:1053–1067

Shiina T, Shimizu Y, Izumi N, Suzuki Y, Asano M, Atoji Y, Nikami H, Takewaki T (2005) A comparative histological study on the distribution of striated and smooth muscles and glands in the esophagus of wild birds and mammals. J Vet Med Sci 67:115–117

Sifrim D, Holloway R (2001) Transient lower esophageal sphincter relaxations: how many or how harmful? Am J Gastroenterol 96:2529–2532

Singaram C, Sengupta A, Sweet MA, Sugarbaker DJ, Goyal RK (1994) Nitrinergic and peptidergic innervation of the human oesophagus. Gut 35:1690–1696

Sivarao DV, Goyal RK (2000) Functional anatomy and physiology of the upper esophageal sphincter. American Journal of Medicine 108 Suppl 4a:27S–37S

Slawik FF (1942) Über das Vorkommen von Muskelspindeln in der Muscularis propria des menschlichen Oesophagus. Anat Anz 93:133–137

Smid SD, Blackshaw LA (2000a) Neuromuscular function of the human lower oesophageal sphincter in reflux disease and Barrett's oesophagus. Gut 46:756–761

Smid SD, Blackshaw LA (2000b) Vagal ganglionic and nonadrenergic noncholinergic neurotransmission to the ferret lower oesophageal sphincter. Auton Neurosci 86:30–36

Smolen AJ (1988) Morphology of synapses in the autonomic nervous system. J Electron Microsc Tech 10:187–204

Söllenböhmer C, Enck P, Häussinger D, Frieling T (1996) Electrically evoked cerebral potentials during esophageal distension at perception and pain threshold. Am J Gastroenterol 91:970–975

Sörensen B, Neuhuber WL, Wörl J (1995) Nitrerge Co-Innervation motorischer Endplatten im Ösophagus bei Mensch und Schwein. Ann Anat Suppl 177:214
Stefanelli A (1938) Considerazioni ed osservazioni sulla struttura microscopica del tessuto nervoso autonomo alla periferia nei vertebrati superiori. Z Zellforsch Mikrosk Anat 28:485–511
Stein HJ, Liebermann-Meffert D, DeMeester TR, Siewert JR (1995) Three-dimensional pressure image and muscular structure of the human lower esophageal sphincter. Surgery 117:692–698
Sterin-Borda L, Ganzinelli S, Berra A, Borda E (2003) Novel insight into the mechanisms involved in the regulation of the m1 muscarinic receptor, iNOS and nNOS mRNA levels. Neuropharmacology 45:260–269
Stöhr J, Ph (1957) Mikroskopische Anatomie des vegetativen Nervensystems. In: Bargmann W (eds) Handbuch der Mikroskopischen Anatomie des Menschen. Springer, Berlin, 1–678
Storr M, Allescher HD (1999) Esophageal pharmacology and treatment of primary motility disorders. Dis Esophagus 12:241–257
Storr M, Geisler F, Neuhuber WL, Schusdziarra V, Allescher HD (2000) Endomorphin-1 and -2, endogenous ligands for the mu-opioid receptor, inhibit striated and smooth muscle contraction in the rat oesophagus. Neurogastroenterol Mot 12:441–448
Storr M, Geisler F, Neuhuber WL, Schusdziarra V, Allescher HD (2001) Characterization of vagal input to the rat esophageal muscle. Auton Neurosci 91:1–9
Suft G, Wörl J, Neuhuber WL (1997) Postnatal development of calretinin-positive nerve fibers in the rat esophagus. Clin Auton Res 7:42
Swithers SE, Baronowsky E, Powley TL (2002) Vagal intraganglionic laminar endings and intramuscular arrays mature at different rates in pre-weanling rat stomach. Auton Neurosci 102:13–19
Takaki M, Jin JG, Lu YF, Nakayama S (1990) Effects of piperine on the motility of the isolated guinea-pig ileum: comparison with capsaicin. Eur J Pharmacol 186:71–77
Teixeira AF, Vives P, Krammer HJ, Kühnel W, Wedel T (2001) Structural organization of the enteric nervous system in the cattle esophagus revealed by wholemount immunohistochemistry. Ital J Anat Embryol 106:313–321
Terenghi G, Polak JM, Rodrigo J, Mulderry PK, Bloom SR (1986) Calcitonin gene-related peptide-immunoreactive nerves in the tongue, epiglottis and pharynx of the rat: occurrence, distribution and origin. Brain Res 365:1–14
Tong Q, Ma J, Kirchgessner AL (2001) Vesicular glutamate transporter 2 in the brain-gut axis. Neuroreport 12:3929–3934
Tottrup A, Svane D, Forman A (1991) Nitric oxide mediating NANC inhibition in opossum lower esophageal sphincter. Am J Physiol 260:G385–389
Toyama T, Yokoyama I, Nishi K (1975) Effects of hexamethonium and other ganglionic blocking agents on electrical activity of the esophagus induced by vagal stimulation in the dog. Eur J Pharmacol 31:63–71
Uddman R, Alumets J, Edvinsson L, Hakanson R, Sundler F (1978) Peptidergic (VIP) innervation of the esophagus. Gastroenterology 75:5–8
Uddman R, Alumets J, Hakanson R, Sundler F, Walles B (1980) Peptidergic (enkephalin) innervation of the mammalian esophagus. Gastroenterology 78:732–737
Uddman R, Grunditz T, Luts A, Desai H, Fernstrom G, Sundler F (1995) Distribution and origin of the peripheral innervation of rat cervical esophagus. Dysphagia 10:203–212
Vanderwinden JM (1994) Role of nitric oxide in gastrointestinal function and disease. Acta Gastroenterol Belg 57:224–229

Vietze S, Neuhuber WL, Wörl J (1995) Postnatale Entwicklung der nitrergen Co-Innervation der quergestreiften Ösophagusmuskulatur bei der Ratte. Ann Anat Suppl 177:213

Wang FB, Powley TL (2000) Topographic inventories of vagal afferents in gastrointestinal muscle. J Comp Neurol 421:302–324

Wang ZJ, Neuhuber WL (2003) Intraganglionic laminar endings in the rat esophagus contain purinergic P2X2 and P2X3 receptor immunoreactivity. Anat Embryol 207:363–371

Wank M, Neuhuber WL (2001) Local differences in vagal afferent innervation of the rat esophagus are reflected by neurochemical differences at the level of the sensory ganglia and by different brainstem projections. J Comp Neurol 435:41–59

Watson N, Reddy H, Eglen RM (1995) Characterization of muscarinic receptor and beta-adrenoceptor interactions in guinea-pig oesophageal muscularis mucosae. Eur J Pharmacol 294:779–785

Wattchow DA, Furness JB, Costa M, O'Brien PE, Peacock M (1987) Distributions of neuropeptides in the human esophagus. Gastroenterology 93:1363–1371

Wei JY, Wang YH, Go VL, Tache Y (1997) Esophageal distension induced gastric relaxation is mediated in part by vagal peripheral reflex mechanism in rats. J Auton Nerv Syst 63:12–18

Weisbrodt NW (1976) Neuromuscular organization of esophageal and pharyngeal motility. Arch Int Med 136:524–531

Whitmore I (1983) The ultrastructure of oesophageal striated muscle in the guinea-pig and marmoset. Cell Tissue Res 234:365–376

Whitmore I, Notman JA (1987) A quantitative investigation into some ultrastructural characteristics of guinea-pig oesophageal striated muscle. J Anat 153:233–239

Wiedner EB, Bao X, Altschuler SM (1995) Localization of nitric oxide synthase in the brain stem neural circuit controlling esophageal peristalsis in rats. Gastroenterology 108:367–375

Wienbeck M (1987) Involvement of enkephalins and other endogenous opioids in the regulation of esophageal motility. Gastroenterol Clin Biol 11:52B–55B

Wörl J, Dütsch F, Neuhuber WL (2002) Development of neuromuscular junctions in the mouse esophagus: focus on establishment and reduction of enteric co-innervation. Anat Embryol 205:141–152

Wörl J, Fischer J, Neuhuber WL (1998) Nonvagal origin of galanin-containing nerve terminals innervating striated muscle fibers of the rat esophagus. Cell Tissue Res 292:453–461

Wörl J, Mayer B, Neuhuber WL (1994) Nitrergic innervation of the rat esophagus: focus on motor endplates. J Auton Nerv Syst 49:227–233

Wörl J, Mayer B, Neuhuber WL (1997) Spatial relationships of enteric nerve fibers to vagal motor terminals and the sarcolemma in motor endplates of the rat esophagus. A confocal laser scanning and electron-microscopic study. Cell Tissue Res 287:113–118

Wörl J, Neuhuber WL (2000) Spatial and temporal organization of TrkB expression in the developing musculature of the mouse esophagus. Histochem Cell Biol 114:229–238

Wörl J, Neuhuber WL (2005a) Enteric co-innervation of motor endplates in the esophagus: state of the art ten years after. Histochem Cell Biol 123:117–130

Wörl J, Neuhuber WL (2005b) Ultrastructural analysis of the smooth-to-striated transition zone in the developing mouse esophagus: emphasis on apoptosis of smooth and origin and differentiation of striated muscle cells. Dev Dynam 233:964–982

Wu M, Majewski M, Wojtkiewicz J, Vanderwinden JM, Adriaensen D, Timmermans JP (2003a) Anatomical and neurochemical features of the extrinsic and intrinsic innervation of the striated muscle in the porcine esophagus: evidence for regional and species differences. Cell Tissue Res 311:289–297

Wu M, Van Nassauw L, Kroese AB, Adriaensen D, Timmermans JP (2003b) Myenteric nitrergic neurons along the rat esophagus: evidence for regional and strain differences in age-related changes. Histochem Cell Biol 119:395–403
Xiang Z, Burnstock G (2004) P2X2 and P2X3 purinoceptors in the rat enteric nervous system. Histochem Cell Biol 121:169–179
Yamamoto Y, Atoji Y, Kuramoto H, Suzuki Y (1998) Calretinin-immunoreactive laminar nerve endings in the laryngeal mucosa of the rat. Cell Tissue Res 292:613–617
Yamamoto Y, Hosono I, Atoji Y, Suzuki Y (1997) Morphological study of the vagal afferent nerve endings in the laryngeal mucosa of the dog. Anat Anz 179:65–73
Yamamoto Y, Kitamura N, Yamada J, Atoji Y, Suzuki Y, Yamashita T (1994) Structure of the enteric nervous system in the sheep omasum as revealed by neurofilament protein-like immunoreactivity. J Anat 184:399–405
Yokota R, Burnstock G (1983) Synaptic organisation of the pelvic ganglion in the guinea-pig. Cell Tissue Res 232:379–397
Yu S, Undem BJ, Kollarik M (2005) Vagal afferent nerves with nociceptive properties in guinea-pig oesophagus. J Physiol 563:831–842
Zagorodnyuk VP, Brookes SJ (2000) Transduction sites of vagal mechanoreceptors in the guinea pig esophagus. J Neurosci 20:6249–6255
Zagorodnyuk VP, Chen BN, Brookes SJ (2001) Intraganglionic laminar endings are mechanotransduction sites of vagal tension receptors in the guinea-pig stomach. J Physiol 534:255–268
Zagorodnyuk VP, Chen BN, Costa M, Brookes SJ (2003) Mechanotransduction by intraganglionic laminar endings of vagal tension receptors in the guinea-pig oesophagus. J Physiol 553:575–587
Zagorodnyuk VP, D'Antona G, Brookes SJ, Costa M (2002) Functional GABAB receptors are present in guinea pig nodose ganglion cell bodies but not in peripheral mechanosensitive endings. Auton Neurosci 102:20–29
Zheng H, Lauve A, Patterson LM, Berthoud HR (1997) Limited excitatory local effector function of gastric vagal afferent intraganglionic terminals in rats. Am J Physiol 273: G661–669

Subject Index

acetylcholine (ACh) 6, 43, 48
achalasia 1, 42
acrylamide 30, 55
antiperistalsis 1
ASIC 29, 36, 55

biotinamide 9, 29
α-bungarotoxin 49

c-fos 7, 30, 31
calbindin 11, 37
calretinin 11, 14, 37
celiac ganglion 1, 37
central pattern generator 1, 30, 43
CGRP 6, 13, 17, 32, 37, 39, 46, 52
ChAT 15
coinnervation 1, 7, 46–50, 55
– ontogeny 49
– rate 48
convergence of spinal afferents 40
cortical representation of esophagus
– motor 44
– sensory 44
cricopharyngeus 3, 47

deglutition 1, 44
deglutitive motor pattern generator (DMPG) 43, 51
DiA 7
DiI 3, 5, 6, 9
dorsal motor nucleus (DMX) 7, 42, 43, 51, 54
dorsal root ganglia 10, 37
dysphagia 1

enteric glia 17
esophageal sphincters 1, 41
– lower (LES) 1, 3, 8, 41, 43
– upper (UES) 1, 3, 41, 47

GABA 30, 42, 55
galanin 15, 48
gastroesophageal reflux disease (GERD) 1, 31, 41, 42, 55
GFAP 17
glutamate 6, 14, 22, 30, 52

IGLEs 8–31, 50, 51, 54, 55
– chemistry 11–14
– development 26
– distribution 8–10
– function 29–31
– myenteric ganglia 14–20
– neurotrophins 26
– regeneration 29
– ultrastructure 18–29
IMAs 10, 30, 42, 46
interstitial cells of Cajal (ICC) 46

lamina muscularis mucosae 2, 3, 8, 45
local effector function 31, 52

mechanosensors 18, 26, 29, 34, 46
medulla oblongata 5, 43
megaesophagus 30, 44
met-enkephalin (M-Enk) 47
metabotropic glutamate receptors (mGluRs) 14, 30
motor endplates 6, 47, 51
mucosa 31
muscarinic acetylcholine receptors (mAChRs) 7, 48, 53
muscle spindles, absence of 8
myenteric ganglia 6–10, 14, 45, 55
myenteric neurons 16, 17, 46
– cholinergic 16, 41, 45
– nitrergic 15, 41, 45, 46, 51
myenteric plexus 2, 45

NADPH diaphorase 15, 42, 46, 48

neurocalcin 12, 22
neurokinin-1 receptor (NK-1R) 40
nicotinic ACh receptors 7
nNOS 15, 37, 46, 48, 49
NO 31, 51, 54
nociceptors
– spinal 39, 44
– vagal 8, 36, 37, 44
nodose ganglion 9, 10, 14
non-cardiac chest pain 1, 36
NPY 15, 47
NT-3 26
nucleus ambiguus (AMB) 5, 6, 41, 43, 51
nucleus tractus solitarii (NTS) 6, 15, 34, 42, 43

opioid receptors 48

peristalsis 1, 44, 45, 47
purinergic receptors 12

receptor matrix 18
recurrent laryngeal nerve (RLN) 4, 8, 32, 41
reflexes 1, 8
– cardiovascular 1
– protective 34, 35
– respiratory 1
– vago–vagal 1, 42
rIGLEs 10
Ruffini endings 34

S100 17
soluble guanylyl cyclase (sGC) 48
spinal afferents 17, 37–41, 55
spinal cord 2, 38
submucosa 31
submucous plexus 2
substance P 16, 22, 37, 39
superior laryngeal nerve (SLN) 6, 30, 32, 41
sympathetic innervation 37
synapses of IGLEs 22
synaptophysin 13, 22, 29

trachealis muscle 46
tracing 3, 9, 14, 29, 34, 37, 42
transformation of tunica muscularis 2, 49
TrkC 26
TRPV1 29, 36, 40, 55
tunica muscularis 2, 8, 46

VAChT 15, 46, 49
VGLUT1 6, 14, 55
VGLUT2 12–14, 55
VIP 15, 42, 47

WGA-HRP 3, 4, 14, 16, 26