Andres Costa • Eugenio Villalba
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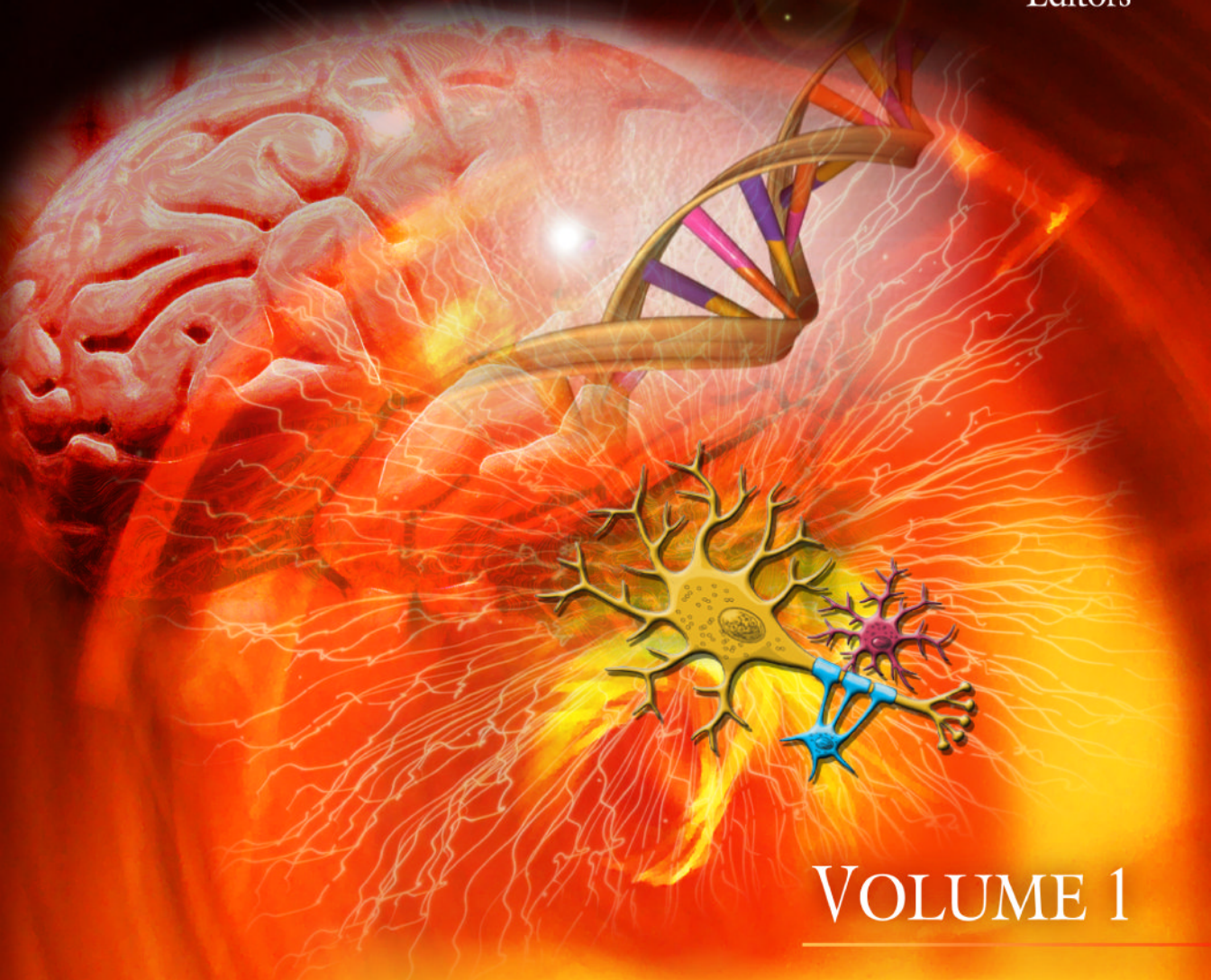

VOLUME 1

HORIZONS IN NEUROSCIENCE RESEARCH

Horizons in Neuroscience Research Series

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HORIZONS IN NEUROSCIENCE RESEARCH SERIES

HORIZONS IN NEUROSCIENCE RESEARCH, VOLUME 1

HORIZONS IN NEUROSCIENCE RESEARCH SERIES

HORIZONS IN NEUROSCIENCE RESEARCH, VOLUME I

Andres Costa and Eugenio Villalba

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HORIZONS IN NEUROSCIENCE RESEARCH SERIES

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AND
EUGENIO VILLALBA
EDITORS

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PREFACE

Neuroscience is the scientific study of the nervous system. The principle mechanism underlying enhanced pain sensitivity is a persistent hyperexcitability of spinal dorsal horn neurons. This book describes cellular mechanisms for the maintained hyperexcitability of spinal dorsal horn neurons mediated by neuron-glia interactions following spinal cord injury. In addition, nerve growth factor (NGF) has a key role not only in the development of sensory and autonomic neurons, but also in the processes of nociception. This book examines the recent evidence of the involvement of NGF in painful diseases and suggests the potential usefulness of anti-NGF strategies as novel analgesics for disabling pathological conditions. Furthermore, neuralgia is a symptom of some neurological disorders and can be found at any age. The pain that accompanies it is usually brief but may be severe. The authors present evidence sustaining the hypothesis that neuralgia (understood as being a shooting/shock-like paroxysmal pain) is a clinical expression of a transcriptional channelopathy. Other chapters in this book review the underlying mechanisms and the anatomical loci/shared circuits between epilepsy and memory, along with the relationship of various neurotransmitters involved. The morphological and functional characteristics of hereditary choroidal dystrophies are also examined, as well as the potential implications of neuroplasticity in dorsal vagal complex (DVC) in the adaptation of gastrointestinal functions.

Chapter 1 - Nerve growth factor (NGF) plays an essential role in the cellular interactions as a member of neurotrophins via controlling the balance between the cell survival and apoptosis, as well as in the differentiation of the motor and sensory neurons. The excessive cell survival may promote the onset of cancers and autoimmune diseases, but the insufficient cell survival could contribute to tissue degeneration and the development of several diseases. Besides of its neuronal origin, NGF is also involved in the inflammatory and immune processes. The cells of the innate and adaptive immune systems are able to secret NGF and bear receptors for NGF. The cytokines that are released from the activated immune cells, influence the activities of the central and peripheral nervous systems. As a growth factor for the sympathetic nervous system, NGF is involved in the catecholamine release by the activation of the hypothalamic-pituitary-adrenal axis. The glands, adipose tissue, muscles, skin and orbital tissues are highly sympathetically innervated organs. Due to the increased sympathoadrenal activity and the immune responses, NGF could be respected as a regulatory factor among the neural, inflammatory, immune and hormonal processes. The autoimmune endocrine diseases reflect a complexity of the neuro-immune-endocrine activities associating with hypertrophy and cardiovascular damages.

The NGF-specific receptor activation via tyrosine kinase (TrkA) differs from the activations induced by hormones and other growth factors, which exhibit mainly G protein-mediated protein kinase activations. Much data have been reported on a cross-talk or transactivation of the NGF receptors with the adrenergic or G protein-coupled receptors, which could be demonstrated in the receptor signaling mechanisms of the different receptor-mediated activations. The receptor $p75^{NTR}$ is involved in the regulation of the programmed cell death during the neurogenesis and the NGF withdrawal.

The main NGF secreting cells are the mast cells, eosinophils, monocytes, lymphocytes, fibroblasts, the smooth muscle and endothelial cells. These cells not only secret NGF but also bear its receptors playing a central role in the inflammatory, allergic and immune diseases. Some of these cells possess receptors for corticotropin-releasing hormone (CRH) and adrenergic receptors. The enhanced sympathoadrenal activity and the catecholamine release thereby may contribute to the activation of these cells resulting in a cytokine release. The cytokines and the NGF interactions can modify the immune reactions leading to a T helper 2 dominance, acting towards the direction of cell survival.

The increased adrenergic receptor activity is responsible for the hypertrophy of the glands, the adipose and muscle tissues. NGF plays a role in the interaction between the target cells and the axon terminals of sympathetic and sensory neurons. On another way, NGF as a trophic factor increases the density of the sympathetic innervation and the adrenergic receptor expression. The increased catecholamine levels induce a resistance to insulin, thereby contributing to the damage of the muscle cells and adipocytes. The catecholamine cytotoxicity and the chronic stimulation of the adrenergic receptors lead to the tissue hypertrophy and an increase in the sympathetic neuron apoptosis. The NGF stimulated angiogenetic effect associates with the hypertrophy and the severity of atherosclerosis. A transactivation between the adenosine and the TrkA receptor activations have been demonstrated after tissue injury caused by hypoxia.

In brief, NGF exerts a relevant role in the tissue repair via improving the sympathetic innervation, the cell survival mechanisms and the inflammatory and immunocompetent cell activations.

The endocrine diseases, particularly the autoimmune disorders, respresent a network of the neural, hormonal and immune processes. The hormones associating with the increased catecholamine release and the enhanced sympathoadrenal activity could contribute to the T helper 2 dominance, the tissue hypertrophy and the cardiovascular damage. It is no doubt that NGF is involved in the development of cardiac diseases, inducing hypertrophy through its modulating effects. The thyroid hormones promote the trophical effect of NGF via the transduction of the cell survival signal. An increased activation of the hypothalamic-pituitary-adrenal axis could be detected in diabetes mellitus. The damages of the adipose and muscle tissues exhibit a complexity of the neural, hormonal and immuno-inflammatory networks. It seems that the trophical effects of several growth factors and the durable stimulation of the adrenergic receptors may associate with the tissue hypertrophy and the enhanced apoptosis. The deterioration of the sympathetic and sensory innervations affects a wide spectrum of the tissues causing thyroid, diabetes and other endocrine diseases. The overloaded sympatho-adrenal activity and the other chronic stimulating factors also affect the cardiovascular tissues. It seems that this affection can not be avoided. Early revealing of the endocrine dysfunctions should be the most important for the prevention of the cardiovascular diseases.

The neural, hormonal, inflammatory and immune processes form a network not only at the tissue levels but also at the cellular levels. NGF plays a modulatory role in this network and contributes to the best homeostasis of the patients.

Chapter 2 - Nerve growth factor (NGF) has a key role not only in the development of sensory and autonomic neurons, but also in the processes of nociception. Several central and peripheral mechanisms have been postulated as the basis of effects of NGF in nociceptive pathways. It has been implicated both in inflammatory and neuropathic pain mechanisms and strategies against NGF, its receptors, belonging to the tyrosine kinase (Trk) family, and downstream intracellular signaling activated by this neurotrophin (NT) have been proposed for the treatment of these pathological conditions.

There is also recent evidence of the involvement of NGF in other painful diseases, such as migraine, in particular in the chronic form, and fibromyalgia. This suggests the potential usefulness of anti-NGF strategies as novel analgesics also for these disabling pathological conditions.

Chapter 3 - It was recently proposed that respiratory sinus arrhythmia (RSA) reflects the ability of the organism to integrate behavioral and metabolic demands, improving its homeostasis efficiency. Since the various anatomical and functional levels of the vagus nerve provide the conceptual basis of this allostatic model, it was designed under the name of the polyvagal theory. Therefore, altered RSA responses to various challenges could help to detect some dysfunctional states. The putative homeostatic roles of this vagal loop, i.e., afferent and efferent pathways, in the domain of psychological and behavioral homeostasis are reviewed. Evaluation of the autonomic activity was issued from the temporal and frequency domain analyses of heart rate variability (HRV). HRV analysis is an elegant noninvasive way to assess autonomic activity via the sympathovagal balance, and is more and more widely used in clinical and fundamental experiments. However, one must be cautious in the interpretation of the various HRV indices to preclude any erroneous conclusion. Keeping this in mind, there is actually a body of evidence arguing for a robust association between changes in vagal activity and (1) fatigue and mood changes during training adaptation and (2) some eating behaviors such as cephalic endocrine and exocrine secretions. They suggest that normal adaptation to psychological or physical loads and energy challenges requires the integrity of the parasympathetic nervous system and an equilibrate sympathovagal balance. Any impaired function of the parasympathetic component of the autonomous nervous system may lead to severe deleterious consequences, either psychological (depression and chronic fatigue) or metabolic (postprandial hyperglycemia). For the purpose of preventing overtraining, a heuristic sequential psychological and sympathovagal evolution are called the “Multistage Psycho-Autonomic Model of Adaptation to Training” (MPAMAT). In conclusion, these results are consistent with an allostatic role of the parasympathetic nervous system in a wide variety of functions, and confirm HRV analyses as promising for improving the detection and prevention of several psychological and metabolic altered states.

Chapter 4 - According to present knowledge, systemic realization of genetic activity in the dynamic spatial organization of the genome in the nucleus provides such a level of plasticity of complex biological systems that allows them to adequately respond to environmental stimuli or signals during the development, modulate and shift the balance of contacting chromatin components and dimensions of their interactions, resulting in structural rearrangements. The chromosome positions within the nucleus determine both normal development and progression of genomic diseases, i.e., changes according to the

environmental requirements, current needs of the organism, and its individual experience. At the same time, the striking output of the evolution of higher organisms, largely ignored to date, is that only 1.2% of the mammalian genome encodes proteins and the vast majority of the expressed information is in RNA. There are hundreds of thousands of non-coding (nc) RNAs, as well as many other yet-to-be-discovered small regulatory RNAs. A new paradigm envisions the interactions between these two worlds, the one of protein and the other of RNA, as providing a dynamic link between the transcriptome and the environment and, therefore, the progressive maturation and functional plasticity of the nervous system in health and disease. Also, a wide repertoire of ncRNAs plays an important role in chromatin organization, gene expression, and disease etiology via a signal cascade of actin remodeling (LIMK1, cofilin, actin). The activity of the protein kinase LIMK1 that controls spine development, local dendritic translation at postsynaptic sites and ionotropic glutamate receptor trafficking is regulated by a brain-specific miRNA miR-134. This miRNA is localized to the synapto-dendritic compartment of rat hippocampal neurons and negatively regulates the size of dendritic spines - postsynaptic sites of excitatory synaptic transmission. Moreover, LIMK1 hemizygosity is considered to cause cognitive defects in a genome disorder Williams syndrome. *Drosophila* is a helpful model organism to determine the sequence of events in this system of hierarchical relationships. Drosophila LIMK1 gene (*agnostic*) with a specific chromosome architecture around the gene capable of generating miRNAs, recapitulates many features both of Williams syndrome and of neurodegenerative disorders. Mutants in the gene have increased expression of LIMK1 and cofilin, modified chromosome packaging and homologous and nonhomologous pairing, implemented in different rates of unequal recombination. Also, they display congofilic inclusions both in the adult brain and larval tissues presumably leading to severe defects in learning and memory during courtship conditioning

Chapter 5 - The principle mechanism underlying enhanced pain sensitivity is a persistent hyperexcitability of spinal dorsal horn neurons. In the central nervous system, the somatosensory information is modulated by the balance between endogenous excitatory and inhibitory circuits, which are modified by glial cells. However, injury to the spinal cord or the peripheral nerve induces maladaptive changes of neuronal circuits in the spinal cord, which result in hyperexcitability of spinal dorsal horn neurons. Once hyperexcitability is developed in the central nervous system following neural injury, the maladaptive neuronal properties produce abnormal pain sensations in response to non-noxious stimuli (called allodynia) as well as enhanced pain sensations in response to noxious stimuli (called hyperalgesia). Thus, maintained hyperexcitability is a fundamental neuronal mechanism representing the persistent pain syndromes, such as neuropathic pain and causalgia following neural injury. Recent literature shows that glial cells are very important players in modulating synaptic circuits for somatosensation. Following neural injury, astrocytic and microglial activation are actively involved in maladaptive modulation of neuronal excitability via release of glutamate, other neurotransmitters and proinflammatory cytokines, termed abnormal glial function, "gliopathy". This chapter describes cellular mechanisms for the maintained hyperexcitability of spinal dorsal horn neurons mediated by neuronal-glial interactions following spinal cord injury and key neuronal intracellular signaling cascades that provide the feed forward cycle that perpetuates maintained hyperexcitaibitly which is persistent pain.

Chapter 6 - Selective activation of brain nicotinic acetylcholine receptors (nAChRs) seems to be a promising treatment strategy for Alzheimer's Disease and related disorders.

Nicotine and its related non-toxic compounds improve cognitive ability (including attention, learning, and memory) by acting directly or indirectly on the cortical neurons. The nicotine effects depend on the anatomical distribution and the cellular localization of the different subtypes of nicotinic acetylcholine receptors and are attributable to modifications induced in a wide variety of cellular mechanisms. Nicotinic receptors in the brain are more commonly associated with the modulation of neuronal function than mediation of synaptic transmission. Enhancement of neurotransmitter release (glutamate, dopamine, noradrenaline) via presynaptic nAChRs and increase of intracellular Ca^{2+} by postsynaptic nAChRs, facilitate the conexion with many intracellular signaling pathways. Moreover, glial cells have nAChRs that regulate their functions. Many of the neuronal changes nicotine induces are poorly understood by this complexity of the colinergic systems. Some of the effects of this drug are positive, such as neuroprotection against neurotoxic agents, ageing and pathological situations, but some neuronal changes can be negative, such as the induction of apoptosis, directly or indirectly through the production of free radicals, cytokines and pro-inflammatory derivatives of arachidonic acid. In a model of rat with subchronic nicotine treatment without neuronal apoptosis, our research group has demonstrated that nicotine increases the turnover of the glycolytic pathway and Krebs cycle in the cortex in a layer dependent manner, and the NGF immunoreactivity in neurons and glial cells in the frontoparietal cortex. Moreover, the increase of COX-2 has been observed in an area-, layer- and neuron type-dependent manner in different regions of the frontoparietal cortex, hippocampus and cerebellar cortex. The up-regulation of these enzymes and the NGF could have beneficial effects on neuronal function by helping neuronal adaptations involved in the performance of cognitive functions. Such results could help in the development of new treatments for cognitive disorders as well as help us understand the mechanism of action of certain drugs of abuse.

Chapter 7 - The enteric nervous system comprises two major cell types: enteric neurons and enteric glia. Enteric glia, which outnumber enteric neurons 4:1, display morphologic and molecular similarities to astrocytes in the central nervous system (CNS); they have irregular shapes, do not synthesize a basal lamina, and have an abundance of glial fibrillary acidic protein. Traditionally, enteric glia have been described as a homogeneous population of cells whose primary function is supporting enteric neurons. Evidence is accumulating challenging the concept that enteric glia are merely passive supports for enteric neurons. This review summarizes the recent advance in calcium signaling events of enteric glia. Enteric glia respond to a variety of neuroligands with intracellular and intercellular calcium signaling. Among these neuroligands are adenosine triphosphate (ATP), endothelins, glutamate and sphingolipids. Mobilization of inositol triphosphate ($InsP_3$) sensitive intracellular calcium stores accounts for the initiation of calcium signaling evoked by activation of these G-protein coupled receptors, while subsequent calcium entry via calcium channels or capacitative calcium entry alters the temporal and spatial patterns of intracellular calcium signaling in enteric glia. Like astrocytes in the central nervous system, enteric glia communicate with each other and with enteric neurons by two mechanisms: (1) propagation of calcium through the gap junction; and (2) release of transmitters triggered by the rise in intracellular calcium. The transmitters released then activate membrane receptors on neighboring cells. The functional consequences following the calcium signaling in enteric glia are also discussed with emphasis toward some of the potential intracellular targets of these calcium transients. We, therefore, propose that enteric glia be considered as an active cellular element participating in the information processing in the enteric nervous system.

Chapter 8 - Cocaine belongs to the psychomotor stimulant drug class, members of which are united by their common action of increasing the synaptic availability of monoamine neurotransmitters. These drugs are also united by the fact that they produce craving in users. However, the means by which psychostimulant effects on normal transmission evolve into a 'hunger for drugs' remains uncertain. Our recent work has begun to elucidate one aspect of this complex process – how drug actions on sensory systems may aid the association of environmental stimuli ('cues'), via classical conditioning, with the effects of the drug, triggering craving and relapse. This chapter shows that administration of cocaine can enhance evoked responses in the primary sensory cortex of experimental animals. Given that the speed of learning in classical conditioning is affected by the intensity of the conditioned stimulus (CS), and that cocaine enhances the neural representation of sensory stimuli in the primary sensory cortex in a manner similar to an increase in intensity, it is proposed that cue-induced craving in human addicts is facilitated by the drug. In short, cocaine speeds the process that leads to craving. This proposal is supported by the fact that cocaine enhances sensory responses in humans and leads to an improvement in attention (the putative intermediary between enhanced sensory responses and facilitated learning). Furthermore, cocaine affects neural loci which are known to play a role in learning and facilitates classical conditioning when present during acquisition. In addition, related drugs like d-amphetamine and ecstasy (which themselves produce craving) affect sensory processing and attention, and in the case of d-amphetamine facilitate human learning. It is therefore possible that cocaine itself plays a – previously under-appreciated – role in the formation of associations between drug and drug-related environmental cues by enhancing primary sensory responses. A corollary of this is that, as with other intense CSs, the established association may be particularly resistant to extinction, potentially explaining why cues continue to elicit craving months or even years after the last cocaine use.

Chapter 9 - Impairment of cognitive functions in patients with epilepsy is a major problem and a very complicated area with many possible causes and implications. The challenge is complex as both the underlying pathology (the disease process) and the therapeutic measures (drugs and surgery) can adversely affect cognitive functions. A close look at the scientific literature reveals an inverse relationship between the mechanisms involved in epilepsy and cognitive functions. Both epilepsy and cognition has been linked to abnormalities in the excitatory amino acid transmission, long-term potentiation and GABAergic inhibition in an opposite manner. Further, epilepsy and memory are reported to share the same anatomical loci in the brain in such a way that the regions of brain, considered important for memory, may provoke a seizure. Improving one condition may thus deteriorate another. Such biological/ pharmacological antagonism has been responsible for compromises in the therapeutic approach towards drug therapy and the management of epilepsy. In this chapter, an attempt has been made to review the underlying mechanisms and the anatomical loci /shared circuits between epilepsy and memory along with the relationship of various neurotransmitters involved.

Chapter 10 - Objective. Leg pain is a common and disabling symptom in lumbar disc herniation. The femoral nerve stretch test (FNST) and straight leg raising (SLR) test have been one of the simplest and surest methods of making a clinical diagnosis of lumbar disc herniation. It is generally believed that nerve root compression is caused by the hernia when the legs are extended and raised, thus resulting in the onset of lumbar pain or leg pain. However, it is unknown whether intraradicular blood flow (IRBF) changes during the FNST

and SLR test in patients with lumbar disc herniation. A nerve root stretch test was conducted in patients with lumbar disc herniation to observe the changes of IRBF, which were then compared with the clinical features. Methods. The subjects were 37 patients with disc herniation who underwent microdiscectomy. Patients were asked to adopt the prone position immediately before surgery, so that FNST test (N=12) was performed to confirm at which anterolateral thigh pain developed or SLR test (N=25) was performed to confirm the angle at which sciatica developed. The needle sensor of a laser Doppler flow meter was inserted into each nerve root immediately above the hernia and the change of IRBF was measured during intraoperative nerve root stretch test. After removal of the hernia, a similar procedure was repeated and IRBF was measured again.

Result. The intraoperative nerve root stretch test showed that the hernia compressed the nerve roots and there was marked disturbance of gliding, which was reduced to only a few millimeters. During the test, intraradicular blood flow showed a sharp decrease at the angle that produced root pain, which lasted for one minute. Intraradicular flow decreased by 92.8~100% in the L4 nerve root and by 40~98% in the L5 or S1 nerve roots relative to the blood flow before the test. After removal of the hernia, all the patients showed smooth gliding of the nerve roots during the test and there was no marked decrease of intraradicular blood flow.

Conclusion. This study demonstrated that the blood flow in the nerve root is reduced when the nerve root is compressed *in vivo*.

Chapter 11 - This chapter involves a survey on the morphological and functional characteristics of hereditary choroidal dystrophies. These belong among a heterogenous group of diseases in which the common feature is pigment epithelium damage and atrophy (loss) of the choriocapillaries, which results in progressive involvement of the photoreceptor layer. There are three characteristic forms of the disease: gyrate atrophy, choroideremia and geographical choroidal dystrophy. The latter can be divided into three subentitites: the central areolar, the peripapillary and the generalized forms. The chapter puts a special emphasis on generalized choroidal dystrophy that is regarded as a rare disease. This chapter describes experiences with the follow-up of 15 patients including a family of seven members. The most important conclusion of our study is that electrophysiological examination methods including multifocal ERG greatly facilitate the diagnosis and care of this disease that seems to be much more frequent than is widely believed.

Chapter 12 - The dorsal vagal complex (DVC), located dorsally in the caudal brainstem, comprises three distinct structures: the sensory nucleus of the solitary tract (NST), the area postrema (AP), and the dorsal motor nucleus of the vagus (DMV). The DVC integrates both peripheral and central signals and is the major center providing innervation to the cardiovascular and gastrointestinal systems to modulate autonomic function. New lines of evidence indicate that DVC neuronal networks undergo neuroplastic changes either in physiological states such as development and aging or as a consequence of pathological conditions. This review summarizes our current knowledge on functional and structural plasticity in the DVC. Emphasis will be given to the following three aspects of neuroplasticity in DVC: (1) changes in neurochemical phenotypes and structural reorganization including synaptic plasticity; (2) alterations in neuronal excitability; and (3) survival and neurogenesis. Several lines of evidence support the presence of proliferative neuronal precursor cells and neurogenesis in adult DVC. These neuronal precursor cells are located in the wall of the fourth ventricle and appear to be closely associated with the glial fibrillary acidic protein

(GFAP) positive radial-like cells. These neuronal precursor cells in the adult DVC respond to several growth factors including epidermal growth factor (EGF), basic fibroblast growth factor (bFGF) and ghrelin with increase in proliferation. The potential implications of neuroplasticity in DVC in the adaptation of gastrointestinal function to physiological and inflammatory conditions are discussed. A better comprehension of neuroplasticity in DVC could provide insights into new therapeutic strategies for patients with gastrointestinal dysfunction.

Chapter 13 - The vagus nerve is the principal exponent of the parasympathetic nervous system. It is originated in the central nervous system and is distributed broadly through the thorax and abdominal viscera. It regulates a wide variety of organic functions such as heart rate, gastric emptying, gastric and intestinal secretion and motility, etc. Regarding microscopic anatomy, it should be highlighted that it is mainly composed of afferent fibres.

Due to its macroscopic and microscopic anatomic characteristics vagus nerve stimulation has been applied in different treatments; some of them broadly used and its possible effects widely demonstrated, such as in the refractory epilepsy (Vagus Nerve Stimulation) and depression. Some others have not been much studied, for instance, in pain, memory enhancement and food intake control.

Based on the exceptional situation of the vagus nerve, specifically the abdominal vagal afferent fibres originate from the conjunction of gastrointestinal tract receptors and they synapse within the central nervous system. This occurs predominately within the nucleus tractus solitarius from which second order fibres connecting to the limbic system and subcortical centres involved with satiety are originated. An attempt is made to act on the perception of satiety by either stimulating/inhibiting afferent and/or efferent vagal fibres in order to reduce food intake and, consequently, body weight.

Up to this point results have been controversial; in rabbits after a 21-day continuous stimulation food intake was reduced. However, in swine despite causing no alteration in food intake pattern, vagal nerve stimulation caused changes in systemic gatrin and insulin concentrations.

During our studies in this research line and based on the results observed in rabbits and swine, many questions have arisen and are still waiting to be answered. For instance, how does the vagus nerve respond to different stimulating voltages? How does the response to the stimulation change in time? Do the microscopical anatomical changes (fibrosis, etc.) have any influence on the response to the stimulation? Should the stimulation parameters be changed in the course of time?

This short communication is intended to show our experience in the abdominal vagus nerve stimulation to control food intake, and response the questions that have arisen during the development of this research line.

Chapter 14 - Recent advancement in molecular biology in the field of taste perception has raised the possibility for ingested nutrients to be *tasted* in the upper gastrointestinal tract as well as tongue. Many works suggest that the individual 20 amino acid including glutamate can be detected by the vagus afferent within the duodenum. Recently, it was reveled that the rat gastric branch of the vagus nerve could specifically detect a non-essential amino acid, glutamate. The glutamate signaling could be transferred to the vagus nerve via mucosal chemical substances such as NO and serotonin. That leaded us to hypothesize that amino acid-sensing pathway exist in the gastric mucosa, like observed in the chemical sensing systems similar to the one functioning in the tongue and intestine. This review summarizes

current status of gut amino acid-sensing research and possible significance of the amino acid induced visceral information in the body nutrient homeostasis.

Chapter 15 - Schizophrenia is a chronic, debilitating psychotic mental disorder that affects approximately 1% of the worldwide population. It is characterized by negative and positive symptoms, resulting from biochemical and environmental factors. Despite remarkable advances achieved in the treatment of schizophrenia as well as the growing advances in molecular diagnosis studies, the biochemical basis of the disease is not completely understood and no biomarkers for molecular diagnosis are avaiable to date.

Despite the comparative proteome analyses of healthy and diseased samples has been extensively used to discover biomarkers, a detailed and careful interpretation of such data may provide a picture of the biochemical integrated systems which will help the comprehension of pathological states, treatments and diagnosis.

The proteome analysis of schizophrenia brain tissues compared to healthy controls has revealed differentially expressed proteins that, not only serve as potential biomarkers, but also compose a scenario that can lead to the comprehension of the dysfunction of neural transmission in schizophrenia. This chapter will show these potential protein role players in schizophrenia pathogenesis.

Chapter 16 - Neuralgia, is a symptom of some neurological disorders and can be found at any age. It is characterized by paroxysmal and lancinating pain that follows the path of the affected nerve. It can be spontaneous or may be triggered by any type of stimuli. This pain is usually brief but may be severe, pain-free intervals being common. This symptom is the main characteristic of some neurological entities and, due to its presence and importance, these diseases are thereby known as trigeminal neuralgia, glossopharyngeal neuralgia or postherpetic neuralgia.

Neuralgia is caused by irritation or nerve damage arising from inflammation, trauma, surgery, compression by adjacent structures such as tumors, infection and chemical or physical irritation of a nerve, even though the cause remains unknown in most cases.

This chapter presents the evidence sustaining the hypothesis that neuralgia is a clinical expression of a transcriptional channelopathy. This will help (in the near future) in designing new drugs orientated towards such target and lead to advances in diagnosing and treating patients who are affected by this important symptom.

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Chapter 1

NERVOUS, IMMUNE, ENDOCRINE REGULATORY SYSTEMS AND DISEASES ASSOCIATED WITH NERVE GROWTH FACTOR CO-SECRETION

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SUMMARY

Nerve growth factor (NGF) plays an essential role in the cellular interactions as a member of neurotrophins via controlling the balance between the cell survival and apoptosis, as well as in the differentiation of the motor and sensory neurons. The excessive cell survival may promote the onset of cancers and autoimmune diseases, but the insufficient cell survival could contribute to tissue degeneration and the development of several diseases. Besides of its neuronal origin, NGF is also involved in the inflammatory and immune processes. The cells of the innate and adaptive immune systems are able to secret NGF and bear receptors for NGF. The cytokines that are released from the activated immune cells, influence the activities of the central and peripheral nervous systems. As a growth factor for the sympathetic nervous system, NGF is involved in the catecholamine release by the activation of the hypothalamic-pituitary-adrenal axis. The glands, adipose tissue, muscles, skin and orbital tissues are highly sympathetically innervated organs. Due to the increased sympathoadrenal activity and the immune responses, NGF could be respected as a regulatory factor among the neural, inflammatory, immune and hormonal processes. The autoimmune endocrine diseases reflect a complexity of the neuro-immune-endocrine activities associating with hypertrophy and cardiovascular damages.

The NGF-specific receptor activation via tyrosine kinase (TrkA) differs from the activations induced by hormones and other growth factors, which exhibit mainly G protein-mediated protein kinase activations. Much data have been reported on a cross-talk or transactivation of the NGF receptors with the adrenergic or G protein-coupled receptors,

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which could be demonstrated in the receptor signaling mechanisms of the different receptor-mediated activations. The receptor $p75^{NTR}$ is involved in the regulation of the programmed cell death during the neurogenesis and the NGF withdrawal.

The main NGF secreting cells are the mast cells, eosinophils, monocytes, lymphocytes, fibroblasts, the smooth muscle and endothelial cells. These cells not only secret NGF but also bear its receptors playing a central role in the inflammatory, allergic and immune diseases. Some of these cells possess receptors for corticotropin-releasing hormone (CRH) and adrenergic receptors. The enhanced sympathoadrenal activity and the catecholamine release thereby may contribute to the activation of these cells resulting in a cytokine release. The cytokines and the NGF interactions can modify the immune reactions leading to a T helper 2 dominance, acting towards the direction of cell survival.

The increased adrenergic receptor activity is responsible for the hypertrophy of the glands, the adipose and muscle tissues. NGF plays a role in the interaction between the target cells and the axon terminals of sympathetic and sensory neurons. On another way, NGF as a trophic factor increases the density of the sympathetic innervation and the adrenergic receptor expression. The increased catecholamine levels induce a resistance to insulin, thereby contributing to the damage of the muscle cells and adipocytes. The catecholamine cytotoxicity and the chronic stimulation of the adrenergic receptors lead to the tissue hypertrophy and an increase in the sympathetic neuron apoptosis. The NGF stimulated angiogenetic effect associates with the hypertrophy and the severity of atherosclerosis. A transactivation between the adenosine and the TrkA receptor activations have been demonstrated after tissue injury caused by hypoxia.

In brief, NGF exerts a relevant role in the tissue repair via improving the sympathetic innervation, the cell survival mechanisms and the inflammatory and immunocompetent cell activations.

The endocrine diseases, particularly the autoimmune disorders, respresent a network of the neural, hormonal and immune processes. The hormones associating with the increased catecholamine release and the enhanced sympathoadrenal activity could contribute to the T helper 2 dominance, the tissue hypertrophy and the cardiovascular damage. It is no doubt that NGF is involved in the development of cardiac diseases, inducing hypertrophy through its modulating effects. The thyroid hormones promote the trophical effect of NGF via the transduction of the cell survival signal. An increased activation of the hypothalamic-pituitary-adrenal axis could be detected in diabetes mellitus. The damages of the adipose and muscle tissues exhibit a complexity of the neural, hormonal and immuno-inflammatory networks. It seems that the trophical effects of several growth factors and the durable stimulation of the adrenergic receptors may associate with the tissue hypertrophy and the enhanced apoptosis. The deterioration of the sympathetic and sensory innervations affects a wide spectrum of the tissues causing thyroid, diabetes and other endocrine diseases. The overloaded sympatho-adrenal activity and the other chronic stimulating factors also affect the cardiovascular tissues. It seems that this affection can not be avoided. Early revealing of the endocrine dysfunctions should be the most important for the prevention of the cardiovascular diseases.

The neural, hormonal, inflammatory and immune processes form a network not only at the tissue levels but also at the cellular levels. NGF plays a modulatory role in this network and contributes to the best homeostasis of the patients.

ABBREVIATIONS

$A_{2A}AR$, $A_{2B}AR$: receptors for adenosine
AC : adenylate cyclase
ACTH : adrenocorticotropic hormone
Akt : serine/threonine kinase
Apaf-1 : adapter protein
APS : autoimmune polyglandular syndrome
AR : adrenergic receptor
Arrestin : regulatory protein
ATP : adenosine triphosphate
BDNF : brain-derived neurotrophic factor
Bad, BAX, Bid : proapoptotic protein
Bcl2, BclXI : antiapoptotic receptor
cAMP : cyclic adenosine monophosphate
C3a, C5 : complement split products
CCR_3 : eotaxin receptor
CD4+ : helper CD4+ T lymphocyte
CD8+ : cytotoxic CD8+ T lymphocyte
CD 27 : tumor necrosis factor (TNF) receptor
CD28, B7 : co-stimulatory molecules
CD30 : cell membrane protein of the tumor necrosis factor (TNF) receptor family
CD40 : co-stimulatory protein on antigen-presenting cells
CD95 : Fas receptor
CD178 : Fas ligand
CGRP : calcitonin gene-related peptide
CNS: : central nervous system
COS-7 : African Green monkey SV40 transformed kidney fibroblast cells
COX : cyclooxygenase
CREB : cyclic AMP response element binding protein
CRH : corticotropin-releasing hormone
CRHR: corticotropin-releasing hormone receptor
CTLA : cytotoxic T-lymphocyte antigen
Cyt C : cytochrome C
DAG : diacylglycerol
DC : dendritic cells
Deiodinase : enzyme for thyroid hormone conversion
E: epinephrine
EGF : epidermal growth factor
ELAM : endothelial cell leukocyte adhesion molecule
Eotaxin : chemoattractant
ER : endoplasmic reticulum
ERK : extracellular signal-regulated kinase
FAK : focal adhesion kinase
Fas : apoptosis antigen

Fcε : IgE receptor
FGF : fibrosis growth factor
Forskolin : cAMP generation stimulating substance
Foxp3 : forkhead box P3
Gab-1 : adaptor molecule
GITR : glucocorticoid-induced TNF receptor-related molecule
GH: growth hormone
GPCR : G protein-coupled receptor
GR : glucocorticoid receptor
Grb2 : growth factor receptor-binding protein
GRK : G protein receptor kinase
GDP : guanosine diphosphate
GTP : guanosine triphosphate
HPA axis : hypothalamic-pituitary-adrenal axis
HR : histamine receptor
5-HT : 5-hydroxytryptamin
ICAM : intercellular adhesion molecule
IFNγ : interferon γ
IgE : immunoglobulin E
IGF-1 : insulin-like growth factor-1
IP_3 : inositol triphosphate
IRS : insulin receptor substrate
Htg: human thyroglobulin
JNK : c-jun NH_2 terminal kinase
LC-NA : locus ceruleus/noradrenergic system
LH: luteinizing hormone
LPA_2 : phospholipase A_2
LPS : lipopolysaccharide
LTB_4 : leukotriene B_4
LTC4 : leukotriene C4
MAPK : mitogen-activated protein kinase
MEK : MAPK or ERK kinase
MHC : major histocompatibility complex
MMP-9 : metalloproteinase-9
NE: norepinephrine
NGF : nerve growth factor
NKA: neurokinin A
NFκB : nuclear factor kappa B
NT-3 : neurotrophin-3
NT-4/5 : neurotrophin-4/5
NPY: neuropeptide Y
$p75^{NTR}$: low-affinity neurotrophin receptor
PAF : platelet-activating factor
PC12 cells: pheochromocytoma cells
PDGF : platelet-derived growth factor
PGD_2, PGE_2, PGI_2: prostanoids

PI-3K : phosphatidylinositol-3 kinase
PIP_3 : phosphatidylinositol 3,4,5-triphosphate
PKA : protein kinase A
PKB : protein kinase B
PKC : protein kinase C
PLCβ : phospholipase Cβ
PLCγ : phospholipase Cγ
PLD : phospholipase D
PPARγ : peroxisome proliferator-activated receptor γ
PRL: prolactin
RAF, RAS : oncogenes
RGS: regulators for G protein signaling
SP: substance P
ROS : reactive oxygen species
Shc : adapter protein
SOS : son of sevenless protein
Sortilin : apoptosis inducing receptor protein
Src : regulatory protein
T_3 : triiodothyronine
T_4 : thyroxine
TCR : T cell receptor
TDZ : thiazolidinedione
TGFβ : transforming growth factor β
Th cell: T helper lymphocyte
TLR : Toll-like receptor
TNF : tumor necrosis factor
TPO: thyroid peroxidase
Tr1 cell : regulatory T lymphocyte releasing IL-10
Treg cell : regulatory T lymphocyte
TRH: thyrotropin-releasing hormone
TRPV1 : transient receptor potential V1
TSH: thyroid-stimulating hormone
TRAIL : TNF-related apoptosis inducing ligand
TRAK : antibodies against TSH receptor
Trk : tyrosine kinase
VCAM : vascular cell adhesion molecule
VEGF : vascular endothelial growth factor
VIP : vasoactive intestinal peptide

INTRODUCTION

Nerve growth factor (NGF) was discovered 50 years ago as a substance, which is nessecary for the survival and differentiation of the sensory and sympathetic neurons.

However, the actions of NGF are more diverse considering the receptor signaling cascades, the various tissue origins and the counterregulating responses.

The NGF is derived from neural and nonneural cells but its effect goes on locally at the nerve terminals surrounded by the target cells. The density of the sympathoadrenal innervation depends on the NGF amount reflecting its protective and restitutive role in the organs. There is little information about the regulatory role of the neural system in the nonneural systems, although a strong connection is no doubt present among these networks.

The partitipation of NGF in these networks can be characterized with respect to the receptor signaling pathways, the NGF producing and binding cell types, as well as the NGF initiated release of the various active substances, e.g. cytokines, chemokines. These secreted factors act directly and indirectly to the immunocompetent and endocrine cells.

There is a cross-talk between the NGF-specific tyrosine kinase and the adrenergic, G protein-coupled receptors. This connection allows us to explain why an increased sympatho-adrenal activity leads to a hypertrophy, a vascular abnormality or an abnormal adiposity.

NGF is an essential regulatory factor among the networks of the neural, immune and endocrine systems. Its large amount is associated with increased cell survival, but its low amount leads to apoptosis with organ atrophy.

The recombinant human NGF allows a new therapeutic application, particularly in wound healing and neurodegeneration or in neuropathy.

1. NERVE GROWTH FACTOR, ITS RECEPTORS AND THE SIGNALING MECHANISMS

1.1. The Characteristics of Nerve Growth Factor

Nerve growth factor (NGF) is a member of the neurotrophin family as well as the family of the small proteins. NGF regulates the neuronal survival and promotes the neurite outgrowth and branching. It is essential in the differentiation of neurons, initiating its axonal transport in the sympathetic and sensory nervous systems [1]. NGF plays a relevant role in the maintenance of sympathetic neuroplasticity, the neuronal density as well as in the restitution after tissue injury [2, 3]. NGF acts as a target-derived growth factor and is secreted from the innervated target tissues.

The mature form of the NGF is generated from its precursor: proNGF [4, 5]. The synthesis of proNGF is derived from two alternative splicing of the preproNGF protein resulting in 25 and 32 kDa isoforms. The glycosylation of these isoforms, leads to the end form, proNGF with the molecular weight of 40 kDa (Figure 1). ProNGF is neurotoxic, so its elevation in the brain associates with neurodegeneration [6]. NGF derives from proNGF after its cleaves possessing a molecular weight of 13 kDa. The release of the proNGF occurs during the nerve stimulation, while the NGF production is caused by plasmin activity generated by proteases in the extracellular spaces [4]. Both forms - proNGF and NGF - can bind to receptors or degrade rapidly by the metalloproteinase-9 (MMP-9). The balance between the neuronal cell death and the growth or survival depends on the receptor signaling mechanisms induced by the binding of proNGF or NGF. Sortilin is an apoptotic receptor

protein expressed broadly in the nervous system. Sortilin influences the balance to the direction of cell death through interacting with the $p75^{NTR}$ receptor [7, 8]. NGF can exert its pleiotropic effect after bindig to the transmembrane receptors: tyrosin kinase A (TrkA) and glycoprotein receptor of $p75^{NTR}$.

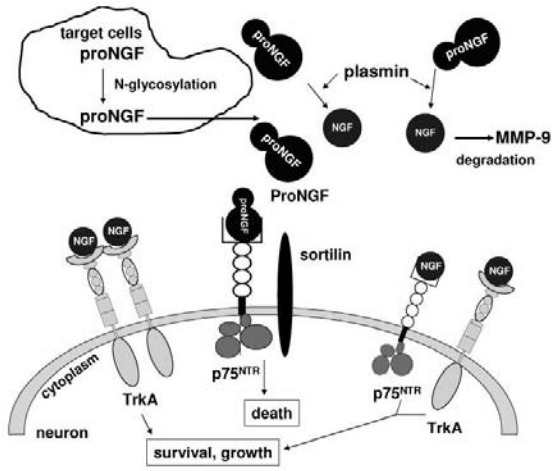

Figure 1. Nerve growth factor maturation and its receptors. Nerve growth factor (NGF) protein arises from proNGF via N-glycosylation and maturates after plasmin cleavage. The nonbinding form of nerve growth factor undergoes degradation via metalloproteinase-9. TrkA: tyrosine kinase receptor A; $p75^{NTR}$: low-affinity tyrosine kinase receptor; sortilin: apoptosis inducing receptor protein; MMP-9: metalloproteinase-9.

1.2. Nerve Growth Factor Receptors

1.2.1. Tyrosin Kinase (TrkA) Receptor

There are three types of tyrosine kinase receptors (TrkA, TrkB and TrkC) that transmit the cellular signaling processes after binding to neurotrophins, such as NGF: nerve growth factor, BDNF: brain-derived neurotrophic factor, NT3: neurotrophin-3 and NT4/5: neurotrophin-4/5 [9, 10]. NGF binds to two different classes of transmembrane receptors: of these the tyrosine kinase type A is the specific, also called high-affinity receptor and it triggers the cell survival signaling events [11]. The genes of tropomyosin related to the tyrosine kinase A is localizated on chromosome 17 [12]. Two forms of TrkA can be demonstrated in the cell extracts: the 110 kDa N-glycosylated form ($gp110^{TrkA}$) and the 140 kDa fully maturated form ($gp140^{TrkA}$) [11, 13]. The NGF binding leads to the dimerization and activation of TrkA, inducing the downstream phophorylation of the tyrosines and the adaptor proteins (Figure 2) [14, 15]. From the synthesis to the degradation, the TrkA receptor undergoes internalization via the endocytic pathway that directs to various localizations within the cells; the TrkA receptor returns to the cell surface via the recycling pathway or degrades in the lysosomes [16, 17, 18]. The extracellular ligand-binding domain of the TrkA receptor contains multiple repeatitions of leucine-rich motifs, two cysteine clusters, and two immunoglobulin-like domains [1, 19]. The binding specifity of the receptor is mostly determined by the second immunoglobulin-like domain. The catalytic and extracellular domains of the cytoplasmic tyrosine kinase show a high degree of identity with the

neurotrophin receptors, approximately 80% for the intra- and 30 % for the extracellular domains, respectively [1].

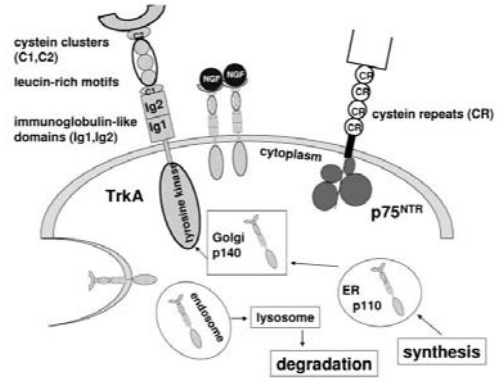

Figure 2. Nerve growth factor receptor trafficking. Tyrosine kinase receptor A (TrkA) undergoes internalization from synthesis to degradation. $p75^{NTR}$: low-affinity tyrosine kinase receptor; ER: endoplasmic reticulum.

The 140 kDa form of TrkA translocates rapidly to the cell surface, its half-life is 138 ± 4 minutes, which is shortened to 86 ± 8 minutes after NGF treatment [11, 16]. NGF induces the clearing of TrkA receptors from the cell surface, by increasing their lysosomal degradation through the internalization. The expressions of both NGF receptors (TrkA and $p75^{NTR}$) exhibit dependency on the cell cycle phases (M: mitotic cell cycle, G1: cell cycle during interphase, S: synthesis phase, G2: cell cycle arrest) [20]. In PC12 cells (derived from pheochromocytoma cells of the rat adrenal medulla), TrkA is expressed in the M and the early G1 phases but not in the late G1, S and G2 phases when $p75^{NTR}$ has expressed on the cell surface. The cellular pools of TrkA are found within the different cellular compartments.

The binding of NGF to TrkA initiates the autophosphorylation of the receptor-specific tyrosine residues; this contributes to the activation of phosphatidylinositol 3-kinase (PI-3K), mitogen-activated protein kinase (MAPK) and phosphorlipase Cγ (PLCγ) [21].

1.2.2. $P75^{ntr}$ Receptor

The $p75^{NTR}$ receptor displays a structural and sequential similarity to the receptors of the tumor necrosis factor (TNF) family, which includes Fas (CD95, apoptosis protein), CD40 (co-stimulatory protein of the antigen-presenting cells), CD30 (cell membrane protein of the tumor necrosis factor receptor family) and CD27 (tumor necrosis factor receptor) [22, 23, 24]. The p75NTR receptor is mainly considered a death receptor, but its exact role has not been highligthed. The activation of the p75NTR receptor can modify the activity of the TrkA receptor due to their co-expression on the cell surface [11, 21]. The ligand-binding extracellular domain of the $p75^{NTR}$ receptor contains repeats of cysteine [1]. None of these receptors exhibit any intrinsic catalytic activity. Their cytoplasmic domain is identical to the death domain.

The Trk-independent effects of $p75^{NTR}$ have been demonstrated including the retrograde transport of the NGF-TrkA complex [25]. The gene expression of proteins involved in the cell migration, the cellular differentiation and the apoptosis events, can be regulated alone by $p75^{NTR}$ [21, 26]. The importance of $p75^{NTR}$ was exhibited during the neurogenesis in the

regulation of the programmed cell death [27, 28]. The role of cell death could be confirmed in the developmental processes of the retina, optic nerve, spinal cord in mice with the generation of the retinal ganglion cells and the initial phase of the axonal elongation [29].

1.3. Nerve Growth Factor Receptor Signaling Mechanisms

1.3.1. Tyrosine Kinase Receptor A Signaling

Three main signaling cascades are activated by TrkA receptors and their substrates [30]. The first activation pathway is the RAS/RAF/MEK/MAPK (RAS, RAF: oncogens; MEK: MAPK or ERK kinase; MAPK: mitogen-activated protein kinase; ERK: extracellular signal-regulated kinase) for the development and differentiation of the neurons. The second is the activation of PI-3K (phosphatidylinositol-3 kinase) for the neuronal survival via PKB/AKT kinase activation (PKB: protein kinase B; Akt: serine/threonine kinase), membrane trafficking, proliferation, and differentiation. The third pathway is the activation of phospholipase Cγ for the neurotrophin-mediated neurotrophin release and the synaptic plasticity [1]. The activation of the same Trk receptors by the different ligands leads to distinct signaling events. The activation of the TrkA receptor alone is sufficient to induce morphogenic and survival signals [15]. Competitive signaling processes, which are responsible for the cell survival, could be demonstrated between the TrkA and $p75^{NTR}$ receptors [31]. The selective interplay between the tyrosine kinases and the cytokine receptors highlights a new, alternate mechanism during the cellular events [32].

a) The binding of NGF to the TrkA receptors triggers the dimerization and recruitment of various adapter molecules, such as the Shc/Grb2/SOS complex (Shc: adapter protein, Grb2: growth factor receptor-binding protein 2; SOS: the son of sevenless protein) for the RAS/RAF/MEK/MAPK cascade (Figure 3) [21]. The adapter molecules and the PI-3K bind to the tyrosine residues located in the juxta membrane region of the TrkA receptor. The phosphorylated tyrosine recruits phosphorlipase Cγ in the C terminal part of the TrkA. NGF promotes the association of TrkA with the Shc adapter protein, eliciting the formation of the Shc/Grb2/SOS complex. The RAS oncogene activation is not receptor bound, and it contributes to the activation of ERKs. Other growth factors, such as the epidermal growth factor (EGF) and insulin, also activate the RAS oncogene and ERKs [33, 34]. The MAPK cascade associates with the activation of the c-fos and c-jun effector transcription factors through the RAF, MEK and ERK cascades [35, 36]. MAPK plays a central role in the control of cell growth. The activation of the RAS/ERK pathway via NGF leads to the induction of the immediate-early genes [37]. The MAPK pathway could be amplified and integrated by signals from extracellular stimuli (growth factors, hormones, inflammatory, environmental and oxidative stress, and cytokines) [32, 38]. The phosphorylated tyrosines of TrkA bind to the Shc adapter protein and one domain of Grb2, which has no catalytic activity at the inner surface of the membrane [21]. Another domain of Grb2 binds to SOS protein. The RAS oncogene, the guanine nucleotide exchanging factor stimulates the replacement of GDP (guanosine diphosphate) with GTP (guanosine triphosphate). The RAS-GTP complex recruits the RAF oncogene to the membrane, promoting it to become an active phospho-

kinase. The active RAF oncogene initiates the MAPK signaling cascade (Figure 4). NGF leads to the activation of two distinct MAPK pathways: RAS/RAF/MEK/ERK and p38. The p38 MAPK pathway contributes to the phosphorylation of the cAMP (cyclic adenosine monophosphate) response element binding protein (CREB). CREB and the RAS/ERK complex potentiate the synthesis of proteins.

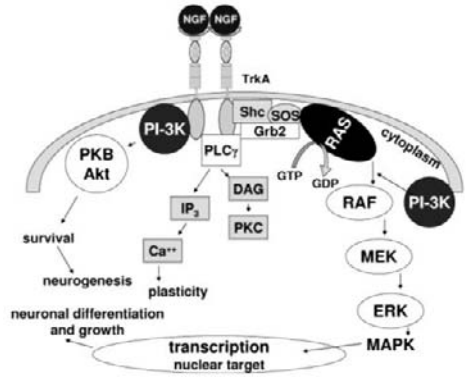

Figure 3. Nerve growth factor signaling cascades through tyrosine kinase A receptors. The binding of nerve growth factor (NGF) to its high-affinity tyrosine kinase receptor (Trk A) triggers the dimerization of the receptors, initiating three signaling pathways: RAS/RAF/MEK/MAPK, PKB/Akt and PKC. PI-3K: phosphatidylinositol 3-kinase; IP_3: inositol phosphate; PLCγ: phospholipase Cγ; PKC: protein kinase C; DAG: diacylglycerol; Shc: adapter protein; SOS: son of sevenless protein; Grb2: growth factor receptor-binding protein; RAF, RAS: oncogenes; MEK: MAPK or ERK kinase**;** MAPK: mitogen-activated protein kinase; ERK: extracellular signal-regulated kinase; PKB: protein kinase B; Akt: serine/threonine kinase; GTP: guanosine triphosphate; GDP: guanosine diphosphate.

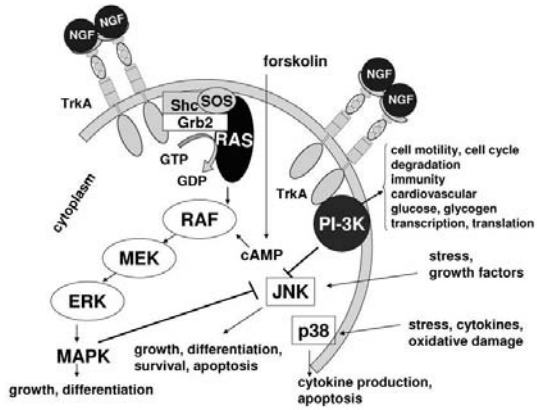

Figure 4. Mitogen-activated protein kinase cascade is modulated by various factors. The nerve growth factor (NGF) initiated mitogen-activated protein kinase (MAPK) signaling pathway is modulated by various factors influencing the cAMP and JNK activities, which may turn the signaling events into apoptosis. PI-3K: phosphatidylinositol 3-kinase; TrkA: tyrosine kinase receptor A; cAMP: cyclic adenosine monophosphate; Shc: adapter protein; SOS: son of sevenless protein; Grb2: growth factor receptor-binding protein; RAF, RAS: oncogenes; MEK: MAPK or ERK kinase**;** ERK: extracellular signal-regulated kinase; GTP: guanosine triphosphate; GDP: guanosine diphosphate; JNK: c-jun NH_2-terminal kinase; p38: mitogen- activated protein kinase; forskolin: cAMP generation stimulating substance; → : action direction; ⊣ : action inhibition.

b) PI-3K in the NGF responses plays an additional signaling route. The insulin receptor substrates - IRS-1 and IRS-2 - binding to the Gab-1 adapter molecule activate PI-3K [39]. PI-3K does not act directly on the TrkA receptors. PI-3K is implicated in the neuronal survival signaling pathway via the activation of protein kinase B/Akt kinase, but it also plays a pivotal role in a wide range of cellular processes mediating a variety of extracellular stimuli, e.g. growth factors and hormones [40, 41, 42]. Akt is characterized as a serine/threonine kinase and a proto-oncogene product playing a role as a second messenger for the PI-3K [43]. Akt takes place in the various effector signaling routes, which control the proliferation, the migration, the invasion and the survival of cells. The PI-3K/Akt signaling pathway seems to be critical for the survival of endothelial cells, the migration and the cord formation which are relevant in angiogenesis [44]. PI-3K is composed of regulatory (p85) and catalytic (p110) subunits. The p85 regulatory subunit displays a common substrate for many upstream regulators. PI-3K phosphorylates the phosphatidylinositol 4,5-bisphosphate (PIP_2), producing phosphatidylinositol 3,4,5-bisphosphate (PIP_3), which acts as a second lipidmessenger in the stimulation of Akt.

c) The association of phospholipase Cγ with TrkA leads to the regulation of the intracellular Ca2+ levels. It also leads to the enzyme activity of protein kinase C (PKC) via cleaving the substrate phosphatidylinositol 4,5-bisphosphate into diacylglycerol (DAG) and inositol trisphosphate (IP_3). The phospholipase Cγ pathway not only regulates the release of neurotrophin and the synaptic plasticity; it also regulates the synthesis of neuron-specific intermediate filament protein, the peripherin [45, 46]. Diacylglycerol induces the activation of protein kinase C, while phosphatidylinositol 3,4,5-bisphosphate leads to a release of calcium from the intracellular stores.

1.3.2. Cell Signaling Via P75^{ntr} Receptor

$p75^{NTR}$ can bind to each neurotrophin and modify the affinity of the Trk receptors [1, 14]. The precursor form of NGF displays high-affinity binding to $p75^{NTR}$, but the receptor is considered having low-affinity in comparison with TrkA. $p75^{NTR}$ serves as a pro-apoptotic receptor during the developmental cell death and after the injury of the nervous system. Apoptosis triggered by $p75^{NTR}$ plays a relevant role in the inflammatory, stress and injury processes [47]. ProNGF exhibits a high-affinity feature in binding to $p75^{NTR}$ proposing that the proteolytic cleavage of proNGF may have a regulatory effect on apoptosis.

The apoptotic events are relevant during the embryonic development, in the loss function of mutations, and in the responses against cell damages caused by environmental stimuli [29, 35]. Apoptosis can be initiated by internal signals, e.g. genetic damage, or oxidative stress and external factors, such as TNFα and the Fas ligand (Figure 5) [48]. The central events of apoptosis are bound to the activity of caspases: the family of proteases. Multiple routes are implicated in the cell death via apoptosis. The binding of TNFα or the Fas ligand to the membrane receptor induces the activation of the caspase 8 initiator. The caspase 8 mediated cleavage causes a downstream activation of caspase 3. The caspase 3 activation promotes the activation of caspase 9 via a cytochrome C (Cyt C) release after the activation of BAX. BAX apoptotic protein allows an influx of ions into mitochondria. Bid and Bad proapoptotic proteins binding to the Bcl2 and BclXI antiapoptotic receptors in the outer mitochondrial membrane can prevent the apoptosis induced by the reaction of the BAX protein with the mitochondrial membrane. The executioner caspases initiate the cleavage of the various target

proteins in the cells, contributing to alterations in the nuclear morphology, fragmentations as well as the degradation of the nuclear DNA via the activated endonucleases.

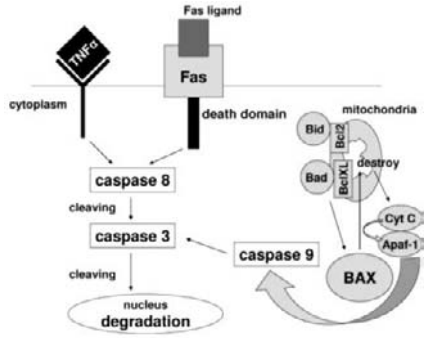

Figure 5. Apoptosis cascades. Apoptosis can be initiated by internal and external factors. The binding of TNFα to $p75^{NTR}$ receptor and/or Fas ligand to Fas membrane receptor initiates the caspase cascades via Bid and Bad proapoptotic proteins of the mitochondrial membranes. Bcl2, BclXI: antiapoptotic receptors; BAX: proapoptotic protein; Cyt C: cytochrome C; Apaf-1: adapter protein; Fas: apoptotic antigen; TNFα : tumor necrosis factor α.

$p75^{NTR}$ is characterized as a member of the Fas/TNF receptor family, but the signaling events are different from those initiated by Fas. The caspase 8 pathway is not required for the cell death mediated by $p75^{NTR}$ [49]. Surprisingly, caspase 6 is implicated in the neurotrophin-induced cell death together with caspase 3 and 9 [50]. In the alternative signaling pathway, caspase 9 activates the caspase 6 and 3 effectors downstream. The caspases are involved in the mitochondrial release of cytochrome C. The adapter protein formed apoptosome (Apaf-1) interacts with cytochrome C. The apoptosome activates caspase 9 causing a downstream activation of caspase 6 and 3. However, the activation of c-jun NH_2-terminal kinase (JNK) is necessary for the loss of mitochondrial cytochrome C (Figure 6).

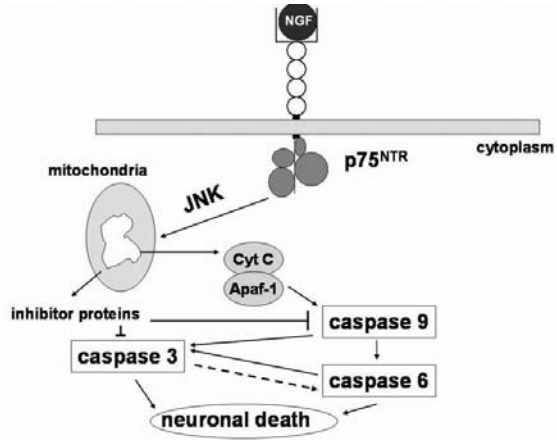

Figure 6. $p75^{NTR}$ mediated neuronal death signaling. Nerve growth factor (NGF) binding to its low-affinity tyrosine kinase receptor, $p75^{NTR}$, activates cytochrome C (Cyt C) via c-jun NH_2-terminal kinase (JNK) and promotes the Apaf-1 adapter protein binding to the caspase 9, downstreaming the caspase 6 and 3 activations. The activations of the caspases are essential for the neuronal death. → : action direction; ⊣ : action inhibition; - - → : decreased action.

Multiple mechanisms affect the cell death via $p75^{NTR}$. In general, NGF induced cell death through $p75^{NTR}$ manifests only in those cells that do not express specific TrkA receptors. The $p75^{NTR}$ activation links to ceramid production and the activation of nuclear factor κB (NFκB) and c-jun NH_2-terminal kinase [51]. The balance between the signaling events of nuclear factor κB in the cell survival and the c-jun NH_2-terminal kinase in the cell death represents the duality of $p75^{NTR}$. The activation of nuclear factor κB can be induced by many cytokines (IL-1β, IFNγ) and ligands of the TNF family, exerting a protective role against apoptosis [41]. The c-jun NH_2-terminal kinase pathway leads to the death of the cell, but the signaling mechanism is influenced by various factors (Figure 7). The following factors modify the activity of c-jun NH_2-terminal kinase:

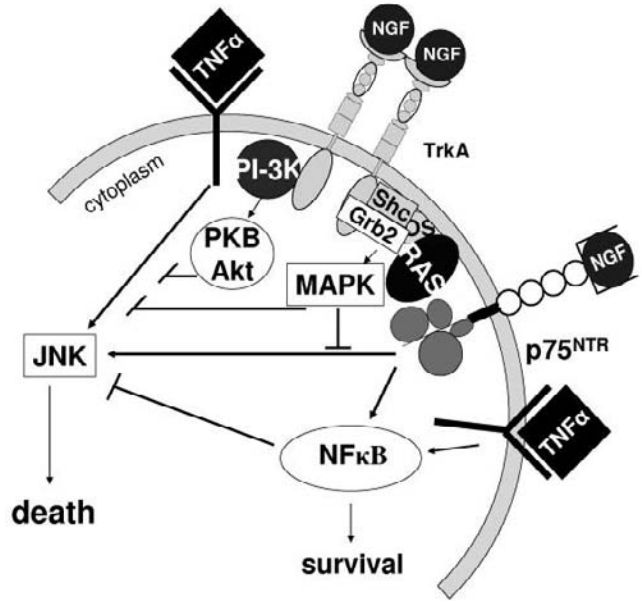

Figure 7. Nerve growth factor receptor-mediated signaling leads to cell death via the c-jun NH_2-terminal kinase activity. The c-jun NH_2 terminal kinase (JNK) pathway, which leads to cell death, may be inhibited by the PKB/Akt, MAPK and NFκB nuclear factor κB (NFκB) signaling mechanisms initiated by the nerve growth factor (NGF). PKB: protein kinase B; Akt: serine/threonine kinase; PI-3K: phosphatidylinositol 3-kinase; TrkA: tyrosine kinase receptor A; Shc: adapter protein; SOS: son of sevenless protein; Grb2: growth factor receptor-binding protein; RAS: oncogene; MAPK: mitogen-activated protein kinase; TNFα: tumor necrosis factor α; → : action direction; ⊣ : action inhibition.

1) The production of TNFα or ceramide during $p75^{NTR}$ activation inhibits the activity of c-jun NH_2-terminal kinase.
2) The co-activation of $p75^{NTR}$ with the TrkA receptor selectively blocks the c-jun NH_2-terminal kinase signaling.
3) The PI-3K/Akt signaling pathway can prevent the TNFα-induced cell death.

These mechanisms support why the $p75^{NTR}$ expression alone is not sufficient to cause cell death. The activation of TrkA does not affect the $p75^{NTR}$ mediated stimulation of nuclear factor κB.

1.3.3. Transactivation Between the Nerve Growth Factor-Bound Tyrosine Kinase A and the G Protein-Coupled Adenosine or Adrenergic Receptor Signalings

1.3.3.1. Transactivation Between the Tyrosine Kinase A and Adrenergic Receptors

Adrenergic receptors (AR) belong to the family of cell membrane G protein-coupled receptors (GPCR). They are mediated by a broad spectrum of extracellular signals in a wide variety of biological processes [52]. G protein-coupled receptors participate directly in the process of endocytosis, intracellular trafficking, and the resensitization of adrenergic receptors. They also modulate the MAPK cascades [53]. The G protein-coupled receptor signaling pathway can be found in several pathological diseases such as hypertension, congestive heart failure, rheumatoid arthritis and endocrine diseases [54, 55, 56].

G protein-coupled receptors at the cell surface are composed of three protein subunits: α, β and γ [57]. In the unstimulated state the α subunit binds to guanosine diphosphate, which makes the G protein inactive. During the activation of the receptor, the α subunit releases its guanosine diphosphate, which leads to the dissociation of the complex into two active Gα and Gβγ subunits. Both subunits modulate distinct effector systems. The G proteins are divided into Gs, Gi, Gq and Go types according to the functional activity or the signaling cascades. The receptor coupling to the G stimulatory (Gs) or G inhibitory (Gi) proteins modulate the adenylate cyclase (AC) activity via generating cAMP, second messenger. The cAMP activates the cAMP-dependent protein kinase A (PKA). The Gq protein-coupled receptor activates phospholipase Cβ (PLCβ) generating the production of diacylglycerol and inositol trisphosphate with the upstream activation of protein kinase C (Figure 8). The Go protein-coupled receptor activates the K+ and Ca2+ channels and phospholipase Cβ**.** The specific G receptor kinases (GRKs) play key roles after the receptor phosphorylation in triggering rapid receptor desensitization [58]. Phosphorylation induced by G receptor kinases promotes the binding of β-arrestins cytosolic regulatory proteins to the receptor, which in turn contributes to an uncoupling of the G receptor kinase from the G proteins**.** G receptor kinases can modulate the cellular functions in a phosphorylation-independent manner due to the interactions of the proteins which are involved in the signaling and trafficking pathways.

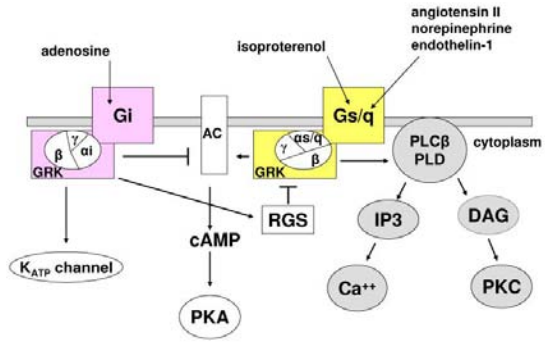

Figure 8. G protein-coupled receptor signaling cascades. The transactivation between the adenosine and G protein-coupled receptor signaling leads to cAMP-dependent protein kinase A (PKA) activation and cAMP-independent protein kinase C (PKC) activation. IP_3: inositol triphosphate; DAG: diacylglycerol; PLCβ: phospholipase Cβ; PLD: phospholipase D; AC: adenylate cyclase; cAMP: cyclic adenosine monophosphate; GRK: G protein receptor kinase; $G_{s/q}$ or G_i : G protein receptors; RGS: regulators for G protein signaling; → : action direction; ⊣ : action inhibition.

The G receptor kinase family consists of seven isoforms, that can be divided into three main groups: the rhodopsin kinase or visual G receptor kinase subfamily (GRK1 and 7), the G receptor kinase 2/3 subfamily termed β-adrenergic receptor kinases (βARK) and the G receptor kinase 4 subfamily (GRK4, 5 and 6) [57]. The cell-type distribution of the different G receptor kinases is physiologically relevant. The G receptor kinase 2 is predominantly present in the vascular endothelial cells, whereas the G receptor kinase 3 mainly resides in the cardiac myocytes; the G receptor kinase 5 is found in both the cardiac myocytes and the endothelial cells.

The tyrosine kinase receptors for epidermal growth factor, insulin-like growth factor-1 (IGF-1), platelet-derived growth factor (PDGF) and the TrkA receptor for NGF can be activated through G protein-coupled receptors [59]. It is well established that the G protein-coupled receptor signaling, which is regulated by adenylate cyclase and cAMP levels, can initiate the phospholipase Cγ and MAPK activities. Therefore, both receptor-signaling pathways - the G protein-coupled receptors and Trk receptors - activate MAPK. The trans-activation mechanism between the G protein-coupled and the Trk receptors plays a central role in the catecholamine - or hormone-induced cell proliferation, differentiation and synaptic plasticity (Figure 9) [60].

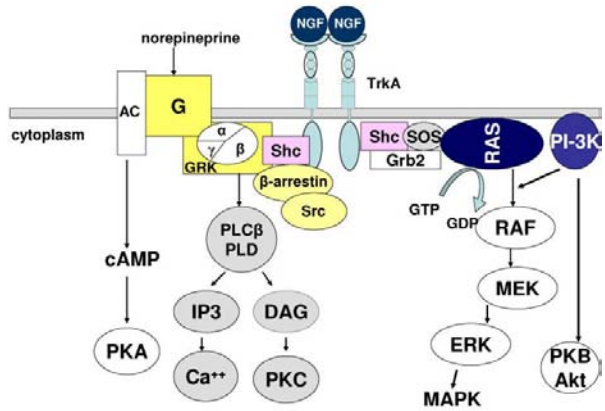

Figure 9. Transactivation between the G protein-coupled and tyrosine kinase A kinase receptors. The tyrosine kinase A (TrkA) receptor activation can be induced by the G protein-coupled receptor (GPCR) activation. The transactivation route is promoted by Shc adapter protein cross-binding between TrkA and GPCR. The phosphatidylinositol 3-kinase (PI-3K) activation leads to cell hypertrophy. PKA: protein kinase A; PKB: protein kinase B; PKC: protein kinase C; Akt: serine/threonine kinase; IP_3: inositol triphosphate; PLCβ: phospholipase Cβ; PLD: phospholipase D; NGF: nerve growth factor; AC: adenylate cyclase; cAMP: cyclic adenosine monophosphate; GRK: G protein receptor kinase; G: G protein receptor; DAG: diacylglycerol; Shc: adapter protein; Src, β-arrestin: regulatory protein; SOS: son of sevenless protein; Grb2: growth factor receptor-binding protein; RAF, RAS: oncogenes; MEK: MAPK or ERK kinase**;** ERK: extracellular signal-regulated kinase; MAPK: mitogen-activated protein kinase; GTP: guanosine triphosphate; GDP: guanosine diphosphate; NGF: nerve growth factor.

There is transactivation between the TrkA and the β2-adrenergic receptors (β2ARs) [61]. The β2AR stimulation in COS-7 (African Green monkey SV40 transformed kidney fibroblast) cells initiates the dimerization of the epidermal growth factor receptor and the autophosphorylation of tyrosine with the activation of the ERK cascades. The Shc adapter protein, which shows Src and collagen homologies, binds to the β2AR-epidermal growth

factor receptor complex. This complex containing β-arrestins and Src family kinases initiates the RAS oncogene-dependent ERK activation. The β2AR mediated ERK activation requires a release of Gβγ subunits from the G proteins. Much data suggest that the epidermal growth factor receptor-mediated mitogenic signals may arise from diverse sources, such as the activations of G protein-coupled, cytokine and prolactin receptors and the stimulations of growth, thyroid hormones, integrins [62, 63].

The heart has a diverse αAR signaling mechanism; it involves multiple signaling pathways regulating the cardiac output as well as the cellular growth responses [64]. There is evidence that PI-3K plays a direct role in cardiac hypertrophy [65]. Studies of cardiac cells, fibroblasts as well as cells of the central nervous system all show that the αAR signaling pathway is involved in the hypertrophic responses [66]. These hypertrophic processes are regulated by the activation of the RAS oncogene and MAPK.

1.3.3.2. Transactivation Between the TrkA and Adenosine A_2 Receptors

Adenosine is viewed as a potentially important signaling molecule in the heart, reflecting the metabolic state of the myocardium [67]. This small molecule regulates the vasomotor tone in the coronary vessels. Two major routes can lead to adenosine production in the cardiac myocytes: (1) the hydrolysis of S-adenosylhomocysteine via transmethylation and (2) the hydrolysis of cAMP during ischemic or hypoxic conditions.

The effects of adenosine are mediated by four distinct receptors which are members of the G protein-coupled receptor family: A_1, A_{2A}, A_{2B}, A_3 [67]. Adenosine is able to inhibit the norepinephrine release from the presynaptic vesicles, attenuate the renin-angiotensin system and decrease the release of endothelin-1. However, its receptors on the T lymphocytes potentiate an antiinflammatory action of adenosine [68, 69]. The activation of Gs in β-adrenergic or of Gq in α-adrenergic protein-coupled receptors, as well as the genetic overexpression of Gs or Gq protein-coupled receptors, lead to cardiac hypertrophy and heart failure. The cardiomyocyte hypertrophy is modulated by adenosine via the stimulation of several neurohormonal factors or adrenergic receptors.

The TrkA and TrkB receptor transactivations could be demonstrated even in the absence of neurotrophins [63, 70]. The adenosine and its agonists, as well as the neuropeptide pituitary adenylate cyclase activating polypeptide (PACAP), are capable of activating the PI-3K/Akt signaling pathway, which in turn enhances the survival response in the PC12 and the hippocampal cells [50]. Lee and co-workers have exhibited that the early (first 10 minutes of) MAPK induction is mediated Trk-independently by the adenosine G protein signaling. The Trk receptor-depending MAPK signaling manifests later, after 60 minutes [53]. Both Akt and MAPK pathways are mediated by the Shc adapter protein binding to the Trk receptor at the tyrosine part. Adenosine induces a long lasting Akt kinase activity. These studies suggest that adenosine may exert a neuroprotective effect. The Src tyrosine kinases, which are nonreceptor tyrosine kinases, are influenced by several MAPK activation and transactivation events mediated by G protein-coupled receptors. The Src protein regulates the catalytic activity of the Trk receptors. The Trk receptor transactivation is slow and is associated with a selective enhancement of the activated Akt [63].

Although the exact signaling mechanisms between the Trk and G protein-coupled receptor transactivations have not yet been revealed, it seems that the adenosine and adrenergic receptor agonists can mimic the trophic responses. These increase the survival of the cell by stimulating the tyrosine kinase receptor.

REFERENCES

[1] Bibel, M; Barbel, YA. *Neurotrophins: key regulators of cell fate and cell shape in the vertebrate nervous system*. Genes & Development 2000; 14:2919-2937.

[2] Mendell, LM; Albers, KM; Davis, BM. *Neurotrophins, nociceptors, and pain*. Microsc Res Tech 1999; 45:252-261.

[3] Murray, E; Kaye, D. *Sympathetic nervous system neuroplasticity*. Hypertension 2006; 47:143-144.

[4] Bruno, MA; Cuello, AC. *Activity-dependent release of precursor nerve growth factor, conversion to mature nerve growth factor, and its degradation by a protease cascade*. Proc Natl Acad Sci USA 2006; 103:6735-6740.

[5] Fahnestock, M; Yu, G; Coughlin, MD. *ProNGF: a neurotrophic or an apoptotic molecule?* Prog Brain Res 2004; 146:107-110.

[6] Al-Shawi, R; Hafner, A; Chun, S; Raza, S; Crutcher, K; Thrasivoulou, C; Simons, P; Cowen, T. *ProNGF, sortilin, and age-related neurodegeneration*. Ann NY Acad Sci 2007; 1119:208-215.

[7] Kaplan, DR; Miller, FD. *A move to sort life from death*. Nature 2004; 427:798-799.

[8] Nykjaer, A; Lee, R; Teng, KK; Jansen, P; Madsen, P; Nielsen, MS; Jacobsen, C; Kliemannel, M; Schwarz, E; Willnow, TE; Hempstead, BL; Petersen, CM. *Sortilin is essential for proNGF-induced neuronal cell death.* Nature 2004; 427: 843-848.

[9] Chao, MV. *Neurotrophins and their receptors: A convergence point for many signaling pathways.* Nat Rev Neurosci 2003; 4:299-309.

[10] Huang, E; Reichardt, L. *Trk receptors: roles in neuronal signal transduction*. Ann Rev Biochem 2003; 73:609-642.

[11] Jullien, J; Guili, V; Reichardt, LF; Rudkin, BB. *Molecular kinetics of nerve growth factor receptor trafficking and activation.* J Biol Chem 2002; 277:38700-38708.

[12] Huebner, K; Isobe, M; Chao, M; Bothwell, M; Ross, AH; Finan, J; Hoxie, JA; Sehgal, A; Buck, CR; Lanahan, A; Nowell, PC; Koprowsky, H; Croce, CM. *The nerve growth factor receptor gene is at human chromosome region 17q12-17q22, distal to the chromosome 17 breakpoint in acute leukemias.* Proc Natl Acad Sci USA 1986; 83:1403-1407.

[13] Kaplan, DR; Hempstead, BL; Martin-Zanca, D; Chao MV; Parada, LF. *The trk proto-oncogene products: A signal transducing receptor for nerve growth factor*. Science 1991; 252:554-558.

[14] Chao, MV; Rajagopal, R; Lee, FS. *Neurotrophin signalling in health and disease*. Clin Science 2006; 110:167-173.

[15] Yan, C; Liang, Y; Nylander, KD; Schor, NF. *TrkA as a life and death receptors: Receptor dose as a mediator of function.* Cancer Res 2002; 62:4867-4875.

[16] Zapf-Colby, A, Olefsky, JM. *Nerve growth factor processing and trafficking events following TrkA-mediated endocytosis*. Endocrinol 1998; 139:3232-3240.

[17] Mufson, EJ; Kroin, JS; Sendera, TJ; Sobreviela, T. *Distribution and retrograde transport of trophic factors in the central nervous system: Functional implications for the treatment of neurodegenerative diseases*. Prog Neurobiol 1999; 57:451-484.

[18] Kholodenko, BN. *Four-dimensional organization of protein kinase signaling cascades: the roles of diffusion, endocytosis and molecular motors*. J Exp Biol 2003; 206:2073-2082.

[19] O'Connell, L; Hongo, JA; Presta, LG; Tsoulfas, P. *TrkA amino acids controlling specificity for nerve growth factor*. J Biol Chem 2000; 275:7870-7877.

[20] Urdiales, JL; Becker, E; Andrieu, M; Thomas, A; Jullien, J; van Grunsven, LA; Menut, S; Evan, GI; Martin-Zanca, D; Rudkin, BB.*Cell cycle phase-specific surface expression of nerve growth factor receptors TrkA and* $p75^{NTR}$. J Neurosci 1998; 18:6767-6775.

[21] Greene, LA; Kaplan, DR. *Early events in neurotrophin signalling via Trk and p75 receptors*. Curr Opin Neurobiol 1995; 5:579-587.

[22] Johnson, D; Lanahan, A; Buck, CR; Sehgal, A; Morgan, C; Mercer, E; Bothwell, M; Chao, M. *Expression and structure of the human NGF receptor*. Cell 1986; 47:545-554.

[23] Mukai, J; Shoji, S; Kimura, MT; Okubo, S; Sano, H; Suvanto, P; Li, Y; Irie, S; Sato, TA. *Structure-function analysis of NADE*. J Biol Chem 2002; 277: 13973-13982.

[24] Nagata, S. *Apoptosis by death factor*. Cell 1997; 88:355-365.

[25] Curtis, R; Adryan, KM; Stark, JL; Park, JS; Compton, DI; Weskamp, G, Huber, IJ; Chao, MV; Jaenisch, R; Lee, KF; DiStephano, P. *Differential role of low-affinity neurotrophin receptor (p75) in retrograde axonal transport of the neurotrophins*. Neuron 1995; 14:1201-1222.

[26] Verdi, JM; Birren, SJ; Ibáñez, CF; Persson, H; Kaplan, DR; Benedetti, M; Chao, MV; Anderson, DJ. $p75^{LNGFR}$ *regulates Trk signal transduction and NGF-induced neuronal differentiation in MAH cells*. Neuron 1994; 12:733-745.

[27] Bamji, SX; Majdan, M; Pozniak, CD; Belliveau, DJ; Aloyz, R; Kohn, J; Causing, CG; Miller, FD. *The p75 neurotrophin receptor mediates neuronal apoptosis and is essential for naturally occurring sympathetic neuron death*. J Cell Biol 1998; 140:911-923.

[28] Belliveau, DJ; Krivko, I; Kohn, J; Lachance, C; Pozniak, C; Rusakov, D; Kaplan, D; Miller, FD*: NGF and neurotrophin-3 both activate TrkA on sympathetic neurons but differentially regulate survival and neuritogenesis*. J Cell biol 1997; 136:375-388.

[29] Frade, JM; Barde, YA. *Genetic evidence for cell death mediated by nerve growth factor and the neurotrophin receptor p75 in the developing mouse retina and spinal cord*. Development 1999; 126: 683-690.

[30] Reichardt, LF. *Neurotrophin-regulated signalling pathways*. Phil Trans R Soc B 2006; 361:1545-1564.

[31] Yoon, SO; Casaccia-Bonnefil, P; Carter, B; Chao, MV. *Competitive signaling between TrkA and p75 nerve growth factor receptors determines cell survival*. J Neurosci 1998; 18:3273-3281.

[32] Kyriakis, JM; Avruch, JA. *Sounding the alarm: Protein kinase cascades activated by stress and inflammation*. J Biol Chem; 271:24313-24316.

[33] Dikic, I; Schlessinger, J; Lax, I. *PC12 cells overexpressing the insulin receptor undergo insulin-dependent neuronal differentiation*. Curr Biol 1994; 4:702-708.

[34] Traverse, S; Seedorf, K; Paterson, H; Marshall, CJ; Cohen, P; Ullrich, A. *EGF triggers neuronal differentiation of PC12 cells that overexpress the EGF receptor*. Curr Biol 1994; 4:694-701.

[35] Palmada, M; Kanwal, S; Rutkoski, NJ; Gufstafson-Brown, C; Johnson, RS, Wisdom, R; Carter, BD. *c-jun is essential for sympathetic neuronal death induced by NGF withdrawal but not by p75 activation*. J Cell Biol 2002; 158:453-461.

[36] Xing, J; Kornhauser, JM; Xia, Z; Thiele, EA; Greenberg, ME. *Nerve growth factor activates extracellular signal-regulated kinase and p38 mitogen-activated protein kinase pathways to stimulate CREB serine 133 phosphorylation*. Mol Cell Biol 1998; 18:1946-1955.

[37] Sheng, M; Greenberg, ME. *The regulation and function of c-fos and other immediate early genes in the nervous system*. Neuron 1990; 4:477-485.

[38] Ballif, BA; Blenis, J. *Molecular mechanisms mediating mammalian mitogen-activated protein kinase (MAPK) kinase (MEK)-MAPK cell survival signals.* Cell Growth Differ 2001; 12:397-408.

[39] Yamada, M; Ohnishi, H; Sano, S; Nakatani, A; Ikeuchi, T; Hatanaka, H. *Insulin receptor substrate (IRS)-1 and IRS-2 are tyrosine-phosphorylated and associated with phosphatidylinositol 3-kinase in response to brain-derived neurotrophic factor in cultured cerebral cortical neurons.* J Biol Chem 1997; 272:30334-30339.

[40] Crowder, RJ, Freeman, RS. *Phosphatidylinositol 3-kinase and Akt protein kinase are necessary and sufficient for the survival of nerve growth factor-dependent sympathetic neurons*. J Neurosci 1998;18:2933-2943.

[41] Takano, R; Hisahara, S; Namikawa, K; Kiyama, H; Okano, H; Miura, M. *Nerve growth factor protects oligodendrocytes from tumor necrosis factor-α-induced injury through Akt-mediated signaling mechanisms.* J Biol Chem 2000; 275:16360-16365.

[42] York, RD; Molliver, DC; Grewal, SS; Stenberg, PE; McCleskey, EW; Stork, PJS. *Role of phosphoinositide 3-kinase and endocytosis in nerve growth factor-induced extracellular signal-regulated kinase activation via RAS and Rap1*. Mol Cell Biol 2000; 20:8069-8083.

[43] Franke, TF; Yang, SI; Chan, TO; Datta, K; Kazlauskas, A; Morrison, DK; Kaplan, DR; Tsichlis, PN.*The protein kinase encoded by the Akt proto-oncogene is a target of the PDGF-activated phsophatidylinositol 3-kinase*. Cell 1995; 81:727-736.

[44] Park, MJ; Kwak, HJ; Lee, HC; Yoo, DH; Park, IC; Kim, MS; Lee, SH; Rhee, CH; Hong, SI. *Nerve growth factor induces endothelial cell invasion and cord formation by promoting matrix metalloproteinase-2 expression through the phosphatidylinositol 3-kinase/Akt signaling pathway and AP-2 transcription factor*. J Biol Chem 2007; 282:30485-30496.

[45] Canossa, M; Griesbeck, O; Berninger, B; Campana, G; Kolbeck, R; Thoenen, H. *Neurotrophin release by neurotrophins: Implications for activity-dependent neuronal plasticity.* Proc Natl Acad Sci 1997; 94:13279-13286.

[46] Loeb, DM; Stephens, RM; Copeland, T; Kaplan, DR; Greene, LA. *A Trk nerve growth factor (NGF) receptor point mutation affecting interaction with phospholipase C-γ1 abolishes NGF-promoted perinephrin induction but not neurite outgrowth*. J Biol Chem 1994; 269:8901-8910.

[47] Dobrowsky, RT; Carter, BD. *p75 neurotrophin receptor signaling: mechanisms for neurotrophic modulation of cell stress?* J Neurosci Res 2000; 61:237-243.

[48] Nagata, S. *Fas ligand-induced apoptosis*. Ann Rev Genet 1999; 33:29-55.

[49] Bossy-Wetzel, E; Green, DR. *Caspases induce cytochrome c release from mitochondria by activating cytosolic factors.* J Biol Chem 1999; 274:17848-17490.

[50] Troy, CM; Friedman, JE; Friedman, WJ. *Mechanisms of p75-mediated death of hippocampal neurons.* J Biol Chem 2002; 277:34295-34302.

[51] Roux, PP; Barker, PA. *Neurotrophin signaling through the p75 neurotrophin receptor. Prog Neurotrophin signaling through the p75 neurotrophin receptor*. Prog Neurobiol 2002; 67:203-233.

[52] Barki-harrington, L; Perrino, C; Rockman,HA. *Network integration of the adrenergic system in cardiac hypertrophy.* Cardiovasc Res 2004; 63:391-402.

[53] Lee, FS; Rajagopal, R; Chao, MV. *Distinctive features of Trk neurotrophin receptor transactivation by G protein-coupled receptors*. Cytokine Growth Factor Rev 2002; 13:11-17.

[54] Iaccarino, G; Dolber, PC; Lefkowitz, RJ; Koch, WJ. *β-adrenergic receptor kinase-1 levels in catecholamine-induced myocardial hypertrophy*. Hypertension 1999; 33:396-401.

[55] Lombardi, MS; Kavelaars, A; Cobelens, PM; Schmidt, RE; Schedlowski, M; Heijnen,CJ. *Adjuvant arthritis induces down-regulation of G protein-coupled receptor kinases in the immune system*. J Immunol 2001; 166:1635-1640.

[56] Metaye, T; Menet, E; Guilhot, J; Kraimps, JL. *Expression and activity of g protein-coupled receptor kinases in differentiated thyroid carcinoma*. J Clin Endocrinol Metab 2002; 87:3279-3286.

[57] Penela, P; Ribas, C; Mayor F Jr. *Mechanisms of regulation of the expression and function of G protein-coupled receptor kinases*. Cell Sign 2003; 15:973-981.

[58] Penn, RB; Pronin, AN; Benovic, JL. *Regulation of G protein-coupled receptor kinases*. Trends Cardiovasc Med 2000; 10:81-89.

[59] Price, RD; Milne, SA; Sharkey, J; Matsuoka, N. *Advances in small molecules promoting neurotrophic function.* Pharmacol Ther 2007; 115:292-306.

[60] Rajagopal, R; Chen, ZY; Lee, FS; Chao, MV. *Transactivation of Trk neurotrophin receptors by G-protein-coupled receptor ligands occurs on intracellular membranes*. J Neurosci 2004; 24:6650-6658.

[61] Maudsley, S; Pierces, KL; Zamah, AM; Miller, WE; Ahn, S; Daaka, Y; Lefkowitz, RJ; Luttrell, LM. *The β_2-adrenergic receptor mediates extracellular signal-regulated kinase activation via assembly of a multi-receptor complex with the epidermal growth factor receptor.* J Biol Chem 2000; 275:9572-9580.

[62] McGeachie, J; Tennant, M. *Growth factor and their implications for clinicians: A brief review*. Australian Dental J 1997; 42:375-380.

[63] Lee, FS; Chao, MV. *Activation of Trk neurotrophin receptors in the absence of neurotrophins.* Proc Natl Acad Sci USA 2001; 98:3555-3560.

[64] Michel, MC; Hanft, G; Gross, G. *Functional studies on alpha 1-adrenoreceptor subtypes mediating inotropic effects in rat right ventricle*. Br J Pharmacol 1994; 111:539-546.

[65] Naga Prasad, SV; Perrino, C; Rockman, HA. *Role of phosphoinositide 3-kinase in cardiac function and heart failure.* Trends Cardiovasc Med 2003; 13:206-212.

[66] Zhang, H; Facemire, CS; Albert J. Banes, AJ; James E. Faber, JE. *Different α-adrenoceptors mediate migration of vascular smooth muscle cells and adventitial fibroblasts in vitro*. Am J Physiol Heart Circ Physiol 2002; 282: H2364-H2370.

[67] Chen, Y; Bache, RJ. *Adenosine. A modulator of the cardiac response to stress*. Circ res 2003; 93:691-693.

[68] Ehrhard, PB; Erb, P; Graumann, U; Schmutz, B; Otten, U. *Expression of functional trk tyrosine kinase receptors after T cell activation*. J Immunol 1994; 152:2705-2709.

[69] Ohta, A; Sitkovsky, M. *Role of G-protein-coupled adenosine receptors in downregulation of inflammation and protection from tissue damage*. Nature 2001; 414:916-920.

[70] Mojsilovic-Petrovic, J; Jeong, GB; Crocker, A; Arneja, A; David, S; Russell, D, Kalb, RG. *Protecting motor neurons from toxic insult by antagonism of adenosine A2a and Trk receptors*. J Neurosci 2006; 26:9250-9263.

2. CELLS SECRETING NERVE GROWTH FACTOR AND THEIR TISSUE LOCALIZATION

2.1. Neuronal Tissues

Nerve growth factor (NGF) is crucial for the development, growth, survival and differentiation of the nervous system. Its effect is mediated by high- (TrkA) and low-affinity ($p75^{NTR}$) receptors which are widespread detectable not only in the normal neural cells, but also in the normal nonneural epithelial, mesenchymal cells, lymphoid tissues and the cells of the bone marrow [1]. Immunohistochemical analysis has proven the expression of NGF receptors in the neuroectodermal, mesenchymal, epithelial and lymphoid malignancies.

In the target organs, the amount of NGF correlates with the density of the sympathetic innervation [2]. NGF released in neurons is detectable in the hippocampus, astrocytes, peripheral sympathetic, sensory and some cholinerg neurons of the central nervous system (CNS) as well as in the sympathetic postganglionic neurons. The detectable levels of NGF in the neurons reflect the intensity of its retrograde transport to the cell body [3] (Figure 10).

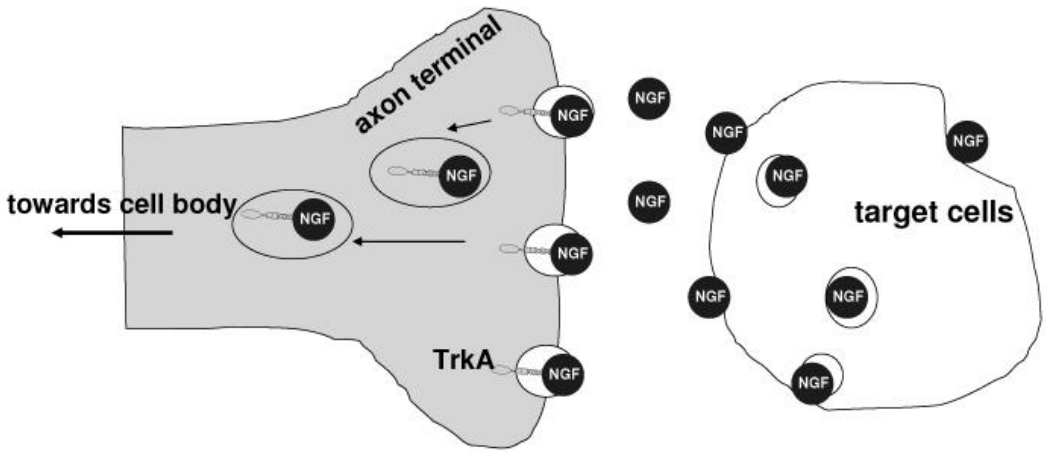

Figure 10. Retrograde transport of nerve growth factor in the neurons. Nerve growth factor (NGF) is a target-derived neurotrophin, and its binding to the high-affinity tyrosine kinase A receptor (TrkA) initiates a retrograde transport of the NGF-TrkA receptor complex into the cell body.

However, the levels of NGF mRNA are associated with the norepinephrine content of the tissues. Shelton and co-workers found that the iris, the heart and the spleen exhibit high NGF mRNA content and these tissues are characterized by heavy sympathetic innervation [3]. The superior cervical and stellata ganglia in rats contain increased levels of NGF similar to the salivary gland, atrium and iris. Heumann and co-workers stressed that the increased levels of NGF are derived from retrograde axonal transport rather than from local synthesis [4]. The synthesis and the release of NGF in the central nervous system relate to astrocytes, microglias and the nonneural cells.

In chickens, the microglia-derived NGF participates in the development of the retina via programmed cell death [5]. In this case, the release of proNGF contributes to the degeneration of the cultured photoreceptor cells. This supports the argument, that the NGF is neuroprotective, but its early form - the proNGF - is cytotoxic. The pathological events associate with the release of the proteolytic enzymes: plasmin and matrix metalloproteinases enhancing the production of NGF from proNGF. Microglias are able to secrete proteases and cleave the proNGF. The receptors of $p75^{NTR}$ without the presence of TrkA are indispensable to the apoptosis of the retinal cells. After light damage, ischemia and reperfusion, the $p75^{NTR}$ receptor density is increased.

Under pathophysiological conditions - such as a tissue damage due to the different neuronal, immunological, inflammatory, and regulatory mechanisms, - the astrocytes become essential [6, 7, 8]. For example, rat astrocytes can produce NGF during various stimulatory conditions [6]. In the cerebral astrocytes in rats, interleukin-1 (IL-1) and transforming growth factor-β1 (TGF-β1) are potent stimulators for the NGF secretion. Norepinephrine, gluco-corticoids and higher concentrations of mineralocorticoids all increase the levels of NGF. The induction of the NGF release after brain injury contributes to a neuroprotection via the elevated levels of cytokines and growth factors [7]. The inflammation in the central nervous system associates with the production of proinflammatory cytokines (TNFα, IL-1, IL-6, TGFβ) leading directly to high NGF production from the astrocytes. The Th2-derived cytokines take part in the pathological events of the central nervous system by suppressing the inflammation and the neurotoxicity [9].

It seems that if the high-affinity NGF receptors - the TrkAs - are dominantly present in the peripheral sympathetic and sensory neurons, this correlates with a protective, neurotrophical effect and a low secretion of NGF in the absence of $p75^{NTR}$. The neuronal cells expressing $p75^{NTR}$ in the absence of TrkA demonstrate an increase of apoptosis. The cerebral astrocytes among the neuronal cells are capable of local NGF secretion, which suggests their beneficial role in the various pathological responses.

2.2. Nonneuronal Tissues

The distribution of the high-affinity neurotrophin receptors has a wide range in the adult, human, normal, nonneuronal tissues [10]. The major NGF targets are the neuronal cells, but the TrkA receptors are also detectable in the gastric parietal cells, and cells of the adrenal cortex, kidneys, testes, prostate, thymus, uterus, ovaries, mammary ducts, skin, salivary glands, esophagus, small intestine, colon, exocrine pancreas, lungs, adipose tissue, orbit, the skeletal muscles and the bone marrow.

The intestinal epithelial cells are regulated by various cytokines and growth factors, as well as NGF. NGF plays a protective role in the responses to injuries and inflammations [11]. The cytokines secreted in mucosal epithelial cells influence the balance between the Th1 and Th2 dominance, exerting a selective upregulatory role for the NGF via the release of IL-10. The mucosal epithelial cells produce NGF in an autocrine fashion **(**Figure 11). These data reflect that the NGF's synthesis and its presence in the surroundings of the mucosa are relevant and implicated in its regulatory function.

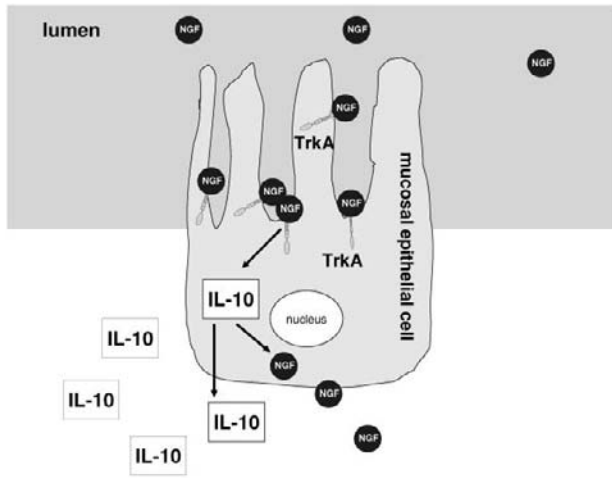

Figure 11. The IL-10 regulated nerve growth factor production is essential in the mucosal epithelial cell integrity. The mucosal epithelial cell produces nerve growth factor (NGF) via IL-10 release and expresses tyrosine kinase receptor A (TrkA) for binding with the NGF.

The salivary glands are the large source of NGF. This fact highlights that the NGF may have a role in the digestive system, and in its healing [12].

Both TrkA and $p75^{NTR}$ NGF receptors are expressed in the pancreatic beta cells. Polak and co-workers reported on a neurotypic response of the pancreatic beta cells to NGF suggesting an overlapping developmental program between the neurons and the pancreatic endocrine cells [13].

The neurotrophins and their receptors are implicated in the folliculogenesis of the mammalian ovaries [14]. Both NGF receptors could be detected in the rat ovaries, but the $p75^{NTR}$ predominantly expressed in the mesenchymal cells. In the ovaries, the amount of NGF is low at the time of the folliculogenesis. The findings show, that the neurotrophin receptors are involved in the organization processes causing follicular assembly.

Human keratinocytes are able to synthesize and release NGF, which stimulates the sprouting of nerve fibers and regulates the expression and the synthesis of several neuro-peptides [15]. The low- and high-affinity receptors expressed on keratinocytes induce apoptosis in the absence of NGF. Psoriatic epidermis associates with the hypertrophy of the peripheral nerves and overexpresses NGF, which increases the size of the neuron, as well as the expression of TrkA receptors on the epidermal cells.

The NGF and TrkA receptor are detectable in human chondrocytes [16]. The elevated NGF level in osteoarthritis suggests elevated NGF level's stimulatory role in the chondrocyte metabolism.

The NGF secretion and the TrkA expression on the mast cells, - such as on the T and B lymphocytes, monocytes, keratinocytes and chondrocytes, - as well as on fibroblasts amplify the cross-talk between the nervous and immune systems.

Skeletal muscle tissue can also produce NGF and express both NGF receptors [1]. The myopathy and the muscle damage initiated by physiological or pathological stress activate the PI-3K/Akt kinase signaling cascades [17, 18].

2.2.1. Bone Marrow

The hematopoetic cells are not immunoreactive for $p75^{NTR}$. The TrkA receptors are detectable on monocytes, mastocytes, B and T lymphocytes, eosinophils, as well as basophils, such as osteoblasts and osteoclasts [19]. These cells not only express TrkA receptors, but also secrete NGF. The pleiotropic effects of NGF manifest in the alterations of the platelet shape and the triggering of monocyte-cytotoxic activity, as well as in the proliferation of B and T lymphocytes. As a survival factor, NGF contributes to increase in the amount of the memory B cells by the Th2 dominance. The growth and the differentiation of the myeloid and the erythroid progenitors are also promoted by NGF. The B lymphocyte activation mediated by the PI-3K/Akt activation plays an important role in the survival of B cells (20). Similar mechanisms are present in the maintenance of survival cascades in fibroblast and epithelial cells.

By expressing TrkA, neutrophils bind to NGF, thus enhancing the survival of the cell, the phagocytosis and the production of superoxid [21]. Therefore, the survival of neutrophils - induced and prolonged by NGF - can lead to a neutrophilia during inflammations or stress situations, and improve the tissue repair processes.

Eosinophils are essential for the production of NGF. More than 90 % of these cells are localized in the mucosal tissues, and they are necessary for the maintenance and restoration of the mucosal homeostatic functions [22]. Eosinophils are potential sources of various cytokines and growth factors. These active substances are produced from the fibrosis, the tissue repair and the vascular endothelial cell damage during inflammatory events caused by allergy, stress, tumor, infections or immune processes. The NGF release from the eosinophils is stimulated by several factors: a./ by Fc-receptor-mediated stimuli via the IgA and IgG immunocomplexes, and b./ by IL-5 cytokines. The widely distributed role of the eosinophil cells illustrates that the immunologic and inflammatory responses may affect the functions of the peripheral nervous system through the production of NGF.

Monocytes, eosinophils, neutrophils, basophils, as well as mast cells arisen from CD34+ progenitors, are detectable in the cord and peripherial blood, and also in the bone marrow [19]. NGF, in synergy with the stem cell factor, supportes the maintenance of CD34+ progenitors. NGF, like other cytokines (IL-3, IL-5, IL-13) potentiates the IgE-mediated histamine and LTC4 releases from the basophils in an autocrine fashion [23]. The secretion of IL-4 and IL-13 from basophils upregulates the level of vascular cell adhesion molecule-1 (VCAM-1) which recruits basophils, eosinophils and Th2 lymphocytes to the site of the inflammation. This recruitment can be promoted by eotaxins released from the fibroblasts upon an IL-4 stimulation, or by eotaxins released from the eosinophils and mast cells (Figure 12). Moreover, the survival of the cell is potentiated by the NGF secretion acting on the TrkA receptors. This mechanism can be illustrated in the allergic inflammation resulting in Th2 dominance with longstanding and perpetuating cellular responses.

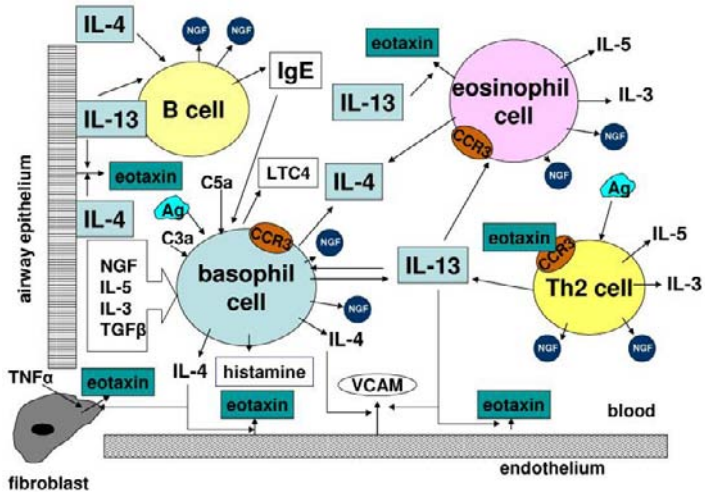

Figure 12. The basophil cell activation and its roles in the airway allergic inflammation. Nerve growth factor (NGF) is implicated in the lung inflammation, in which several factors play a role together with the immunocompetent cells. The activations of the basophil and eosinophil cells, plus the B and T helper 2 (Th2) lymphocytes lead to a release of chemokines such as eotaxin causing a cell recruitment with the fibroblast activation. VCAM: vascular cell adhesion molecule; LTC4: leukotriene C4; Ag: antigen; C3a, C5 : complement split products; eotaxin: chemoattractant; TNFα: tumor necrosis factor α; TGFβ: transforming growth factor β; Th2 cell: T helper 2 lymphocyte; IgE: immunoglobulin E; CCR_3: eotaxin receptor.

Mast cells are prominent sources of NGF. Indeed, NGF increases the number of the mast cells, modifies their cytokine releases and leads to an alterations in the TNFα and IL-6 productions [24]. TNFα is a neurotoxic agent. The NGF- mediated decrease in the secretion of TNFα not only associates with its antiinflammatory effect, but it also represents a neuroprotection. NGF can initiate the release of histamine from mast cells and basophils with the chemotactic migration of the polymorphonuclear leukocytes [25]. Sawada and co-workers demonstrated that the action of NGF on the migration is mediated by the MAPK and PI-3K signaling pathways [25].

Mast cells are in the center of allergic disorders, tumors, the healing of wounds and the host defense responses against certain infections, such as helminth parasites and ectoparasites. The local accumulation of the mast cells could be reached by the release of NGF or by the NGF binding to its receptors. The secreted cytokines (IL-3, IL-4, IL-10, and IL-13) are essential for the allergic and the nonallergic inflammatory conditions; they are also vital in the tissue repair events.

2.2.2. Lung Tissues

Human lungs synthesize and release NGF, and express TrkA receptors [26]. The bronchial epithelial cells are immunoreactive for NGF but not for the TrkA receptor. However, the alveolar cells can express the TrkA receptor, while the alveolar and interstitial macrophages exhibit NGF. The IL-1β and TNFα cytokines are potent stimulators for NGF secretion in the bronchial epithelial cells. In the lungs, NGF may promote the hyperplasia of the respiratory smooth muscles, because there is a cross-talking effect between the NGF and G protein-coupled receptors on the immune cells, which leads to a progressive fibrosis [27]. NGF is released by various types of cells in the lungs, such as cells of the immune system -

mast cells, eosinophils, basophils, lymphocytes, monocytes, macrophages, - as well as fibroblasts, epithelial cells and smooth muscle cells [28]. The serum levels of NGF are elevated in allergic diseases. The hyperresponsiveness of the airways may be induced by NGF as a result of its effect on the sensory nerves [29, 30, 31]. The NGF-mediated airway hyper-responsiveness can be evoked by aggravating the airway with capsaicin or histamine. The mechanism may be similar to skin and muscle hyperalgesia, or bladder hyperactivity.

Fibroblasts in the human lungs are prominent NGF and cytokine secreting cells, playing an active role in the inflammation of the airways [32]. The IL-1β and TNFα cytokines are released from the neighbouring macrophages, mast cells and lymphocytes, which are also prominent stimulators for the secretion of NGF in the lungs.

2.2.3. Adipose Tissues

According to new data, the white (WAT) and brown (BAT) adipose tissues are potent sources of NGF. The white and brown adipose tissues not only secrete NGF, but they are also targets of the immune and inflammatory responses [33, 34] (Figure 13). Contrary to their role in the storage of energy, nowadays their endocrine and secretory features should be stressed. The proinflammatory cytokines, such as TNFα are the major stimulators for NGF in the white and brown adipose tissues [35]. The peroxisome proliferator-activated receptor-γ (PPARγ) agonists and steroids inhibit the production of NGF and its release from the fat cells, thus demonstrating their antiinflammatory effects. The white and brown adipose tissues are organs very highly innervated with the sympathetic nerves and the β-adrenergic receptor expressions. The stress, and the autoimmune and allergic inflammatory processes maintain a state of a low-grade inflammation with increased NGF levels. The prostaglandin derivatives of PGD_2 are potent NGF inducers. Norepinephrine can initiate the trophic function through the β-adrenergic receptors.

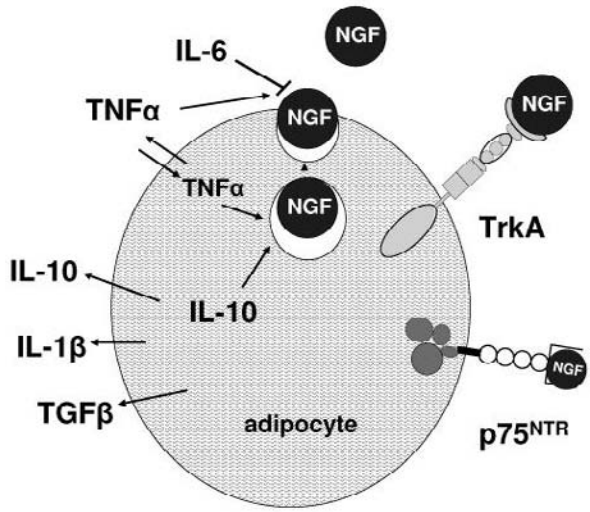

Figure 13. Adipocyte-derived cytokines and nerve growth factor release. Adipocyte itself releases nerve growth factor (NGF), which acts in an autocrine manner. Various cytokines (IL-10, IL-1β, TNFα, TGFβ) are produced by adipocytes. TNFα: tumor necrosis factor α; TGFβ: transforming growth factor β; TrkA: high-affinity tyrosine kinase A receptor; $p75^{NTR}$: low-affinity tyrosine kinase receptor; → : action direction; ⊣ : action inhibition.

Both high- and low-affinity NGF receptors are expressed in the white and brown adipose tissues. The brown adipose tissue is present in the interscapular, perirenal, axillary and cervical regions of infants, while in the omental, perirenal, and periadrenal regions in adults. The sympathetic nerves directly innervate the brown adipose tissue, in which norepinephrine acts predominantly [33]. Obesity is generally associated with a drop in the sympathetic neuronal activity, while cold temperatures enhances the sympathetical activity and induces a hypertrophy in brown adipose tissue. Nisoli and co-workers demonstrated that the NGF synthesis in the brown adipose tissue is under an inhibitory effect of norepinephrine itself [33].

Lipopolysaccharide (LPS**)** increases the secretion of NGF in the fat cells via the Toll-like receptors-4 (TLR4). In obesity and diabetes, the synthesis of IL-6 and TNFα is enhanced and is associated with the NGF production, reflecting the presence of a chronic inflammatory feature.

2.2.4. Orbital Tissues

The orbital tissues represent special sources for NGF release and express both NGF receptors (TrkA and $p75^{NTR}$). The orbital tissues make up a very highly innervated organ, and the orbital developments are regulated under strong neuronal effects. The cornea, iris, ciliary body and the lens can express NGF and the high-affinity TrkA receptor [36]. NGF modulates the development and differentiation of the retina and the optic nerve promoting the survival and recovery of the ganglion cells, photoreceptors and the optic nerve. Intraocular injury leads to an overproduction of NGF with the release of cytokines. The iris is innervated by sympathetic, parasympathetic and sensory nerves, and like the ciliary body, it is a major source of NGF.

The autoimmune and allergic diseases regularly affect the ocular surfaces and associate with its proliferative, inflammatory responses. The secretion of NGF during the orbital inflammation leads to the proliferation and differentiation of the corneal epithelium, blood vessels and the release of different cytokines from the immunocompetent cells, macrophages, eosinophils, basophils, mast cells, fibroblasts, and endothelium (TNFα, IL-6, IL-1β, IL-4, IL-10, and IL-13) [37, 38]. High intraocular pressure increases the amount of NGF. NGF can act as a modulator of the inflammatory responses of the orbital tissues. Moreover, the conjunctival and the corneal epithelium express TrkA receptors. The profibrogenic effect of NGF could be demonstrated on fibroblasts derived from vernal keratoconjunctivitis [38].

2.2.5 Endocrine Tissues

2.2.5.1. Adrenal Glands

Mammalian adrenal glands consist of two endocrine organs: a steroid-producing adrenal cortex and an adrenal medulla, the latter of which secretes catecholamines, neuropeptides, ATP (adenosine triphosphate) and other small molecules. The adrenal cortex is under a hormonal control of the hypothalamic-pituitary-adrenal axis (HPA), while the adrenal medulla is innervated by sympathetic neurons - predominantly cholinergic innervated preganglionic neurons whose cell bodies are localized in the spinal cord (Figure 14) [39]**.** In the adrenal medulla, the sympathetic and chromaffin cells secrete neurotrophins, such as NGF. The adrenal medullary cells are capable of TrkA and $p75^{NTR}$ in addition to TrkB and

TrkC receptor expression. The preganglionic sympathetic neurons innervate the postganglionic noradrenergic neurons, as well as the noradrenergic and adrenergic chromaffin cells in the adrenal medulla. Tai and co-workers reported that the adrenergic phenotype expression in the adrenal medulla is regulated by NGF together with the neuropeptide pituitary adenylate cyclase-activating polypeptide [40]. The adrenergic phenotype expression is controlled by phenylethanolamine N-methyltransferase at the posttranscriptional level, which is the final enzyme of the catecholamine biosynthesis [40]. In the adrenal medulla as well as in the chromaffin cells and the preganglionic nerves, the TrkA signaling stimulates the activity of the postnatal acethylcholinesterase (AchE) enzyme [41]. Only the TrkB, TrkC and $p75^{NTR}$ receptors are expressed on the motoneurons; the TrkA receptor is not. The adrenal chromaffin cells could be stimulated by NGF through their TrkA receptors. The nerve fiber outgrowth induced from adrenal chromaffin cells in rats associates with a relevant increase in the volume of the sympathetic ganglia [42]. The glucocorticoids exert an inhibitory effect on the NGF induced fiber outgrowth from the chromaffin cells, indicating a negative feedback, which can play an important role in the adrenal medulla under physiological conditions.

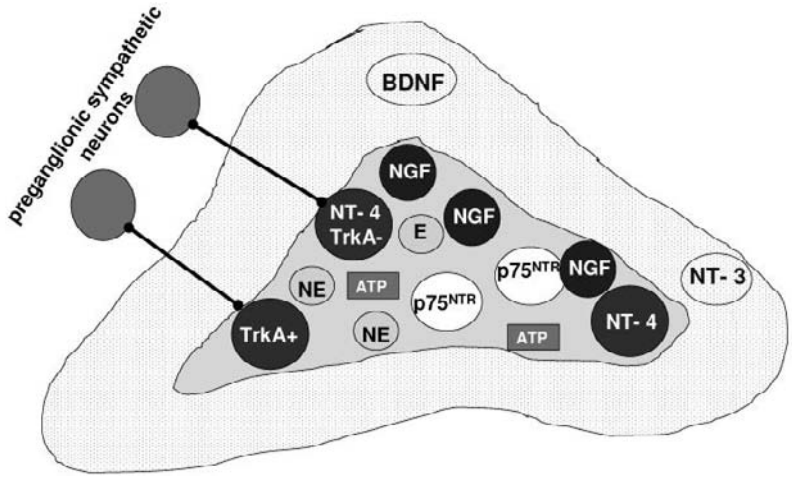

Figure 14. The expression of neurotrophins and their receptors in the adrenal gland. The adrenal medulla is innervated by sympathetic neurons, predominantly preganglionic sympathetic neurons and postganglionic noradrenergic neurons with the liberation of catecholamines (NE, E). The adrenal medulla contains chromaffin cells secreting neurotrophins (NGF, NT-3, NT-4, BDNF). NGF: nerve growth factor; NT-3: neurotrophin-3; NT-4: neurotrophin-4; BDNF: brain-derived neurotrophic factor; NE: norepinephrine; E: epinephrine; ATP: adenosine triphosphate; TrkA: high-affinity tyrosine kinase A receptor ; $p75^{NTR}$: low-affinity tyrosine kinase receptor.

2.2.5.2Pituitary Gland

Stress situations in the pituitary initiate an increase of NGF levels. The hypothalamic-pituitary-adrenal activation is triggered by some stress and is implicated in the inflammatory and autoimmune diseases [43]. The NGF gene transcript and NGF protein have been studied by immunofluorescence analysis in the anterior pituitary (44). These investigations lead to the surprising findings that NGF was predominantly detectable in the mammotroph and somatotroph cells, and the results showed a co-secretion of prolactin in these cells. The anterior pituitary thought of as a target for NGF via its TrkA receptors [45]. NGF increases the ACTH (adrenocorticotropic hormone) and cortisol levels through the activation of the

hypothalamic-pituitary-adrenal axis. The variance in the NGF contents of the pituitary cells suggests that NGF has a modulatory role on the pituitary hormone secretion in an autocrine or a paracrine manner. Immunoreactivities against NGF were measured in 10 % of the cells containing ACTH, 64 % of the cells with TSH (thyroid-stimulating hormone), 75 % of the cells with LH (luteinizing hormone), 51 % of the cells with GH (growth hormone) and 42 % of the cells with prolactin (Figure 15). A TrkA expression was detected in 33 % of ACTH containing cells, 45 % of TSH containing cells, 44 % of LH containing cells, 23 % of GH containing cells and 41 % of prolactin containing cells. The strong link between the NGF secreting and NGF targeting cells in the anterior pituitary demonstrates the complexity of the neuroendocrine-immune networks.

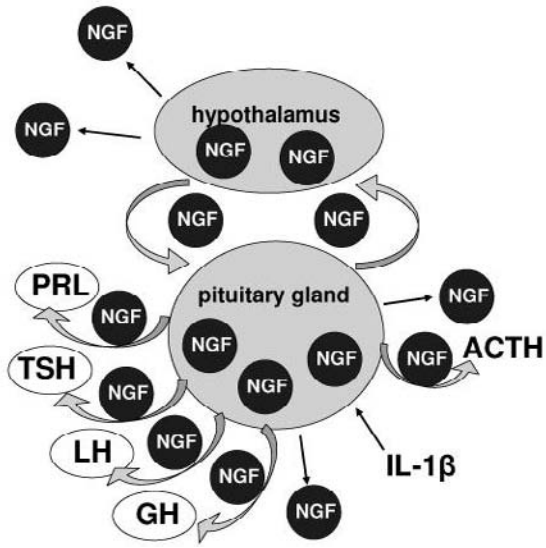

Figure 15. Nerve growth factor co-secretion of the hormones stored in the pituitary gland. PRL: prolactin; TSH: thyroid-stimulating hormone; LH: luteinizing hormone; GH: growth hormone; ACTH: adrenocorticotropic hormone; NGF: nerve growth factor.

2.2.5.3.Thyroid Glands

Thyroid and parathyroid glands exhibit NGF mRNA and protein [46]. Very little data were reported on the levels of NGF in human thyroid diseases. Recent findings confirm that the hyperthyroid Graves' patients possess elevated serum levels of NGF; this highlights thyroid autoimmunity as a source of NGF [47]. The thyroid hormones are essential in the formation of myelin, the development and survival of oligodendrocytes that act as a growth factor and as an NGF inducing material in the central nervous system [48]. The thyroid hormones can stimulate the NGF production in the salivary glands and modulate the secretion profile of biologically active peptides [49].

Receptors binding to TSH and thyroid hormones are expressed on lymphocytes. The activations of these receptors change the release of IL-6 and IFNγ cytokines (Figure 16) [50]. The thyroid hormone interactions among the immune and nervous systems, and cross-talk between the adrenergic receptors emphasize the complexity of the responses during increased sympathetic activity.

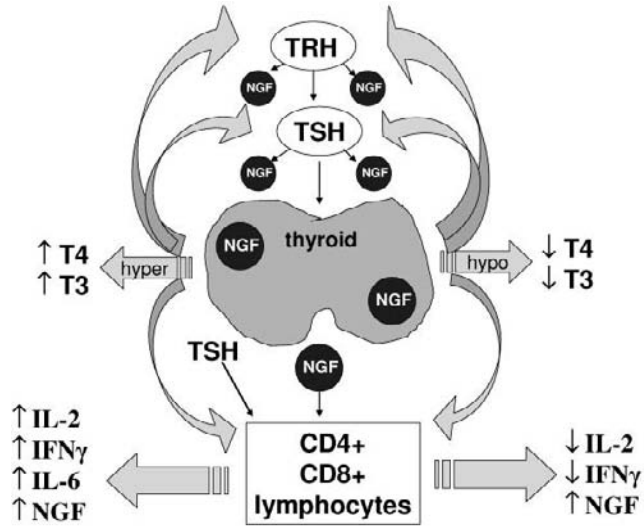

Figure 16. Thyroid hormones induce nerve growth factor secretion during the thyroid autoimmune responses. Nerve growth factor (NGF) co-secretes from TRH and TSH as well as from the released substances from the activated lymphocytes. The nerve growth factor production in the thyroid glands can modulate the cytokine release, the inflammatory reactions and the tissue restitution. TSH: thyroid-stimulating hormone; TRH: thyrotropin-releasing hormone; IFNγ: interferon γ; hyper: hyperthyroidism; hypo: hypothyroidism.

REFERENCES

[1] Chesa, PG; Retting, JWJ; Thomson, TM; Old, LJ; Melamed, MR. *Immunohistochemical analysis of nerve growth factor receptor expression in normal and malignant human tissues*. J Histochem Cytochem 1988; 56:383-389.

[2] Korsching, S; Thoenen, H. *Nerve growth factor in sympathetic ganglia and corresponding target organs of the rat: Correlation with density of sympathetic innervation.* Proc Natl Acad Sci USA 1983; 80:3513-3516.

[3] Shelton, DL; Reichardt, LF. *Expression of the β-nerve growth factor gene correlates with the density of sympathetic innervation in effector organs*. Proc Natl Acad Sci USA 1984; 81:7951-7955.

[4] Heumann, R; Korsching, S; Scott, J; Thoenen, H. *Relationship between levels of nerve growth factor (NGF) and its messenger RNA in sympathetic ganglia and peripheral target tissues.* Eur Mol Biol Org J 1984; 3:3183-3189.

[5] Srinivasan, B; Roque, CH; Hempstead, BL; Al-Ubaidi, MR; Roque, RS. *Microglia-derived pronerve growth factor promotes photoreceptor cell death via p75 neurotrophin receptor.* J Biol Chem 2004; 279:41839-41845.

[6] Zafra, F; Lindholm, D; Castrén, E; Hartikka, J; Thoenen, H. *Regulation of brain-dericed neurotrophic factor and nerve growth factor mRNA in primary cultures of hippocampal neurons and astrocytes*. J Neurosci 1992; 12:4793-4799.

[7] Brodie, C. *Differential effects of Th1 and Th2 derived cytokines on NGF synthesis by mouse astrocytes*. Fed Eur Biochem Societ Letters 1996; 394:117-120.

[8] Nance, DM; Sanders, VM. *Autonomic innervation and regulation of the immune system* (1987-2007). Brain Behav Immun 2007; 21:736-745.

[9] Rahimi,O; Juliano, SL. *Transplants of NGF-secreting fibroblasts restore stimulus-evoked activity in barrel cortex of basal-forebrain-lesioned rats*. J Neurophysiol 2001; 86:2081-2096.

[10] Shibayama, E; Koizumi, H. *Cellular localization of the neurotrophin receptor family in human non-neuronal tissues.* Am J Pathol 1996; 148:1807-1818.

[11] Ma, D; Wolvers, D; Stanisz, AM; Bienenstock, J. *Interleukin-10 and nerve growth factor have reciprocal upregulatory effects on intestinal epithelial cells*. Am J Physiol Regul Integr Comp Physiol 2003; 284:R1323-R1329.

[12] Murphy, RA; Saide, JD; Blanchard, MH; Young, M. *Nerve growth factor in mouse serum and saliva: Role of the submandibular gland*. Proc Natl Acad Sci USA 1977; 74:2330-2333.

[13] Polak, M; Scharfmann, R; Seilheimer, B; Eisenbarth, G; Dressler, D; Verma, IM; Potter, H. *Nerve growth factor induces neuron-like differentiation of an insulin-secreting pancreatic beta cell line*. Proc Natl Acad Sci USA 1993; 90:5781-5785.

[14] Dissen, GA; Hirschfield, AN; Malamed, S; Ojeda, SR. *Expression of neurotrophins and their receptors in the mammalian ovary is developmentally regulated*: *Changes at the time of folliculogenesis.* Endocrinology 1995; 136:4681-4692.

[15] Pincelli, C. *Nerve growth factor and keratinocytes: a role in psoriasis*. Eur J Dermatol 2000; 10:85-90.

[16] Iannone, F; De Bari, C; Dell'Accio, F; Covelli, M; Patella, V; Lo Bianco, G; Lapadula, G. *Increased expression of nerve growth factor (NGF) and high affinity NGF receptor (p140 TrkA) in human osteoarthritic chondrocytes*. Rheumatology 2002; 41:1413-1418.

[17] Wojtaszewski, JFP; Lynge, J; Jakobsen, AB, Goodyear, LJ; Richter, EA. *Differential regulation of MAP kinase by contraction and insulin in skeletal muscle: metabolic implications*. Am J Physiol Endocrinol Metab 1999; 277:E724-732.

[18] Del Aguila, LF, Krishnan, RK; Ulbrecht, JS; Farrell, PA; Correll, PH; Lang, CH; Zierath, JR; Kirwan, JP. *Muscle damage impairs insulin stimulation of IRS-1, PI 3-kinase, and Akt-kinase in human skeletal muscle*. Am J Physiol Endocrinol Metab 2000; 279:E206-E212.

[19] Labouyrie, E; Dubus, P; Groppi, A; Mahon, FX; Ferrer, J; Parrens, M; Reiffers, J; de Mascarel, A; Merlio, JP. *Expression of neurotrophins and their receptors in human bone marrow.* Am J Pathol 1999; 154:405-415.

[20] Pogue, SL; Kurosaki, T; Bolen, J; Herbst, R. *B cell antigen receptor-induced activation of Akt promotes B cell survival and is dependent on Syk kinase*. J Immunol 2000; 165:1300-1306.

[21] Kannan, Y; Ushio, H; Koyama, H; Okada, M; Oikawa, M; Yoshihara, T; Kaneko, M; Matsuda, H. *Nerve growth factor enhances survival, phagocytosis, and superoxide production of murine neutrophils*. Blood 1991; 77:1320-1325.

[22] Kobayashi, H; Gleich, GJ; Butterfield, JH; Kita, H. *Human eosinophils produce neurotrophins and secrete nerve growth factor on immunologic stimuli*. Blood 2002; 99:2214-2220.

[23] Falcone, FH; Haas, H; Gibbs, BF. *The human basophils: a new appreciation of its role in immune responses.* Blood 2000; 96:4028-4038.

[24] Marshall, JS; Gomi, K; Blennerhassett, MG; Bienenstock, J. *Nerve growth factor modifies the expression of inflammatory cytokines by mast cells via a prostanoid-dependent mechanism.* J Immunol 1999; 162:4271-4276.

[25] Sawada, J; Itakura, A; Tanaka, A; Furusaka, T; Matsuda, H. *Nerve growth factor functions as a chemoattractant for mast cells through both mitogen-activated protein kinase and phosphatidylinositol 3-kinase signaling pathways*. Blood 2000; 95:2052-2058.

[26] Ricci, A; Felici, L; Mariotta, S; Mannino, F; Schmid, G; Terzano, C; Cardillo, G; Amenta, F; Bronzetti, E. *Neurotrophin and neurotrophin receptor protein expression in the human lung*. Am J Respir Cell Mol Biol 2004; 30:12-19.

[27] Micera, A; Vignetti, E; Pickholtz, D; Reich, R; Pappo, O; Bovini, S; Maquart, FX; Aloe, L; Levi-Schaffer, F. *Nerve growth factor displays stimulatory effects on human skin and lung fibroblasts, demonstrating a direct role for this factor in tissue repair*. Proc Natl Acad Sci USA 2001; 98:6162-6167.

[28] Höglund, CO; de Blay, F; Oster, JP; Duvernelle, C; Kassel, O; Pauli, G; Frossard, N. *Nerve growth factor levels and localization in human asthmatic bronchi*. Eur Respir J 2002; 20:1110-1116.

[29] Hoyle, G; Graham, RM; Finkelstein, JB; Nguyen, KP; Gozal, D; Friedman, M. *Hyperinnervation of the airways in transgenic mice overexpressing nerve growth factor*. Am J Respir Cell Mol Biol 1998; 18:149-157.

[30] Sanico, AM; Stanisz, AM; Gleeson, TD; Bora, S; Proud, D; Bienenstock, J; Koliatsos, VE; Togias, A. *Nerve growth factor expression and release in allergic inflammatory disease of the upper airways*. Am J Respir Crit Care Med 2000; 161:1631-1635.

[31] Woolf, CJ; Safieh-Garabedian, B; Ma, QP; Crilly, P, Winter, J. *Nerve growth factor contributes to the generation of inflammatory sensory hypersensitivity*. Neuroscience 1994; 62:327-331.

[32] Olgart, C; Frossard, N. *Human lung fibroblasts secrete nerve growth factor: effect of inflammatory cytokines and glucocorticoids*. Eur Respir J 2001; 18:115-121.

[33] Nisoli, E; Tonello, C; Benarese, M; Liberini, P; Carruba, MO. *Expression of nerve growth factor in brown adipose tissue: Implications for thermogenesis and obesity*. Endocrinology 1996; 137:495-503.

[34] Bulló, M; Peeraully, MR; Trayhurn, P. *Stimulation of NGF expression and secretion in 3T3-L1 adipocytes by prostaglandins PGD_2, PGJ_2, and Δ^{12}-PGJ_2.* Am J Physiol Endocrinol Metab 2005; 289:E62-E67.

[35] Peeraully, MR; Jenkins, JR; Trayhurn, P. *NGF gene expression and secretion in white adipose tissue: regulation in 3T3-L1 adipocytes by hormones and inflammatory cytokines.* Am J Physiol Endocrinol Metab 2004; 287:E331-E339.

[36] Lambiase, A; Bonini, S; Manni, L; Ghinelli, E; Tirassa, P; Rama, P; Aloe, L. *Intraocular production and release of nerve growth factor after iridectomy*. Invest Ophthalmol Vis Sci 2002; 43:2334-2340.

[37] Lambiase, A; Bonini, S; Micera, A; Rama, P; Bonini, S; Aloe, L. *Expression of nerve growth factor receptors on the ocular surface in healthy subjects and during manifestation of inflammatory diseases.* Invest Ophthalmol Vis Sci 1998; 39:1272-1275.

[38] Micera, A; Lambiase, A; Stampachiacchiere, B; Sgrulletta, R; Normando, EM; Bonini, S; Bonini, S. *Nerve growth factor has a modulatory role on human primary fibroblast*

cultures derived from vernal keratoconjunctivitis-affected conjunctiva. Molecular Vision 2007; 13:981-987.

[39] Schober, A; Wolf, N; Kahane, N; Kalcheim, C; Krieglstein, K; Unsicker, K. *Expression of neurotrophin receptors trkB and trkC and their ligands in rat adrenal gland and the intermediolateral column of the spinal cord.* Cell Tissue Res 1999; 296:271-279.

[40] Tai, TC; Wong-Faull, DC; Claycomb, R; Wong, DL. *Nerve growth factor regulates adrenergic expression.* Mol Pharmacol 2006; 70:1792-1801.

[41] Schober, A; Minichiello, L; Keller, M; Huber, K; Layer, PG; Roig-López, JL; Garcia-Arrarás, JE; Klein, R; Unsicker, K. *Reduced acethylcholinesterase (AchE) activity in adrenal medulla and loss of sympathetic preganglionic neurons in TrkA-deficient, but not TrkB-deficient, mice*. J Neurosci 1997; 17:891-903.

[42] Unsicker, K, Krisch, B; Otten, U; Thoenen, H. *Nerve growth factor-induced fiber outgrowth from isolated rat adrenal chromaffin cells*: *Impairment by glucocorticoids.* Proc Natl Acad Sci USA 1978; 75:3498-3502.

[43] Elenkov, IJ; Chrousos, GP. *Stress, hormones, proinflammatory and antiinflammatory cytokines, and autoimmunity.* Ann NY Acad Sci 2002; 966:290-303.

[44] Missale, C; Boroni, F; Sigala, S; Buriani, A; Fabris, M; Leon, A; Dal Toso, R; Spano, PF. *Nerve growth factor in the anterior pituitary: Localization in mammotroph cells and cosecretion with prolactin by a dopamine-regulated mechanism.* Proc Natl Acad Sci USA 1996; 93: 4240-4245.

[45] Patterson, JC; Childs, GV. *Nerve growth factor and its receptor in the anterior pituitary.* Endocrinology 1994; 135:1689-1696.

[46] Dicou, E; Lee, J; Brachet, P. *Synthesis of nerve growth factor mRNA and precursor protein in the thyroid and parathyroid glands of the rat.* Proc Natl Acad Sci USA 1986; 83:7084-7088.

[47] Molnár, I; Bokk, Á. *Decreased nerve growth factor levels in hyperthyroid Graves' ophthalmopathy highlighting the role of neuroprotective factor in autoimmune thyroid diseases.* Cytokine 2006; 35:109-114.

[48] Fernandez, M; Giuliani, A; Pirondi, S; D'Intino, G; Giardino, L; Aloe, L; Levi-Montalcini, R; Calza, L. *Thyroid hormone administration enhances remyelination in chronic demyelinating inflammatory disease*. Proc Natl Acad Sci USA 2004; 101:16363-16368.

[49] Black, MA; Pope, L; Lefebvre, FA; Lefebvre, YA; Walker, P. *Thyroid hormones precociously increase nerve growth factor gene expression in the submandibular gland of neonatal mice.* Endocrinology 1992; 130:2083-2090.

[50] Klecha, AJ; Genaro, AM; Gorelik, G; Arcos, MLB; Silberman, DM; Schuman, M; Garcia, SI; Pirola, C, Cremaschi, GA. *Integrative study of hypothalamus-pituitary-thyroid-immune system interaction: thyroid hormone-mediated modulation of lymphocyte activity through the protein kinase C signaling pathway*. J Endocrinol 2006; 189:45-55.

3. NERVE GROWTH FACTOR IN INFLAMMATORY, ALLERGIC AND AUTOIMMUNE PROCESSES

3.1. Nerve Growth Factor Modulates the Inflammatory Responses Via Prostaglandins

NGF secretion shows an increase during inflammation due to various causes of the inflammatory responses. A number of cytokines are released, such as IL-1β, IL-6, IL-8, and TNFα, from the immune cells together with NGF. NGF acts in either an autocrine or a paracrine manner and induces the pain associated with the inflammation [1].

Adipocytes are prominent sources of NGF. NGF secretion from the adipocytes depends on several factors: catecholamines, cytokines (IL-6, TNFα, IL-1β, IL-10, and IL-18), lipopolysaccharide, insulin, glucocorticoids and prostaglandins, substantia P, neurokinin A (NKA), and neuropeptide Y (NPY) [2].

Prostaglandins belong to hydroxyacids similarly to leukotrienes; they are called prostanoids, which is involved in the inflammation and hyperalgesia. The biosynthesis of prostaglandin is regulated by three steps:1. The liberation of arachidonic acid from the membrane phospholipids due to the lysophosphatidic acid-2 (LPA_2) isoenzyme. 2. The conversion of arachidonic acid (AA) to PGH_2 via cyclooxygenase (COX) isoforms. 3. The terminal conversion of PGH_2 via the lypoxygenase enzyme to bioactive prostanoids, such as PGD_2, PGE_2 prostaglandins and PGI_2 prostacyclin or thromboxane A_2 [3]. These bioactive lipid mediators activate cAMP, the second messenger or G protein-coupled receptors; they also contribute to different biological effects on the vascular tissues and the sensory, nociceptive neurons [4].

The prostaglandin (EP) and prostacyclin (IP) receptors are present in the cells of the central nervous system, as well as in the sensory neurons, sympathetic fibers, astrocytes, endothelial cells, adipocytes, and mast cells. Bulló and co-workers demonstrated that the expression of NGF in the adipocytes may be stimulated by PGD_2 and its metabolite: PGJ_2 [5]. Both PGE_2 and PGI_2 are secreted in human adipocytes. TNFα could stimulate the production of NGF via PGD_2 and this prostaglandin is downregulated by the drugs indomethacin or rosiglitazone. It is well known that both indomethacin (an inhibitor of the prostaglandin synthesis and rosiglitazone (a PPARγ agonist) downregulate the NGF levels. By another way, they could also block the TNFα-induced lipolysis.

Mast cells represent a potent regulatory function in the chronic and allergic inflammations (Figure 17). It should be emphasized, that NGF not only releases but also modifies the prostaglandin production of mast cells. Prostaglandins PGE_2 and PGD_2 are released from the mast cells during the degranulation processes [6]. However, apart from the mast cells and the adipocytes, PGE_2 synthesis could also be detected in macrophages, neutrophils, fibroblasts, and follicular dendritic cells [7, 8]. The PGE_2 production can be decreased by TNFα and increased by IL-6 cytokines via the induction of cyclooxygenase into the mast cells; this represents an autocrine antiinflammatory effect of NGF [6]. The ability of NGF to induce the release of a PGE_2 is independent of the degranulation of mast cells (Figure 18). The NGF-induced cyclooxygenase expression in the mast cells may associate with PGD_2 generation depending on the secretory phospholipase A_2 ($sPLA_2$) activity [3].

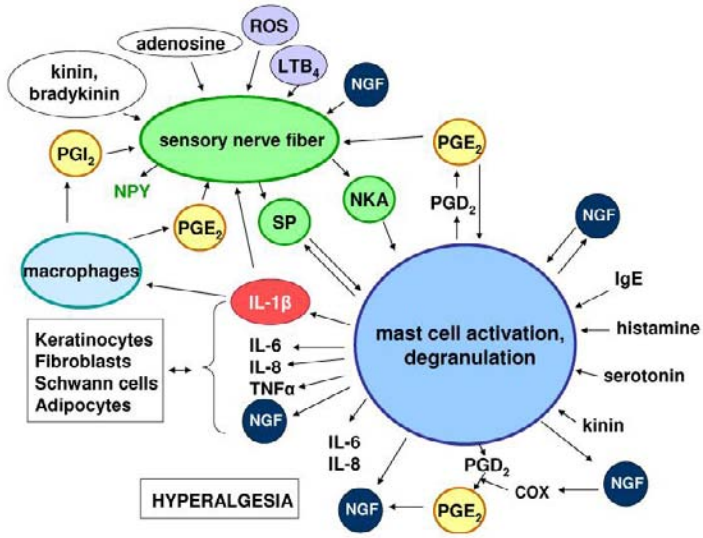

Figure 17. NGF potentiates the mast cell regulated inflammatory processes. Nerve growth factor (NGF) is capable of the degranulation of mast cells and the release of several active substances and cytokines. Therefore, nerve growth factor may initiate the inflammation, the hyperalgesia and the different cell activations. SP: substance P; NKA: neurokinin A; NPY: neuropeptide Y; ROS: reactive oxygen species; LTB_4 : leukotriene B_4 ; COX: cyclooxygenase; PGI_2 , PGE_2 , PGD_2 : prostanoids; IgE: immunoglobulin E; TNFα: tumor necrosis factor α.

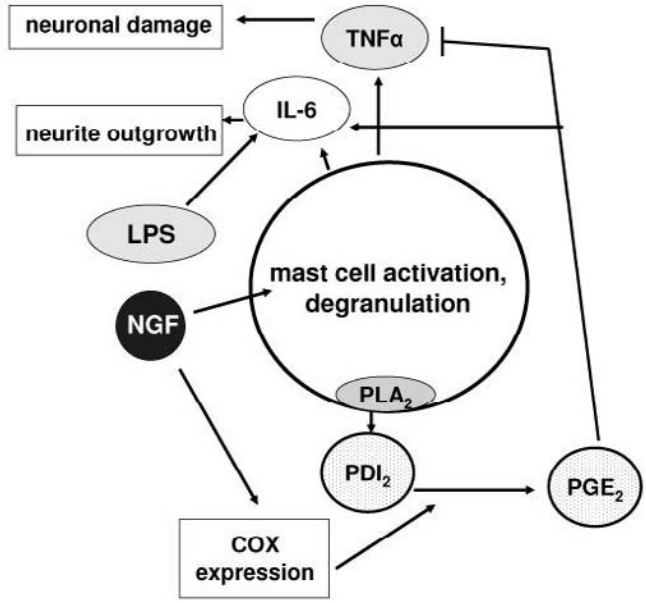

Figure 18. NGF induces PGE_2 release independently of the mast cell degranulation. Nerve growth factor (NGF) initiates the mast cell degranulation and increases the cyclooxygenase (COX) expression. The mast cell is capable of PGE_2 secretion via its phospholipase A_2 (PLA_2) enzyme independently of the degranulatory events. The PGE_2 production is potentiated by the nerve growth factor via the increased cyclooxygenase expression. LPS: lipopolysaccharide; TNFα: tumor necrosis factor α; PGI_2 , PGE_2 : prostanoids; → : action direction; ⊣ : action inhibition.

3.1.1. Nerve Growth Factor Associates with the Inflammatory Pain

PGE_2 and other mediators -, such as bradykinin, histamine, neuropeptides, and ions like potassium or hydrogen - change the sensitivity of the high threshold nociceptors [9, 10]. However, endotoxin is also able to induce an elevation of IL-1β levels derived from immune cells. The co-secreted NGF levels initiate the development of hyperalgesia in the sensory nerve terminals [11]. This endotoxin-induced hyperalgesia happens in a PGE_2-dependent fashion.

PGE_2, PGD_2 and PGF_2 all participate in the modulation of the neurotransmitter relase [12, 13]. A transient pain, which can be induced by a brief, high intensity stimulus, affects the fine afferent C- and A-δ fibres. As a neurotrophic factor, NGF is responsible for the increased afferent excitability and hyperalgesia. However, the delayed burning pain and hyperalgesia is caused by the release of the inflammatory mediators, such as bradykinin, PGE_2, nitric oxid (NO), calcitonin gene-related peptide (CGRP) and kinin, ATP, serotonin, and histamine [1]. NGF may regulate the proton- and capsaicin-mediated sensory neuron activations. In these cases, the inflammatory mediators enhance the responsiveness of the nociceptor, and these mediators are transmitted by the G protein-mediated processes. The protein kinase C and A play relevant roles in this inflammation-initiated hyperalgesia (Figure 19). The cAMP/ protein kinase A signaling cascade was demonstrated by the pain and hyperalgesia caused by heat [14]. The stimulation of the prostaglandin receptor can also transactivate the cAMP/protein kinase A signaling pathway. There are special primary sensory neurons, such as sensory ganglia or the dorsal root ganglia (DRG), which are activated during the transduction of the pain-producing stimuli. The transient receptor potential V1 (TRPV1) is the receptor for the heat- and capsaicin-mediated hyperalgesia via the inducion of ion channel activations. TRPV1 is not restricted merely to the peripheral neuronal tissues, but can also be found in some nonneuronal tissues, such as keratinocytes, smooth muscle, as well as skin, gastric and urothelium epithelial cells. The dorsal root ganglia expresses several receptors, such as TrkA, TRPV1, and G protein-coupled receptors and adenylate cyclase. TRPV1 can be stimulated by various inflammatory mediators: bradykinin, ATP, NGF, insulin, IGF-1 and prostaglandin PGE_2, as well as prostacyclin PGI_2. The TRPV1 receptor mediates the physical and chemical stimuli through the nociceptors. The TRPV1 receptors represent potential targets for the phosphorylation-mediated protein kinases, including protein kinase A and C, phosphatidylinositol biphosphate, and the Src regulatory protein [15]. In the sensitization of TRPV1, multiple effects are displayed and various signaling cascades are stimulated. G protein-coupled receptors can be activated by bradykinin, ATP, chemokines, 5-hydroxytryptamin, PGE_2 and PGI_2. These signals lead to the downstream activation of protein kinase C and A, with the transactivation of the TRPV1 signaling pathway. NGF, like insulin and IGF-1, exerts its effects on the TrkA receptors and promotes the activation of the PI-3K pathway via TRPV1 sensitization. In the PI-3K signaling pathway, the Src kinase phosphorylates the TRPV1 receptor. Therefore, NGF plays a principal role in thermal hyperalgesia as a mediator of the inflammatory pain. The capsaicin-sensitive receptors on dorsal root ganglia neurons together with the TRPV1 receptor expressions are the targets for NGF and are mediated by TrkA-dependent MAPK pathways.

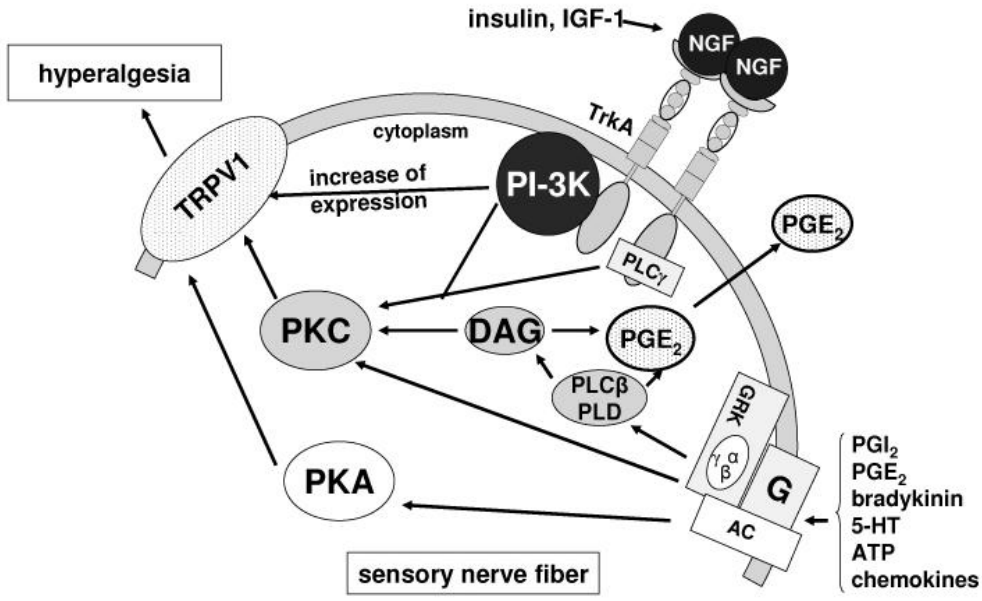

Figure 19. Inflammatory mediators induce hyperalgesia. Hyperalgesia, similar to the pain caused by heat, is mediated by a transient receptor potential V1 (TRPV1) receptor activation at the sensory nerve fiber terminals. Prostaglandins and other G protein-coupled receptor (GPCR) stimulators can transactivate the TRPV1 receptor via protein kinase C (PKC) and protein kinase A (PKA) signaling pathways. Nerve growth factor (NGF) increases the TRPV1 receptor density via tyrosine kinase A (TrkA) receptors. PI-3K: phosphatidylinositol 3-kinase; PLCγ: phospholipase Cγ; PLCβ: phospholipase Cβ; PLD: phospholipase D; AC: adenylate cyclase; GRK: G protein receptor kinase; G: G protein receptor; DAG: diacylglycerol; 5-HT: 5-hydroxytryptamin; IGF-1: insulin-like growth factor-1; ATP: adenosine triphosphate; PGI_2 , PGE_2 : prostanoids.

3.2. Nerve Growth Factor in Allergic Diseases

3.2.1. Characterization of the Allergic Responses

Allergic diseases, such as allergic rhinitis, conjunctivitis, food allergy, atopic dermatitis and asthma, are characterized by Th2 dominance and chronic inflammation in the airway tissues, as well as hypersensitivity against the allergens [16]. The factors at cause are complex and have genetic, environmental and neurohormonal origins. The atopic feature (an accepted phenomenon in allergy) is considered as a specific immunopathological stage for the production of the allergen specific IgE antibodies, and Th2-derived cytokines (IL-4, IL-5, and IL-13), both of which associate with the excessive inflammatory responses [17, 18]. The cytokines IL-4 and IL-13 are the most important inducers of the IgE release. The process of the allergic inflammation can be divided into two steps: 1. The immediate onset of the hypersensitive reactions, 2. The late-phase reactions with the clinical signs: skin edema, redness, indurated swelling and bronchial smooth muscle contraction, increased vascular permeability, mucus hypersecretion, and the consequent immune cell accumulation of lymphocytes, monocytes, eosinophils, neutrophils and basophils (Figure 20).

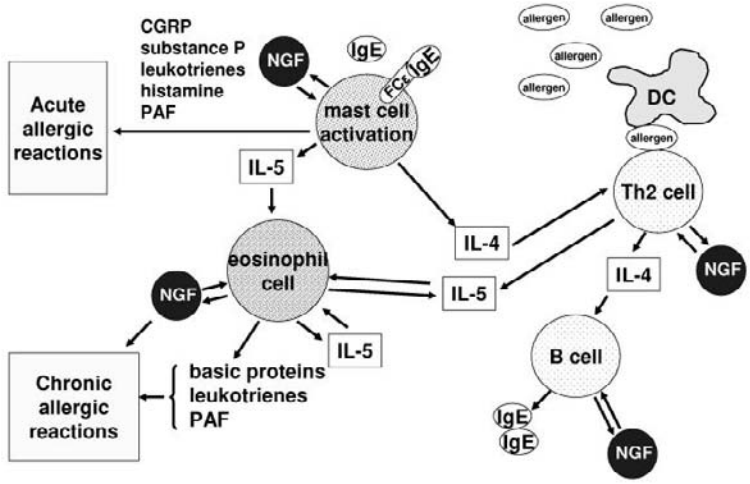

Figure 20. Cells and mediators in allergic reactions. Allergic reactions can manifest in acute or chronic forms. The mast and eosinophil cells respresent the major targets for IL-4, IL-5 (T helper 2 derived cytokines) and the nerve growth factor (NGF). The mast and eosinophil cell activations contribute to the release of several active substances, inducing inflammation, pain, and increased vascular permeability. CGRP: calcitonin gene-related peptide; DC: dendritic cell; PAF: platelet-activating factor; Th2: T helper 2 lymphocyte; IgE: immunoglobulin E; Fcε: IgE receptor.

The late-phase is IgE-independent. The allergic reactions are initiated by a mediator release from the eosinophils and mast cells. These substances are responsible for the injury of the mucosal surface, the tissue damage and the recruitments of the inflammatory cells. Eosinophils display a wide spectrum of cytokine receptors (IL-5R, IL-3R, IL2R, IL-6R, IL-9R, IL-13R, TNFαR, IFNγR, and TGFβR) as well as cytokine productions (IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, INFγ, TGFβ, and TNFα). These factors support the priming of the eosinophils in the circulation, their rolling along the endothelial cells and their adhesion to the endothelium in consequence of their transendothelial diapedesis, and chemotaxis to the inflammatory site [19].

The mast cells bear high-affinity IgE receptors, which bind to the allergen-induced IgE and lead to the degranulation of the mast cells and the release of an assortment of substances: histamine, PGD_2, major basic protein, serotonin, IL-3, IL-4, IL-6, IL-10, and TNFα [20].

Recent data suggest that this immune defect may be confirmed by regulating the immune responses mediated by the effector T cells [21, 22, 23, 24]. The regulatory T cells (Tregs) may prevent the development of autoimmune diseases and the sensitization against allergens. A variety of Tregs cells have been revealed, which can inhibit the T cell responses [25, 26, 27, 28]. The suppressive effect of the Treg cells is implicated in the inhibition of the autoimmunity initiated by self-antigens, as well as in the positive autoantigen selection during the ontogenesis or the postnatal period. Two main categories of Treg cells can be distinguished: natural and adaptive. Both kinds of Treg cells originate from the thymus and possess a phenotype of CD4+ Treg or CD8+ Treg. The CD4+ Treg cells express an IL-2 receptor, so they are CD25+. The natural CD4+CD25+ Treg cells suppress the immune reactions against the self-antigens in the thymus during the ontogeny. The adaptive CD4+CD25+ Treg cells regulate the responses against the nonself-antigens in the periphery of immune system. Two subsets of the adaptive Treg cells can be further distincted: 1. The

Th3 cells, which exert an inhibitory effect via the release of a TGFβ suppressive cytokine. 2. The Tr1 cells, which exert suppression by releasing IL-10 cytokine. The regulatory activities of the natural and the adaptive Treg cells are antigen specific but the effectory ways are nonspecific and mediated by cell-to-cell contact or by suppressive cytokines [29, 30, 31].

During allergic reaction, the responses of Th2 cells to the allergens are normally suppressed by both CD4+CD25+ Treg cells and IL-10-producing Tr1 Treg cells. These two subtypes of Treg cells occur in decreased amounts in allergic individuals [32, 33, 34]. The allergic inflammation can be prevented by Th1 cells due to the inhibitory effects of Th1-derived cytokines on the Th2 cells [35, 36] (Figure 21).

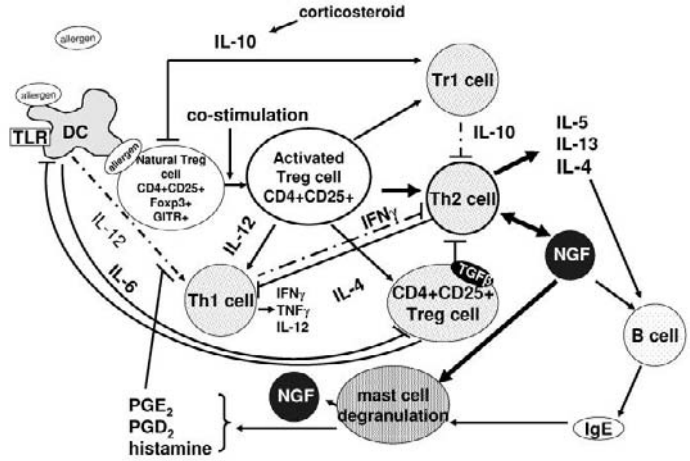

Figure 21. Pathways directing towards T helper 2 dominance in atopic patients. Nerve growth factor (NGF) derived from immunocompetent cells during the immune and/or allergic reactions leads to the mast cell degranulation and the T helper 2 (Th2) dominance. Treg cell: regulatory T lymphocyte; Th1 cell: T helper 1 lymphocyte; Th2 cell: T helper 2 lymphocyte; TLR: Toll-like receptor; DC: dendritic cell; Tr1 cell: regulatory T lymphocyte releasing IL-10; PGE_2 , PGD_2 : prostaglandins; IgE: immunoglobulin E; TNFα: tumor necrosis factor α; IFNγ: interferon γ; TGFβ: transforming growth factor β; GITR: glucocorticoid-induced TNF receptor-related molecule; Foxp3: forkhead box P3; → : action direction; ⊣ : action inhibition; - - → : decreased inhibition.

No difference has been found in the suppressive ability of the CD4+CD25+ Treg cells between atopic and nonatopic patients [37]. In fact, there are substances that promote Th2 responses: a low dose of endotoxin, the TLR2 ligand, glucocorticoids, G protein-coupled receptor agonists (histamine, PGE_2, PGD_2). These substances also inhibit or reduce the production of IL-12 and promote the IL-4 regulatory pathway [38, 39, 40].

The procedure of the allergen-specific immunotherapy is based on the recent knowledge of T cell tolerance and the behavior of the Treg cell subsets [41]. The balance between Th2 and the allergen-specific Treg cells in allergic reactions shifts towards Th2 dominance in consequence of the production of IL-13 cytokine, IL-4 and IL-5. On the other hand, allergen-specific immunotherapy (SIT therapy) leads to the suppression of Th2 cells and their cytokine responses. Th2 cells are activated by aeroallergens, food antigens, autoantigens and bacterial superantigens during the allergic inflammations. Mediators that are associated with the cAMP activated G protein-coupled receptors - like histamine receptor 2 (H2R), - contribute to the development of the peripheral tolerance. Multiple suppressive factors are implicated in the mechanism of the peripheral tolerance: e.g. IL-10, TGFβ, CTLA-4, and apoptosis. In

allergen-specific immunotherapy, IL-10 does not only generate tolerance in the T cells, but it also regulates a specific skewing of the immunoglobulin isotype from IgE to mainly IgG4 and to small amounts of either IgG1 or IgA.

3.2.2. The Sensory Neuronal Innervation and Nerve Growth Factor in Allergy

The effector organs of allergy, particularly the airways, are highly innervated. The major respiratory effector system is composed of the classical sympathetic and parasympathetic innervations, as well as the sensory nerve fibers. The sensory neurons stay in the center of the local neuronal mechanisms due to the neuropeptides secreted at the fiber terminal, for instance calcitonin gene-related peptide, substance P, tachykinin, vasoactive intestinal polypeptide (VIP), and neuropeptide Y [42]. These neuropeptides have been detected in the vessel walls, the bronchial smooth muscles, the mucus gland and the airway epithelial areas. The release of neuropeptides during the inflammation leads to hyperemia, edema, mucus hypersecretion and the contraction of the bronchial smooth muscles. In addition to the proinflammatory neuropeptides, neurotrophin interactions are also implicated in the allergic neurogen inflammation. The quantities of neurotrophins arising from the various cell types, are increased in allergic diseases. The inflammatory activities of NGF stress its ability for the stimulation of cytokines and the promotion of cell survival through chronic manifestation. In some studies, high levels of NGF have been demonstrated in the sera and bronchoalveolar lavage fluids of patients who suffered from allergic diseases. NGF propagates the release of substance P, tachykinin, and neurokinin A originating from the sensory nerve terminals. However, the NGF release can be induced by IL-1, IL-4, IL-5, and TNFα arised from mast cells, eosinophils, fibroblasts, epithelial cells. These facts support, that the airway hyperinnervation may be modified by NGF.

NGF contributes to the hyperresponsiveness of the airways through the secretion of substancia P and tachykinin [43]. The airway resistance is induced by the release of histamine from the mast cells. This release is mediated not only by IgE but also by NGF. In guinea pigs, NGF can enhance the neurogenic inflammation by altering the neuropeptide that come from the sensory nerve terminals.

Tachykinins binding to the neurokinin receptors set off a multitude of symptoms and activities; they are responsible for the bronchoconstriction, the mucus secretion, the microvascular leakage, chemotaxis, the activation of various cells and the stimulation of cytokine productions. Capsaicin is a well known stimulator for the release of tachykinin from the sensory C-fiber afferents [44]. Substance P could be considered as a marker for the tachykinin-containing sensory nerve fibers, and its presence in the respiratory system leads to an increase in the density of the innervation.

3.2.3. Nerve Growth Factor is Involved in the Allergic Inflammation Associated with Fibrosis

NGF accumulation in the allergic diseases can be detected by the hypersecreted mucus in the airways, by the skin eruption (e.g. in vessel walls) and by the circulation [45]. NGF activates the immune responses via its TrkA receptors expressed on the T and B lymphocytes, mast cells, eosinophils, basophils, neutrophils and macrophages or epithelial cells; this leads to the perpetuation of these reactions on an autocrine or a paracrine manner. In addition to NGF, these cells - particularly the mast cells and eosinophils - secrete a large spectrum of

other mediators and cytokines. The distinct steps in the inflammatory responses of the allergy are the following: 1. The high NGF level associates with the allergic inflammation and induces an IgE-mediated mediator release from the basophils or the mast cells. 2. The release of chemokines from the eosinophils leads to the chemotaxis of neutrophils and the activation of fibroblasts (Figure 22). The early-phase reactions of allergy are caused by allergen and IgE-dependent reactions and they associate with an enhancement of NGF in the acute airway inflammation [20]. NGF induces a mast cell hyperplasia or hypertrophy and promotes its maturation and degranulation. The mast cell degranulation results in the release of serotonin, IL-6 and IL-4 besides other mediators. The eosinophil activation following the stimulation of its NGF receptors results in the release of IL-4, IL-5 and chemotaxins directing to a local cell recruitment into the site of the inflammation. The sensory neurons are able to produce substantia P and other neuropeptides due to the NGF binding to their TrkA receptors and due to various other mechanical, thermal, and chemical stimuli. Substantia P exerts prominent inflammatory responses leadig to allergic early-phase reactions and the hyperreactivity of the airways. In fact, NGF may play a relevant role in the allergic late-phase reactions contributing to tissue repair.

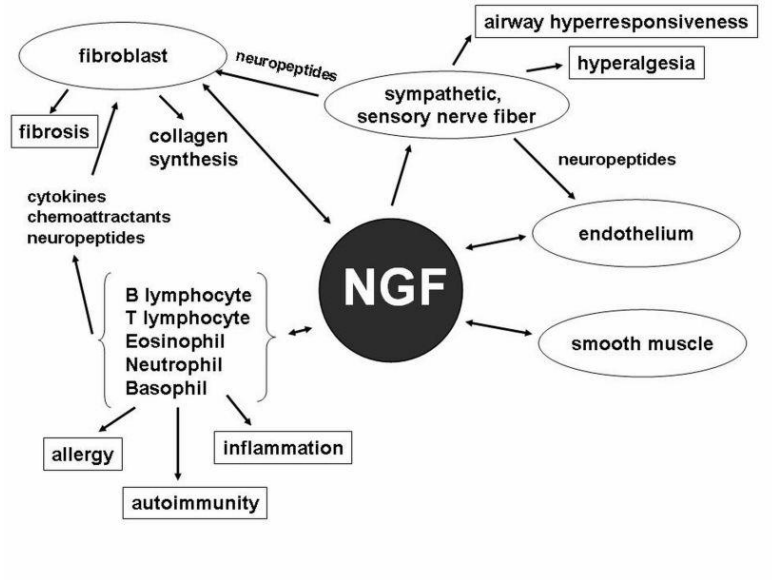

Figure 22. The regulatory role of nerve growth factor in the cell interactions during the inflammation. Nerve growth factor (NGF) binding to its receptors on the various cell types is involved in the airway hyperresponsiveness, hyperalgesia, inflammation, autoimmunity, allergy and fibrosis.

In the allergic late-phase reactions, the activation and a hyperplasia of the fibroblasts come into prominence. Fibroblasts are the targets and effectors during the inflammation (Figure 23). The fibroblasts as target cells secrete a large amounts of cytokines (IL-1β, IL-6, IL-8, IL-11, and TNFα), NGF and extracellular matrix. As effector cells they express NGF receptors and the major histocompatibility complex class II (MHC II) antigens. The role of NGF in the allergic inflammation can be considered as a neurotrophic factor, which influences the survival of the mast cells, eosinophils, fibroblasts through the inhibition of their apoptosis associating later with fibrosis [46].

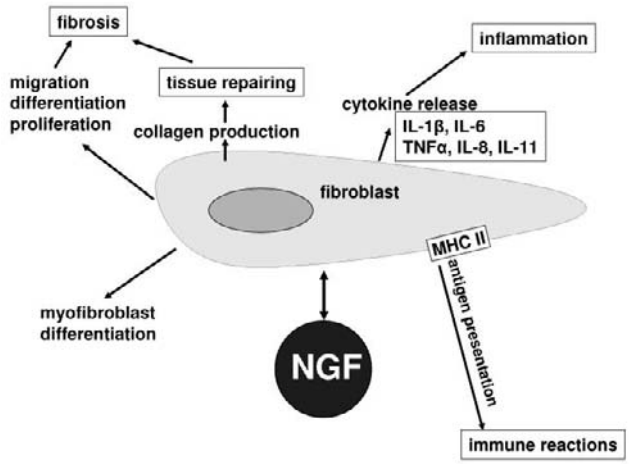

Figure 23. Fibroblasts mediated events in the inflammation. Fibroblasts are activated by various cytokines, and themselves release proinflammatory cytokines, collagens, as well as express major histocompatibility complex class II antigens. They also possess the ability for myofibroblast transformation. NGF: nerve growth factor; TNFα: tumor necrosis factor α.

Allergic diseases represent an integrative complexity among the neuronal, inflammatory and immune responses. The role of NGF in the allergic reactions is multilevel; moreover, it demonstrates NGF's importance in the maturation, differentiation and activation of the immunocompetent cells, as well as NGF's ability to initiate inflammatory mediators from the cells, such as cytokines, chemoattractants, and neuropeptides. The interactions among NGF, the mast cells and the eosinophils are characteristic for the allergic early-phase responses. The neurotrophic activity of NGF supports the Th2 dominance in allergic reactions due to the enhancement of the TrkA receptor mediated by the cell survival. The neuronal innervation of the airways is influenced indirectly by the NGF potentiated hyperresponsiveness. At the final phase of the inflammatory processes, the interactions among NGF, the fibroblast and endothelial cells may conclude with tissue repair or chronic fibrosis.

3.3. Relationship Between Nerve Growth Factor and Apoptosis

Apoptosis - programmed cell death - is a physiological death form in the multicellular organisms supporting the renewal of cells and a prevention against self-structures. Apoptosis differs from necrosis, which is a pathological death form, and which associates with the acute inflammatory responses. The cell destruction in apoptosis is characterized by DNA fragmentations, the blebbing of the plasma membrane, the shrinkage of the cell volume, the rupture of the cell membranes, and the leakage of the cell plasma. The causes of apoptosis include many factors; many physiological and pathological stimuli are required: a CD95 or TNFα receptor, an IL-12 receptor, TRAIL, TNFα, many stress forms, growth factor withdrawal, irradiation, ultraviolet light, drugs, infections, and/or hormones [47, 48, 49, 50, 51]. It is not surprising that defects in the apoptotic cell death may explain the development of many human diseases. The types of the defects may be the following: 1. The absence of growth factors (e.g. NGF, IGF, epidermal growth factor, or platelet-derived growth factor, 2. Inactivating mutation of the CD95 receptor (Fas receptor), 3. High levels of inhibitors against

apoptosis (e.g. secretion of protease inhibitors), 4. Depletion of CD4+ T cells, 5. Factors, which are responsible for human degenerative diseases, such as neurodegenerative diseases during or after hypoxia [50]. 6. There are cytokines that suppress apoptosis via distinct routes and affect various cell types [52].

Autoimmunity can be described not only as immune responses against the self-antigens but also as reactions with a defect in apoptosis. The over- or underproduction of IL-1β and TNFα proapoptotic cytokines may be consequence factors for autoimmune diseases [53]. The defects in apoptosis in the autoimmunity could be the following: 1. The affection of the lymphocyte switch-off system resulting in the break down of the immune tolerance and causing the survival of those T and B lymphocyte clones that would direct to self-antigens, 2. The induction of new, apoptosis related antigens [54]. 3. In another way, the diminished clearence of the apoptotic cells (e.g. C1q deficiency) can associate with a prolonged autoantigen presentation by dendritic cells. However, the diminished apoptosis of the autoreactive lymphocytes (e.g. Fas mutation) can facilitate the autoimmune responses [35, 55]. With respect to the signaling mechanisms, the upregulation of the lymphocyte activation can occur via three activation routes of MAPK cascades: ERK, JNK and p38 [56].

The allergic immunoreactions lead to a Th2 dominance, which displays an increased apoptosis affecting the memory cells and the effector Th1 cells [57]. Apoptosis is crucial in the development of the atopic features and the allergic diseases. The cytotoxic T lymphocyte-associated antigen positive (CTLA+) and CD45RO+ memory/effector T cells undergo apoptosis in consequence of the PI-3K upregulation. This associates with the inhibition of the caspase 8 cleavage at the death-inducing complex as well as with the resistance of Th2 cells to the Fas mediated apoptosis [58]. NGF acts on the immunocompetent cells and possesses a modulatory effect on the B lymphocyte development and on other cell functions of the immune system via their TrkA receptors [59]. The defect of NGF's action in the immune system can appear as an increase of certain immunoglobulins due to the deterioration of the TrkA signaling events. The role of NGF appears in the antigen presentation processes exhibiting NGF's inhibitory effect on the expression of the major histocompatibility complex class II antigens. The microglias are antigen-presentig cells and are targets for the actions of NGF in the brain; however, they are also able to produce NGF, thereby playing a preventory role in the autoimmunity of the central nervous system [60]. The inhibition of the expression of the major histocompatibility complex class II antigen is mediated by p75 NGF receptors. It seems that the MAPK signal transduction is crucial in the expression of major histocompatibility complex class II antigen, which is initiated by the stimulation of the antigen binding receptors on B lymphocytes [61].

The hormonally controlled apoptosis represents a special mechanism in the endocrine diseases: it is mediated by the interactions of hormones, cytokines and growth factors. Steroids and thyroid hormones have been the most investigated regulators that are involved in apoptosis. The steroid-dependent cell death can occur in the adrenal glands, mammary glands, prostate, ovaries and testes. Several growth factors, such as epidermal growth factor, NGF, platelet-derived growth factor, and IGF-1 can inhibit the apoptosis acting as survival factors, but their withdrawal associates with apoptosis [62]. The development of autoimmune endocrine diseases demonstrates an interaction between the survival and the apoptotic events. The classical signal transduction pathways that lead to apoptosis, affect the following signaling events: calcium channels, G protein-coupled receptors, tyrosines kinases, tyrosine phosphatates, protein kinase C, heat-shock proteins, and cAMP. The growth factors –

including NGF - regulate the apoptosis via the Trk receptors. Cytokines possess a regulatory feature for apoptosis, such that a high dose of IL-2 can induce apoptosis, but IL-4 with a low dose of IL-2 can inhibit it [63, 64]. Nevertheless, prolactin (PRL), human chorionic gonadotrophin (hCG), adrenocorticotrophic hormone (ACTH), parathyroid hormone-related peptide and thyroxine can play a preventing role in the apoptosis.

Receptors of the TNF/NGF family lead to tissue damage and/or apoptosis [65]. The causes of apoptosis are well studied in the thyroid diseases suggesting that the thyroid hormone is important in the programmed cell death [66, 67, 68, 69]. The autoimmune thyroid diseases are characterized by the autoantibodies, an enhancement of the mononuclear cell infiltrations and changes in the thyroid volume. The thyrocytes undergo apoptosis initiated by various factors. The Fas mediated form is the more frequent apoptotic cascade in which the thyroid cells are affected. The Fas expression in thyroid diseases is influenced by TSH, TSH receptor activity, IL-1β and TNFα [70]. The thyroid epithelial cells and the thyroid infiltrated T cells are able to express Fas ligands and Fas antigens. The metalloproteinases exert antiapoptotic effects, because they are responsible for the production of soluble Fas (sFas). The cytokines, such as IL-1β, IFNγ and TNFα, are also involved in the thyrocytes' apoptosis as well as in the increase of Fas antigen expression. In Graves' disease – a thyroid autoimmunity – high levels of soluble Fas could be detected, which inhibits apoptosis and promotes the proliferation of thyrocyte as well as the production of anti-TSH receptor antibodies (TRAK) [68]. In another autoimmune thyroid disease: in Hashimoto's thyroiditis a high percentage of thyrocytes demonstrate Fas-mediated apoptosis, which is rarely present among the lymphocytes infiltrating the thyroids. However, in Graves' disease, the Fas-mediated apoptosis of the thyrocytes is rare but it is relevant in the lymphocytes infiltrating the thyroids.

The goitre nodularity highlights a new aspect of apoptosis in the thyroid abnormalities. Mezosi and co-workers revealed that the thyroid epithelial cells are resistant to apoptosis induced by TRAIL (TNF-related apoptosis inducing ligand) or Fas. The resistance to the apoptosis could be blocked with the additing of TNFα/IL-1β or IFNγ/ IL-1β [70, 71]. This blocking effect can be explained by the downregulation of the MAPK (ERK p44/42) pathway [72]. The data confirm that the survival factors against the TRAIL mediated apoptosis act through the MAPK signaling cascade. Similarly to the TRAIL mediated thyrocyte apoptosis, the Fas mediated form is also blocked by the MAPK signaling pathway [69].

Recent findings that have been published about the actions of thyroid hormones show that they act via a nongenomic manner. The nongenomic effects of the thyroid hormones represent their rapid, nontranscriptional and physiological activities (Figure 24). The nongenomic actions are more often displayed by the T_4 rather than the T_3 hormones. The biological processes of the thyroid hormones can manifest in the cellular respiration, cell morphology, vascular tone and ion homeostasis. The nongenomic effects are induced by the thyroid hormones via the MAPK pathway, and they potentiate the antiviral, and IFNγ immunmodulatory actions as well as activate the protein kinase A or C pathway [73, 74]. Recent data suggest that the nongenomic action of the thyroid hormones is characterized by them binding to the integrins of the plasma membrane [75, 76]. Intergins are involved in the suppression of tumors; they interact with the substrates of the Shc adapter protein and the focal adhesion kinase (FAK), which leads to the transactivation between the integrins and growth factors [77]. The TSH binding to the G protein-coupled receptor initiates a MAPK activation and causes a transactivation of the cAMP signaling pathway [78].

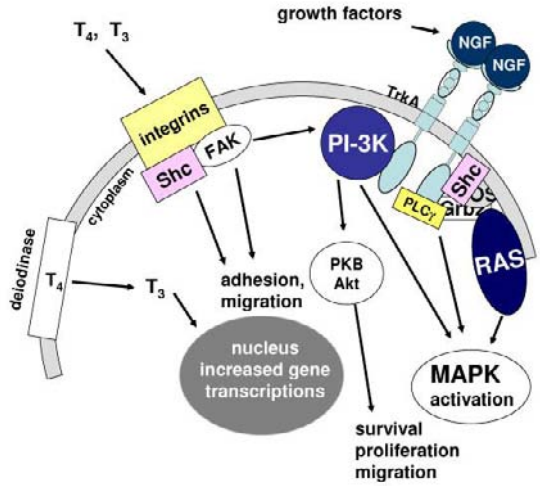

Figure 24. The nongenomic pathway of thyroid hormones in the cells. The nongenomic effect of thyroid hormones exerts itself via the hormones binding to integrins of the cell membrane. Their bindings lead to rapid and nontranscriptional receptor activities. The nongenomic actions initiate the MAPK and PI-3K/Akt signaling pathways, resulting in decreased apoptosis. MAPK: mitogen-activated protein kinase; Akt: serine/threonine kinase; PKB: protein kinase B; PI-3K: phosphatidylinositol 3-kinase; Shc: adapter protein; SOS: son of sevenless protein; RAS: oncogene; PLCγ: phospholipase Cγ; TrkA: tyrosine kinase receptor A; FAK: focal adhesion kinase; Grb2: growth factor receptor-binding protein; SOS: son of sevenless protein; NGF: nerve growth factor; T_3: triiodothyronine; T_4: thyroxine; deiodinase: enzyme for thyroid hormone conversion.

The cardiovascular effects of thyroid hormones suggest that the thyroid response element (TRE) mediated activation can crosslink to the PI-3K/Akt signaling pathway [79]. The PI-3K/Akt pathway mediates the acute vasodilatory and neuroprotective effects of the thyroid hormones. Besides the nongenomic effects of the thyroid hormones, a crosslink between the PI-3K signaling pathway and the steroid hormone receptors has also been demonstrated [80, 81]. It is an established fact, that the vascular endothelial cells are activated rapidly via the PI-3K/Akt mediated pathway in the presence of thyroid hormones. The manifestation of the nongenomic actions of the thyroid hormones requires higher thyroid hormone levels than what are necessary for the genomic actions. The nongenomic effects of thyroid hormones affect their neuronal protection and result in the reduction of the cerebral infarct volume and the systemic blood pressure, which results in a neuroprotection against the cerebral ischemia. The above mentioned favourable effects support that the application of thyroid hormones are useful for the healing cardiovascular damages.

In another way, the nongenomic actions of thyroid hormones stimulate the superoxide production of the polymorphonuclear leukocytes, and therefore potentiate the cellular defense mechanisms [82].

Thyroid hormones increase the expression of the cell membrane proteins on oligodendrocytes, which contribute to alterations in the cell differentiation and maturation. Therefore, the administration of thyroxine can restore diminished NGF levels in the central nervous sytem, thereby protecting it from TNFα, inflammatory cytokines and hypoxia induced by injury.[83].

The programmed cell death plays a relevant role in the development of the inner ear; this is influenced by NGF and IGF-1 [84]. The low-affinity NGF receptor - p75 - is expressed in

the inner ear, which possesses an ectoderm origin like the retinal and ocular structures. The afferens neurons of the inner ear migrate from the cochleo-vestibular ganglion. The organogenesis of the inner ear is controlled by the NGF mediated apoptosis. IGF-1 is also a pleiotrophic growth factor for the epithelial and neuronal cells in the inner ear. There is a balance between the NGF-mediated apoptosis and the IGF-1-mediated survival; this affects the sensory neuronal development of the inner ear. A similar cross-talk exists between the TRPV1 and IGF-1 activities in myopathy, and in the actions of the endothelial and synovial cells.

Myopathies representing a wide spectrum of neuromuscular diseases are characterized by the atrophy and loss of muscle fibers [85]. The skeletal muscles are multinucleated, allowing for a special, long-time disease process of myopathy. It should be emphasized that the apoptotic events do not affect the whole muscle fiber, because the fiber apoptosis occurs segmentally. The apoptotic cascades are induced by the upregulation of mitochondria-associated factors, which activates the downstream caspase 9; this causes a release of cytochrome C and Apaf-1 adapter proteins. In myopathies both apoptosis and necrosis are present, especially in diseases with autoimmune and/or endocrine origins. Exercise increases the muscle mass, thereby initiating apoptosis and necrosis as well as a metabolic insufficiency (e.g. glycolytic or respiratory defects due to the depletion of ATP). Exercise also triggers the MAPK signal cascade. The favourable effects of exercise are time-dependent and ties to the changes in the gene expressions involved in muscle metabolism [86]. In myopathies caused by denervation, the apoptotic muscle fibers express both apoptosis-promoting and -inhibiting proteins. Therefore, neuronal damage can lead to either an increase or a degradation of NGF levels, as well as the resorption of contractile sarcoplasmic elements. In myofibrillar myopathy, the secondary neuromuscular tissue damages associate with endocrine and autoimmune causative factors (e.g. thyroid-associated ophthalmopathy, adrenal gland insufficiency, and sepsis).

a) Smooth muscle cells, like the skeletal muscle cells, express p75 and Trk receptors (particularly TrkB and TrkC), allowing the possibility for a vascular injury. The role of neurotrophins in the vascular alterations is dual.
b) The changes could be initiated by the migration and the proliferation of smooth muscle cells into the intima via Trk receptors. They could also be initiated by the apoptosis of endothelial and smooth muscle cells via p 75 receptors [87, 88].

In rheumatoid arthritis, the survival and apoptosis of synoviocytes depends on the balance between the effects of proinflammatory cytokines (IL-1β, TNFα) and those with antiapoptotic effects ones (IL-4, IGF-1) [89]. The proliferation of synovium and the destruction of cartilage provide a model for the duality of survival and apoptosis in the development of arthritis. IL-4 suppresses the detrimental effects of IL-1β and TNFα in the presence of IL-1Ra with the several consequences. This causes the downregulation of the expression of TNF receptor; this promotes the transcription of metalloproteinases. Also, it results in the synovial proliferation [90, 91, 92]. The antiapoptotic effects of IL-4 are mediated by the PI-3K and protein kinase C signaling pathways highlighting the role of NGF receptor transactivations. NGF also acts indirectly on the cell survival in arthritis due to the Th2 dominance.

3.4. Nerve Growth Factor in the Autoimmune Diseases

3.4.1. Suppressor Regulatory T Cells in the Autoimmune Diseases

The autoimmune diseases represent an abnormality of the Treg cells resulting in an activation of self-reactive T cells [93]. Nowadays, it is established that self-reactive T cells are detectable even in the circulation of healthy individuals without any evidence of a disease [94]. Recently, a new piece of knowledge was brought into this research field: the central tolerance was responsible for the selection among the repertoire of T cells which recognize self-antigens, while the peripheral tolerance was mediated by suppressor T cells and/or cytokines [95].

The human CD4+CD25+ regulatory T cells inhibit autoreactive T cells. Sakaguchi and co-workers demonstrated that the depletion of CD4+CD25+ Treg cells lead to the onset of systemic autoimmune diseases [96]. In fact, autoreactive T cells could be demonstrated in the human autoimmune diseases [97]. Multiple sclerosis, autoimmune polyglandular syndromes, autoimmune thyroid diseases, diabetes mellitus type 1, rheumatoid arthritis and autoimmune bowel diseases could develop due to defects in the regulatory T cell functions [98, 99, 100, 101].

The task of the immune system is the following: to protect the organisms from the harmful agents that can come from outside (e.g. infections, toxins) or inside (e.g. self-antigens, antibodies). The protective mechanisms are initiated by distinct effector routes, such as inflammation, the production of antibodies and the activation of killer cells [95]. The immunohomeostatic control mechanisms are regulated by antigen-specific T cell receptors (TCRs), regulatory T cells (CD4+ and/or CD8+ Treg cells), Th1 and Th2 cells and cytokine effects. The CD4+ Treg cells are activated by co-stimulatory molecules expressed on the cell-surface (molecules, such as CD25+, CD28+, cytotoxic T lymphocyte antigen-4 (CTLA-4), glucocorticoid-induced TNF receptor-related molecule (GITR), or CD40L). They interact with the antigen-presenting cells (APCs) or dendritic cells (DCs), both of which express counterresponding ligands or their receptors (CD40, B7) (Figure 25). GITR molecules are expressed mainly on CD4+ rather than CD8+ T cells, but they also can be found on B cells, macrophages and dendritic cells. GITR is an apoptosis promoting molecule with an inhibitory feature of Treg cell activity. It co-stimulates responder T cells as well as exacerbates any existing autoimmune responses [102]. The differentiation of T cells into the Th2 or Th1 subsets is mediated by cytokines (e.g. IL-4 and IL-12/ IFNγ) derived from the activated CD4+CD25+ Treg cells. It seems that compared to Th1 cells, Th2 cells are less susceptible to the suppressive effects of the CD4+CD25+ regulatory thymocytes [103]. The suppression of CD8+ Treg cells takes part in the development of the experimental autoimmune encephalo-myelitis: a disease that is respected as a model for multiple sclerosis [104].

The CD4+CD25+ Treg cells nonetheless express Toll-like receptors (TLRs) like dendritic cells, macrophages, and B cells. The importance of Toll-like receptors is that they recognize both exogen pathogen-associated molecular patterns shared among large groups of microbes (e.g. lipopolysaccharide, which is a major component of the gram-negative bacteria) and endogenous molecules released during the inflammation (e.g. heat shock protein (HSP)). The activation of Treg cells exerts their suppressive reaction because the IL-10 cytokine inhibits the activities of the macrophage and the effector T lymphocytes (Figure 26).

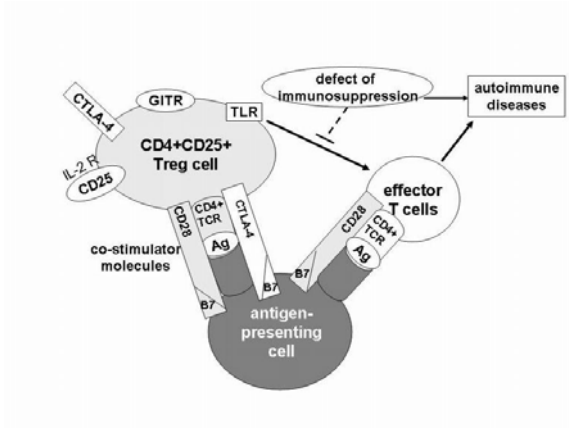

Figure 25. The suppressive effect of the regulatory T cells fails in autoimmunity. The autoimmune reactions are suppressed by the regulatory T cells (Treg) in healthy subjects. Any defect in this suppression enhances the effector T lymphocyte activity and leads to the development of autoimmune diseases. Several co-stimulator molecules participate in the initiation of the immune responses, and they are necessary for the antigen-presenting cell activation. Ag: antigen; CD28, B7: co-stimulatory molecules; CD25, IL-2R: IL-2 receptor; TLR: Toll-like receptor; GITR: glucocorticoid-induced TNF receptor-related molecule; CTLA-4: cytotoxic T lymphocyte antigen-4; TCR: T cell receptor; → : action direction; ⊣ : decreased inhibition.

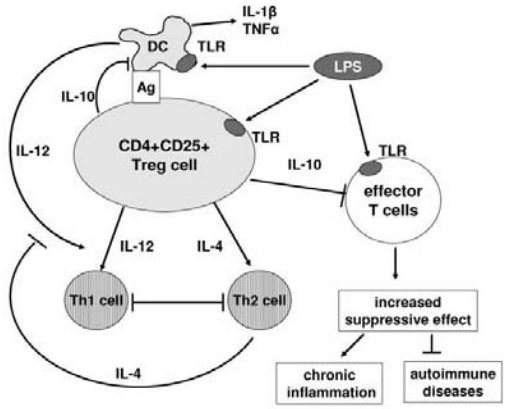

Figure 26. The role of Toll-like receptors in the localization of inflammation. Toll-like receptors (TLR) are essential for the prevention of the bacterial infections. The lipopolysaccharide (LPS) in the bacterial wall initiates reactions with the antigen-presenting dendritic cells (DC), regulatory T (Treg) cells and the effector T cells. The Treg cell activity leads to generalized immunosuppression with local inflammation. Th1 cell: T helper 1 lymphocyte; Th2 cell: T helper 2 lymphocyte; Ag: antigen; TNFα: tumor necrosis factor α; → : action direction; ⊣ : action inhibition.

After being triggered by Toll-like receptors, macrophages, dendritic cells and Treg cells react with the co-stimulatory molecules leading to releases of cytokines (IL-1β, TNFα, and IL-10) [105]. IL-10 is responsible for the local maintenance of the inflammation in infectious diseases [106]. Lipopolysaccharide induces the production of IL-12 from the dendritic cells or macrophages, which in turn initiates the differentiation of Treg cells towards Th1 cells; however, the release of IL-12 is blocked by IL-4 [107]. Toll-like receptors are expressed

selectively on CD4+ CD25+ Treg cells. Their expressions are activated by lipopolysaccharide confirming their protective roles in the generalized immunopathologies [108].

The adenosine receptors (of type A_{2A}) represent another way of inflammatory and immune protection. Adenosine receptors are expressed on T cells [109, 110]. The stimulation of the A_{2A} adenosine receptors on Treg cells leads to immunosuppression and intracellular cAMP accumulation. New data confirming the transactivation between the adenosine and G protein-coupled receptors highlight a new signaling pathway for histamine, prostaglandins and β-adrenergic receptor agonists: one that induces a cAMP-mediated immunosuppression (Figure 27). The cross-talk between the adenosine or G protein-coupled receptors and the TrkA receptors suggests a common signaling pathway for the NGF transactivated cell survival and the cAMP-mediated immunosuppression.

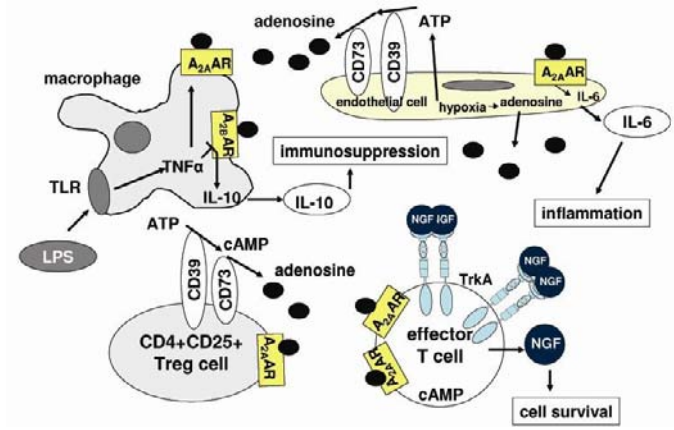

Figure 27. Anti- and proinflammatory effects are mediated by the activation of A_{2A} adenosine receptors. The adenosine receptors expressing on T lymphocytes possess an immunomodulatory effect. Adenosine binding to their receptors on the effector T cells contributes to a decreased susceptibility to apoptosis. Adenosine binding to their receptors on regulatory T (Treg) cells and/or macrophages leads to immunosuppression via IL-10 production. Adenosine binding to the endothelial cells causes inflammation. CD39, CD73: ecto-nucleotidases; LPS: lipopolysaccharide; TLR: Toll-like receptor. A_{2A}AR: receptors for A_{2A} ; A_{2B}AR: receptors for A_{2B} ; ATP: adenosine triphosphate; TNFα: tumor necrosis factor α; cAMP: cyclic adenosine monophosphate; TrkA: tyrosine kinase A; NGF: nerve growth factor.

The autoimmunity very often affects more than one endocrine organ, thereby manifesting the clinical signs of polyglandular diseases. The autoimmune polyglandular syndromes (APS) are divided into two types: 1**.**/ APS type I caused by a loss of central tolerance and 2./ APS type II caused by a defect in the suppressive capacity of peripheral Treg cells [111]. The defect in the central tolerance may explain the early onset of APS-I due to the mutation of the autoimmune regulator gene (AIRE) and its clinical manifestations: Addison's disease, hypoparathyroidism and the susceptibility to mucocutaneous candidiasis. APS-II manifests later in adult patients and forms a combination of endocrinopathies: Addison's disease, diabetes mellitus type 1 and autoimmune thyroid diseases.

The various regulatory Treg cells are detectable in the peripheral blood and the thyroid tissue from patients with thyroid autoimmunity: high portions of CD4+GITR+, CD4+ forkhead box P3 (Foxp3+) and CD4+IL-10+ lymphocytes and enhanced IL-10- and TGFβ-positive lymphocytes (CD69+CD25+) [101].

Under modern living conditions, inflammatory bowel disease (IBD) may reflect a defect in the maturation of Treg cells. Rook and co-worker highlighted a crucial role of certain relative harmless microorganisms (saprophytic, mycobacteria, lactobacilli, and helminths) in the maturation of Treg cells. This specific, bystander immunregulation is based on the increased activities of dendritic and Treg cells, which leads to a suppressive cytokine release, such as IL-10 and TGFβ [100]. Therefore, the absence of a normal gut flora may trigger the allergic or autoimmune diseases due to the defect in the suppressor Treg cells activities.

The neurotrophins and their receptors are involved in the homeostasis of the immune system. The regulatory CD4+ T cells – along with other immune cells (B cells, macrophages, and monocytes) - produce and release NGF and express TrkA receptors [112, 113]. NGF could be detected in the cell subpopulations of the primary and secondary lymphoid organs suggesting its regulatory role in the density of sympathetic innervations and the survival of immunocompetent cells. The actions of neutrophins, such as NGF, are confirmed in the development of the thymus and the survival of thymocytes (Figure 28) [114].

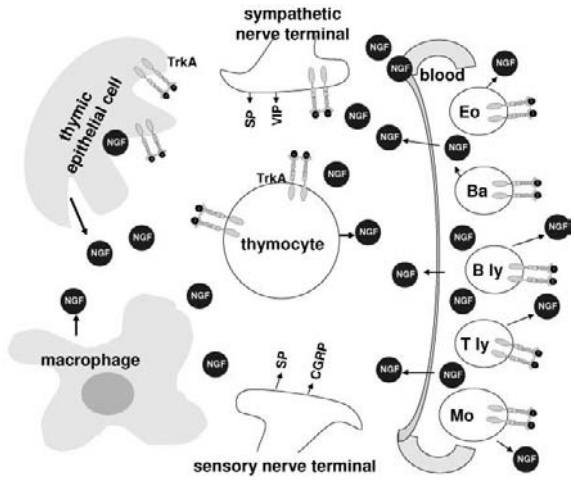

Figure 28. Thymus acts as the source and the target for nerve growth factor. Nerve growth factor (NGF) not only acts on the thymic epithelial cells and thymocytes, but it also releases from these cells and from the blood circulating cells in the thymus. The relationship confirms a network between the immune and neural systems. TrkA: tyrosine kinase A; SP: substance P; CGRP: calcitonin gene-related peptide; VIP: vasoactive intestinal peptide; Eo: eosinophil cell; Ba: basophil cell; B ly: B lymphocyte; T ly: T lymphocyte; Mo: monocytes.

3.4.2. Neurogenic Inflammation

NGF modulates the function of B lymphocytes via the calcitonin gene-related peptide synthesis, which occurs in the sensory neuronal cells. However, NGF also acts directly on the B lymphocytes influencing their immunoglobulin secretion [115]. Tthe calcitonin gene-related peptide and the substance P are the two main factors affecting the immune cell's functions. This fact, has been established by their elevated levels in the synovial fluid and the circulation. Data confirm that the lymphocytes are capable to produce both the calcitonin gene-related peptide and the substance P, and express the calcitonin gene-related peptide receptor. Lipopolysaccharide actions, akin to NGF, increase the density of the calcitonin gene-related peptide receptors. The calcitonin gene-related peptide can be considered as an inhibitor for the mitogen - or antigen - stimulated T cell proliferation [116]. The calcitonin

gene-related peptide prevents the release of inflammatory mediators (leukotriene 4, and histamine), which induce the edema and the hypersensitivity reactions (Figure 29) [117].

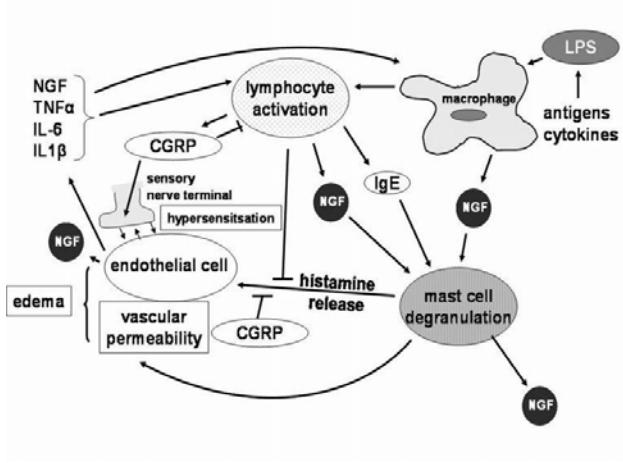

Figure 29. The role of calcitonin gene-related peptide in the neurogenic inflammation. Calcitonin gene-related peptide (CGRP) causes an endothelial cell injury with an increased vascular permeability. CGRP can be derived from activated lymphocytes and hypersensitive reactions after mast cell degranulation as well as lipopolysaccharide (LPS) induced macrophage activation. NGF: nerve growth factor; IgE: immunoglobulin E; TNFα: tumor necrosis factor α.

3.4.2.1. Nerve Growth Factor Regulatory Role in the Autoimmune Diseases

A strong anatomical and physiological connection has been revealed between the nervous and immune systems [118]. The role of neurotrophic factors in the mechanism of autoimmunity was studied by several research groups. In these studies, the autoimmune encephalomyelitis was accepted as a model for multiple sclerosis [119]. Multiple sclerosis represents a chronic disease of the central nervous system, in which the white matter is infiltrated by mononuclear cells and macrophages; this in turn causes demyelinisation with the proliferation of astrocytes, gliosis and the death of oligodendrocytes. The immune reactions are directed against the neural structures, and are based on the autoaggressive T cell reactions against the myelin antigens. NGF exerts an antiinflammatory effect in the autoimmune encephalomyelitis via downregulating the IFNγ secretion of the T cells and upregulating the IL-10 secretion of the glial cells. It is known that the oligodendrocytes are responsible for the myelin synthesis in the central nervous system, whereas the Schwann cells are responsible for that in the peripheral neurons. The myelin production is supported by NGF, which acts in the central nervous system as a neuronal trophic and survival factor by binding to the TrkA receptors in the affected cells. The Th2 derived immunosuppressive cytokines (IL-4, IL-5, IL-10, and TGFβ) display favourable effects in the development of disease [120]. The beneficial effect of NGF can be potentiated by its deteriorating action on the major histocompatibility complex class II expression and the modulation of cytokine releases. Multiple sclerosis frequently associates with thyroid autoimmunity, particularly with Graves' disease [121].

Several experiences were reported on the favourable effects of locally used NGF therapy. Rheumatoid and juvenile chronic arthritis exhibit elevated levels of NGF in the sera, synovial fluid and joint tissues in correlation with the disease activity [122]. The sources of NGF in rheumatoid arthritis may be the synoviocytes, lymphocytes and other mononuclear cells

involved in the local inflammation (Figure 30). The NGF release is caused by the stimulatory and proinflammatory cytokines - such as IL-1β and TNFα – that arise from monocytes, synoviocytes and fibroblasts. The high amounts of NGF are responsible for the local pain, the maintenance of the inflammatory or autoimmune reactions, and the tissue restitution [123, 124].

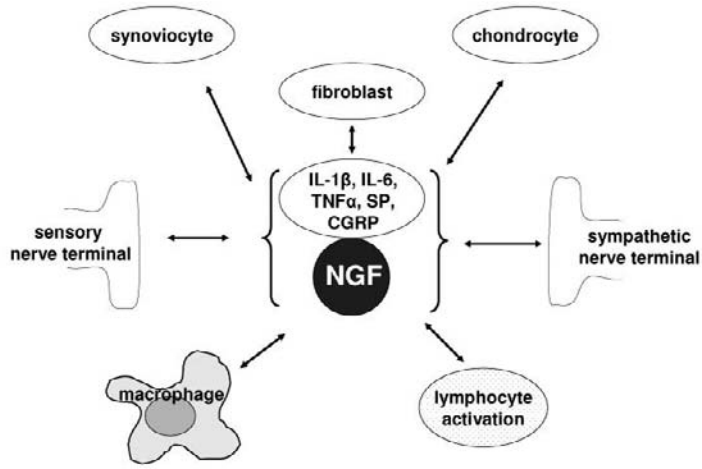

Figure 30. Neuro-inflammatory processes in arthritis. The neurogen pain is induced by at the sympathetic and sensory nerve terminals released cytokines and nerve growth factor (NGF). The main mediators are the substance P (SP) and the calcitonin gene-related peptide (CGRP), which induce a local inflammation with the sensory fiber activation. TNFα: tumor necrosis factor α.

Chronic vasculitic ulcers very often associate with the systemic autoimmune diseases, for example with rheumatoid arthritis. The development of chronic ulcers is preceded by the vasculitic necrosis and ulceration due to the local immune reactions, which lead to the tissue damage and the release of mediator from the affected endothelial cells, keratinocytes, fibroblasts and sympathetic and sensory neuronal fibers. The missing trophical effect of NGF leads to the tissue atrophy. The local NGF secretion of the target cells - which in this manner act both as sources and targets of NGF - induces a trophic improvement via the neoangiogenesis [125]. NGF locally activates the fibroblasts, keratinocytes and endothelial cells in an autocrine manner; this associates with the proliferation of these cells, the vascular neoangiogenesis, the production of extracellular matrix, collagens and proteases; these events are summarized with the term "fibrotic events". The treatment of vasculitic ulcers with NGF is commonly used in a local solution form after the removal of the fibrin and scab. This local NGF treatment results in a rapid and less painful healing of the ulcers in the patients [125].

In other cases, the immune abnormalities are associated with the endothelial injury and the fibroblast activation. This is the case for systemic sclerosis, which consists of damages to the microvasculature and connective tissue. The study by Matucci-Cerinic and co-workers demonstrated increased NGF and vasoactive intestinal peptide levels in the serum, suggesting the role of these substances in the autoimmune inflammatory processes of systemic sclerosis [126]. In this way, the origins of NGF seem to be analogous to other systemic autoimmune diseases: NGF arises from the involved immunocompetent cells but it exhibits specific features according to the localisation and the affected organs. These specific features are connected to the fibrotic events based on the activities of fibroblasts, mast cells, lymphocytes,

macrophages, endothels and keratinocytes or other epithelial cells in the diseased organs. Research data confirm that the neuropeptides released from the nerve terminals can mediate the neurogen inflammation. Their production is induced by NGF, but it should be stressed that the immunocompetent and endothelial cells are also capable of releasing neuropeptides [123]. The modulating role of the neuropeptides can manifest in the different cellular functions. The elevated serum levels of NGF and the vasoactive intestinal peptide highlight their pathogenic roles in systemic sclerosis. The NGF levels correlates with the progression of the disease, such as the vasoactive intestinal peptide levels, particularly in patients who show skin and lung manifestations. The skin lesions in systemic sclerosis potentiate the involvement of mast cells in the immune and inflammatory responses. The high levels of vasoactive intestinal peptide are also associated with the skin damage and the esophageal dysmotility in systemic sclerosis; this emphasizes the role of vasoactive intestinal peptide and NGF neurogen factors in the inflammatory reactions. Finally, NGF may lessen the local tissue injury due to its trophic action on the vascular and neuronal pathology.

The effects of NGF on the target cells may be mediated by other factors expressed on the collaborative cells like the platelets. In the platelet-associated mast cell activation, the role of lysophosphatidylserine was demonstrated, the expression of which is induced by the platelet activation [127]. The expression of lysophosphatidylserine on the platelets could be initiated by the release of 5-hydroxytryptamin from the mast cells, as well as the additional NGF stimulation. However, 5-hydroxytryptamin alone is insufficient for the platelet activation. The dual factorated platelet stimulation reflects a new pathway for vascular injuries and reveals a new interaction between the neuronal and vascular responses.

In conclusion, the allergy and the autoimmunity represent a dysregulation of the immune system, in which the local inflammatory events lead to the activations of sensory and nocicept neurons, endohelial cells, fibroblasts, lymphoid, myeloid cells, and of epithelial cells of various origins.

3.5. Nerve Growth Factor Involvement in the Stress

3.5.1. Stress Induced Endocrine Alterations

Stress is the summarized response against extrinsic or intrinsic factors, in which the threatened homeostasis could lead to various diseases. Several physiologic and behavioral adaptive responses are initiated by the stress in order to reestablish the required equilibrium of the body. The adaptation is based on the integrative complexity of the neuroendocrine, immune, cellular and molecular responses. The cardiovascular, respiratory, gastrointestinal, renal, endocrine and immune systems are regulated by the sympathetic and parasympathetic neurons of the autonomic nervous system. The stress system is composed of central and peripheral parts. The central nervous system forms one of the two central parts; here the main regulatory components are the corticotropin-releasing hormone (CRH) and the arginin-vasopressin neuropeptide (AVP), which both secrete from the paraventricular nuclei of the hypopthalamus (Figure 31) [128, 129]**.** In the hypothalamus, the locus ceruleus-noradrenergic system (LC-NA) represents the other central part of the autonomic sympathetic neurons. The peripheral parts of the stress system are composed of two major components: the hypo-thalamic-pituitary-adrenal (HPA) axis and the autonomic nervous system. The activation of hypothalamic-pituitary-adrenal axis is initiated by CRH resulting in an increase of ACTH,

which induces a systemic elevation of glucocorticoids from the adrenal glands. CRH binds to its specific receptors: the CRH-1 and CRH-2 subtypes. The CRH-1 subtype is detectable in the brain (e.g. anterior pituitary), adrenal gland, skin, ovaries and testes, while the subtype of CRH-2 is expressed in the peripheral vascular tissues, skeletal muscles, gastrointestinal tract, heart and brain (e.g. hypothalamus). CRH could be considered as a major anorexiogenic peptide inhibiting the locus ceruleus-noradrenergic sympathetic sytem [130]. One could induce the secretion of ACTH from the anterior pituitary by getting CRH into the hypophyseal portal system. In nonstressful situations, the release of CRH and ACTH shows a circardian rhythm in the portal system. ACTH is a potent stimulator of the adrenal cortex, regulating the glucocorticoids in the zona fasciculata and the adrenal androgens in the zona reticularis, but it has a mild effect on the aldosterones in the zona glomerulosa.

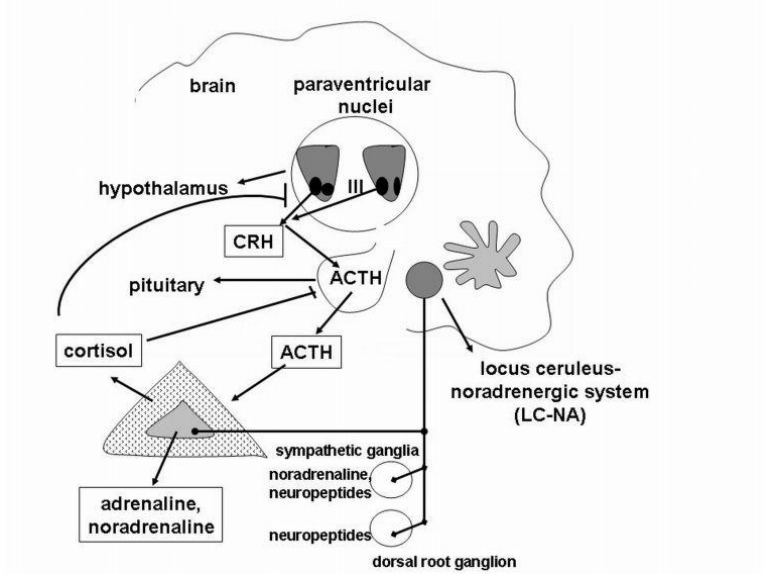

Figure 31. The main parts of the central and peripheral stress systems. The stress system is composed of two central and two peripheral parts. The two central parts are the paraventricular area of the hypothalamus and the locus ceruleus-noradrenergic system (LC-NA). The hypothalamic-pituitary-adrenal axis and the sympathetic nerve system make up the peripheral parts. ACTH: adrenocorticotropic hormone; CRH: corticotropin-releasing hormone; → **:** action direction; ⊣ : action inhibition.

The activation of the autonomic sympathetic nervous system leads to the increase of catecholamines from the adrenal glands. The chronic activation of the stress system causes a suppression of the immune system, the gonadal, growth hormone and thyroid function in consequence of the increased cortisol. However, the high levels of catecholamines may also be induced by the IL-6 production. In this aspect, the stress system can be considered as an intergrator of the neurosensory, visual, auditory, somatosensory, nociceptive and visceral signals.

In chronic stress, the high glucocorticoid levels associate with the metabolic alterations in the organs as well as the congenitive and mood disturbances of the brain. The metabolic syndrome affects the adipose tissues, the liver, skeletal muscles, and blood vessels; also, it represents metabolic alterations with insulin resistance, visceral obesity, hypertension, dyslipidaemia and cardiovascular diseases (Figure 32) [131].

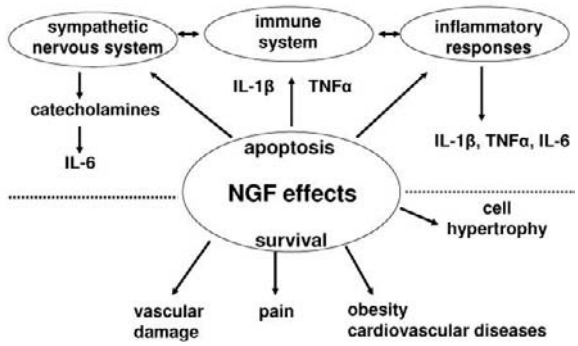

Figure 32. The regulatory and integrative roles of nerve growth factor among the stress, immune and inflammatory responses. Nerve growth factor (NGF) may regulate the balance between the cell survival and cell death processes. The activation of the stress system turns the reactions into apoptosis, while the cell survival dominance links to cardiovascular damages, pain and cell hypertrophy. TNFα: tumor necrosis factor α.

In acute stress, the clinical signs of the sympathetic activities become dominant, which causes vasoconstriction, and aggravates hypertension, hypercoagulation, arrhythmia and myocardial infarction or stroke. The neurotransmitters of sympathetic neurons are strongly influenced by both the central and the local factors.

Surprising, the adipose tissues are considered as a highly active endocrine gland, which is able to produce a wide variety of hormones and factors. Factors derived from the adipose tissue maintain a chronic inflammation and lead to life-threatening cardiovascular diseases [132].

It has been demonstrated, that the NGF levels change during the emotional and physical stress. Aloe and co-workers found that anxiety, such as during parachute jumping, triggers the synthesis of NGF and its release into the circulation [133]. The increased NGF in the serum precedes the increase in the levels of cortisol and ACTH. The exact mechanism is not clear, but much data point to that NGF may be involved in the early phase of the adaptation (Figure 33).

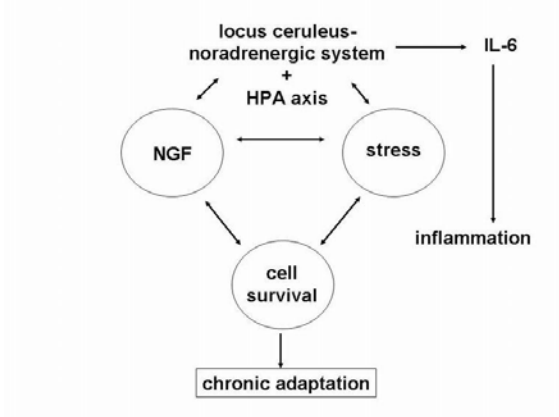

Figure 33. Network between the nerve growth factor and stress system. Nerve growth factor (NGF) represents a linkage between the stress responses and the cell survival contributing to a chronic adaptation. HPA axis: hypothalamic-pituitary-adrenal axis.

3.5.2. The Relationship Between the Stress Induced Endocrine and Cytokine Processes

The hormones, neuropeptides and neurotransmitters participate in both innate and adaptive immune responses. In turn, the lymphoid organs are innervated predominantly by sympathetic noradrenergic and/or neuropeptide nerve fibers [134]. Serotonergic, cholinergic and catecholaminergic systems upregulate the growth hormone, prolactin, ACTH and CRH. Surprisingly, the immune cells are able to secrete various types of hormones and neuropeptides, as well as express neurotransmitter receptors, adrenergic receptors, and receptors for histamine and CRH [135, 136]. The gonadotrophin-releasing hormone (GnRH) and sex steroids play an important stimulatory (e.g. estrogen) or inhibitory (e.g. androgens) role in the maturation of the immune system and the bone marrow. The activation of the hypothalamic-pituitary-thyroid axis during chronic stress leads to a decrease of T_3 levels, but the immune cells express TSH and thyroid hormone receptors [137]. Consequently, the immune system can also regulate the neuronal system via cytokines.

The catecholamines are able to inhibit the production of proinflammatory cytokines, such as IL-12, TNFα and IFNγ, and to stimulate the antiinflammatory cytokines, such as IL-10 and TGFβ [138]. The activation of the hypothalamic-pituitary-adrenal axis results in an elevation of glucocorticoid levels, thereby exerting a suppressive action on the immune responses [139, 140]. The activation of the stress system modulates the immune reactions towards the chronic and local directions, which are supported by the selective suppression of Th1 responses and the promotion of Th2 responses (Figure 34). The peripheral nervous system seems to be crucial in the local regulation of the inflammation via the neuropeptides, such as substance P, CRH, and vasoactive intestinal peptide [141, 142]. The interactions of the cytokines and NGF at the sensory and sympathetic nerve terminals represent a new integrative regulatory form among the neuronal, immune and endocrine systems for the local tissue area. The alterations manifest clinically in pain, neurogen inflammation and tissue restitution.

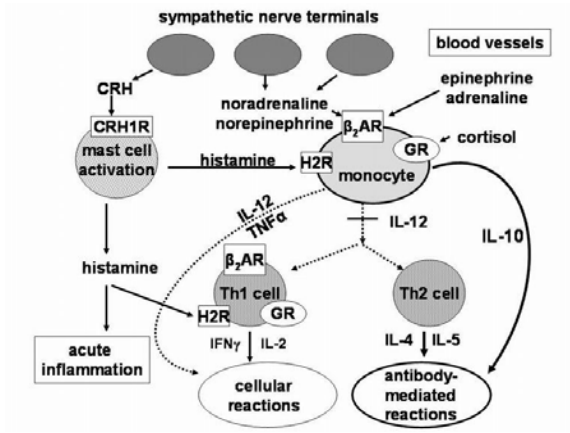

Figure 34. Endocrine actions modulate the immune responses towards T helper 2 dominance. Corticotropin-releasing hormone (CRH), cortisol and catecholamines, all of which are secreted at the sympathetic nerve terminals, bind to their receptors on the immunocompetent cells, furthermore they induce an acute inflammation, as well as antibody-mediated immune reactions. The released cytokines promote the balance into T helper 2 dominance (Th2) as opposed to T helper 1 (Th1). CRH1R: corticotropin-releasing hormone receptor 1; GR: glucocorticoid receptor; H2R: histamine receptor 2; β_2AR: β_2-adrenergic receptor; TNFα: tumor necrosis factor α; IFNγ: interferon γ; → : action direction; - - → : decreased action.

The IL-1β, IL-6 and TNFα cytokines that are produced in brain, are the main initiators for the stimulation of the hypothalamic-pituitary-adrenal axis [134, 143]. Several exogen factors - infective agents, bacterial products and tissue damage alone or with macrophage activation - could stimulate the release of proinflammatory cytokines. But TNFα and IL-1β may serve as the triggers for the central stress systems, causing an elevation of CRH, which subsequently increases the ACTH in the anterior pituitary gland (Figure 35). However, TNFα inhibits the release of norepinephrine at the nerve terminals in the adrenal glands and the skeletal muscles. The CRH stimulation, which leads to the high secretion of glucocorticoids and catecholamines, takes part in several infectious, allergic and autoimmune diseases [144].

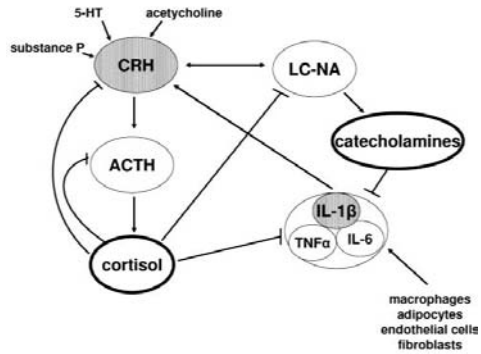

Figure 35. Stress and cytokine networks. Locally released cytokines (IL-1β, IL-6, and TNFα), derived from the immunocompetent cells, act on the corticotropin-releasing hormone (CRH) secretion and potentiate the sympathoadrenal activity; this leads to an increase in the levels of cortisol and catecholamines. ACTH: adrenocorticotropic hormone; LC-NA: locus ceruleus-noradrenergic system; 5-HT: 5-hydroxytryptamin; TNFα: tumor necrosis factor α; → : action direction; ⊣: action inhibition.

The hormonal alterations highlight that the hyperactive stress may promote Th2 dominance. The hypoactive stress facilitates Th1 dominance associating with low levels of cortisol and the increase of IL-1β, IL-6 and TNFα. The reduction of the sympathetic nerve fibers contributes to a concomitant hypofunction of the hypothalamic-pituitary-adrenal and locus ceruleus-noradrenergic systems. A similar relationship between the endocrine and autoimmune interactions could be demonstrated in the Th1 mediated rheumatoid arthritis and in multiple sclerosis [145]

The inflammation, ischemia and tissue injury cause the release of ATP and adenosine; it also attenuates the local reactions via cAMP/protein kinase A, which contributes to the productions of IL-10 and IL-12 as well as the inhibition of TNFα [146].

The histamine receptors (H1 or H2) are expressed on the immunocompetent cells that exert immunregulatory functions. The histamine receptor-1 activation may facilitate the acute inflammation and allergic reactions. However, the histamine receptor-2 activation inhibits the effects of IL-12 and TNFα but promotes the productions of IL-10 and IL-6 from the monocytes/macrophages and APC cells [147].

The catecholamines possess a direct modulating effect on the IL-1β reactions in the alveolar macrophages that are induced by lipopolysaccharide; they also upregulate the IL-6 production in the adipocytes, like insulin [148]. It was demonstrated that the recruitment of polymorphonuclear cells, monocytes, epithelial and endothelial cells could be potentiated by the catecholamines during the inflammation [149].

It should be emphasized that the vulnerability due to autonomic dysfunctions - like myocardial infarction, brain stroke or diabetes mellitus - associates with impaired immune reactions. In these cases, the neuroendocrine control of the inflammatory reactions manifests not only in the allergic and/or autoimmune diseases but also in obesity, depression and atherosclerosis.

3.5.3. Nerve Growth Factor During Stress Links to the Endocrine and Immune Networks

The neurotrophic feature of NGF is known to be crucial for the development of the sympathetic nervous system, yet NGF also possesses a widespread nonneuronal aspect. The systemic appearence of the neurotrophic effects could be explained by the fact that NGF affects the endocrine and immune systems at the several points. The previous chapters described the pleiotrophic effects of NGF in the cell responses during the inflammatory and immune events. It was previously detailed that the production of NGF is regulated by the cytokines (mainly by IL-1β, IL-6, and TNFα) and that there is a transactivation between the TrkA and adrenergic or adenosine receptor signaling pathways. The endocrine and immune cells not only secrete NGF but also bear its high-affinity TrkA receptors; however, a lot of cells express both TrkA and p75 receptors on their surfaces.

In the pituitary gland, IL-1β and the vasoactive intestinal peptide are established as stimulators for NGF, while TNFα inhibits the NGF secretion during the stress responses [150]. The autonomous nerve system and the activation of the hypothalamic-pituitary-adrenal axis are accounted for as the sources of NGF production [151, 152]. Both endogen and exogen stimulating factors are responsible for the local NGF production, which is directly or indirectly mediated by the cytokines. The crucial role of NGF is in no doubt its effect of promoting survival (Figure 36). The cell survival effect of NGF manifesting on the lymphoid cells associates with the proliferation of T and B lymphocytes, the production of immunoglobulin, the shift into Th2 dominance, as well as the recruitment of target cells caused by the release of chemoattractant mediators from the mast and eosinophil cells.

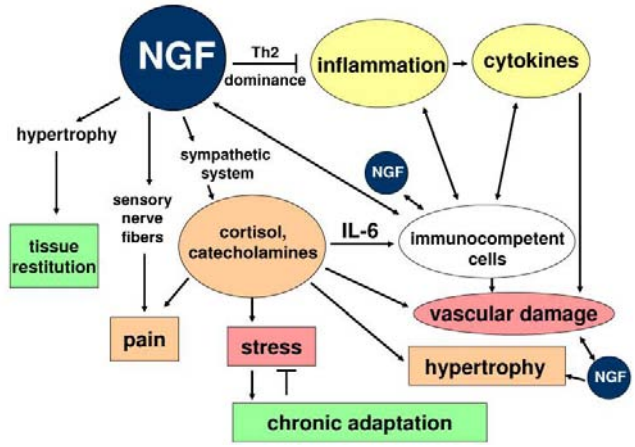

Figure 36. Nerve growth factor modulates the immune and inflammatory reactions during stress. In the stress, nerve growth factor (NGF) is implicated in the cortisol and catecholamine increase and in the inflammatory, cytokine reactions contributing to the pain, tissue hypertrophy, vascular damage and the tissue restutition. Th2 : T helper 2 lymphocyte; → : action direction; ⊣ : action inhibition.

The IL-1β, IL-6 and TNFα cytokines are potent inducers for the secretion of NGF and CRH in the brain and the peripheral tissues, as well as for the production of catecholamines in the adrenal medulla [134, 153]. The antigen-presenting cells (monocytes or macrophages) and CD4+CD25+ Treg cells are able to secrete IL-10, which is an NGF stimulator like the Th2 derived cytokines: IL-4 and IL-5. Lymphocytes, astrocytes, mast cells can also produce these cytokines. The shift towards Th2 dominance acts in the direction of NGF mediated survival.

The glucocorticoids possess both protective and destructive effects on the nervous system. Recently, Jeanneteau and co-workers demonstrated that the actions of the glucocorticoid and Trk receptors overlap [154].

It is not surprising, that NGF plays a special importace in the development and the postnatal function of the ovaries. NGF is essential for the early development of the primordial follicles and also for the initiation of the FSH receptor synthesis [155]. The findings highlight that NGF is an essential mediator for the organogenesis of the ovaries at the stage when the early growing follicules become gonadotropin dependent in the postnatal life.

The adipose tissues reveal a special connection between the endocrine and the neural systems. The highly innervated adipose tissues reflect an increased sympathetic activity and a connection with the hypothalamic-pituitary-adrenal activity. The integrative regulatory levels between the endocrine and nervous systems reflect a connection among the inflammatory, immune and metabolic events [152]. The elevated NGF levels in obesity and the metabolic syndrome emphasizes the role of neurotrophic factors in the adipocyte functions during inflammation [156]. The adipose tissues as endocrine organs display distinct functions: controlled by appetite, energy balance, lipid metabolism, insulin sensitivity, glucose and vascular homeostasis, as well as immune and inflammatory actions. More than 50 different adipokines are known, of which TNFα and IL-1β are potent NGF stimulators. Bulló and co-workers demonstrated a positive correlation among the circulating NGF levels and the body mass index (BMI) as well as the morbidity of obese patients [156]. The lipolysis inducing cytokines (IL-1β, IL-6, and TNFα) may lead to an increase in the amount of NGF, suggesting the importance of the neuronal protection in the adipose tissues. It seems that the macrophage infiltration in the adipose tissues becomes more important for the amplification of the inflammatory events [157].

In the clinical aspects, the participation of adipose tissues in the neuroendocrine processes can justify, why the cardiovascular diseases connect so frequently to the different endocrine diseases, particularly to those in which the chronic stress comes into prominence (Figure 37). In addition to the chronic stress and environmental factors, the psychoemotional stress may also induce an elevation of NGF levels. The stimulation of the hypothalamic-pituiutary-adrenal axis and the sympathetic nervous system associates with the hypertrophy and hypercortisolemia of the adrenal gland, as well as metabolic changes [152]. Consequently, the increased proinflammatory cytokines and the NGF enhancement caused by the degradation of mast cells lead to the activation of the stress system along with hypercortisolemia, catecholaminemia and dislipidemia. The hypercortisolemia and the sympathicotony caused by immunosuppression create a predisposition for infections. The metabolic alterations due to the blocking effect of hypercortisolemia could manifest in impaired thyroid and gonadal functions, or in hyperprolactinemia. The high cortisol and catecholamine levels induce lipolysis in the adipose tissues with the secretion of adipokines, which amplify the metabolic changes; this results in hyperinsulinemia, insulin resistance, lipogenesis, obesity, leptin resistance, and dyslipidemia. The early stage of the metabolic

syndrome is characterized by a concomitant NGF elevation in the circulation, while the generalized state of this syndrome links to decreased NGF levels. The metabolic alterations precede the vascular manifestation and the onset of cardiovascular diseases.

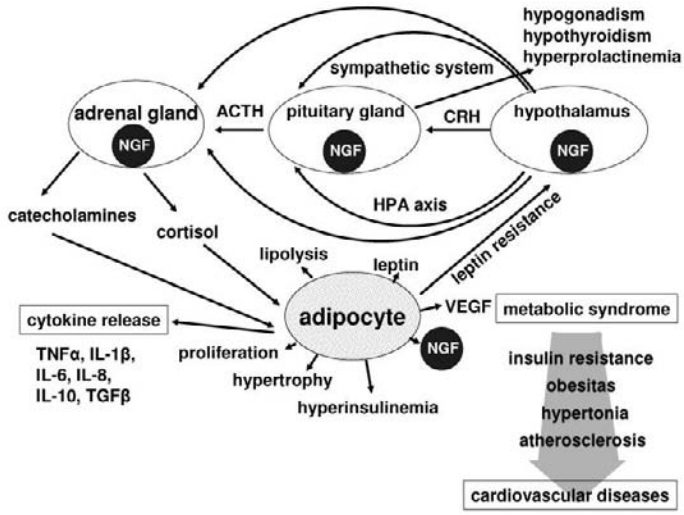

Figure 37. The involvement of adipose tissue in stress leads to cardiovascular diseases. In the chronic stress, an increase in the hypothalamic-pituitary-adrenal (HPA) axis activity leads to obesity, metabolic syndrome and cardiovascular diseases via hormonal, cytokine and neurotrophin actions. ACTH: adrenocorticotropic hormone; CRH: corticotropin-releasing hormone; VEGF: vascular endothelial growth factor; NGF: nerve growth factor; TNFα: tumor necrosis factor α; TGFβ: transforming growth factor β.

3.5.4. The Network Between the Stress Induced Inflammation and the Actions of the Nerve Growth Factor. The Neurogenic Pain

Stress is a trigger for inflammatory and metabolic alterations resulting in a high susceptibility to infections. The chronic stress-induced systemic inflammation affects several organs and manifests clinically in the following alterations: inflammation of the endothelium, Th2 dominance, osteoporosis, hypercoagulability, dyslipidemia, insulin resistance, lipogenesis, vasodilation with increased permeability, cytokinemia, neurogen hyperalgesia and pain [145]**.** The effector substances, - which initiate the systemic inflammation, - are the cytokines releasing from macrophages, fibroblasts, mast cells, endothelial cells, T and B lymphocytes, monocytes, eosinophils, basophils, adipocytes and epithelial cells. The peripheral nerve terminals of the sympathetic and sensory nerve fibers are the active sources of several neuropeptides, such as CRH, norepinephrine, epinephrine, substance P, and calcitonin gene-related peptide. The peptides liberated from the nerve terminals react to their specific cell receptors, which leads to the modulation of distinct immune, endocrine and inflammatory responses. The consequence of the stress interactions is the increased cytokine release in addition to NGF production. These active mediators contribute to the perpetuation of the inflammatory responses with the desire to localize and prolong the reactions. The integrative responses to the stress and the concomitantly secreted NGF may help the local manifestation of the inflammatory events (Figure 38). One of the important moments of this process is the degranulation of mast cells – mediated by NGF – which links to the local

chemoattractant release. In this way, NGF promotes the recruitment of the tissue-infiltrating cells. The cell migration mechanism to the area of the tissue damage is crucial. The expressions of the adhesion molecules on the leukocytes (endothelial cell leukocyte adhesion molecule-1 (ELAM-1), E selectin) and endothelial cells (intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1) represent the substances for the cell to cell contact in the migration. Moreover, NGF acts locally on the angiogenesis, which is mediated by the cell survival signaling pathway and the adrenoreceptor mediated cell hypertrophy [158]. The action of NGF on the endothelial cells is direct, and it plays an essential role with the consequent keratinocyte proliferation in the tissue granulation of the wound healing.

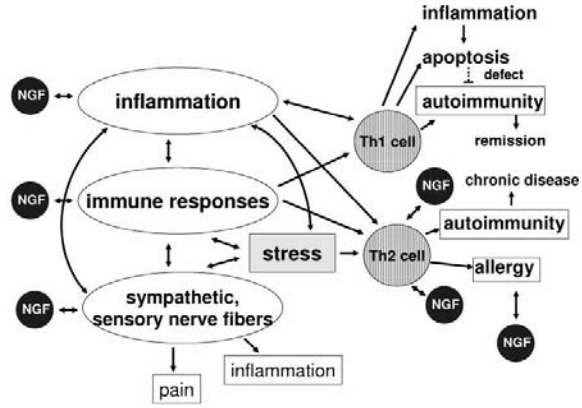

Figure 38. Nerve growth factor involvement in the neural-immune-endocrine network. The activity of the sympathetic system affects the neural, immune and endocrine responses. As a regulator, nerve growth factor (NGF) orchestrates among the networks: autoimmunity, allergy, inflammation, pain and stress. Th1: T helper 1 lymphocytes; Th2: T helper 2 lymphocytes; → : action direction; ⟞ : decreased inhibition.

The sympathetic system participates in the neurogenic inflammation and hyperalgesia [159]. Studies regarding the endotoxin-induced hyperalgesia revealed that the process was independent of the peripheral and central sympathetic mechanisms. In turn, this endotoxin-induced hyperalgesia links to the local production of proinflammatory cytokines and is mediated by NGF. In these events, the sensitizations of the nociceptors are implicated to play a secondary role in the activation of the sympathetic efferens.

Damages to the peripheral nervous system often manifest themselves in the forms of chronic and neuropathic pain. Different factors are involved in the pathogenesis of the neuropathic pain: reactions among the immunocompetent cells, cytokines and the locally released mediators [160, 161, 162]. The locally accumulated immunocompetent cells directly lead to the neuropathic pain by setting the degranulation of the mast cells in the center; this is then followed by neutrophil-initiated phagocytosis and macrophage infiltration. The immuno-competent cells and the sensory nerves release various types of cytokines (IL-1β, IL-6, and TNFα), chemokines, histamine, prostaglandins, NO, bradykinin, NGF, neuropeptides (substance P, and calcitonin gene-related peptide). These substances induce different cell activations, as well as T lymphocyte activations connecting to the increase of the vascular permeability. Under the inflammatory conditions, the pain initiates membrane alterations in the senzitized nociceptive neurons. In the pain pathomechanism, the Schwann cells play an

important role, which is supported by the following: 1. They have a strong connection with the sensory neurons, 2. They are capable to synthesize biologically active mediators.

In fact, NGF is implicated in the development of pain. In some published studies, TrkA receptor mutation associated with the insensitivity of pain [163]. This supports that the systemic administration of NGF caused thermal and mechanical hyperalgesia. The nociceptor sensitization induced by NGF exerts a modulatory role for the immunocompetent cells acting directly or indirectly on them. However, nowadays the role of NGF is controversial in the treatment of the neuropathic pain.

3.6 Nerve Growth Factor Exerts an Effect Towards the Direction of T Helper 2 Dominance

Th2 dominance may be accepted as a way for the organism to avoid the rapid organ or tissue damages that are caused by infections, cancer, exogen agents and are mediated by Th1 and/or natur killer (NK) cell responses. The Th2 immune responses lead to a prolonged formation and a chronic duration of immune reactions, during which the production of antibodies is essential. As a neurotrophic factor, NGF potentiates the survival and the differentiation of the sympathetic and sensory, nociceptive neurons. However, besides its neurogenic activity, NGF can modulate several cell functions via its TrkA and/or p75 receptors, particularly, in the immunocompetent cells. The shift towards Th2 highlights the interaction between the endocrine and immune systems, which are controlled and regulated by the sympathetic nervous system. Four different factors could be established, which may contribute to the Th2 shift: 1. The interactions between the endocrine and hormonal actions during the stress responses promote the appearance of Th2 dominance during hypercortisolemia; also, the interactions promote increased immunosuppression mediated by catecholamines as well as CRH. 2. The direct hormonal actions of glucocorticoids and catecholamines binding to their receptors on the immunocompetent cells. 3. The increased apoptosis-susceptibility of the Th1 memory/effector cells and the Th2 apoptosis-resistance mediated by NGF. 4. Cytokines (IL-4, IL-5, and IL-13) which increase the differentiation and the maturation of Th2 cells.

3.6.1 The Effects of Stress, Glucocorticoids, Norepinephrines and CRH on the Direction Towards Th2 Dominance

The central and peripheral parts of the stress system act as neural controls on the endocrine-immune reponses. However, the activation of hypothalamic-pituitary-adrenal axis and the autonomous sympathetic neuronal fibers contribute to the increase of glucocorticoids and catecholamines. These substances possess generally immunosuppressive effects and cause an inhibiton of inflammations [135, 139]. The glucocorticoids regulate a wide variety of the immune-related genes, as well as the gene expressions and functions of the immunocompetent cells. The shift of dominance from Th1 to Th2 is influenced by glucocorticoids and is based on the cytokine levels, adhesion molecules, chemoattractans and cell migration, cell trafficking, immune cell maturation and differentiation [164]. The glucocorticoid receptor activations - expressed on several cells - affect the immune and inflammatory responses and cause an immunomodulation. By another way, catecholamines

can inhibit the production of proinflammatory cytokines (IL-12, TNFα, and IFNγ) during the stimulation of the immunosuppressive cytokine productions (IL-10, TGFβ) [165]. Data show that catecholamines may directly contribute to the shift from Th1 to Th2 by modulating the cytokine productions that are mediated by the adrenergic receptors on the surface of the immunocompetent cells.

CRH is the main stress mediator and possesses a dual function in respect to its systemic immunosuppressive effects: 1. It is a regulator of the stress system 2. It has immunoreactive effects that appear locally in the peripheral inflammatory areas [166, 167]. Surprisingly, the majority of the plasma CRH is not derived from the hypothalamic sources during the inflammatory reactions but rather from the peripheral organs, such as the adrenal medulla, spinal cord, cardiovascular system, gastrointestinal tract, lungs, skin, endometrium, placenta, ovaries, testes [168]. It seems, that the CRH release can only reach a measurable degree under certain circumstances, like in the insulin induced hypoglycemia, or pregnancy. The decrease in the hypothalamic CHR release may be due to its rapid receptor binding or degradation. Two types of CRH receptors are known, which belong to the G protein-coupled receptor family. CRH receptor expression is detectable on the immune and endothelial cells, epithelial and stromal cells of various tissues. Therefore, the peripheral CRH may directly influence the immune responses [166]**.** The CRH receptors on the mast cells directly take part in the inflammatory reactions. The sensory and postganglionic sympathetic neurons of the peripheral nervous system secrete CRH locally, suggesting CRH's role in the tissue damages during the local immune and inflammatory reactions [128]. In the peripheries, CRH initiates the degranulation of mast cells by binding to their CRH-1 receptors; thereby attenuating the liberation of histamine, IL-4 and IL-10. However, the histamine can bind to the histamine receptor-2 expressed on the immunocompetent cells, monocytes/macrophages and Th1 lymphocytes. Therefore, histamine - binding to its histamine receptor-2 - inhibits the IL-12 and TNFα productions of monocytes/macrophages, as well as the IL-2 and IFNγ productions of Th1 cells [145]. Contrary to histamine receptor-1, the activation of histamine receptor-2 is potentiated by the IL-10 and IL-6 cytokines produced by monocytes/macrophages, but it is not potentiated by IL-4 derived cytokines from Th2 lymphocytes. The histamine activation affects the histamine receptor-1 expressed on the vascular walls, which contributes to the increased vascular permeability and vasodilatation. It was also demonstrated that the histamine promotes the releases of IL-6 and IL-8 from the endothelial cells through the histamine receptor-2.

In this respect, CRH can modulate the immune responses towards Th2 dominance, because its receptors are expressed on the mast cells and activate the stress system.

3.6.2 The Immunocompetent Cells Express Glucocorticoid and Catecholamine Receptors

Both glucocorticoids and catecholamines directly inhibit the IL-12 production of the antigen-presenting cells, but the catecholamines also potentiate their IL-10 production [128, 144]. The thyroid autoimmunity represents a special field for stress influenced events. The shift towards Th2 dominance is characteristic for the course of the thyroid diseases [169]. For example, Graves' disease is an autoimmune thyroid disease with Th2 dominance. As an exception, Hashimoto's thyroiditis has Th1 dominance. The regulation of the immune responses is based on the distinct cytokine patterns that are derived from the antigen-presenting and -infiltrating immunocompetent cells in the thyroid. The influencing factors for

the shift into Th2 in Graves' disease are the following: a/ The dominant effect of IL-10, b/ The inhibition of natural killer cell (NK) and Th1 cell activations due to the diminished IL-12 release. In this case, the balance between the Th1 and Th2 cytokine profiles can turn into apoptosis induced by the activation of the effector T lymphocyte, or into apoptosis resistance in the presence of antibody production.

The NGF neurotrophic factor co-secretes during the immune processes and acts directly on the processes that lead towards the Th2 direction during the activation of the stress system.

3.6.3 The Th1 Apoptosis-Susceptibility and the Th2 Apoptosis-Resistance Mediated by NGF

The expressions of the major histocompatibility complex class I and II are crucial for the antigen presentation and the effector immune reactions. In particular, the major histocompatibility complex class II expression on the antigen-presenting cells and T lymphocytes is essential for the cell activation associating with the high amounts of proinflammatory cytokines. In the brain, the microglia could be respected as the antigen-presenting cell causing immune and inflammatory events. The major histocompatibility complex class II expression on the microglia is inhibited by NGF via p75 receptor activation [60]. This deficit of the major histocompatibility complex class II expression on the microglias makes them resistant against neuronal injury.

The high-affinity TrkA receptor ablation (total lack of) leads to the elevation in the quantity of immunoglobulins, reflecting that the endogenous NGF in the nonneuronal cells associates with the functional abnormalities [59]. The apoptosis-susceptibility of Th1 lymphocytes gives a new possibility for Th2 dominance [57]. The TrkA receptor signaling pathway via the PI-3K upregulation seems to be involved in the Th2 resistance [58]. The Fas-mediated apoptosis is required for the maintenance of the T cell homeostasis and the self-tolerance. The role of caspases in the PI-3K/Akt signaling processes has been demonstrated: it prevents the phosphorylated caspase-9 cleavage due to the caspase-3. Both caspases are components of the mitochondrial-dependent pathway of the Fas-mediated apoptosis. The distinct intensities of the PI-3K activation in the Th1 and Th2 cells explain their distinct vulnerabilities for apoptosis. The activation of PI-3K in the Th1 cells is weak, but it associates with their increased susceptibility to apoptosis. The enhancement in the apoptosis affects the Th1 memory cells [57]. The atopic feature in allergic individuals is based on the novel mechanism of the PI-3K upregulation.

3.6.4 Cytokines Promote the Th2 Dominance

Some cytokines are characterized by a suppressive feature that acts on multiple pathways and leads to apoptosis. They include antioxidants, Ca^{2+}-mobilizing compounds and protease inhibitors [52]. When NGF binds to its high-affinity TrkA receptors on nonimmune cell surfaces during the immune-endocrine inflammatory reactions, it associates with the release of several cytokines. The Th2-like cytokines modulate the Fas expression on the surface of the activated CD4+ T cells and contribute to the persistence of the allergic immune responses [35]. However, the apoptosis of T lymphocytes could be prevented by IL-4 and IL-5 cytokines, which upregulate the Bcl2 and BclXL antiapoptotic proteins.

NGF dose-dependently increases cytokine genes (IL-13, IL-4, IL-10, and TNFα) in the mast cells and initiates the degranulation of the mast cell with the release of mediators and IL-4 [170]. According to these findings, the action of NGF towards the Th2 shift is driven by the release of IL-4.

REFERENCES

[1] Dray, A. *Inflammatory mediators of pain*. Br J Anaesth 1995; 75:125-131.

[2] Peeraully, MR; Jenkins, JR; Trayhurn, P. *NGF gene expression and secretion in white adipose tissue: regulation in 3T3-L1 adipocytes by hormones and inflammatory cytokines*. Am J Physiol Endocrinol Metab 2004; 287:E331-E339.

[3] Tada, K; Murakami, M; Kambe, T; Kudo, I. *Induction of cyclooxygenase-2 by secretory phospholipases A_2 in nerve growth factor-stimulated rat serosal mast cells is facilitated by interaction with fibroblasts and mediated by a mechanism independent of their enzymatic functions.* J immunol 1998; 161:5008-5015.

[4] Rowlands, DK; Kao, CL; Wise, H. *Regulation of prostacyclin and prostaglandin E2 receptor mediated responses in adult rat dorsal root ganglion cells, in vitro*. Br J Pharmacol 2001; 133:13-22.

[5] Bulló, M; Peeraully, MR; Trayhurn, P. *Stimulation of NGF expression and secretion in 3T3-L1 adipocytes by prostaglandins PGD_2, PGJ_2, and Δ^{12}-PGJ_2*. Am J Physiol Endocrinol Metab 2005; 289:E62-E67.

[6] Marshall, JS; Gomi, K; Blennerhassett, MG; Bienenstock, J. *Nerve growth factor modifies the expression of inflammatory cytokines by mast cells via a prostanoid-dependent mechanism.* J Immunol 1999; 162:4271-4276.

[7] Herlong, JL; Scott, TR. *Positioning prostanoids of the D and J series in the immunopathogenic scheme*. Immunol Lett 2006; 102:121-131.

[8] Holgate, ST; Burns, GB; Robinson, C; Church, MK. *Anaphylactic- and calcium-dependent generation of prostaglandin D2 (PGD2), thromboxane B2, and other cyclooxygenase products of arachidonic acid by dispersed human lung cells and relationship to histamine release.* J Immunol 1984; 133:2138-2144.

[9] Linhart, O; Obreja, O; Kress, M. *The inflammatory mediators serotonin, prostaglandin E2 and bradykinin evoke calcium influx in rat sensory neurons*. Neuroscience 2003;118:69-74.

[10] Perkins, M; Dray, A. *Novel pharmacological strategies for analgesia*. Ann Rheum Dis 1996; 55:715-722.

[11] Safieh-Garabedian, B; Kanaan, SA; Haddad, JJ; Jaoude, PA; Jabbur, SJ; Saadé, NE. *Involvement of interleukin-1β, nerve growth factor and prostaglandin E2 in endotoxin-induced localized inflammatory hyperalgesia*. Br J Pharmacol 1997; 121:1619-1626.

[12] Takechi, H; Matsumura, K; Watanabe, Y; Kato, K; Noyori, R; Suzuki, M; Watanabe, Y. *A novel subtype of the prostacyclin receptor expressed in the central nervous system*. J Biol Chem 1996; 271:5901-5906.

[13] Nakae, K; Saito, K; Iino, T; Yamamoto, N; Wakabayashi, M; Yoshikawa, S; Matsushima, S; Miyashita, H; Sugimoto, H; Kiba, A; Gupta, J. *A prostacyclin receptor*

antagonist inhibits the sensitized release of substance P from rat sensory neurons. J Pharmacol Exp Ther 2005; 315:1136-1142.

[14] Rathee, PK; Distler, C; Obreja, O; Neuhuber, W; Wang, GK; Wang, SY; Nau, C; Kress, M. *PKA/AKAP/VR-1 module: A common link of Gs-mediated signaling to thermal hyperalgesia*. J Neurosci 2002; 22:4740-4745.

[15] Huang, J; Zhang, X; McNaughton, PA. *Inflammatory pain: The cellular basis of heat hyperalgesia.* Curr Neuropharmacol 2006; 4:197-206.

[16] Georas, SN; Guo, J; De Fanis, U; Casolaro, V. *T-helper cell type-2 regulation in allergic disease*. Eur Resp J 2005; 26:1119-1137.

[17] Kay, AB. *Allergy and allergic diseases*. N Engl J Med 2001; 344:30-37.

[18] Ngoc, LP; Gold, DR; Tzianabos, AO; Weiss, ST; Celedón, JC. *Cytokines, allergy, and asthma*. Curr Opin Allergy Clin Immunol 2005; 5:161-166.

[19] Lampinon, M; Carlson, M; Håkansson, LD; Venge, P. *Cytokine-regulated accumulation of eosinophils in inflammatory disease*. Allergy 2004;59:793-805.

[20] Päth, G; Braun, A; Meents, N; Kerzel, S; Quarcoo, D; Raap, U; Hoyle, GW; Nockher, WA; Renz, H. *Augmentation of allergic early-phase reaction by nerve growth factor*. Am J Respir Crit Care Med 2002; 166:818-826.

[21] Baecher-Allan, C; Brown, JA; Freeman, GJ; Hafler, DA. *$CD4^+CD25^{high}$ regulatory cells in human peripheral blood*. J Immunol 2001; 167:1245-1253.

[22] Jonuleit, H; Schmitt, E. *The regulatory T cell family: Distinct subsets and their interrelations.* J Immunol 2003; 171:6323-6327.

[23] O'Neill, EJ; Sundstedt, A; Mazza, G; Nicolson, KS; Ponsford, M; Saurer, L; Streeter, H; Anderton, S; Wraith, DC. *Natural and induced regulatory T cells*. Ann N Y Acad Sci 2004; 1029:180-192.

[24] Romagnani, S. *Regulatory T cells: which role in the pathogenesis and treatment of allergic disorders?* Allergy 2006; 61:3-14.

[25] Kuniyasu, Y; Takahashi, T; Itoh, M; Shimizu, J; Toda, G; Sakaguchi, S. *Naturally anergic and suppressive $CD25^+CD4^+$ T cells as a functionally and phenotypically distinct immunoregulatory T cell subpopulation*. Int Immunol 2000; 12:1145-1155.

[26] Alyanakian, MA; You, S; Damotte, D; Gouarin, C; Esling, A; Garcia, C; Havouis, S; Chatenoud, L; Bach, JF. *Diversity of regulatory $CD4^+$ T cells controlling distinct organ-specific autoimmune diseases.* Proc Nat Acad Sci USA 2003; 100:15806-15811.

[27] Fehérvári, Z; Sakaguchi, S. *$CD4^+$ Tregs and immune control.* J Clin Invest 2004; 114:1209-1217.

[28] Jiang, H; Chess, L. *An integrated view of suppressor T cell subsets in immunoregulation.* J Clin Invest 2004; 114:1198-1208.

[29] Thornton, AM; Shevach, EM. *Suppressor effector function of $CD4^+CD25^+$ immunoregulatory T cells is antigen nonspecific*. J Immunol 2000; 164:183-190.

[30] Kearley, J; Barker, JE; Robinson,DS; Lloyd, CM. *Resolution of airway inflammation and hyperreactivity after in vivo transfer of $CD4^+CD25^+$ regulatory cells is interleukin 10 dependent.* J Exp Med 2005; 202:1539-1547.

[31] Walker, MR; Carson, BD; Nepom, GT; Ziegler, SF; Buckner, JH. *De novo generation of antigen-specific $CD4^+CD25^+$ regulatory T cells from human $CD4^+CD25^-$ cells*. Proc Nat Acad Sci USA 2005; 102:4103-4108.

[32] Robinson, DS; Larché, M; Durham, SR. *Tregs and allergic disease*. J Clin Invest 2004; 114:1389-1397.

[33] Hawrylowicz, CM. *Regulatory T cells and IL-10 in allergic inflammation*. J Exp Med 2005; 202:1459-1463.

[34] Lewkowich, IP; Herman, NS; Schleifer, KW; Dance, MP; Chen, BL; Dienger, KM; Sproles, AA; Shah, JS; Köhl, J; Belkaid, Y; Wills-Karp, M. *$CD4^+CD25^+$ T cells protect against experimentally induced asthma and alter pulmonary dendritic cell phenotype and function.* J Exp Med 2005; 2002: 1549-1561.

[35] Spinozzi, F; Agea, E; Fizzotti, M; Bassotti, G; Russano, A; Droetto, S; Bistoni, O; Grignani, F; Bertotto, A. *Role of T-helper type 2 cytokines in down-modulation of Fas mRNA and receptor on the surface of activated $CD4^+$ T cells: molecular basis for the persistence of the allergic immune response*. FED Am Societ Exp Biol J 1998; 12:1747-1753.

[36] Jaffar, Z; Sivakuru, T; Roberts, K. *$CD4^+CD25^+$ T cells regulate airway eosinophilic inflammation by modulating the Th2 cell phenotype*. J Immunol 2004; 172:3842-3849.

[37] Ling, EM; Smith, T; Nguyen, XD; Pridgeon, C; Dallman, M; Arbery, J; Carr, VA; Robinson, DS. *Relation of $CD4^+CD25^+$ regulatory T-cell suppression of allergen-driven T-cell activation to atopic status and expression of allergic disease*. Lancet 2004; 363:608-615.

[38] Kondo, N; Matsui, E; Kaneko, H; Fukao, T; Teramoto, T; Inoue, R; Watanabe, M; Aoki, M; Kasahara, K; Morimoto, N. *Atopy and mutations of IL-12 receptor beta 2 chain gene*. Clin Exp Allergy 2001; 31:1189-1193.

[39] Pace, L; Pioli, C; Doria, G. *IL-4 modulation of $CD4^+CD25^+$ T regulatory cell-mediated suppression*. J Immunol 2005; 174:7645-7653.

[40] Velasco, G; Campo, M; Manrique, OJ; Bellou, A; He, H; Arestides, RSS; Schaub, B; Perkins, DL; Finn, PW. *Toll-like receptor 4 or 2 agonists decrease allergic inflammation.* Am J Respir Cell Mol Biol 2005; 32:218-224.

[41] Jutel, M; Akdis, M; Blaser, K, Akdis, CA. *Mechanisms of allergen specific immunotherapy – T-cell tolerance and more*. Allergy 2006; 61:796-807.

[42] Groneberg, DA; Quarcoo, D; Frossard, N; Fischer, A. *Neurogenic mechanisms in bronchial inflammatory diseases*. Allergy 2004; 59:1139-1152.

[43] de Vries, A; Dessing, MC; Engels, F; Henricks, PAJ; Nijkamp, FP. *Nerve growth factor induces a neurokinin-1 receptor-mediated airway hyperresponsiveness in Guinea pigs*. Am J Respir Crit Care Med 1999; 159:1541-1544.

[44] Hoyle, GW; Graham, RM; Finkelstein, JB; Nguyen, KPT; Gozal, D; Friedman, M. *Hyperinnervation of the airways in transgenic mice overexpressing nerve growth factor*. Am J Respir Cell Mol Biol 1998; 18:149-157.

[45] Bonini, S; Lambiase, A; Bonini, S; Angelucci, F; Magrini, L; Manni, L; Aloe, L. *Circulating nerve growth factor levels are increased in humans with allergic diseases and asthma*. Proc Natl Acad Sci USA 1996; 93:10955-10960.

[46] Micera, A; Puxeddu, I; Aloe, L; Levi-Schaffer, F. *New insights on the involvement of nerve growth factor in allergic inflammation and fibrosis.* Cytokine Growth Factor Rev 2003; 14:369-374.

[47] Smith, CA; Farrah, T; Goodwin, RG. *The TNF-receptor superfamily of cellular and viral proteins: activation, costimulation, and death*. Cell 1994; 76:959-962.

[48] Stevenson, MA; Pollock, SS; Coleman, CN; Calderwood, SK. *X-irradiation, phorbol esters and H_2O_2 stimulate mitogen activated protein kinase activity in NIH-3T3-cells through the formation of reactive oxygen intermediates.* Cancer Res 1994; 54:12-15.

[49] Collins, M. *Potential roles of apoptosis in viral pathogenesis*. Am J Respir Crit Care Med 1995; 152:S20-S24.

[50] Gulbins, E; Jekle A; Ferlinz, K; Grassmé, H; Lang, F. *Physiology of apoptosis*. Am J Physiol Renal Physiol 2000; 279:F605-F615.

[51] Nutku, E; Zhuang, Q; Soussi-Gounni, A; Aris, F; Mazer, BD; Hamid, Q. *Functional expression of IL-12 receptor by human eosinophils: IL-12 promotes eosinophil apoptosis*. J Immunol 2001; 167:1039-1046.

[52] Lotem, J; Sachs, L. *Cytokines as suppressors of apoptosis*. Apoptosis 1999; 4:187-196.

[53] Beutler, B; Bazzoni, F. *TNF, apoptosis and autoimmunity: A common thread?* Blood cells, Molecules & Diseases 1998; 24:216-230.

[54] Pittoni, V; Valesini, G. *The clearence of apoptotic cells: implications for autoimmunity*. Autoimmunity Rev 2002; 1:154-161.

[55] Stuart, L; Hughes, J. *Apoptosis and autoimmunity*. Nephrol Dial Transplant 2002; 17:697-700.

[56] DeSilva, DR; Jones, EA; Favata, MF; Jaffee, BD; Magolda, RL; Trzaskos, JM; Scherle, PA. *Inhibition of mitogen-activated protein kinase kinase blocks T cell proliferation but does not induce or prevent anergy*. J Immunol 1998; 160:4175-4181.

[57] Akdis, M; Trautmann, A; Klunker, S; Daigle, I; Küçüksezer, UC; Deglmann, W; Disch, R; Blaser, K; Akdis, CA. *T helper (Th) 2 predominance in atopic diseases is due to preferential apoptosis of circulating memory/effector Th1 cells*. Fed Am Societ Exp Biol J 2003; 17:1026-1035.

[58] Varadhachary, AS; Peter, ME; Perdow, SN; Krammer, PH; Salgame, P. *Selective up-regulation of phosphatidylinositol 3'-kinase activity in Th2 cells inhibits caspase-8 cleavage at the death-inducing complex: A mechanism for Th resistance from Fas-mediated apoptosis.* J Immunol 1999; 163:4772-4779.

[59] Coppola, V; Barrick, CA; Southon, EA; Celeste, A; Wang, K; Chen, B; Haddad, EB; Yin, J; Nussenzweig, A; Subramaniam, A; Tessarollo, L. *Ablation of TrkA function in the immune system causes B cell abnormalities.* Development 2004; 131:5185-5195.

[60] Neumann, H; Misgeld, T; Matsumuro, K; Wekerle, H. *Neurotrophins inhibit major histocompatibility class II inducibility of microglia: Involvement of the p75 neurotrophin receptor*. Proc Natl Acad Sci USA 1998; 95:5779-5784.

[61] Richards, JD; Davé, SH; Chou, CHG; Mamchak, AA; DeFranco, AL. *Inhibition of the MEK/ERK signaling pathway blocks a subset of B cell responses to antigen*. J immunol 2001; 166:3855-3864.

[62] Kiess, W; Gallaher, B. *Hormonal control of programmed cell death/apoptosis*. Eur J Endocrinol 1998; 138:482-491.

[63] Migliorati, G; Nicoletti, I; Nocentini, G; Pagliacci, MC; Riccardi, C. *Dexamethasone and interleukins modulate apoptosis of murine thymocytes and peripheral* T-lymphocytes. Pharmacol Res 1994; 30:43-52.

[64] Migliorati, G; Nicoletti, I; D'Adamio, F; Spreca, A, Pagliacci, MC; Riccardi, C. *Dexamethasone induces apoptosis in mouse natural killer cells and cytotoxic T lymphocytes*. Immunol 1994; 81:21-26.

[65] Wallach, D; Boldin, M; Ezer, S; Goltsev, Y; Goncharov, T; Mett, I; Malinin, N; Adar, R; Kovalenko, A; Varfolomeev, E. *Exploring cell death mechanisms by analyzing signaling cascades of the TNF/NGF receptor family*. Behring Inst Mitt 1996; 97:144-155.

[66] Andrikoula, M; Tsatsoulis, A. *The role of Fas-mediated apoptosis in thyroid disease*. Eur J Endocrinol 2001; 144:561-568.

[67] Lin, JD. *The role of apoptosis in autoimmune thyroid disorders and thyroid cancer*. Brit Med J 2001; 322:1525-1527.

[68] Salmaso, C; Bagnasco, M; Pesce, G; Montagna, P; Brizzolara, R; Altrinetti, V; Richiusa, P; Galluzzo, A; Giordano, C. *Regulation of apoptosis in endocrine autoimmunity. Insights from Hashimoto's thyroiditis and Graves' disease*. Ann NY Acad Sci 2002; 966:496-501.

[69] Mezosi, E; Wang, sH; Utsugi, S; Bajnok, L; Bretz, JD; Gauger, PG; Thompson, NW; Baker, JR. *Induction and regulation of Fas-mediated apoptosis in human thyroid epithelial cells*. Mol Endocrinol 2005; 19:804-811.

[70] Wang, SH; Bretz, JD; Phelps, E; Mezosi, E; Arscott, PL; Utsugi, S; Baker, JR. *A unique combination of inflammatory cytokines enhances apoptosis of thyroid follicular cells and transforms nondestructive thyroiditis in experimental autoimmune thyroiditis*. J Immunol 2002; 168:2470-2474.

[71] Mezosi, E; Yamazaki, H; Bretz, JD; Wang, SH; Arscott, PL; Utsugi, S; Gauger, PG; Thompson, NW; Baker, JR. *Aberrant apoptosis in thyroid epithelial cells from goiter nodules.* J Clin Endocrinol Metab 2002; 87:4264-4272.

[72] Mezosi, E; Wang, SH; Utsugi, S; Bajnok, L; Bretz, JD; Gauger, PG; Thompson, NW; Baker, JR. *Interleukin-1β and tumor necrosis factor (TNF)-α sensitize human thyroid epithelial cells to TNF-related apoptosis-inducing ligand-induced apoptosis through increases in procaspase-7 and Bid, and the down-regulation of p44/42 mitogen-activated protein kinase activity.* J Clin Endocrinol Metab 2004; 89:250-257.

[73] Brent GA. *Thyroid hormone action: down novel paths. Focus on „Thyroid hormone induces activation of mitogen-activated protein kinase in cultured cells".* Am J Physiol 1999; 276:C1012-C1013.

[74] Lin HY; Davis, FB; Gordinier, JK; Martino, LJ; Davis, PJ. *Thyroid hormone induces activation of mitogen-activated protein kinase in cultured cells*. Am J Physiol 1999; 276:C1014-C1024.

[75] Yen, PM. *Thyroid hormones and 3,5-diiodothyropropionic acid: New keys for new locks.* Endocrinol 2006; 147:1598-1601.

[76] Bergh, JJ; Lin, HY; Lansing, L; Mohamed, SN; Davis, FB; Mousa, S; Davis, PJ. *Integrin αVβ3 contains a cell surface receptor site for thyroid hormone that is linked to activation of mitogen-activated protein kinase and induction of angiogenesis.* Endocrinol 2005; 146:2864-2871.

[77] Yamada,KM; Araki, M. *Tumor suppressor PTEN: modulator of cell signaling, growth, migration and apoptosis.* J Cell Sci 2001; 114: 2375-2382.

[78] Pomerance, M, Abdullah, HB; Kamerji, S; Corréze, C; Blondeau, JP. *Thyroid-stimulating hormone and cyclic AMP activate p38 mitogen-activated protein kinase cascade.* J Biol Chem 2000; 275:40539-40546.

[79] Hiroi,Y; Kim, HH; Ying, H; Furuya, F; Huang, Z; Simoncini, T; Noma, K; Ueki, K; Nguyen, NH; Scanlan, TS; Moskowitz, MA; Cheng,S; Liao, JK. *Rapid nongenomic actions of thyroid hormone.* Proc Natl Acad Sci USA 2006; 103:14104-14109.

[80] Simoncini, T; Hafezi-Moghadam, A; Brazil, DP; Ley, K; Chin, WW; Liao, JK. *Interaction of oestrogen receptor with the regulatory subunit of phosphatidylinositol-3-OH kinase*. Nature 2000; 407: 538-541.

[81] Limbourg, FP; Huang, Z; Plumier, JC; Simoncini, T; Fujioka, M; Tuckermann, J; Schutz, G; Moskowitz, MA; Liao, JK. *Rapid nontranscriptional activation of endothelial nitric oxide synthase mediates increased cerebral blood flow and stroke protection by corticosteroids.* J Clin Invest 2002; 110:1729-1738.

[82] Mezosi, E; Szabo, J; Nagy, EV; Borbely, A; Varga, E; Paragh, G; Varga Z. *Nongenomic effect of thyroid hormone on free-radical production in human polymorphonuclear leukocytes*. J Endocrinol 2005; 185:121-129.

[83] Calza, L; Fernandez, M; Giuliani, A; Aloe, L; Giardino, L. *Thyroid hormone activates oligodendrocyte precursors and increases a myelinforming protein and NGF content in the spinal cord during experimental allergic encephalomyelitis*. Proc Natl Acad Sci USA 2002; 99:3258-3263.

[84] Frago, LM; Cañón, S; de la Rosa, EJ; León, Y; Varela-Nieto, I. *Programmed cell death in the developing inner ear is balanced by nerve growth factor and insulin-like growth factor I*. J Cell Sci 2002; 116:475-486.

[85] Tews, DS. *Role of apoptosis in myopathies*. Basic Appl Myol 2003; 13:181-190.

[86] Krook, A; Widegren, U; Jiang, XJ; Henriksson, J; Wallberg-Henriksson, H; Alessi, D; Zierath, JR. *Effects of exercise on mitogen- and stress-activated kinase signal transduction in human skeletal muscle*. Am J Physiol Regulatory Intergrative Comp Physiol 2000; 279:R1716-R1721.

[87] Harrington, EO; Smeglin, A; Parks, N; Newton, J; Rounds, S. *Adenosine induces endothelial apoptosis by activating protein tyrosine phosphatase: a possible role of p38α*. Am J Physiol Lung Cell Mol Physiol 2000; 279:L733-L742.

[88] Wang, S; Bray, P; McCaffrey, T; March, K; Hempstead, BL; Kraemer, R. *$p75^{NTR}$ mediates neurotrophin-induced apoptosis of vascular smooth muscle cells.* Am J Pathol 2000; 157:1247-1258.

[89] Relić, B; Guicheux, J; Mezin, F; Lubberts, E; Togninalli, D; Garcia, I; van den Berg, WB; Guerne, PA. *IL-4 and IL-13, but not IL-10, protect human synoviocytes from apoptosis.* J Immunol 2001; 166:2775-2782.

[90] Dechanet J, Taupin JL, Chomarat P, Rissoan MC, Moreau JF, Banchereau J, Miossec P. *Interleukin-4 but not interleukin-10 inhibits the production of leukemia inhibitory factor by rheumatoid synovium and synoviocytes.* Eur J Immunol 1994; 24:3222-3228.

[91] Seitz, M; Loetscher, P; Dewald, B; Towbin, H; Ceska, M; Baggiolini, M. *Production of interleukin-1 receptor antagonist, inflammatory chemotactic proteins, and prostaglandin E by rheumatoid and osteoarthritic synoviocytes: regulation by IFN-γ and IL-4.* J Immunol 1994; 152:2060-2065.

[92] Borghaei, RC; Rawlings, PL; Mochan, E. *Interleukin-4 suppression of interleukin-1-induced transcription of collagenase (MMP-1) and stromelysin 1 (MMP-3) in human synovial fibroblasts*. Arthritis Rheum 1998; 41:1398-1406.

[93] Asano, M; Toda, M; Sakaguchi, N; Sakaguchi, S. *Autoimmune disease as a consequence of developmental abnormality of a T cell subpopulation.* J Exp Med 1996; 184:387-396.

[94] Baecher-Allan, C; Hafler, DA. *Suppressor T cells in human diseases*. J Exp Med 2004; 200:273-276.

[95] Jiang, H; Chess, L. *An integrated view of suppressor T cell subsets in immunoregulation.* J Clin Invest 2004; 114:1198-1208.

[96] Sakaguchi, S; Sakaguchi, N; Asano, M; Itoh, M; Toda, M. *Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune disease*. J Immunol 1995; 155:1151-1164.

[97] Danke, NA; Koelle, DM; Yee, C; Beheray, S; Kwok, WW. *Autoreactive T cells in healthy individuals.* J Immunol 2004; 172:5967-5972.

[98] Viglietta, V; Kent, SC; Orban, T; Hafler, DA. *GAD65-reactive T cells are activated in patients with autoimmune type 1a diabetes.* J Clin Invest 2002; 109:895-903.

[99] Gambineri, E; Torgerson, TR; Ochs, HD. *T1-immune dysregulation, polyendocrinopathy, enteropathy, and X-linked inheritance (IPEX), a syndrome of systemic autoimmunity caused by mutations of FOXP3, a critical regulator of T-cell homeostasis*. Curr Opin Rheumatol 2003; 15:430-435.

[100] Rook, GA; Brunet, LR. *Microbes, immunoregulation, and the gut*. Gut 2005; 54:317-320.

[101] Marazuela, M; Garcia-López, MA; Figueroa-Vega, N; de la Fuente, H; Alvarado-Sánchez, B; Monsiváis-Urenda, A; Sánchez-Madrid, F; González-Amaro, R. *Regulatory T cells in human autoimmune thyroid disease*. J Clin Endocrinol Metab 2006; 91:3639-3646.

[102] Nocentini, G; Riccardi, C. *GITR: a multifaceted regulator of immunity belonging to the tumor necrosis factor superfamily*. Eur J Immunol 2005; 35:1016-1023.

[103] Cosmi, L; Liotta, F; Angeli, R; Mazzinghi, B; Santarlasci, V; Manetti, R; Lasagni, L; Vanini, V; Romagnani, P; Maggi, E; Annunziato, F; Romagnani, S. *Th2 cells are less susceptible than Th1 cells to the suppressive activity of $CD25^+$ regulatory thymocytes because of their responsiveness to different cytokines.* Blood 2004; 103:3117-3121.

[104] Jiang, H; Zhang, SI; Pernis, B. *Role of $CD8^+$ T cells in murine experimental allergic encephalomyelitis.* Science 1992; 256:1213-1215.

[105] Sakaguchi, S. *Control of immune responses by naturally arising $CD4^+$ regulatory T cells that express Toll-like receptors.* J Exp Med 2003; 197:397-401.

[106] O'Garra, A; Vieira, PL; Vieira, P; Goldfeld, AE. *IL-10-producing and naturally occurring CD4+ Tregs: limiting collateral damage*. J Clin Invest 2004; 114:1372-1378.

[107] Kaliński, P; Smits, HH; Schuitemaker, JH;Vieira, PL; van Eijk, M; de Jong, EC; Wierenga, EA; Kapsenberg, ML. *IL-4 is a mediator of IL-12p70 induction by human Th2 cells: Reversal of polarized Th2 phenotype by dendritic cells*. J Immunol 2000; 165:1877-1881.

[108] Caramalho, I; Lopes-Carvalho, T; Ostler, D; Zelenay, S; Haury, M; Demengeot, J. *Regulatory T cells selectively express Toll-like receptors and are activated by lipopolysaccharide*. J Exp Med 2003; 197:403-411.

[109] Linden, J. *New insights into the regulation of inflammation by adenosine*. J Clin Invest 2006; 116:1835-1837.

[110] Sitkovsky, M; Lukashev, D; Deaglio, S; Dwyer, K; Robson, SC; Ohta, A. *Adenosine A2A receptor antagonists: blockade of adenosinergic effects and T regulatory cells*. BrJ Pharmacol 2008; 153:S457-S464.

[111] Kriegel, MA; Lohmann, T; Gabler, C; Blank, N; Kalden, JR; Lorenz, HM. *Defective suppressor function of human $CD4^+$ $CD25^+$ regulatory T cells in autoimmune polyglandular syndrome type II.* J Exp Med 2004; 199:1285-1291.

[112] Lambiase, A; Bracci-Laudiero, L; Bonini, S; Bonini, S; Starace, G; D'Elios, MM; De Carli, M; Aloe, L. *Human CD4$^+$ T cell clones produce and release nerve growth factor and express high-affinity nerve growth factor receptors.* J Allergy Clin Immunol 1997; 100:408-414.

[113] Besser, M; Wank, R. *Cutting edge: Clonally restricted function of the neurotrophins brain-derived neurotrophic factor and neurotrophin-3 mRNA by human immune cells and Th1/Th2-polarized expression of their receptors*. J Immunol 1999; 162:6303-6306.

[114] Vega, JA; Garcia-Suárez, O; Hannestad, J; Pérez-Pérez, M; Germana, A. *Neurotrophins and the immune system*. J Anat 2003; 203:1-19.

[115] Bracci-Laudiero, L; Aloe, L; Buanne, P; Finn, A; Stenfors, C; Vigneti, E; Theodorsson, E; Lundeberg, T. *NGF modulates CGRP synthesis in human B-lymphocytes: a possible anti-inflammatory action of NGF?* J Neuroimmunol 2002; 123:58-65.

[116] Umeda, Y; Takamiya, M; Yoshizaki, H; Arisawa, M. *Inhibition of mitogen-stimulated T lymphocyte proliferation by calcitonin gene-related peptide*. Biochem Biophys Res Commun 1988; 154:227-235.

[117] Raud, J; Lundeberg, T; Brodda-Jansen, G; Theodorsson, E; Hedqvist, P. *Potent anti-inflammatory action of calcitonin gene-related peptide*. Biochem Biophys Res Commun 1991; 180:1429-1435.

[118] Steinman, L. *Connections between the immune system and the nervous system*. Proc Natl Acad Sci USA 1993; 90:7912-7914.

[119] Villoslada, P; Hauser, SL; Bartke, I; Unger, J; Heald, N; Rosenberg, D; Cheung, SW; Mobley, WC; Fisher, S; Genain, CP. *Human nerve growth factor protects common marmosets against autoimmune encephalomyelitis by switching the balance of T helper cell type 1 and 2 cytokines within the central nervous system*. J Exp Med 2000; 191:1799-1806.

[120] Brodie, C. *Differential effects of Th1 and Th2 derived cytokines on NGF synthesis by mouse astrocytes*. Fed Eur Biochem Societ Letters 1996; 394:117-120.

[121] Sloka, JS; Phillips, PW; Stefanelli, M; Joyce, C. *Co-occurrence of autoimmune thyroid disease in a multiple sclerosis cohort.* J Autoimmune Diseases 2005; 2:1-6.

[122] Falcini, F; Cerinic, MM; Lombardi, A; Generini, S; Pignone, A; Tirassa, P; Ermini, M; Lepore , L; Partsch, G; Aloe, L. *Increased circulating nerve growth factor is directly correlated with disease activity in juvenile chronic arthritis*. Am Rheum Dis 1996; 55:745-748.

[123] Matucci-Cerinic, M. *Sensory neuropeptides and rheumatic diseases.* Rheum Dis Clin North Am 1993; 19:975-991.

[124] Foreman, JC; Jordan, CC. *Neurogenic inflammation.* Trends Pharmacol Sci 1984; 5:116-119.

[125] Tuveri, M; Generini, S; Matucci-Cerinic, M; Aloe, L. *NGF, a useful tool in the treatment of chronic vasculitic ulcers in rheumatoid arthritis*. Lancet 2000; 356:1739-1740.

[126] Marucci-Cerinic, M; Giacomelli, R; Pignone, A; Cagnoni, ML; Generini, S; Casale, R; Cipriani, P; Del Rosso, A; Tirassa, P; Konttinen, YT; Kahaleh, BM; Fan, PS; Paoletti, M; Marchesi, C; Cagnoni, M; Aloe, L. *Nerve growth factor and neuropeptides circulating levels in systemic sclerosis (scleroderma).* Am Rheum Dis 2001; 60:487-494.

[127] Kawamoto, K; Aoki, J; Tanaka, A; Itakura, A; Hosono, H; Arai, H; Kiso, Y; Matsuda, H. *Nerve growth factor activates mast cells through the collaborative interaction with lysophosphatidylserine expressed on the membrane surface of activated platelets*. J Immunol 2002; 168:6412-6419.

[128] Chrousos, GP. *The stress response and immune function: Clinical implications. The 1999 Novera H. Spector Lecture*. Ann NY Acad Sci 2000; 917:38-67.

[129] Chrousos, GP; Gold, PW. Editorial: *A healthy body in a healthy mind – and vice versa – The damaging power of „uncontrollable" stress*. J Clin Endocrinol Metab 1998; 83:1842-1845.

[130] Chrousos, GP. *Stressors, stress, and neuroendocrine integration of the adaptive response. The 1997 Hans Selye Memorial Lecture.* Ann NY Acad Sci 1998; 851:311-335.

[131] Tsatsoulis, A; Fountoulakis, S. *The protective role of exercise on stress system dysregulation and comorbidities.* Ann NY Acad Sci 2006; 1083:196-213.

[132] Kyrou, I; Chrousos, GP; Tsigos, C. *Stress, visceral obesity, and metabolic complications.* Ann NY Acad Sci 2006; 1083:77-110.

[133] Aloe,L; Bracci-Laudiero, L; Alleva, E; Lambiase, A; Micera, A; Tirassa, P. *Emotional stress induced by parachute jumping enhances blood nerve growth factor levels and the distribution of nerve growth factor receptors in lymphocytes.* Proc Natl Acad Sci USA 1994; 91:10440-10444.

[134] Elenkov, IJ; Wilder, RL; Chrousos, GP; Vizi, ES. *The sympathetic nerve – An integrative interface between two supersystems: The brain and the immune system.* Pharmacol Rev 2000; 52:595-638.

[135] Eskandari, F; Webster, JI; Sternberg, EM. *Neural immune pathways and their connection to inflammatory diseases.* Arthritis Res Ther 2003; 5:251-265.

[136] Jara, LJ; Navarro, C; Medina, G; Vera-Lastra, O; Blanco, F. *Immune-neuroendocrine interactions and autoimmune diseases.* Clin Dev Immunol 2006; 13:109-123.

[137] Bağriaçik, EU; Klein, JR. *The thyrotropin (Thyroid-stimulating hormone) receptor is expressed on murine dendritic cells and on a subset of CD45RBhigh lymph node T cells: functional role for thyroid-stimulating hormone during immune activation*. J Immunol 2000; 164:6158-6165.

[138] Vizi, ES, Elenkov, IJ. *Nonsynaptic noradrenaline release in neuro-immune responses*. Acta Biol Hung 2002; 53:229-244.

[139] Sternberg, EM. *Neuroendocrine regulation of autoimmune/ inflammatory disease*. J Endocrinol 2001; 169:429-435.

[140] Miller, GE; Cohen, S; Ritchey, AK. *Chronic psychological stress and the regulation of pro-inflammatory cytokines: A glucocorticoid-resistance model*. Health Psychology 2002; 21:531-541.

[141] Verge, VMK; Richardson, PM; Wiesenfeld-Hallin, Z; Hökfelt, T. *Differential influence of nerve growth factor on neuropeptide expression in vivo: A novel role in peptide suppression in adult sensory neurons*. J Neurosci 1995; 15:2081-2096.

[142] Bucelli, RC; Gonsiorek, EA; Kin, WY; Bruun, D; Rabin, RA; Higgins, D; Lein, PJ. *Statins decrease expression of the proinflammatory neuropeptides calcitonin gene-related peptide and substance P in sensory neurons*. J Pharmacol Exp Ther 2008; 324:1172-1180.

[143] Straub, RH; Miller, LE; Schölmerich, J; Zietz, B. *Cytokines and hormones as possible links between endocrinosenescence and immunosenescence*. J Neuroimmunol 2000; 109:10-15.

[144] Elenkov, IJ; Chrousos, GP. *Stress, hormones, proinflammatory and antiinflammatoty cytokines, and autoimmunity*. Ann NY Acad Sci 2002; 966:290-303.

[145] Elenkov, IJ. *Neurohormonal-cytokine interactions: implications for inflammation, common human diseases and well-being*. Neurochem Int 2008; 52:40-51.

[146] Link, AA; Kino, T; Worth, JA; McGuire, JL; Crane, ML; Chrousos, GP, Wilder, RL; Elenkov IJ. *Ligand-activation of the adenosine A2a receptors inhibits IL-12 production by human monocytes*. J Immunol 2000; 164:436-442.

[147] Falus, A; Merétey, K. *Histamine: an early messenger in inflammatory and immune reactions.* Immunol Today 1992; 13:154-156.

[148] Goyarts, E; Matsui, M; Mammone, T; Bender, AM; Wagner, JA; Maes, D; Granstein, RD. *Norepinephrine modulates human dendritic cell activation by altering cytokine release.* Exp Dermatol 2008; 17:188-196.

[149] Ortega, E; Marchena, JM, Garcia, JJ; Barriga, C; Rodríguez, AB. *Norepinephrine as mediator in the stimulation of phagocytosis induced by moderate exercise*. Eur J Appl Physiol 2005; 93:714-718.

[150] Denef, C. *Paracrinicity: The story of 30 years of cellular pituitary crosstalk.* J Neuroendocrinol 2008; 20:1-70.

[151] Hadjiconstantinou, M; McGuire, L; Duchemin, AM; Laskowski, B; Kiecolt-Glaser, J; Glaser, R. *Changes in plasma nerve growth factor levels in older adults associated with chronic stress.* J Neuroimmunol 2001; 116:102-106.

[152] Hristova, M; Aloe, L. *Metabolic syndrome – Neurotrophic hypothesis*. Med Hypotheses 2006; 66:545-549.

[153] Kiecolt-Glaser, JK; Preacher, KJ; MacCallum, RC; Atkinson, C; Malarkey, WB; Glaser, R. *Chronic stress and age-related increases in the proinflammatory cytokine IL-6.* Proc Natl Acad Sci USA 2003; 100:9090-9095.

[154] Jeanneteau, F; Garabedian, MJ; Chao, MV. *Activation of Trk neurotrophin receptors by glucocorticoids provides a neuroprotective effect*. Proc Natl Acad Sci USA 2008; 105:4862-4867.

[155] Romero, C; Paredes, A; Dissen, GA; Ojeda, SR. *Nerve growth factor induces the expression of functional FSH receptors in newly formed follicles of the rat ovary*. Endocrinol 2002; 143:1485-1494.

[156] Bulló, M; Peeraully, MR; Trayhurn, P; Folch, J; Salas-Salvadó, J. *Circulating nerve growth factor levels in relation to obesity and the metabolic syndrome in women*. Eur J Endocrinol 2007; 157:303-310.

[157] Trayhurn, P. *Adipose tissue in obesity – An inflammatory issue*. Endocrinol 2005; 146:1003-1005.

[158] Rayhaudhuri, SK; Raychaudhuri, SP, Weltman, H; Farber, EM. *Effect of nerve growth factor on endothelial cell biology: proliferation and adherence molecule expression on human dermal microvascular endothelial cells.* Arch Dermatol Res 2001; 293:291-295.

[159] Safieh-Garabedian, B; Poole, S; Haddad, JJ; Massaad, CA; Jabbur, SJ; Saadé, NE. *The role of the sympathetic efferents in endotoxin-induced localized inflammatory hyperalgesia and cytokine upregulation.* Neuropharmacol 2002; 42:864-872.

[160] Thacker, MA; Clark, AK; Marchand, F; McMahon, SB. *Pathophysiology of peripheral neuropathic pain: Immune cells and molecules.* Anesth Analg 2007; 105:838-847.
[161] Bracci-Laudiero, L; Aloe, L; Buanne, P; Finn, A; Stenfors, C; Vigneti, E; Theodorsson, E; Lundeberg, T. *NGF modulates CGRP synthesis in human B-lymphocytes: a possible anti-inflammatory action of NGF?* J Neuroimmunol 2002; 123:58-65.
[162] Ruiz, G; Baňos, JE. *The effect of endoneurial nerve growth factor on calcitonin gene-related peptide expression in primary sensory neurons*. Brain Res 2005; 1042:44-52.
[163] Indo, Y; Tsuruta, M; Hayashida, Y; Karim, MA; Ohta, K; Kawano, T; Mitsubuchi, H; Tonoki, H; Awaya, Y; Matsuda, I. *Mutations in the TRKA/NGF receptor gene in patients with congenital insensitivity to pain with anhidrosis*. Nat Genet 1996; 13:485-488.
[164] Barnes, PJ. *Anti-inflammatory actions of glucocorticoids: molecular mechanisms*. Clin Sci (Lond) 1998; 94:557-572.
[165] Elenkov, IJ; Chrousos, GP. *Stress hormones, Th1/Th2 patterns, pro/anti-inflammatory cytokines and susceptibility to disease.* Trends Endocrinol Metab 1999; 10:359-368.
[166] Karalis, K; Sano, H; Redwine, J; Listwak, S; Wilder, RL; Chrousos, GP. *Autocrine or paracrine inflammatoty actions of corticotropin-releasing hormone in vivo*. Science 1991; 254:421-423.
[167] Chrousos, GP. *The hypothalamic-pituitary-adrenal axis and immune-mediated inflammation.* N Eng J Med 2005; 332: 1351-1362.
[168] Mastorakos, G; Karoutsou, EI; Mizamtsidi, M. *Corticotropin releasing hormone and the immune/inflammatory response.* Eur J Endocrinol 2006; 155:S77-S84.
[169] Tsatsoulis, A. *The role of stress in the clinical expression of thyroid autoimmunity*. Ann NY Acad Sci 2006; 1088:382-395.
[170] Bullock, ED; Johnson, EM. *Nerve growth factor induces the expression of certain cytokine genes and bcl-2 in mast cells*. J Biol Chem 1996; 271:27500-27508.

4. ENHANCED SYMPATHOADRENAL AND NERVE GROWTH FACTOR ACTIVITIES IN THE TISSUE HYPERTROPHY

4.1. The Enhanced Sympathoadrenal Activity and the Modulatory Effects of Catecholamines on the Nerve Growth Factor

The beneficial effects of NGF on the nervous system are characterized by an increase of the sympathetic innervation, as well as by the intactness of sympathetic and sensory neurons. It is established that the mammalian sympathetic and sensory effector organs express NGF; therefore, they have the ability to synthesize them [1]. The sympathetic neural control of the heart represents a special aspect. The high expression of NGF contributes to an enhancement in the neuronal re-uptake of norepinephrine [2]. The causalgia and the pathological pain suggest an abnormal sympathetic function and an increased expression of the α-adrenergic receptor [3].

The cardiac sympathetic nerves regulate the heart rate, the contractility and the conduction velocity. The mechanical stretch or the pressure overload initiates the productions

of growth factors and cytokines; this then triggers hypertrophy as well as hyperplasia, reduced norepinephrine uptake and synthesis, and fetal gene expression [4]. The pressure overload induces cardiac hypertrophy and sympathetic hyperinnervation along with the simultaneously deteriorating neuronal cellular function. NGF modulates the synaptic transmissions between the sympathetic neurons and the target cells, including the cardiac myocytes. NGF acts via TrkA receptors and plays both acute and long-term roles in the regulation of the sympathetic synapse formation in the cardiac system [5].

However, catecholamines may regulate the synthesis or secretion of NGF in the astroglial and fibroblast cells [6, 7]. The influencing effects of catecholamines on the NGF content from the target cells are growth-dependent. Epinephrine or dopamine elevates the levels of NGF only in quiescent cells; in contrast, decreases NGF levels in growing cells [6]. But some findings suggest that a direct effect of the catechol ring stays in the backround of the NGF stimulation. The NGF production promoted by the catechol ring is not mediated by adrenergic receptors [7].

The cardiac sympathetic efferents at the postganglionic nerve terminals are abnormal in chronic heart failure, demonstrating an evidence of the decreased norepinephrine uptake activity. Therefore, an increase in the norepinephrine content could be detected in the cardial tissue interstitium. But norepinephrine may reduce the NGF content, inhibiting its ownprotein synthesis and mRNA expression [8]. These data emphasize that the protection of NGF is necessary for normal cardiac function. Kaye and co-workers found that the degree of the NGF reduction may reach 40% of the myocardial production, and it associates with cardiac sympathetic overactivity [9]. The lower NGF content in the diabetic myocardium is due to similar causes [10].

By another way, the activation of the β-adrenergic receptors can stimulate the NGF production in the astrocytoma cells, highlighting the cAMP mediated mechanism in the neuronal protection [11].

Glucocorticoids are involved in the neuronal plasticity by increasing the synthesis of NGF [12]. The trophic effects of the corticosteroids are mediated by an increase in NGF synthesis, and are enforced through the maturation of the postnatal cholinergic neurons, which causes a hypertrophy of the medial septum in the heart. NGF is a trophic factor for the cholinergic neurons in the basal forebrain and striatum. After adrenalectomy, NGF expression decreases in the hippocapmus and the cerebral cortex.

The trophic effects of NGF could be manifested in the angiogenesis and the arteriogenesis; moreover, the catecholamines also potentiate these effects. Ischemia is a relevant factor for the release of NGF and catecholamines; it accelerates the neovascularization and stimulates the proliferation of vascular endothelial cells [13, 14]. Ischemia increases NGF production and the expression of TrkA receptors. These neural and vascular stimulatory features of ischemia promote the tissue repair. NGF itself acts as a local vasodilator but has no systematic effects on blood pressure.

Catecholamines exert a growth-factor-like activity via the activation of the α1-adrenergic receptors, leading to hyperplasia and hypertrophy of the arterial smooth muscle cells and the adventitial fibroblasts [14]. Norepinephrine also acts as a trophic mediator for the normal and the diseased vascular walls; furthermore, it is responsible for the intimal expansion and the loss of lumen after the injury [15]. The α1-adrenoreceptor stimulation leads to the hypertrophy and stiffness of the wall, as well as to the severity of atherosclerosis. Recent studies highlight that norepinephrine may also increase the collagen content of the neointima.

Thyroid hormones provide a special route for the cardiac hypertrophy and arrhythmia initiated by excess catecholamines. Thyroid hormones directly influence (increase or decrease) the expression or the function of the stimulatory elements of the β-adrenergic cascade [16]. Their regulatory effects on the β-adrenergic signaling can modulate the adrenergic receptor-mediated adenylate cyclase activity. Therefore, thyroid hormones may act directly on the contractile elements of the myocytes such as the endoplasmic reticulum Ca^{++} pump and phosphorlamban (SERCA) [17].

4.2. Relationhip Between the Enhanced Sympathoadrenal Activity and the Tissue Hypertrophy

4.2.1. Catecholamines-Induced Cardiac Hypertrophy

Cardiac hypertrophy could be viewed as an adaptive response that associates with an enhancement of the sympathetic activity as well as with an impairment of contractility, often leading to heart failure. Both α- and β-adrenergic receptor stimulations can contribute to the myocardial hypertrophic events. The cardiac hypertrophy is an abnormal increase of the heart's muscle mass, which associates with changes to the shape and volume of myocyte, as well as with functional, mechanistical, and histiological impairments [18]. Stress stimulates the physiological and pathological cell hypertrophy. The genes of the brain natriuretic peptide, the angiotensin-coverting enzyme, the endothelin receptor and the β-adrenergic receptor kinase are upregulated in the pathological form but not by exercise. The PI-3K/Akt pathway represents a signaling route for both the physiological and the exercise induced hypertrophy. The cross-talk between the PI-3K and protein kinase C signaling cascades explains the mechanism of the pathological hypertrophy.

The adrenergic signaling pathway of the α- or β-adrenergic receptors is triggered by the activation of the sympathetic nervous system. [19, 20]. The β-adrenergic receptors are the predominant ones in the heart [21]. In turn, autoantibodies againts the second extracellular loop of the β1-adrenergic receptors can induce myocardial hypertrophy and the desensitization of the β-adrenergic receptors [22].

The apoptosis events become more relevant in the heart failure, disrupting the balance between the hypertrophic and death cascades [23]. MAPK activities are implicated in the receptor signaling of the cardiac hypertrophy. A distinct action of p38 kinases that affects β rather than α adrenergic receptor signaling, could be demonstrated in the increase of the cardiac mass and volume, but not in the apoptosis caused by the heart failure. It seems that a certain minimal adrenergic tone is necessary for the myocyte survival [24]. The high levels of norepinephrines possess a stimulatory effect on the myocyte apoptosis, which activates the β-adrenergic pathway [25]. The β1- and β2-adrenerg receptors expressed on the myocytes lead to opposing effects in the apoptosis events [26]. The β1-adrenergic receptor activation increases the apoptosis via a cAMP- dependent mechanism, while the β2-adrenergic receptor activation inhibits the apoptosis via the Gi coupled pathway.

Recently, a common signaling pathway of the β-adrenergic receptors was found to be responsible for the cardiac hypertrophy and the apoptotic heart failure [27]. The crucial point of the mechanism is represented by the Gq signaling cascade directing to cardiac hypertrophy. The concomitant activation of Gαq leads to apoptosis. The cross-talking activation between

the adrenergic receptor stimulations – in this case the G protein-coupled receptors - exert a dual modulatory effect on the cell survival and the cell death [28]. In fact, the β2-adrenergic receptor signaling can initiate apoptosis via a Gs mediated protein kinase A dependent mechanism, but it also activates the PI-3K dependent survival cascade. This duality is displayed by a switching in the coupling of the β2-adrenergic receptor (to a different G protein, e.g. Gs to Gi) so that the protein kinase A phosphorylation is activated by a different G protein [29] (Figure 39).

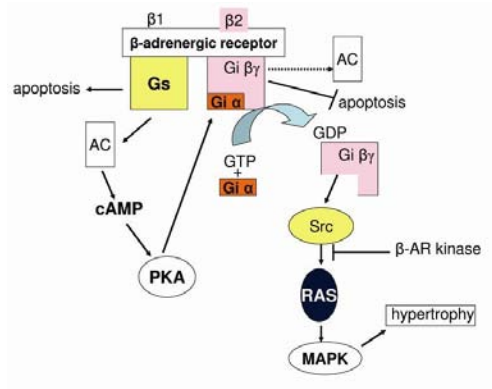

Figure 39. Switching of the coupling between β1 and β2 adrenergic receptors. The cross-talk in the signaling pathways between the adrenergic or G protein-coupled receptors (GPCR) represents a dual modulatory effect in the cell survival and cell death. Gs in the GPCR activates protein kinase A (PKA) via the cyclic adenylate monophosphate (cAMP) depentent route. The binding of PKA to the Giα part of the receptor cleaves the Giβγ part from the GPCR and causes mitogen-activated protein kinase (MAPK) activation via a cAMP independent route. The switching leads to the desensitization of the β-adrenergic receptor (β-AR) and it potentiates the hypertrophy via the cell survival signaling cascades. AC: adenylate cyclase; Src: regulatory protein; RAS: oncogene; GTP: guanosine triphosphate; GDP: guanosine diphosphate; Gsα or Giα and Gβγ : the parts of G protein receptors; → : action direction; ⊣ : action inhibition; - - → : decreased action.

The stimulation of the Gs protein-coupled receptor leads to protein kinase A activation via a cAMP dependent route. However, the protein kinase A mediated β-adrenergic receptor phosphorylation is the key for the desensitization of the heterologous receptors and the reduction of the cAMP activities. In the desensitization mechanism, the β2-adrenergic receptor activation switches from Gs protein signaling to Gi protein signaling, so that the protein kinase A phosphorylation will be less able to stimulate Gs than Gi [30,31]. The Gi protein-coupled signaling pathway is cAMP independent and the MAPK activation leads to cell proliferation, differentiation and growth. The β-adrenergic receptor desensitization and the myocardial hypertrophy have occurred due to the increased levels of the β-adrenergic receptor kinase-1 (βARK1) activation [32]. β-adrenergic receptor kinase-1 is a member of G receptor kinases.

4.2.2. Catecholamines in the Skeletal Muscle Hypertrophy

4.2.2.1. Phosphatidylinositol 3-Kinase (PI-3K) in the Myogenesis

PI-3K activation is a common element of the different signaling cascades in the regulation of the hormonal effects and other metabolic affections that can lead to cell

proliferation, survival, trafficking, migration, and apoptosis [33]. A special role of PI-3K has been highlighted by the myogenesis [34]. In the myogenesis, the differentiation is dependent on the activation of integrins - the membrane proteins of the myoblasts. It should be mentioned that the thyroid hormones exhibit a direct nongenomic action that acts through the binding to integrins. The integrin is a member of the membrane receptor proteins. The insulin-like growth factor-1 (IGF-1) is considered to be a positive regulator of the skeletal muscle differentiation. Its myogenic effects are mediated by PI-3K activation. IGF-1 and its receptors are expressed on the skeletal and heart muscles, and also on the fibroblasts. In addition to IGF-1, IGF-2 is also a potent stimulator of the muscle differentiation. Both are satellite cell regulators. Satellite cells lie on the periphery of the muscle fibers. IGF-1 stimulation results in myofiber hypertrophy, so that it is considered as a crucial growing factor for the myofibers. PI-3K not only activates the muscle cell differentiation, but it also plays a central role in the muscle regeneration after the injury. In fact, the expression of IGF-1 receptors on the myocytes emphasizes the role of IGF-1 in the cardiac hypertrophy.

Furthermore, IGF-1's action seems to be beneficial during the regenerating events after an acute myocardial infarction.

The signaling cascade initiated by IGF-1 protects the myocytes and the fibroblasts from the apoptosis [35]. The activation of the Trk receptors is essential for the antiapoptotic actions. IGF-1 receptors belong to the Trk receptors. The binding of IGF-1 to its receptor causes its autophosphorylation, which recruits the insulin receptor substrate proteins. The binding reaction is critical for the proliferative and differentative actions of IGF-1 [36]. The downstream signaling pathway of PI-3K results in Akt/protein kinase B and MAPK activations, which are required for the protection against apoptosis. There are differences between the MAPK cascade stimulations induced by epidemal growth factor and IGF-1. This difference is explained by their distinct apoptotic features, because the epidemal growth factor induced MAPK action does not prevent the apoptosis.

4.2.2.2. Skeletal Muscle Hypertrophy

The size, mass and volume of the skeletal muscles are modulated by several factors, and are associated with aging [37]. The properties of the muscle fibers are characterized by the tendency to the shift towards faster fiber types in the acute atrophy; whereas, chronic atrophy manifests itself in a decrease in the total fiber number, with the selective atrophy of the faster types. The myofibers are postmitotic, multinucleated cells; their regeneratory processes are initiated by satellite cells activated by IGF-1 on the peripheries of the muscle cells.

Paul and co-workers reported on the distinct types of the skeletal muscle fiber hypertrophy [38]. Two types of muscle hypertrophy could be distinguished depending on whether the nerve terminals had one or two endplate. Having one endplate band on the muscle fibers increases the fiber diameter while having two endplate band leads to the elongation of the muscle fiber.

The action of catecholamines on the skeletal muscles is complex [39]. Mainly, they affect the energy metabolic routes via the catabolic effects – which decrease the glycogen and lipid levels, - or they contribute to the muscle contraction and the blood flow via the anabolic effects. The effects of catecholamines can manifest themselves on the skeletal muscle cells through the stimulation of G protein-coupled receptors. Epinephrine may regulate several gene expressions of the human skeletal muscles, some of which are involved in the immune and inflammatory responses [40]. It is not surprising that catecholamines have profound

effects on the protein metabolism of the skeletal muscles. By potentiating the β2-adrenergic receptor activation, catecholamines induce a skeletal muscle hypertrophy.

IGF-1 is essential for normal growth and development, but it also represents a major mediator for the postnatal growth promoting actions. The liver is the predominant source of IGF-1, but large amounts of IGF-1 are also expressed in the muscle, brain, and kidney tissues. The production of IGF-1 by the local tissue is involved in the normal growth and muscle-repairing processes [41]. IGF-1 acts directly on the muscle fibers, consequently increasing the protein synthesis and the muscle mass; but its stimulation of the satellite cells may further contribute to the fusion of the existing muscle fibers.

REFERENCES

[1] Shelton, DL; Reichardt, LF. *Expression of the β-nerve growth factor gene correlates with the density of sympathetic innervation in effector organs*. Proc Natl Acad Sci USA 1984; 81:7951-7955.

[2] Esler, M; Kaye, D. *Sympathetic nervous system neuroplasticity*. Hypertension 2006; 47:143-144.

[3] Perl, ER. *Causalgia, pathological pain, and adrenergic receptors*. Proc Natl Acad Sci USA 1999; 96:7664-7667.

[4] Kimura, K; Ieda, M; Kanazawa, H; Yagi, T; Tsunoda, M; Ninomiya, S; Kurosawa, H; Yoshimi, K; Mochizuki, H; Yamazaki,K; Ogawa, S; Fukuda, K. *Cardiac sympathetic rejuvenation. A link between nerve function and cardiac hypertrophy*. Circ Res 2007; 100:1755-1764.

[5] Lockhart, ST; Turrigiano, GG; Birren, SJ. *Nerve growth factor modulates synaptic transmission between sympathetic neurons and cardiac myocytes*. J Neurosci 1997; 17:9573-9582.

[6] Furukawa, S; Furukawa, Y; Satoyoshi, R; Hayashi, K. *Regulation of nerve growth factor synthesis/secretion by catecholamine in cultured mouse astroglial cells.* Biochem Biophys Res Commun 1987; 147:1048-1054.

[7] Furukawa, Y; Tomioka, N; Sato, W; Satoyoshi, E; Hayashi, K; Furukawa, S. *Catecholamines increase nerve growth factor mRNA content in both mouse astroglial cells and fibroblast cells*. Fed Eur Biochem Societ 1989; 247:463-467.

[8] Qin, F; Vulapalli, RS; Stevens, SY; Liang, CS. *Loss of cardiac sympathetic neurotransmitters in heart failure and NE infusion is associated with reduced NGF*. Am J Physiol Heart Circ Physiol 2002; 282:E363-E371.

[9] Kaye, DM; Vaddadi, G; Gruskin, SL; Du, XJ; Esler, MD. *Reduced myocardial nerve growth factor expression in human and experimental heart failure*. Circ Res 2000; 86:80-84.

[10] Schmid, H; Forman, LA; Cao, X; Sherman, PS; Stevens, MJ. *Heterogeneous cardiac sympathetic denervation and decreased myocardial nerve growth factor in Streptozotocin-induced diabetic rats*. Diabetes 1999; 48:603-608.

[11] Mocchetti, I; De Bernardi, MA; Szekely, AM; Alho, H; Brooker, G; Costa, E. *Regulation of nerve growth factor biosynthesis by β-adrenergic receptor activation in*

astrocytoma cells: A potential role of c-Fos protein. Proc Natl Acad Sci USA 1989; 86:3891-3895.

[12] Shi, B; Rabin, SJ; Brandoli, C; Mocchetti, I. *Dexamethasone induces hypertrophy of developing medial septum cholinergic neurons: Potential role of nerve growth factor*. J Neurosci 1998; 18:9326-9334.

[13] Emanuali, C; Salis, MB; Pinna, A; Graiani, G; Manni, L; Madeddu, P. *Nerve growth factor promotes angiogenesis and arteriogenesis in ischemic hindlimbs.* Circulation 2002; 106: 2257-2262.

[14] Chalothorn, D; Zhang, H; Clayton, JA, Thomas, SA; Faber, JE. *Catecholamines augment collateral vessel growth and angiogenesis in hindlimb ischemia*. Am J Physiol Heart Circ Physiol 2005; 289:H947-H959.

[15] Erami, C; Zhang, H; Ho, JG; French, DM; Faber, JE. *α1-adrenoreceptor stimulation directly induces growth of vascular wall in vivo.* Am J Physiol Heart Circ Physiol 2002; 283:H1577-H1587.

[16] Kim, B; Carvalho-Bianco, SD; Larsen, PR. *Thyroid hormone and adrenergic signaling in the heart*. Arq Bras Endocrinol Metab 2004; 48:171-175.

[17] Lohse, MJ; Engelhardt, S; Eschenhagen, T. *What is the role of β-adrenergic signaling in heart failure?* Circ Res 2003; 93:896-906.

[18] Dorn II, GW. *The fuzzy logic of physiological cardiac hypertrophy*. Hypertension 2007; 49:962-970.

[19] Du, XJ. *Sympathoadrenergic mechanisms in functional regulation and development of cardiac hypertrophy and failure: findings from genetically engineered mice*. Cardiovasc Res 2001; 50:443-453.

[20] Barki-Harrington, L; Perrino, C; Rockman, HA. *Network integration of the adrenergic system in cardiac hypertrophy*. Cardiovasc Res 2004; 63:391-402.

[21] Engelhardt, S; Hein, L; Wiesmann, F; Lohse, MJ. *Progressive hypertrophy and heart failure in β_1-adrenergic receptor transgenic mice*. Proc Natl Acad Sci USA 1999; 96:7059-7064.

[22] Iwata, M; Yoshikawa, T; Baba, A; Anzai, T; Nakamura, I; Wainai, Y; Takahashi, T; Ogawa, S. *Autoimmunity against the second extracellular loop of β_1-adrenergic receptors induces β-adrenergic receptor desensitization and myocardial hypertrophy in vivo*. Circ Res 2001; 88:578-586.

[23] Wang, Y; Huang, S; Sah, VP; Ross, J; Brown, JH; Han, J; Chien, KR. *Cardiac muscle cell hypertrophy and apoptosis induced by distinct members of the p38 mitogen-activated protein kinase family*. J Biol Chem 1998; 273: 2161-2168.

[24] Henaff, M; Hatem, SN; Mercadier, JJ*: Low catecholamine concentration protect adult rat ventricular myocytes against apoptosis through cAMP-dependent extracellular signal-regulated kinase activation.* Mol Pharmacol 2000; 58:1546-1553.

[25] Communal, C; Singh, K; Pimentel, DR; Colucci, WS. *Norepinephrine stimulates apoptosis in adult rat ventricular myocytes by activation of the β-adrenergic pathway*. Circulation 1998; 98:1329-1334.

[26] Communal, C; Singh, K; Sawyer, DB; Colucci, WS. *Opposing effects of β_1- and β_2-adrenergic receptors on cardiac myocyte apoptosis. Role of a Pertussis toxin-sensitive G protein*. Circulation 1999; 100:2210-2212.

[27] Adams, JW; Sakata, Y; Davis, MG; Sah, VP; Wang, Y; Liggett, SB; Chien, KR; Brown, JH; Dorn, GW. *Enhanced Gαq signaling: A common pathway mediates cardiac*

hypertrophy and apoptotic heart failure. Proc Natl Acad Sci USA 1998; 95:10140-10145.

[28] Zhu, WZ; Zheng, M; Koch, WJ; Lefkowitz, RJ; Kobilka, BK; Xiao, RP. *Dual modulation of cell survival and cell death by β_2-adrenergic signaling in adult mouse cardiac myocytes*. Proc Natl Acad Sci USA 2001; 98:1607-1612.

[29] Daaka, Y; Luttrell, LM; Lefkowitz, RJ. *Switching of the coupling of the β_2-adrenergic receptor to different G proteins by protein kinase* A. Nature 1997; 390:88-91.

[30] Zou, Y; Komuro, I; Yamazaki, T; Kudoh, S; Uozumi, H; Kadowaki,T; Yazaki, Y. *Both G_s and G_i proteins are critically involved in isoproterenol-induced cardiomyocyte hypertrophy*. J Biol Chem 1999; 274:9760-9770.

[31] Zamah, AM; Delahunty, M; Luttrell, LM; Lefkowitz, RJ. *Protein kinase A-mediated phosphorylation of the β_2-adrenergic receptor regulates its coupling to G_s and G_i*. J Biol Chem 2002; 277:31249-31256.

[32] Iaccarino,G; Dolber, PC; Lefkowitz, RJ; Koch, WJ. *β-adrenergic receptor kinase-1 levels in catecholamine-induced myocardial hypertrophy. Regulation by β- but not α_1-adrenergic stimulation.* Hypertension 1999; 33:396-401.

[33] Hirsch, E; Costa, C; Ciraolo, E. *Phosphoinositide 3-kinases as a common platform for multi-hormone signaling.* J Endocrinol 2007; 194:243-256.

[34] Kaliman, P; Zorzano, A. *Phosphatidylinositol 3-kinase in myogenesis*. Trends Cardiovasc Med 1997; 7:198-202.

[35] Kulik, G; Klippel, A; Weber, MJ. *Antiapoptotic signalling by the insulin-like growth factor I receptor, phosphatidylinositol 3-kinase, and Akt.* Mol Cell Biol 1997; 17:1595-1606.

[36] Philippou, A; Halapas, A; Maridaki, M; Koutsilieris, M. *Type I insulin-like growth factor receptor signaling in skeletal muscle regeneration and hypertrophy*. J Musculoskelet Neuronal Interact 2007; 7:208-218.

[37] Barton, E; Morris, C. *Mechanisms and strategies to counter muscle atrophy*. J Gerontol 2003; 58:M923-926.

[38] Paul, AC; Rosenthal, N. *Different modes of hypertrophy in skeletal muscle fibers*. J Cell Biol 2002; 156:751-760.

[39] Arner, P. *Editorial: Novel target genes for catecholamines in skeletal muscle*. J Clin Endocrinol Metab 2004; 89:1998-1999.

[40] Viguerie, N; Clement, K; Barbe, P; Courtine, M; Benis, A; Larrouy, D; Hanczar, B; Pelloux, V; Poitou, C; Khalfallah, Y; Barsh, GS; Thalamas, C; Zucker, JD; Langin, D. *In vivo epinephrine-mediated regulation of gene expression in human skeletal muscle*. J Clin Endocrinol Metab 2004; 89:2000-2014.

[41] Barton, ER. *The ABCs of IGF-I isoforms: impact on muscle hypertrophy and implications for repair*. Appl Physiol Nutr Metab 2006; 31:791-797.

5. NERVE GROWTH FACTOR IN TISSUE RESTITUTION

5.1. Nerve Growth Factor in Wound Healing

The injury of the skin initiates inflammatory reactions, the formation of a new tissue and tissue remodeling in order to reconstruct the wounded area [1]. Several factors are released during the healing processes, such as growth factors, cytokines, low-molecular-weight compounds. The disruption of a blood vessel leads to a blood clot composed of cross-linked fibrin and extracellular matrix proteins. A few hours after the injury, inflammatory and immune cells invade the wound area. The growth factors that are secreted from the invading cells are necessary at the later stage of the healing processes. In the intermediary stage, a massive angiogenesis and nerve sprouting are accelerated and form the granulation tissue. The final stage is characterized by the migration and proliferation of keratinocytes the transformation of fibroblast → to myofibroblast, as well as the deposition of collagen (Figure 40) [2].

NGF is implicated in the cutaneous wound repair, while the exogenous NGF can accelerate the healing processes. The role of NGF in the tissue repair is the stimulation of the nerve ingrowth. The newbuilding of the sensory nerve is required for the angiogenesis, in which contains several processes: the interaction among the new dermal microvascular endothelial cells, fibroblasts and smooth muscle cells, as well as the hypertrophic effects of NGF [3].

The submandibular glands secrete a high amounts of NGF it into the saliva, which highlights their wound healing promoting effects [4].

However, the activation of the β2-adrenergic receptors can delay the wound healing [5]. β-adrenergic receptors are expressed in a wide variety of tissues of cardiac, pulmonary, vascular, endocrine and central nervous origins. The β2-adrenergic receptor subtypes are expressed mainly in cells that make up the skin, such as keratinocytes, fibroblasts, and melanocytes. Keratinocytes not only respond to catecholamines but they are also capable of synthesizing them. Hence, the aberrations in keratinocytes or β2-adrenergic function associate with skin diseases, as well as with atopic ekzema [6]. The β-adrenergic receptor agonists prevent the wound healing through a decrease of keratinocytes, as well as inhibit the cell migration and proliferation. The function of keratinocytes deteriorates as a consequence of ERK binding to the β-adrenergic receptor agonists, which reduces the phosphorylation of ERK. Impaired wound healing is a growing clinical problem. Chronic skin ulcers represent enormous costs for the public health.

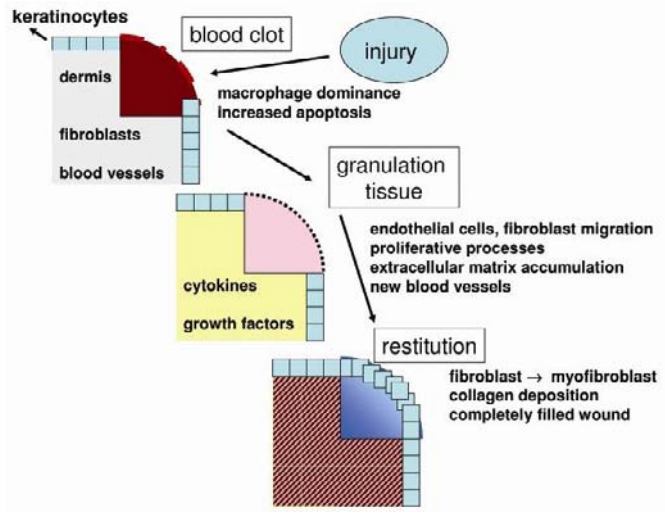

Figure 40. Steps of wound repair. After the injury, the blood clot fills in the wounded area in consequence of the disrupted blood vessels (Step 1). Later inflammatory reactions, cytokine and growth factor releases and cell recruitments are initiated. Massive angiogenesis and nerve sprouting are accelerated in order to form the granulation tissue (Step 2). The keratinocyte migration and proliferation, the collagen deposition and the appearence of myofibroblasts lead to the total restitution (Step 3).

5.2. Nerve Growth Factor Therapy

The nerve growth factor treatment has been applied for chronic ulcers of the feet and cornea. The recombinant human nerve growth factor (rhNGF) exerts a neurotrophic activity, and in contrast to the murine NGF, it is widespreadly used for human therapy [7]. A systemic application of NGF may be dangerous because of its hypertrophic effects, but there have been experiences with its subcutaneous application in diabetic neuropathy.

Pressure ulcers affect many people, emphasizing its relevant importance in the clinical therapy. The ability to healing these ulcers is poor, but patients are mainly elderly and immobilized. The worsening ulcer leads to chronic infections, osteomyelitis and a life threatening stage. NGF seems to be an ideal choice for the restitution of the tissues and the functional activity. The topical application of NGF in the pressure ulcers reduced the duration of the recovery [8].

The orbital tissues, particularly the cornea, express both NGF receptors. Similarly to substance P and IGF-1, the protectting role of NGF is crucial in the corneal epithelial barrier function [9]. In patients with thyroid associated ophthalmopathy, the NGF levels were significantly decreased compared to those without eye symptoms. These observations stress the neuroprotective effect of NGF in the endocrine orbitopathy [10]. In fact, the trophic effect of NGF is essential for the health of the cornea and /or the orbit. Considering the ectodermal origins of the retina and the optic nerve, the neurotrophic importance of NGF is without doubt in the sensory and the sympathetic nerve functions of the orbit. Barouch and co-workers demonstrated the participation of autoimmunity in the neurotrophin production and the rat optic nerve protection [11]. During the autoimmune responses, T and B cells, as well as

microglias/macrophages are significantly increased in the injured nerves; they represent crucial sources for the neurotrophic factors, such as NGF.

Many studies have proven that the exogenous NGF application accelerates the healing of the corneal epithelium [12]. NGF is stored and produced by the normal corneas, highlighting its necessity for the integrity and the innervation of the corneal epithelium.

REFERENCES

[1] Werner, S; Grose, R. *Regulation of wound healing by growth factors and cytokines*. Physiol Rev 2003; 83:835-870.

[2] Micera, A; Vigneti, E; Pickholtz, D; Reich, R; Pappo, O; Bonini, S; Maquart, FX; Aloe, L; Levi-Schaffer, F. *Nerve growth factor displays stimulatory effects on human skin and lung fibroblasts, demonstrating a direct role for this factor in tissue repair*. Proc Natl Acad Sci USA 2001; 98:6162-6167.

[3] Gibran, NS; Tamura, R; Tsou, R; Isik, FF. *Human dermal microvascular endothelial cells produce nerve growth factor: Implications for wound repair*. Shock 2003; 19:127-130.

[4] Li, AKC; Koroly, MJ; Schattenkerk, ME; Malt, RA; Young, M. *Nerve growth factor: Acceleration of the rate of wound healing in mice.* Proc Natl Acad Sci USA 1980;77:4379-4381.

[5] Pullar, CE; Grahn, JC; Liu, W; Isseroff, RR. *β2-adrenergic receptor activation delays wound healing.* Fed Am Societ Exp Biol J 2006; 20:76-86.

[6] Schallreuter, KU. *Epidermal adrenergic signal transduction as part of the neuronal network in the human epidermis*. J Investig Dermatol Symp Proc 1997; 2:37-40.

[7] Colangelo, AM; Finotti, N; Ceriani, M; Alberghina, L; Martegani, E; Aloe, L; Lenzi, L; Levi-Montalcini, R. *Recombinant human nerve growth factor with a marked activity in vitro and in vivo.* Proc Natl Acad Sci USA 2005; 102:18658-18663.

[8] Landi, F; Aloe, L; Russo, A; Cesari, M; Onder, G; Bonini, S; Carbonin, PU; Bernabei, R. *Topical treatment of pressure ulcers with nerve growth factor. A randomized clinical trial.* Ann Intern Med 2003; 139:635-641.

[9] Nakamura, M; Kawahara, M; Nakata, K; Nishida, T. *Restoration of corneal epithelial barrier function and wound healing by substance P and IGF-1 in rats with capsaicin-induced neurotrophic keratopathy*. Invest Ophthalmol Vis Sci 2003; 44:2937-2940.

[10] Molnár, I; Bokk, Á. *Decreased nerve growth factor levels in hyperthyroid Graves' ophthalmopathy highlighting the role of neuroprotective factor in autoimmune thyroid diseases.* Cytokine 2006; 35:109-114.

[11] Barouch, R; Schwartz, M. *Autoreactive T cells induce neurotrophin production by immune and neural cells in injured rat optic nerve: implications for protective autoimmunity.* Fed Am Societ Exp Biol J 2002; 16:1304-1306.

[12] Lambiase, A; Manni, L; Bonini, S; Rama, P; Micera, A; Aloe, L. *Nerve growth factor promotes corneal healing: Structural, biochemical, and molecular analyses of rat and human corneas.* Invest Ophthalmol Vis Sci 2000; 41:1063-1069.

6. AUTOIMMUNE AND ENDOCRINE DISEASES WITH ENHANCED SYMPATHOADRENAL ACTIVITY

6.1. Graves'ophthalmopathy

6.1.1. Autoimmune Processes in Graves' Ophthalmopathy

The autoimmune thyroid diseases, such as Graves' disease and Hashimoto's thyroiditis are viewed as organ specific autoimmune disorders. The multifactorial pathways that lead to the development of these diseases comprise of the genetic and environmental factors, as well as the autoimmune responses against thyroid and nonthyroid tissues [1]. In Hashimoto's thyroiditis, thyroid tissue damages caused by the autoimmune responses contribute to hypothyroidism, while in Graves' disease, the presence of anti-TSH receptor antibodies associates with hyperthyroidism and a diffuse goitre. The ophthalmopathy and the dermopathy appear in 50 to 75 percent and 1 to 2 percent of Graves' patients, respectively [2]. Graves'ophthalmopathy is characterized by proptosis, eyelid retraction, chemosis and periorbital edema, and rarely by opticus nerve lesion. The alterations act directly on the increase in the volume of fibroadipose connective and/or extraocular muscle tissues of the orbit. Inflammatory and immune responses, as well as a cell accumulation, are present in the affected tissues containing predominantly T lymphocytes, mast cells, and macrophages with an excessive glycosaminoglycan production [3]. The released cytokines, growth factors, chemoattractants and autoantibodies are responsible for the pathological changes in the orbit, skin and the thyroid glands [4, 5, 6].

Research supports that the stimulatory factors are important in the orbital adipogenesis, which occur in consequence of the macrophage derived cytokines (IL-1β, TNFα, IL-10) [7, 8]. Except for TSH receptor expression, which is essential for the initiation of Graves' disease, an increase in leptin, adiponectin and peroxisome proliferator-activated receptor γ (PPARγ) could be demonstrated in the orbital tissues of patients with ophthalmopathy [9]. The preadipocyte fibroblasts were only recently regarded as candidates for the immune reactions, which could express the potential autoantigens (TSH receptor, IGF-1 receptor) and secrete several cytokines, growth factors (IGF-1, NGF, fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF)) adipogenesis marker protein, and soluble frizzled-related protein-1(sFRP-1) [10, 11, 12, 13, 14]. There is dominance between the cytokine profiles of the extraocular and orbital adipose tissues, which could be observed during the onset [15]. The Th1 cytokine profiles, such as IL-1β and IL-6, are the most common cytokines detected in the extraocular muscles whereas IL-4 and IL-10 are the ones in the orbital fat tissues. The expression of cyclooxygenase-2 is a marker for the inflammation, for the severity of orbital diseases, and is upregulated by IL-1β [16, 17]. The cyclooxygenase-2 expression is implicated in the cell growth, cell apoptosis and the tumor genesis in various cell types. The orbital fibroadipose tissues exhibit increased cyclooxygenase-2 levels corresponding to the degree of the orbital inflammation.

In fact, the immunoglobulins of Graves' patients may stimulate the hyaluronan synthesis of orbital fibroblasts. These new findings are confirmed by the ability of the immuno-globulins to secrete IGF-1 in these patients [18]. Recent data highlight that the immunoglobulin activation of T cells leads to a chemoattractant expression on the fibroblasts

via IGF-1 receptor stimulation [19]. The production of IL-16 and RANTES chemokines in the orbit, thyroid, skin and the synovial membrane are mediated by the cytokine activation of fibroblasts. These chemokines may induce the activation of monocytes and T lymphocytes.

The allergic events and/or the elevated IgE levels may modify the disease activity of Graves' patients. The remission or recurrence of hyperthyroidism is linked to the elevation of IgE and/or IL-13 levels [20]. Other researchers found an aggravation of Graves' disease after allergic rhinitis and a lower remission rate in the patients with high IgE levels [21, 22]. Our previous study also confirmed that the IgE levels closely associate with the presence of ophthalmopathy compared to those who have no eye symptoms in Graves' disease [23].

The novel aspect of Graves' ophthalmopathy highlights the role of two antigens as indicated by the strong association between the disease activity and the autoantibodies againts TSH and IGF-1 receptors. Having more knowledge about the chemokines and their receptors involved in the endocrine autoimmune diseases may represent previously unseen pathways and lead to new therapeutic application.

Various types of growth factors play a range of roles in the development of Graves' ophthalmopathy. IGF-1, NGF, fibroblast growth factor and vascular endothelial growth factor are all associated with the disease activity and are expressed in the retroocular connective tissues [12, 13]. IGF-1, like NGF may directly stimulate the cell proliferation and survival, and contributes to hypertrophy [24, 25]. Trophic factors have established effects on the impact extraocular muscle strength [26]. Macrophages and macrophage-derived cytokines - IGF-1 and IL-10 - seem to be activating factors for Graves' ophthalmopathy [27]. IGF-1 may stimulate the fibroblast activity and proliferation, causing fibrosis by promoting the collagen matrix synthesis [28]. Th2 derived cytokines possess stimulatory effects on the orbitopathy, but the Th1 derived ones inhibit the expression of IGF-1 on the macrophages. Nonetheless, IGF-1 stimulates the IL-10 production of T cells in an autocrine manner and exerts immuno-modulatory effects [29]. As a proinflammatory mediator, IGF-1 leads to the expression of the cell adhesion molecules on the endothelial cells; this consequently enhances the adhesion of monocytes. T and B lymphocytes are IGF-1 receptor bearing cells [30]. IGF-1 receptor signaling is required for the B lymphocyte responses; this effects on the development and function of lymphocytes with the concomitant secretion of Th2 cytokines. On the other hand, the chemokine actions affect predominantly the Th1 lymphocytes, because these cells bear large amounts of chemokine receptors. During the inflammation, the release of IFNγ and TNFα cytokines may contribute to the chemokine expression of the thyrocytes, retrobulbar fibroblasts, preadipocytes, this perpetuates the inflammatory reactions in patients with Graves' ophthalmopathy [31]. Iodine-131 treatment in hyperthyroidism decreases the chemokine levels after 6 months of therapy [32]. Measurements of the CXCL10 chemokines in Graves' disease indicate that the clinical activity associates with the chemokine levels; therefore the chemokine level could be a useful marker in the follow-up of the patients [33].

6.1.2. Enhanced Sympathoadrenal Activity in the Thyroid, Adipose and Eye Muscle Tissue Hypertrophy

An increase in the activity of the sympathetic system links to the autoimmune thyroid diseases, particularly to Graves' ophthalmopathy. The higher sympathoadrenal activity is potentiated by the effects of the thyroid hormones in hyperthyroidism. The cells of the orbital tissues have ectodermal (retina, opticus nerve) and mesodermal origins. The neuronal innervation of the orbital tissues is high in density considering the sympathetic and sensory

neurons. The survival and the developmental processes, as well as the density, are connected to the amount of NGF. The innervation of the eye muscles and the adipose tissue in the orbits differ from those of other skeletal muscles and the body adiposity. Both the orbits and the thyroid glands display high density vascularity, highlighting the vascular affection in the pathognomic events. The autoimmune responses against the thyroid, eye muscles and other self-antigens, together with the increased levels of hormones, cytokines, growth factors, chemokines and other active peptide substances, induce the clinical signs in Graves' ophthalmopathy.

Smith and co-workers described a tissue remodelling in the thyroid-associated ophthalmopathy, which represents interplay among the endothelium, vascular smooth muscle, extraocular muscle, fibroblasts and adipocytes [34]. In this remodelling, the monocytes and their released cytokines, chemokines and growth factors play central roles. The initiating factor is the IL-1β proinflammatory cytokine, which leads to fibroblast activation and the accumulation of hyaluronan and glycosaminoglycan. However, the IL-1β also influences the synthesis of prostaglandin E_2 in the skin, thyroid, and retrobulbar fibroblasts; this associates with the modulation of the mast cells, as well as of the T and B lymphocyte functions. The growth factors, such as IGF-1, may induce hyaluronan synthesis and TGFβ promotes the fibroblast → myofibroblast differentiation.

Recent data suggest that the thyroid autoimmunity, mainly Graves' ophthalmopathy, can not be explained as only an immune and endocrine disease. The nervous system, particularly the sympathetic and sensory innervations, is involved in the pathognomic events. Mediators that act on the nerve terminals, such as substance P, calcitonin-gene related proteins, catecholamines and NGF, possess direct effects on the nonneural immunocompetent cells.

The complexity among the nervous, immune and endocrine systems - orchestrated by NGF - may represent a novel hypothesis in the pathognomic responses of the thyroid-associated ophthalmopathy (Figure 41). The prominent sympathetic activation could explain the tissue hypertrophy, and it contributes to the Th2 dominance and the local inflammatory reactions initiating hyperthyroidism. The unanswered question is: what are the causes of the high levels of the proinflammtory Th1 cytokines and their relation to the Th2 ones in the pathomechanism of Graves' ophthalmopathy? In fact, the causative factors of the inflammation seem to be crucial. If the direct cell destruction in the thyroid autoimmunity was mediated mainly by the inflammation, then it may be related to a Th1 dominant disease: Hashimoto's thyroiditis. However, the sympathetic overexpression in the thyroid glands, the retrobulbar adipose tissue, extraocular muscles, and skin associates with a large release of catecholamines, CRH, cortisol and NGF; this induces direct cellular actions via the adrenergic, glucocorticoid and TrkA receptors. The receptor activations of the immunocompetent cells lead to the release of cytokine in the Th2 direction, the sensitisation of the Th1 lymphocytes, as well as the resistance of the Th2 lymphocytes for apoptosis. The Th2 direction enables the production of various autoantibodies, some of which possesses pathognomic roles, for example the autoantbodies against TSH receptor, thyroid peroxidase and thyroglobulin or IGF-1. The thyroid hormones may act as survival substances, which promote the local maintenance of the immune and inflammatory responses. It is a fact that the transactivation among G-protein-coupled, adrenergic and Trk receptors could promote the development of tissue hypertrophy, neurogen inflammation and pain. The vascular affection via NGF and other growth factors and peptides is associated with the thyroid autoimmunity and the euthyroid goiter; this reflects the importance of these substances in the development

of the cardiovascular diseases. The muscle cells are regulated by the growth factors at the different events. For example, the skeletal muscles and/or extraocular muscles are regulated by IGF-1, but low levels of NGF could contribute to muscle fibrosis and atrophy due to the impaired innervation.

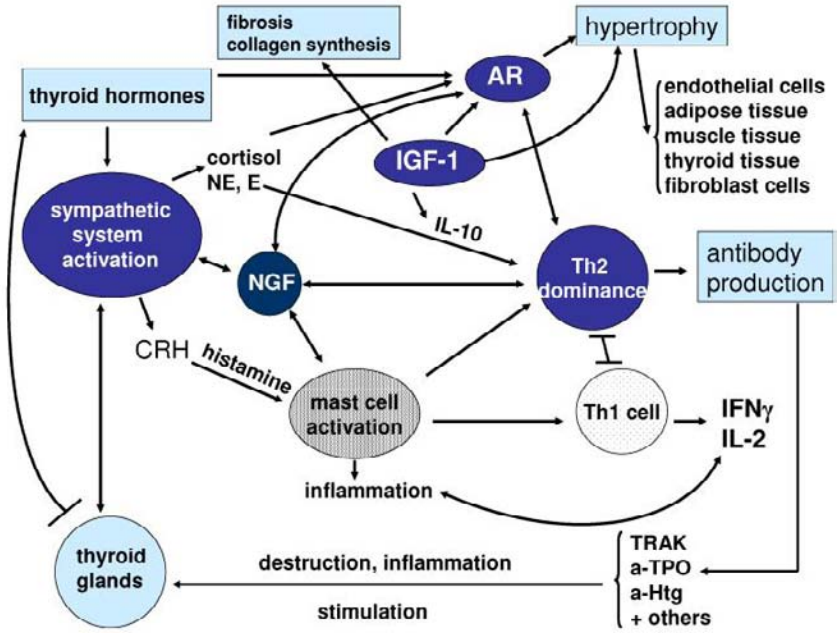

Figure 41. The main directions of the neuro-immuno-endocrine responses in the pathomechanism of Graves' ophthalmopathy. The sympathoadrenal activation induces cortisol, epinephrine (E), norepinephrine (NE), nerve growth factor (NGF), and corticotropin-releasing hormone (CRH) production; this increases adrenergic receptor (AR), mast cell and Th2 cell activation. The released cytokines and NGF lead to Th2 cell activation and a local inflammation. The antibody production against TSH receptor (TRAK) initiates hyperthyroidism with the release of thyroid hormones. The adrenergic receptor stimulation contributes to the hypertrophy of the affected tissues (endothelial cells, fibroblasts, adipose, thyroid, and muscle tissues). Insulin-like growth factor-1 (IGF-1) potentiates the inflammatory, fibrotic and hypertrophic processes, and results in collagen accumulation. A complex network of the neuro-immune-endocrine responses stays in the background of the pathomechanism of Graves' ophthalmopathy. a-TPO: antibody against thyroid peroxidase; a-Htg: antibody against human thyroglobulin; Th2 cell: T helper 2 lymphocytes; Th1 cell: T helper 1 lymphocytes; IFNγ: interferon γ; → : action direction; ⊣ : action inhibition.

This complexity of the different regulatory systems in Graves' ophthalmopathy can better describe the distinct network sytems that display pathomechanisms.

6.2. Diabetes Mellitus

6.2.1. Regulation of the Sympathoadrenal System and the Hypothalamic-Pituitary-Adrenal Axis in Diabetes Mellitus

Diabetes mellitus is a chronic disease that is caused by an inherited and/or acquired deficiency in the production of insulin. The deficiency of insulin could result from

autoimmune reactions or from the resistance to insulin's effects. The diminishing effect of insulin leads to high concentrations of glucose and other substances, as well as to metabolic alterations causing damages particularly in the eyes, kidney, nerves, heart and blood vessels. Two major types of diabetes mellitus could be distinguished: type 1 diabetes, also called insulin-dependent diabetes (IDDM) and type 2, called noninsulin-dependent diabetes (NIDDM).

The increased hypothalamic-pituitary-adrenal activity and the impaired stress responsiveness are characteristic for diabetes. The dysfunction of the central nervous system can be seen in the elevated levels of ACTH and cortisol and the lower epinephrine levels. The increased glucocorticoid levels mobilize the glucose from the liver, free fatty acid (FFA), as well as adipocyte stores; this associates with the inhibition of the glucose uptake and utilization in the local cerebral neurons [35]. Insulin treatment restores the increased hypothalamic-pituitary-adrenal activity [36]. The brain contains two types of glucocorticoid receptors: type 1 is the mineral receptor (MR) and type 2 is the glucocorticoid receptor (GR). The effects of insulin manifest themselves centrally and peripherally, whereby its action on the hypothalamic-pituitary-adrenal activity is independent of hypoglycaemia. The increased basal activity of the hypothalamic-pituitary-adrenal axis in diabetes may represent incapability with the responses to the stress challenge [37]. The interactions between insulin and hypothalamic-pituitary-adrenal axis are displayed in the energy homeostasis, insulin resistance, and obesity in consequence of the expression of anorexigenic neuropeptide CRH in the hypothalamus. The hyperinsulinemia- and euglycemia-caused activation on the hypothalamic-pituitary-adrenal axis is delayed in diabetes due to the decreased pituitary and adrenal sensitivities.

The release of ACTH from the anterior pituitary could be induced by the stimulation of the β-adrenergic receptor and also the CRH release of insulin [38].

Hypoglycemia induces various counterregulatory responses promoting the activation of the sympathoadrenal and the hypothalamic-pituitary-adrenal axis. Glucagon, epinephrine, norepinephrine and corticosterone responses are implicated in the counterregulatory events. These counterregulatory responses initiated by hypoglycemia are impaired in diabetes [39]. The decrease in the sympathoadrenal regulation may be explained by defects in the catecholamine synthesis; for example, in the enzyme activities of the adrenal tyrosine hydroxylase and phenylethanolamine-N-methyltransferase.

6.2.2. Diabetic Neuropathy and Nerve Growth Factor

The neuropathy is the most insidious complication of both type 1 and type 2 diabetes. The diabetic neuropathy affects the small myelinated and nonmyelinated fibers, as well as the large myelinated fibers which are involved in the sensory and autonomic functions [40]. Histologically, the diabetic neuropathy is characterized by the axonal degeneration, demyelination and atrophy, associating with the failed axonal regeneration. The clinical manifestations of the neurological disturbances are the following: electrodiagnostic and sensory test abnormalities, diffuse, distal symmetric sensorimotor and autonomic syndromes, and the focal syndromes. The small fiber affections lead to pain and hyperalgesia and later turn into the loss of the thermal, vibration and mechanical sensitivity.

Several mechanisms could lead to neuropathy in diabetes: chronic hyperglycemia, sorbitol pathway, accumulation of advanced glycation end products, vascular abnormalities,

and decrease in the neurotrophic growth factors, oxidative stress, or autoantibodies againts neuronal antigens.

NGF seems to be responsible for the development of neuropathy. Data show that NGF levels are low in diabetes. Moreover, along with the production of NGF, its retrograd transport is also diminished. NGF mediates the survival of sympathetic and sensory neurons, as well as their resistance againts apoptosis. The reduced levels of other neuropeptides, such as substance P and calcitonin-gene related protein, contribute to the clinical symptoms. The maintenance of the cutaneous fiber survival via NGF is essential for the protection of the skin. In diabetes, the cutaneous myelinated and nonmyelinated fiber loss could link to hyperglycemia [41]. The reduction in NGF levels could be induced by oxidative stress and autoimmunity. NGF may also increase the sensitivity of myelinated fibers in the diabetic skin.

An interaction between NGF and IGFs are revealed in the neuronal survival. The similirity between the structures of NGF and proinsulin (from 40% to 50%) highlights overlapping mechanisms of both growth factors in their cellular actions and their early signaling cascades [42]. IGF-1 and IGF-2 growth factors support the normal peripheral nervous system, such as sensory and sympathetic neurons. The circulating levels of IGFs are decreased in diabetic neuropathy, which could be corrected by insulin [43].

6.2.3. The Role of Cellular Integrity and Function in Diabetic Vascular Complications

The chronic hyperglycemia is the major initiator of the micro- and macrovascular complications in diabetes. The pathognomic events induced by hyperglycemia affect the diacylglycerol - protein kinase C activation. In diabetes, this diacylglycerol-protein kinase C pathway regulates several vascular abnormalities in the retinal, renal, and cardiovascular tissues. This signaling mechanism is involved in the regulation of the endothelial permeability, vasoconstriction, extracellular matrix synthesis, cell growth, angiogenesis, cytokine releases and cell adhesion [44]. An increased activation of the diacylglycerol - protein kinase C pathway could be demonstrated in diabetes and insulin resistance. Different stimulators are implicated in this activation, such as hyperglycemia, oxidative stress, growth factors, and cytokines (Figure 42). However, the vascular damages caused by protein kinase C activation in diabetes are independent of diacylglycerol accumulation – also known as glycolysis. The growth factors, such as insulin, IGFs, NGF and cytokines may induce signaling cascades which lead to the metabolisms of phophatidylinositides and diacylglycerol via the phospholipase isoenzymes. The increased protein kinase C activation - due to hyperglycemia and/or hypothalamic-pituitary-adrenal stimulation - associates with the alteration of the blood flow, the thickening of the basement membrane, the expansion of the extracellular matrix, the vascular permeability, the angiogenesis, the cell growth and the alterations of the enzymatic effects. Several growth factor actions could be distinguished according to the vascular abnormalities.

The changes in the blood flow and the vascular contractility result in hemodynamic alterations of the kidneys, retina, skin, arteries and nerves. Ischemia increases the overexpression of angiogenic growth factors, which can induce edema and proliferative processes. NGF may play a crucial preventing role in the neuro- and hypertrophic processes, as well as in the skin and the neurogen inflammatory responses in diabetes. The inhibition of the protein kinase C activity provides a new therapeutic possibility for treating the vascular complications of diabetes.

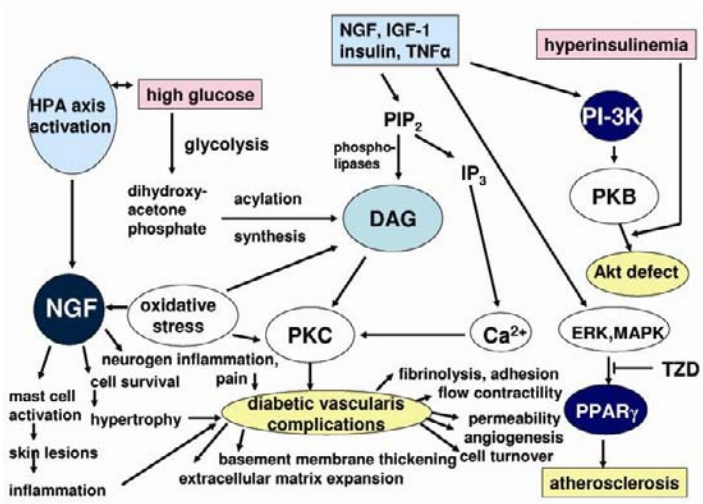

Figure 42. Signaling pathways induced by hyperglycemia and growth factors in diabetes. Diacylglycerol (DAG) accumulation is induced by hyperglycemia via the protein kinase C (PKC) and by hyperinsulinemia via the protein kinase B (PKB) signaling pathways. Hyperinsulinemia causes a defect in the Akt signaling and enhances the apoptosis. The hypothalamic-pituitary-adrenal (HPA) axis activation, oxidative stress and growth factors can potentiate the diabetic vascular complications via DAG accumulation. The peroxisome proliferator-activated receptor γ (PPARγ) agonists restore the balance between mitogen-activated protein kinase (MAPK)/ extracellular signal-regulated kinase (ERK) and phosphatidylinositol 3-kinase (PI-3K)/PKB/ serine/threonine kinase (Akt) activities. PIP_3: phosphatidylinositol 3,4,5-triphosphate; IP_3: inositol triphosphate; TDZ: thiazolidinedione; NGF: nerve growth factor; IGF-1: insulin-like growth factor-1; TNFα: tumor necrosis factor α; → : action direction; ⊣ : action inhibition.

The Akt kinase/protein kinase B signaling pathway is also diminished in diabetes and insulin resistance. The protein kinase B acts as a regulator of the glucose transport via the PI-3K dependent cascade [45]. The protein kinase B-mediated signaling of the insulin is accelerated by the utilization of glucose, affecting the adipocytes and the skeletal muscles. The protein kinase B potentiates the translocation of the insulin-regulated glucose transporter (GLUT4) from its intracellular storage pool to the plasma membrane. The Akt protein mediates a wide variety of growth factor and cytokine signals [46]. The increased glucose levels cause secondary defects in diabetes; for example, defects in the Akt activation. The glucosamine, such as the polyol pathway, oxidative stress and TNFα lead to a relevant reduction of the Akt phosphorylation. The PI-3K/protein kinase B/Akt dependent responses affect the neovascularisation, the cell migration and the endothelial NO production. The insulin resistance enhances the baseline activity of Akt. The defect in the Akt activation in knock-out mice exhibits a mild hyperglycemia, an impaired glucose tolerance, hyperinsulinemia and an enhanced apoptosis. Thiazolidinediones can stimulate the Akt phosphorylation via the peroxisome proliferator-activated receptor γ. Therefore, the peroxisome proliferator-activated receptor γ agonists provide a beneficial effect, so that nowadays they are used in the treatment of diabetic cardiovascular and renal complications. The peroxisome proliferator-activated receptor γ agonists (thiazolidinediones) restore the balance between the PI-3K and ERK, MAPK activities, thus protecting the atherosclerosis. Thiazolidinedions lead to the normalization of insulin resistance and the diabetes milieu causing an inhibition of the vascular cell growth and movement [47].

REFERENCES

[1] Weetman, AP. *Autoimmune thyroid disease: propagation and progression*. Eur J Endocrinol 2003; 148:1-9.

[2] Weetman, AP. *Graves' disease*. N Engl J Med 2000; 343:1236-1248.

[3] Aniszewski, JP; Valyasevi, RW; Bahn, RS. *Relationship between disease duration and predominant orbital T cell subset in Graves' ophthalmopathy*. J Clin Endocrinol Metab 2000; 85:776-780.

[4] Li, Wang; Weiping, T; Zhongyan, S. *Effect of IFN-γ and IL-4 on proliferation and synthesis of hyaluronic acid and collagen in cultured human retroorbital fibroblasts in vitro*. Chinese Med J 2000; 113:907-910.

[5] Xia, N; Zhou, S; Liang, Y; Xiao, C; Shen, H; Pan, H; Deng, H; Wang, N; Li, QQ. *$CD4^+$ T cells and the Th1/Th2 imbalance are implicated in the pathogenesis of Graves' ophthalmopathy*. Int J Mol Med 2006; 17:911-916.

[6] Gianoukakis, AG; Khadavi, N; Smith, TJ. *Cytokines, Graves' disease and thyroid-associated ophthalmopathy*. Thyroid 2008; 18:953-958.

[7] Kumar, S; Bahn, RS. *Relative overexpression of macrophage-derived cytokines in orbital adipose tissue from patients with Graves' ophthalmopathy*. J Clin Endocrinol Metab 2003; 88:4246-4250.

[8] Kumar, S; Coenen, MJ; Scherer, PE; Bahn, RS. *Evidence for enhanced adipogenesis in the orbits of patients with Graves' ophthalmopathy*. J Clin Endocrinol Metab 2004; 89: 930-935.

[9] Valyasevi, RW; Harteneck, DA; Dutton, CM; Bahn, RS. *Stimulation of adipogenesis, peroxisome proliferator-activated receptor-γ (PPARγ), and thyrotropin receptor by PPARγ agonist in human orbital preadipocyte fibroblasts.* J Clin Endocrinol Metab 2002; 87:2352-2358.

[10] Bahn, RS; Dutton, CM; Natt, N; Joba, W; Spitzweg, C, Heufelder, AE. *Thyrotropin receptor expression in Graves' orbital adipose/connective tissues: Potential autoantigen in Graves' ophthalmopathy.* J Clin Endocrinol Metab 1998; 83:998-1002.

[11] Kumar, S; Leontovich, A; Coenen, MJ; Bahn, RS. *Gene expression profiling of orbital adipose tissue from patients with Graves' ophthalmopathy: A potential role for secreted frizzled-related protein-1 in orbital adipogenesis.* J Clin Endocrinol Metab 2005; 90:4730-4735.

[12] Molnár, I; Bokk, Á. *Decreased nerve growth factor levels in hyperthyroid Graves' ophthalmopathy highlighting the role of neuroprotective factor in autoimmune thyroid diseases.* Cytokine 2006; 35:109-114.

[13] Matos, K; Manso, PG; Marback, E; Furlanetto, R; Alberti, GN; Nosé, V. *Protein expression of VEGF, IGF-1 and FGF in retroocular connective tissues and clinical correlation in Graves' ophthalmopathy.* Arq Bras Oftalmol 2008; 71:486-492.

[14] Tsui, S; Naik, V; Hoa, N; Hwang, CJ; Afifiyan, NF; Hikim, AS; Gianoukakis, AG; Douglas, RS; Smith TJ. *Evidence for an association between thyrod-stimulating hormone and insulin-like growth factor 1 receptors: A tale of two antigens implicated in Graves' disease*. J Immunol 2008; 181:4397-4405.

[15] Hiromatsu, Y; Yang, D; Bednarczuk, T; Miyake, I; Nonaka, K; Inoue, Y. *Cytokine profiles in eye muscle tissue and orbital fat tissue from patients with thyroid-associated ophthalmopathy*. J Clin Endocrinol Metab 2000; 85:1194-1199.

[16] Han, R; Tsui, S; Smith, TJ. *Up-regulation of prostaglandin E_2 synthesis by interleukin-1β in human orbital fibroblasts involves coordinate induction of prostaglandin-endoperoxide H synthase-2 and glutathione-dependent prostaglandin E_2 synthase expression*. J Biol Chem 2002, 277:16355-16364.

[17] Konuk, EBY; Konuk, O; Misirlioglu, M; Menevse, A; Unal, M. *Expression of cyclooxygenase-2 in orbital fibroadipose connective tissues of Graves' ophthalmopathy patients*. Eur J Endocrinol 2006; 155:681-685.

[18] Smith, TJ; Hoa, N. *Immunoglobulins from patients with Graves' disease induce hyaluronan synthesis in their orbital fibroblasts through the self-antigen, insulin-like growth factor-I receptor*. J Clin Endocrinol Metab 2004; 89:5076-5080.

[19] Pritchard, J; Han, R; Horst, N; Cruikshank, WW; Smith, TJ. *Immunoglobulin activation of T cell chemoattractant expression in fibroblasts from patients with Graves' disease is mediated through the insulin-like growth factor I receptor pathway*. J Immunology 2003; 170:6348-6354.

[20] Komiya, I; Yamada, T; Sato, A; Kouki, T; Nishimori, T; Takasu, N. *Remission and recurrence of hyperthyroid Graves' disease during and after methimazole treatment when assessed by IgE and interleukin 13*. J Clin Endocrinol Metab 2001; 86:3540-3544.

[21] Hidaka, Y; Amino, N; Iwatani, Y; Itoh, E; Matsunaga, M; Tamaki, H. *Recurrence of thyrotoxicosis after attack of allergic rhinitis in patients with Graves' disease*. J Clin Endocrinol Metab 1993; 77:1667-1670.

[22] Yamada, T; Sato, A; Komiya, I; Nishimori, T; Ito, Y; Terao, A; Eto, S; Tanaka, Y. *An elevation of serum immunoglobulin E provides a new aspect of hyperthyroid Graves' disease*. J Clin Endocrinol Metab 2000; 85:2775-2778.

[23] Molnár, I; Horváth, S; Balázs, C. *Detectable serum IgE levels in Graves' ophthalmopathy*. Eur J Med Res 1996; 1:543-546.

[24] Adams, GR; McCue, SA. *Localized infusion of IGF-I results in skeletal muscle hypertrophy in rats.* J Appl Physiol 1998; 84:1716-1722.

[25] Navarro, M; Baserga, R. *Limited redundancy of survival signals from the type 1 insulin-like growth factor receptor.* Endocrinology 2001; 142: 1073-1081.

[26] Chen, J; von Bartheld, CS. *Role of exogenous and endogenous trophic factors in the regulation of extraocular muscle strength during development*. Invest Ophthalmol Vis Sci 2004; 45: 3538-3545.

[27] Douglas, RS; Gianoukakis, AG; Kamat, S; Smith, TJ. *Aberrant expression of the insulin-like growth factor-1 receptor by T cells from patients with Graves' disease may carry functional consequences for disease pathogenesis*. J Immunol 2007; 178:3281-3287.

[28] Wynes, MW; Riches, DWH. *Induction of macrophage insulin-like growth factor-I expression by the Th2 cytokines IL-4 and IL-13*. J Immunol 2003; 171:3550-3559.

[29] Kooijman, R; Coppens, A. *Insulin-like growth factor-I stimulates IL-10 production in human T cells.* J Leukoc Biol 2004; 76:862-867.

[30] Baudler, S; Baumgartl, J; Hampel, B; Buch, T; Waisman, A; Snapper, CM; Krone, W; Brüning, JC. *Insulin-like growth factor-1 controls type 2 T cell-independent B cell response.* J Immunol 2005; 174:5516-5525.

[31] Antonelli, A; Rotondi, M; Ferrari, SM; Fallahi, P; Romagnani, P; Franceschini, SS; Serio, M; Ferrannini, E. *Interferon-γ-inducible α-chemokine CXCL10 involvement in Graves' ophthalmopathy: Modulation by peroxisome proliferator-activated receptor-γ agonists.* J Clin Endocrinol Metab 2006; 91:614-620.

[32] Antonelli, A; Rotondi, M; Fallahi, P; Grosso, M; Boni, G; Ferrari, SM; Romagnani, P; Serio, M; Mariani, G; Ferrannini, E. *Iodine-131 given for therapeutic purposes modulates differently interferon-γ-inducible α-chemokine CXCL10 serum levels in patients with active Graves' disease or toxic nodular goiter*. J Clin Endocrinol Metab 2007; 92:1485-1490.

[33] Rotondi, M; Chiovato, L; Romagnani, S; Serio, M; Romagnani, P. *Role of chemokines in endocrine autoimmune diseases.* Endocrine Reviews 2007; 28:492-520.

[34] Smith, TJ; Tsai, CC; Shih, MJ; Tsui, S; Chen, B; Han, R; Naik, V; King, CS; Press, C; Kamat, S; Goldberg, RA; Phipps, RP; Douglas, RS; Gianoukakis, AG. *Unique attributes of orbital fibroblasts and global alterations in IGF-1 receptor signaling could explain thyroid-associated ophthalmopathy*. Thyroid 2008; 18:983-988.

[35] Chan, O; Chan, S; Inouye, K; Vranic, M; Matthews, SG. *Molecular regulation of the hypothalamo-pituitary-adrenal axis in Streptozotocin-induced diabetes: Effects of insulin treatment*. Endocrinology 2001; 142:4872-4879.

[36] Chan, O; Chan, S; Inouye, K; Shum, K; Matthews, SG. *Diabetes impairs hypothalamo-pituitary-adrenal (HPA) responses to hypoglycemia, and insulin treatment normalizes HPA but not epinephrine responses.* Diabetes 2002; 51:1681-1689.

[37] Chan, O; Inouye, K; Akirav, E; Park, E; Riddell, MC; Vranic, M; Matthews, SG. *Insulin alone increases hypothalamo-pituitary-adrenal activity, and diabetes lowers peak stress responses*. Endocrinology 2005; 146:1382-1390.

[38] Mezey, E; Reisine, TD; Brownstein, MJ; Palkovits, M; Axelrod, J. *β-adrenergic mechanism of insulin-induced adrenocorticotropin release from the anterior pituitary*. Science 1984; 226:1085-1087.

[39] Inouye, KE; Chan, O; Yue, JTY; Matthews, SG; Vranic, M. *Effects of diabetes and recurrent hypoglycemia on the regulation of the sympathoadrenal system and hypothalamo-pituitary-adrenal axis.* Am J Physiol Endocrinol Metab 2005; 288: E422-E429.

[40] Pittenger, G; Vinik, A. *Nerve growth factor and diabetic neuropathy*. Experimental Diab Res 2003; 4:271-285.

[41] Christianson, JA; Ryals, JM; Johnson, MS; Dobrowsky, RT; Wright, DE. *Neurotrophic modulation of myelinated cutaneous innervation and mechanical sensory loss in diabetic mice.* Neuroscience 2007; 145:303-313.

[42] Saltiel, AR. *Diverse signaling pathways in the cellular actions of insulin*. Am J Physiol 1996; 270:E375-E385.

[43] Schmidt, RE; Dorsey, DA; Beaudet, LN; Plurad, SB; Parvin, CA, Miller, MS. *Insulin-like growth factors I reverses experimental diabetic autonomic neuropathy.* Am J Physiol 1999; 155:1651-1660.

[44] Evcimen, ND; King, GL. *The role of protein kinase C activation and the vascular complications of diabetes*. Pharmacol Res 2007; 55:498-510.

[45] Hajduch, E; Litherland, GJ; Hundal, HS. *Protein kinase B (PKB/Akt) – a key regulator of glucoe transport?* Fed Eur Biochem Societ Letters 2001; 492:199-203.

[46] Ždychová, J; Komers, R. *Emerging role of Akt kinase/protein kinase B signaling in pathophysiology of diabetes and its complications*. Physiol Res 2005; 54:1-16.

[47] Hsueh, WA; Law, RE. *PPARγ and atherosclerosis. Effects on cell growth and movement.* Arterioscler Thromb Vasc Biol 2001; 21:1891-1895.

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Chapter 2

NERVE GROWTH FACTOR AND PAIN: EVIDENCE FROM EXPERIMENTAL MODELS AND HUMANS, AND NEW PROSPECTIVES FOR TREATMENT

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ABSTRACT

Nerve growth factor (NGF) has a key role not only in the development of sensory and autonomic neurons, but also in the processes of nociception. Several central and peripheral mechanisms have been postulated as the basis of effects of NGF in nociceptive pathways. It has been implicated both in inflammatory and neuropathic pain mechanisms and strategies against NGF, its receptors, belonging to the tyrosine kinase (Trk) family, and down-stream intracellular signaling activated by this neurotrophin (NT) have been proposed for the treatment of these pathological conditions.

There is also recent evidence of the involvement of NGF in other painful diseases, such as migraine, in particular in the chronic form, and fibromyalgia. This suggests the potential usefulness of anti-NGF strategies as novel analgesics also for these disabling pathological conditions.

Keywords: nerve growth factor, neuropathic pain, inflammatory pain, migraine, fibromyalgia, NGF targeting strategies.

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INTRODUCTION

Nerve growth factor (NGF) was the first neurotrophin (NT) to be identified, and its role in the development and survival of both peripheral and central sensory neurons has been well characterized [Wiesmann et al, 2001]. Among its effects in the post-natal period, a great deal of attention has focused on the involvement of NGF in pain mechanisms [Nicol et al, 2007].

NGF is produced by a number of cell types including keratinocytes [Di Marco et al, 1993], mast cells [Leon et al., 1994], B-lymphocytes [Torcia et al, 1996], fibroblasts [Lindholm et al, 1990], bronchial epithelial cells [Kassel et al, 2001], renal mesangial cells [Steiner et al, 1991], smooth muscle cells [Ueyama et al, 1993], and skeletal muscle myotubes [Schwartz et al, 2002]. This NT up-regulates the expression of sensory neuropeptides in nociceptive neurons, and its activity is mediated through two different membrane-bound receptors, the tyrosine kinase A (TrkA) receptor and the p75 receptor [Pezet & McMahon, 2006]. The latter receptor is structurally related to other members of the tumor necrosis factor (TNF) receptor family, which have been found on a variety of cell types also outside of the nervous system [Pezet & McMahon, 2006].

NGF appears to act by multiple mechanisms which include: i) changes in the peripheral and central sensory neurons including nociceptive neurons; ii) changes in the expression of genes responsible for nerve activation and conduction such as ion channels and some pain-related receptors; iii) axonal growth and nerve degeneration; iv) induction of brain-derived neurotrophic factor (BDNF), which, in turn, intervenes in pain signal processing within the spinal cord [Pezet & McMahon, 2006].

The above evidence supports, therefore, the central role of NGF in nociception, in particular, in inflammatory pain or neuropathic pain. In addition, elevated levels of NGF have been detected in tissues, cerebrospinal fluid (CSF), and central venous blood in a number of painful diseases, such as visceral pain, post-surgical pain, fibromyalgia and also migraine, manly in chronic migraine [Giovengo et al, 1999; Sarchielli et al, 2001; Sarchielli et al, 2002; Sarchielli et al, 2007b]. The ability of this NT to increase nociception had raised the idea that compounds targeting NGF, its TrkA receptor and down-stream intracellular signaling would be a promising alternative treatment for acute and chronic intractable pain [Sarchielli et al, 2004a]. Their administration has been proposed for potentiation of the effects of opioid analgesics on severe pain, with consequent reduction of dosage and undesirable side effects [Cahill et al, 2003]. In alternative, antagonistic compounds of NGF were also considered for mild-moderate pain or for the preventive treatment of various chronic painful diseases [Owolabi et al, 1999].

The present review highlights the role of NGF in nociception during adulthood and, beginning with experimental and clinical evidence, illustrates the most relevant knowledge concerning the pathogenic mechanisms of inflammatory and neuropathic pain, as well as migraine and fibromyalgia, with particular regard to the involvement of NGF. Finally, it provides the most recent findings on the potential role of therapeutic strategies targeting NGF in inflammatory and neuropathic pain and, potentially, in migraine and fibromyalgia.

NGF AND NOCICEPTION

NGF has been clearly shown to be involved in the embryonic development of sensory neurons, including nociceptive sensory neurons [Ritter et al, 1991]. In addition, an altered expression of this NT factor and its receptors contributes to plastic changes in primary afferents during the early neonatal period consequent to peripheral inflammatory events [Chien et al, 2007].

Nociceptive neurons require this NT to maintain their phenotype also in the post-natal period. Moreover, TrkA receptors continue to be expressed in these neurons, which synthesize this NT also after embryonic development [Mendell et al, 1999]. Even in adulthood, NGF regulates specification of the nociceptive phenotype [Koltzenburg, 1999; Bennett, 2001].

Experimental evidence showed that NGF is released by several cell types, including mast cells [Bienenstock et al, 1987; Skaper et al, 2001], monocytes/macrophages [Bracci-Laudiero et al, 2005], lymphocytes [Bonini et al, 2003] and also Schwann cells [Créange, 1997] in response to tissue inflammation. In addition, an increase in NGF production can be triggered by a variety of stimuli, which include proinflammatory cytokines, such as interleukin (IL)-1β, tumor necrosis factor (TNF-α), IL-4 and platelet-derived growth factor (PDGF) produced by inflammatory cells [Levi-Montalcini et al, 1996; Woolf et al, 1997].

NGF is responsible for hyperalgesia when administered locally, systemically or intrathecally in different animal species [Brodie, 1995; Hao et al, 2000]. This hyperalgesic effect of NGF emerged both from experiments investigating behavioral responses in humans and from pain perception models in animals and humans [Pezet & McMahon, 2006; Della Seta et al, 1994; Amann et al, 1995]. In animal models NGF-induced thermal hyperalgesia occurs within an hour after its administration. Both this short latency and the local effect of NGF support the involvement of a prevalent peripheral mechanism [Shu & Mendell, 1999]. NGF-induced mechanical hyperalgesia develops, in contrast, with a latency of some hours, suggesting the activation of more complex central mechanisms [Ma & Woolf, 1997; Hathway et al, 2006].

Pain-related behavioral responses to NGF in animals also manifest within minutes but last hours or days, depending on the dose and route of administration [Zahn et al, 2004; Lewin et al, 1994]. Similar effects have been shown in human volunteers in whom administration of small amounts of NGF, subcutaneously or intramuscularly, induces pain and tenderness at the site of injection, which start within minutes and persist for several hours. Likewise, small intravenous NGF doses are responsible for widespread deep pain and tenderness that may persist for several days [Svensson et al, 2003].

INTRACELLULAR MECHANISMS OF NGF ACTION

The role played by NGF in peripheral nociception is emphasized by the evidence of the up-regulation and increased delivery of this NT and over-expression of high-affinity TrkA receptors in the nociceptive terminals in the injured areas after inflammatory insults [Pezet et al, 1999]. The hyperalgesic action of NGF in inflammatory pain models may, in part, be the consequence of the increased sensitivity of peripheral nociceptive terminals. This increased

sensitivity is due to a direct action of NGF on TrkA-expressing Aδ ad C fibers via the enhancement of the release of substance P (SP), calcitonin gene-related peptide (CGRP), and other sensitizing mediators from TrkA-expressing cells, post-ganglion sympathetic neurons, mast cells and immune cells in the site of inflammatory insult or after peripheral NGF administration [Woolf et al, 1996; Malcangio et al, 1997, 2000].

TrkA activation, after interaction with NGF, leads to the induction of several intracellular signaling cascades affecting the sensitivity of nociceptive afferents. The retrograde signal mediated by the internalized TrkA-NGF complex transported in sensory neurons to the dorsal root ganglion (DRG) alters the transcription of several proteins and peptides [Wehrman et al, 2007]. Genes differentially expressed include sensory neuropeptides, receptors and voltage-regulated ion channels [Chao et al, 2006; Malik-Hall et al, 2005; Molliver et al, 2005].

In particular, the exogenous NGF administration *in vivo* or the addition of NGF *in vitro* to cultured sensory neurons induces the increase in messenger RNA (mRNA) and protein levels of both neuropeptides SP and CGRP in cell bodies [Vedder et al, 1993]. The remodeling of CGRP fibers surrounding NGF-immunoreactive cell bodies, the increase in CGRP and SP as well as the over-expression of neurokinin K1 (NK1) receptor due to NGF is a slow process, requiring from hours to days to manifest itself. These modifications confirm, therefore, the implication of transcriptional mechanisms, which may account for a long-lasting sensitization that contributes to the maintenance of painful sensation [McMahon, 1996].

A number of ligand-gated ion channels, normally expressed throughout the sensory neurons and concentrated at both peripheral and central axonal arbors, are also regulated by NGF in neurons bearing TrkA receptors. They include transient receptor potential vanilloid 1 ($TRPV_1$), acid-sensing ion channels (ASIC3), purinergic receptors of adenosine triphosphate (ATP) 2X3 (P2X3), G protein–coupled receptors, such as bradykinin (BK) B_2 receptors, and the μ opiate receptors [Xue et al, 2007; Amaya et al, 2004; Mamet et al, 2003; Ramer et al, 2001; Lee et al, 2002]. Specifically, sensitization of nociceptive afferents seems to be related to the enhanced responsiveness of $TRPV_1$ receptor due to its phosphorylation [Cortright & Szallasi, 2004]. The intracellular cascades related to $TRPV_1$ sensitization have not been completely clarified, but phosphatidylinositol 3-kinase (PI3K) and the mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) pathways seem to be the most likely candidates [Zhang et al, 2005; Zhuang et al, 2004; Zhu & Oxford, 2007].

A further effect of NGF is the regulation of the voltage-gated ion channel genes, which include calcium (Ca^{2+}), potassium (K^+) and Na^+ channels [Pezet & McMahon, 2006]. Both tetrodotoxin-resistant (TTX-r) and tetrodotoxin-sensitive (TTX-s) Na^+ currents are increased in DRG neurons by NGF [Woodall et al, 2007; Park et al, 2003; Okuse et al, 1997]. Multiple α subunits, including Nav 1.3, 1.7, 1.8, and 1.9 are regulated by NGF, and this effect of endogenously produced or exogenously administered NGF has functional consequences on the sensory transduction/transmission of nociceptive information [Gould et al, 2000; Leffler, 2002; Fjell et al, 1999; Kerr et al, 2001; Amaya et al, 2006].

Through the above mechanism, NGF-induced changes have a great impact on the excitability of nociceptive neuron excitability, as shown by increased spontaneous activity registered in DRG neurons in the presence of NGF [Djouhri et al, 2001; Kitamura et al, 2005].

A further pivotal effect of NGF is the upregulation of BDNF in DRG and the central afferents of nociceptive neurons [Michael et al, 1997]. This NT, which is normally expressed in approximately 30-40% of DRG neurons [Apfel et al, 1996] and trigeminal neurons

[Ichikawa et al, 2006], is induced in virtually all TrkA-expressing neurons after NGF treatment [Michael et al, 1997].

PATHOPHYSIOLOGICAL MECHANISMS OF INFLAMMATORY AND NEUROPATHIC PAIN

Available information obtained from experimental animal models suggests that both inflammatory and neuropathic pain result from cellular and neuroplastic changes occurring in both the peripheral and central nervous system (CNS). A series of processes have been observed that, singly or in combination, may explain the symptoms pointed out in individual patients [Scholz & Woolf, 2002]. In the periphery, following tissue damage or an event that causes direct nerve damage, an inflammatory response ensues. Both inflammatory cells and peripheral neurons in the site of nerve insult release various chemical mediators, including 5-hydroxytryptamine (5-HT), BK, SP, CGRP, histamine, ATP, proinflammatory cytokines, and products from the cyclooxygenase and lipoxygenase pathways of arachidonic acid metabolism. The above substances induce the activation of peripheral nociceptive afferents, and the severity of nociceptive pain is correlated with the extent of tissue damage, and if tissue healing occurs, pain tends to lessen or disappear [Sutherland et al, 2000].

Conversely, neuropathic pain may be initiated by a relatively modest physical insult, and the severity of neuropathic pain does not always correlate with the extent of neuronal damage. As a consequence, significant negative neurologic symptoms such as sensory deficit, weakness and reflex changes, indicative of significant nerve injury, can be, in some cases, prevalent with scarce or no pain. In other cases, acute pain can occur and be the prevailing symptom. In addition, there may be paresthesias, dysesthesias (abnormal or unpleasant sensations without true pain), or spontaneous pain [Bowsher & Haggett, 2005]. In both conditions of inflammatory and neuropathic pain, sensitization of peripheral nociceptive afferents results in an increased responsiveness to thermal and mechanical stimuli, which is mediated through thin, unmyelinated C-fiber primary afferent neurons. This is the most accepted hypothesis regarding the mechanism of hyperalgesia [Siddall & Cousins, 1997; Woolf & Chong, 1993]. If demyelination of the nerve occurs (e.g., Aβ or Aδ fibers), ectopic discharges along the length of the nerve fiber may provide sustained afferent input to the spinal cord. These ectopic signals may persist for extended periods of time and are believed to play a role in the initiation and maintenance of neuropathic pain. Continuous discharge in C fibers may produce sensations of burning pain, whereas intermittent spontaneous discharge in Aβ or Aδ fibers is responsible for lancinating dysesthesias or paresthesias [Petersen & Rowbotham, 2000]. In addition, a neuroma may form at the site of injury, which is responsible not only for spontaneous pain, but also for hyperalgesia and paresthesias [Wall & Gutnick, 1974; Govrin-Lippmann & Devor, 1978].

Neuropathic pain syndromes may be divided into two groups, central and peripheral, based on the location of the nervous system lesion. Following nerve damage, the neuropathic mechanisms can expand during the disease and lead to both peripheral and central pathological changes, making the distinction between peripheral and central pain less distinct than once believed. An example is post-herpetic neuralgia (PHN), which is a persistent pain in the area of a herpes zoster outbreak. PHN involves damage to the peripheral nervous

system (DRG), but this can result in anatomic changes in the DH in the CNS, thus making PHN a neuropathic pain with mixed central and peripheral components [Oaklander, 2008].

Changes in the expression of ion channels and receptors have been observed in peripheral and spinal cord afferent pathway underlying inflammatory and neuropathic pain. Specifically, an alteration in voltage-dependent Na^+ and Ca^{2+} channel subunits has been shown after chronic nerve injury associated with neuropathic pain [Matzner & Devor, 1994]. These include an increase in the expression of Nav1.3 channels and Na^+ channel β3 (Navβ3), Ca^{2+} channel α2αδ1 (Cavα2δ-1) subunits in DRG neuron cell bodies, and in the expression of Nav1.3 in second-order nociceptive neurons in the spinal cord dorsal horn (DH) of the spinal cord [Cummins et al, 2001]. The TTX-r Na^+ channel subunits Nav1.8 and Nav1.9 are also redistributed from DRG neuron cell bodies to peripheral axons and pain receptors at the site of injury [Cummins & Waxman, 1997]. Nav 1.9, in particular, is the more relevant effector of the hypersensitivity produced by multiple inflammatory mediators on nociceptive peripheral terminals [Amaya et al, 2006]. A role for the TTX-r Na^+ channel Nav 1.8 in NGF-induced hyperalgesia has also been demonstrated [Kerr et al, 2001].

Abnormal ectopic discharges from neuromas also seem to be caused by alterations in the expression of the different Na^+ ion channel subtypes that are involved in lowering the threshold for mechanical activation [Ro & Chang, 2005]. Furthermore, a pivotal role emerged for TRP channels, a family of evolutionarily conserved ligand-gated ion channels. Six TRPs ($TRPV_1$, $TRPV_2$, $TRPV_3$, $TRPV_4$, $TRPM_8$ and TRPA1) are expressed in primary nociceptive neurons, where they act as transducers for thermal, chemical and mechanical stimuli. They have been demonstrated to contribute to pain hypersensitivity associated with peripheral inflammatory and neuropathic pain states [Levine & Alessandri-Haber, 2007]. Among these receptors, the nociceptor-specific ion channel $TRPV_1$ serves as the molecular target of capsaicin. It can also be activated by noxious heat (with a thermal threshold >43° C) or low pH and endogenous lipids, and all these stimuli are known to cause pain *in vivo* [Palazzo et al, 2008]. Furthermore, studies using $TRPV_1$-deficient mice have shown that $TRPV_1$ is essential for selective modalities of pain sensation and thermal hyperalgesia [Roberts & Connor, 2006].

Sensitization of $TRPV_1$ is one mechanism that has been recognized to initiate pain by tissue damage/inflammation [Huang et al, 2006]. Phenotypic change of primary afferents with respect to expression of $TRPV_1$ channels may also account for some paroxysmal symptoms and signs in neuropathic pain [Katsura et al, 2006]. $TRPV_1$ receptors are expressed in the CNS, where they seem to play a pivotal role in pain mediated by central sensitization. They can also be the target of specific agonists, whose penetration into the CNS is essential for producing a broad-spectrum analgesia [Cui et al, 2006]. However, if the $TRPV_1$ receptor plays a pro-nociceptive role in certain models of acute tissue injury, under chronic polyneuropathic conditions it can initiate anti-nociceptive counter-regulatory mechanisms possibly mediated by somatostatin released from sensory neurons [Helyes et al, 2007].

In addition to $TRPV_1$, the other five thermosensitive ion channels in mammals, $TRPV_2$, $TRPV_3$, $TRPV_4$, $TRPM_8$ and $TRPA_1$ act within the noxious range of temperatures and may further contribute to pain due to inflammatory injury [Numazaki & Tominaga, 2004; Bölcskei et al, 2003].

Further molecular sensors detecting adverse stimuli can be activated by inflammatory signals, causing primary hyperalgesia [Coutaux et al., 2005]. Among them, the PX receptor channels, the molecular sensor for ATP, are implicated in hyperalgesia accompanying tissue

damage [Donnelly-Roberts et al, 2008]. Their expression is increased in animal models of neuropathic pain [Chen et al, 2005]. Moreover, the specific 5-HT receptor subtype expressed in nociceptive endings, 5-HT_{3R}, seems to be involved in the activation of nociceptive neurons by 5-HT released from activated platelets and enterochromaffin cells [Meuser et al, 2002].

The increased susceptibility to firing and the increased firing frequency of sensitized peripheral nociceptive afferents are responsible for the so-called phenomenon of "central sensitization" that refers to neuroplastic changes involving second-order nociceptive neurons, which is believed to account for the clinical phenomenon of allodynia [Garcia-Larrea & Magnin, 2008].

Plastic changes of the DH neurons is implicated in the increase in receptive field size, the increase in the magnitude and duration of the response to stimuli, and the reduction in their excitatory threshold. Under these conditions, central neurons that normally receive high-threshold sensory input may begin to receive input from low-threshold mechanoreceptors. This information may be interpreted as nociceptive [Katz & Rothenberg, 2005].

An alternative hypothesis is that allodynia is caused by a decrease in central inhibition of the mechanically-induced nociceptive input. Furthermore, within the spinal cord, collateral sprouting of primary afferent neurons may occur [Garcia-Larrea & Magnin, 2008]. Accordingly, nerve fibers in deeper laminae that do not normally transmit pain sprout into more superficial regions of the DH (e.g., laminae I and II) and become receptive to nociceptive input. Another possible mechanism for central sensitization is ephaptic conduction, also known as "crosstalk" between neurons [Soderling & Derkach, 2000]. Chemical mediators lower, in fact, the threshold for cross-excitation, which becomes sufficient to evoke ectopic firing from a biochemical and molecular point of view. Experimental findings suggest that central sensitization is mediated through the release of various neurotransmitters [e.g., SP, glutamate, CGRP, γ-aminobutyric acid (GABA), and NKA, production of nitric oxide (NO) by NO synthase, through activation of the N-methyl-D-aspartate (NMDA) receptor, increased Ca^{2+} flux, possibly mediated by N-type calcium channels], and prostaglandin synthesis [Petrenko et al, 2003; Omoigui, 2007].

In the last few years, the intervention of some NTs in persisting pain states has been emphasized. In particular, NGF has been demonstrated to play a pivotal role in diffuse inflammatory and neuropathic pain conditions by regulating sensory neuron phenotype and excitability.

EXPERIMENTAL EVIDENCE FOR THE INVOLVEMENT OF NGF IN INFLAMMATORY AND NEUROPATHIC PAIN

The increase of both NGF mRNA and protein has been demonstrated in different experimental models of inflammatory pain, where this NT acts as an algogenic mediator produced by skin keratocytes, Schwann cells, macrophages, mast cells and T cells. Examples of inflammatory conditions in experimental animals, in which NGF has been implicated, include models of cutaneous carragenin, lipopolysaccharide (LPS), complete Freund's adjuvant (CFA), skin wounds, muscle formalin, acute injury, arthritis, spinal cord injury disc herniation, acute burn injury, bone cancer-pain, acute pancreatitis and incisional pain [Kanaan et al, 1998; Safieh-Garabedian et al, 2002; Talhouk et al, 2004; Woolf et al, 1997; Djouhri et

al, 2001; Cruise et al, 2004; Ueda et al, 2002; Krenz &Weaver, 2000; Obata et al, 2004a; Toma et al, 2000; Saban et al, 2002; Wu et al, 2007; Zahn et al, 2004].

In contrast, in several neuropathic conditions characterized by sensory loss, peripheral nerve injury leads to a down-regulation of NGF, which is responsible for some changes occurring in TrkA-expressing neurons, typically over several days or weeks. This has been demonstrated in the case of axotomy and includes a down-regulation of the neuropeptides SP and CGRP and, conversely, an up-regulation of galanin, vasoactive intestinal peptide, and activating transcription factor 3. The above changes can be prevented by a few micrograms of NGF delivered via an osmotic pump onto the end of the sciatic nerve [Fitzgerald et al, 1985] or intrathecally [Verge et al, 1992]. Similarly, a down-regulation of $TRPV_1$, Nav 1.8 and Nav 1.9 is induced by axotomy, and is reversed by NGF [Gould et al, 2000]. Other transmitters and receptors in non-TrkA neurons, (mainly large mechanosensitive cells and small non peptidergic neurons), constitutively expressing the P2X3 receptor, are unaffected by NGF treatment [Averill et al, 2004]. NGF also protects damaged sensory neurons from high doses of capsaicin, which is recognized to induce a loss of axon terminals and a decrease of sensory neuropeptide expression [Donnerer et al, 2005]

A neuroprotective effect of NGF was also demonstrated following damage to the central branch of the primary sensory neurons, such as in the case of dorsal root avulsion [Romero et al, 2001]. They undergo wallerian degeneration and DH neurons lose their afferent input. Injured sensory neurons can regenerate within the DH and this phenomenon is dependent on NGF availability [Romero et al, 2001].

Further recent evidence suggests that regeneration of the peripheral branches of sensory neurons and neurite outgrowth of subpopulations of injured sensory neurons due to NGF occur, as with NT-3. This seems to be mediated by α-7 integrin and laminin signaling [Gardiner et al, 2005]. NGF also restores the *in vivo* expression of voltage gated Na^+ channels, which is reduced in the case of peripheral nerve damage in some neuropathic models in rats, such as the L5/6 thigh nerve ligation or axotomy [Okuse et al, 1997].

Furthermore, experimental findings support a role for NGF in preventing semaphorin 3A-mediated growth cone collapse in adult neurons, in which this chemorepellent guides centrally projecting axons of DRG. A similar effect is shared by glial cell line-derived neurotrophic factor (GDNF), neurturin or cyclic adenosine 5'-monophosphate (cAMP). This novel converging signaling pathway could possibly be used as a therapeutic option for neuropathic pain, and needs to be explored in future research [Wanigasekara & Keast, 2006].

In contrast, recent data show that the NGF precursor (pro-NGF) may function as a death-inducing ligand mediating its apoptotic effects via p75NTR. Pro-NGF-induced apoptosis seems to be dependent upon membrane expression of the sortilin receptor, which interacts with p75NTR to promote a high-affinity binding site for pro-NGF, as demonstrated in the model of sciatic nerve transection. The relevance of this finding for sensory neuron loss needs to be established in experimental neuropathic models [Arnett et al, 2007].

NGF dramatically upregulates pituitary adenylate cyclase-activating polypeptide (PACAP) expression in TrkA-positive neurons in both intact and injured DRGs in the model of chronic constriction injury (CCI). Also, galanin expression in adult sensory neurons was modulated by NGF in injured neurons and after inflammatory insult. The elevated expression of both PACAP and galanin in injured neurons is mitigated by NT-3, suggesting a role for this NT, but not NGF, in returning the 'injured phenotype' back towards an 'intact phenotype'

without modifying TrkA mRNA expression due to CCI of the sciatic nerve [Wilson-Gerwing & Verge, 2006].

Diabetic neuropathy is a prototypic condition in which peripheral sensory nerves are compromised in their ability to transport NTs, mainly NGF [Apfel, 1999], and in which a reduction of $TRPV_1$ levels has been observed as a result of NGF decrease [Facer et al, 2007; Anand et al, 2006]. The depletion of NGF in human diabetic neuropathy skin has been correlated with the dysfunctioning of nociceptive fibers [Anand, 2004]. Similar mechanisms can be hypothesized even for Human Immunodeficiency Virus (HIV) neuropathy.

The above observations prompted the design of NGF administration trials involving both diabetic and HIV patients, which failed, however, to show any significant effect on antagonizing the sensory alterations in patient outcome [McArthur et al, 2000; Larkin, 1998; Schifitto et al, 2001], perhaps because of the difficulty in identifying appropriate doses and the probable need for multiple trophic factors, in contrast to the beneficial effects observed in animal models [Apfel, 2002; Christianson et al, 2007].

In experimental models of diabetic neuropathy, the majority of studies addressing the loss of axons focused on C-fiber depletion in the epidermis, but little information was obtained regarding myelinated fibers, in which an ultrastructural damage was shown in both experimental animals and patients [Weis et al, 1995; Mizisin et al, 1998]. Electrophysiological studies in diabetic rats have suggested that increased sensory input from myelinated afferents plays an important role in diabetic neuropathic pain, and that NGF can have a pivotal function in inducing the increased input [Khan et al, 2002].

Inconsistent results, however, have been obtained for NGF in animal diabetic neuropathy models, depending on animal strains. In particular, the finding of mechanical hypoalgesia, which has been demonstrated in streptozotocin (STZ)-induced diabetic mice, and can be restored by NGF, is in line with the insensate neuropathy occurring in the majority of humans with diabetic neuropathy [Christianson et al, 2007, 2003]. This is in contrast with the tactile allodynia and increased sensitivity shown in STZ-induced diabetic rats. Depletion of capsaicin-sensitive C-fibers in this model does not alter the development of tactile allodynia, supporting the view that A-fibers, as a potential source of NGF, play a relevant role in increased mechanical sensitivity in the latter model like that presenting in some diabetic patients as a potential source of NGF [Khan et al, 2002]. These latter findings stress the complex role played by NGF in diabetic neuropathy.

Despite the discrepant results obtained in diabetic neuropathy, a local increase of NGF can be hypothesized to underlie several conditions of neuropathic pain, such as post-herpetic neuralgia, cisplatinum and taxol-induced neuropathies, as well as post-traumatic injury to peripheral nerves, DRG or spinal cord [Anand, 2004]. If NGF levels are acutely reduced in injured nerve trunks, in these neuropathic painful conditions, skin hyperalgesia and allodynia may be due to a marked local increase of this NT.

The majority of preclinical models that have been developed for neuropathic pain are traumatic injury models (sciatic constriction, spinal nerve ligation, sciatic axotomy, ventral root section), and in all of them, an increase of NGF has been detected [Kanaan et al, 1998; Safieh-Garabedian et al, 2002; Talhouk et al, 2004; Woolf et al, 1997; Djouhri et al, 2001; Cruise et al, 2004; Ueda et al, 2002; Krenz &Weaver, 2000; Obata et al, 2004a; Toma et al, 2000; Saban et al, 2002].

Figure 1 displays the main pathophysiological events underlying neuropathic pain involving NGF.

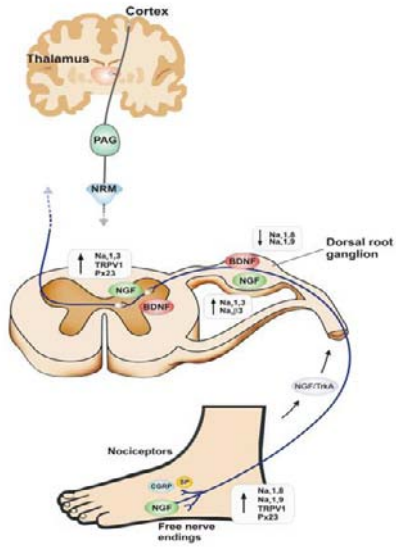

Figure 1. Potential mechanisms of NGF in the pathophysiological events underlying neuropatic pain. The figure illustrates the modifications induced by NGF released by inflammatory cells, Schawann cells and keratinocytes surrounding the injured nociceptive terminals in the case of neuropathic pain. NGF interacts with own high affinity receptors with TRkA on nociceptive neurons . By this interaction it stimulates the release and the synthesis of the sensory neuropeptides SP and CGRP. These peptides are released both in periphery and in spinal cord DH neurons where they interact with their own receptors, NK1 and CGRP receptors, on second order nociceptive neurons. The most significant changes which occur in peripheral nociceptive neurons as a consequence of NGF are: an upregulation of Na^+ channel subunits Nav 1.3 (tipo III), a redistribution of Na^+ channel subunits Nav 1.8 e Nav 1.9 from the somata to the axons of nocieptive neurons and an increase in the auxillary subunit of Na^+ β3 channel. An enhanced responsiveness of TRPV1 is also induced by increasing its phosphorylation. An increased excitability of nociceptive neurons follows the above modifications within the DRG and nerve root; where NGF induces BDNF synthesis through interaction TrkA and intracellular ERK-MAPK pathway activation. The BDNF is transported and released in the DH where interacts with its specific receptor TRkB on second-order neurons. This neurotrophin has a variety of postsynaptic effects. It induces a rapid increase in TrkA phosphorylation and an increase of c-fos, c-jun, and krox 24 expression, and of cAMP responsive element binding protein (CREB) phosphorylation, as well as phosphorylation of the mitogen-activated kinase ERK in second-order nociceptors which are important steps in the development of central sensitization underlying pain maintenance. A further effect contributing to central sensitization due to the TrkB phosphorylation, is the increase of NMDA-mediated responses. This increase is due to the phosphorylation of NR1 and NR2B subunits which enhance a three-fold increase in NMDA receptor open time.TrkA: Tropomyosin-Related Kinase A, TrkB: Tropomyosin-Related Kinase B, NK1: Neurokinin 1SP: Substance P, DRG: Dorsal Root Ganglion, PAG: periaqueductal gray region; NRM: magnus raphae nucleus, CGRP: Calcitonin Gene Related Peptide, DH: Dorsal Horn, MAPK:Mitogen-Activated Protein Kinase, ERK: Extracellular Signal-Regulated kinases, CREB: cAMP responsive element binding protein, NR1: subunit 1 of N-methyl-D-aspartic acid receptor, NR2: subunit 2 of N-methyl-D-aspartic acid receptor, NMDA: N-methyl-D-aspartic acid, BDNF Brain-Derived Neurotrophic Factor, NGF: Nerve Growth Factor, TRPV1: Transient receptor potential vanilloid 1.

As in inflammatory pain, recent findings indicate that both SP and CGRP are upregulated in a small population of large- and medium-sized primary sensory neurons after peripheral injury (due to the increase of NGF) in some animal models of neuropathic pain [Ruiz & Baños, 2005]. An increase of CGRP and activin A, which act in concert with NGF, has also been shown in nociceptive neurons that are involved in the sensitization of $TRPV_1$ in primary sensory neurons after injury [Xu & Hall, 2007].

Differential expression of TRP receptors has also been confirmed *in vitro* by the addition of NGF to DRG culture [Anand et al, 2004]. This NGF-dependent mechanism can be hypothesized to occur *in vivo* in neuropathic pain. In injured human DRG sensory neurons, co-expression of $TRPV_1$ and $TRPV_3$ has also been demonstrated. In models of post-traumatic neuropathy, the accumulation of $TRPV_1$ and $TRPV_3$ in peripheral nerves after injury indicates that these receptors continue to be exported from the ganglion and/or accumulate in the nerve proximal to injury, despite overall reduced support from peripheral trophic factors, e.g., NGF/GDNF. An increased availability of trophic factors to spared nerve fibers may be similar to the finding in the animal model of partial nerve injury, where undamaged fibers have more available NT because of the reduced uptake by damaged fibers. $TRPV_1$-immunoreactive nerves are also present in injured dorsal spinal roots and DH neurons in the spinal cord, but not in ventral roots, while $TRPV_3$ and $TRPV_4$ were detected in spinal cord motor neurons [Facer et al, 2007]. Furthermore, NGF derived from damaged Schwann cells, or infiltrating macrophages at the site of injury enhances the expression of $TRPV_1$ through the p38 MAPK pathway, which can be antagonized by anti-NGF, p38 MAPK inhibitor, or $TRPA_1$ antisense oligodeoxynucleotides [Obata et al, 2005]. In addition, *de novo* expression of P2X3 receptors is induced by NGF in both spinal cord lamina I and outer lamina II and to the ventro-medial afferent bundle beneath the central canal [Ramer et al, 2001]. In contrast with TRP and purinergic receptors, increased voltage-gated sodium channel activity seems to only partially contribute to NGF-induced hyperexcitability in neuropathic pain, according to Okuse et al (1997). The above phenotypic changes of nociceptive sensory neurons due to increased NGF expression are responsible for an increased excitability of nociceptive terminals.

Another mechanism mediated by increased levels of NGF is the collateral sprouting of sensory neurons, specifically the A-δ-axons involved in nociception and the C-fiber mediating heat nociception, which is antagonized by anti-NGF antibodies, and is responsible for the expansion of pinch and heat field in the skin innervated by damaged neurons [Diamond et al, 1992].

Sympathetic sprouting within axotomized DRGs may also contribute to neuropathic pain. This is related to upregulation of NGF, which is retrogradely transported in the DRG in uninjured sensory axons, and its overexpression, at least in the CCI model, is driven by a glial protein promoter (GFAP) [Ramer et al, 1997, 1998].

In models of thermal injury-induced mechanical hyperalgesia, which mimics the prolonged duration of clinical burn injury pain, NGF was elevated in burn-injured tissue and produced mechanical hyperalgesia [Summer et al, 2006]. It also activated PKC- ε, a key mediator in inflammatory and neuropathic pain. This was confirmed by the evidence that intrathecal administration of antisense oligodeoxynucleotides to TrkA and PKC- ε, starting 3 days before inducing a burn injury, caused a dose-related decrease of burn-induced primary mechanical hyperalgesia. In addition, intradermal injection of a PKC- ε -selective inhibitor eliminated hyperalgesia, supporting the potential usefulness of strategies directed against

receptors or downstream pathways activated by NGF as analgesics in pain due to burn injury in humans.

NGF, like NT-3, GDNF and BDNF, has also been implicated in marked and long-lasting mechanical hypernociception following brachial plexus avulsion (BPA). Antibodies against all the above NTs, including NGF, were, in fact, able to post-pone its development in mice when administered locally, systemically or intrathecally at the time of surgery [Quintão et al, 2008].

As a consequence of increased expression of NGF, TrkA expression was elevated and *c-fos* was upregulated in the spinal DH. If *c-fos* expression plays a crucial role in the development of hyperalgesia in the early stages of neuropathic pain, TrkA receptors may, instead, be involved in the long-lasting adaptive changes of the central pathway in neuropathic pain, as suggested by findings in the CCI sciatic nerve model in rats [Wan et al, 2005].

In addition, in models of both inflammatory pain induced by CFA and neuropathic pain due to axotomy, the enhancement in mechanical allodynia related to the increase of NGF has been shown to be associated with the increase in ERK phosphorylation in TrkA-expressing small- and medium-size neurons. This increase was inhibited by MAPK 1/2 inhibitor, U0126, and was mediated through Trk receptors, because intrathecal treatment with the Trk inhibitor, K252a, reduced the activation of ERK, whose labelling occurred through P2X3 receptors in the terminals [Noguchi et al, 2004]. Likewise, activation of the MAPK pathway in primary nociceptive afferents may be involved in the pathophysiological mechanisms of inflammation-induced radiculopathy or trasverse section-induced neuropathy, which can be potential targets for pharmacological intervention [Obata et al, 2004b].

It has been demonstrated that, after an inflammatory insult as well as sciatic nerve transection, NGF synthesized within the nerve root and DRG induces BDNF expression through TrkA receptors and intracellular ERK-MAPK pathway activation [Obata et al, 2004b].

The crucial role played by BDNF in neuropathic pain is, furthermore, supported by the demonstration that the local inactivation of this NT decreases the incidence and the severity of autotomy and neuroma formation, but did not influence neuron regeneration [Marcol et al, 2007].

BDNF also contributes to the increased excitability of spinal dorsal neurons, as shown in models of CCI and underlies central sensitization related to neuropathic pain [Lu et al, 2007]. Actions of BDNF on excitatory synaptic drive to putative excitatory, 'delay' firing neurons in the substantia gelatinosa, which are exclusively presynaptic and involve increased miniature excitatory postsynaptic current (mEPSC) frequency and amplitude without changes in the function of postsynaptic AMPA receptors. In contrast, BDNF exerts both pre- and postsynaptic actions on 'tonic' cells. These selective and differential actions of BDNF on excitatory and inhibitory neurons contributes to the global increase in DH network excitability, as assessed by the amplitude of depolarization-induced increases in intracellular Ca^{2+} [Lu et al, 2007].

In recent years, emerging evidence has suggested that NGF, other than regulating sensory neuron phenotype by elevating expression of ion channels and receptors, is able to upregulate the number and efficacy of sensory μ opioid receptors (MORs), which are colocalized with CGRP, TrkA and p75 receptors within DRG, rendering sites of painful inflammation more

susceptible to better pain control. These findings support the potential of this NT to overcome the unresponsiveness to opioids of certain neuropathic pain states [Mousa et al, 2007].

PATHOPHYSIOLOGICAL MECHANISMS OF MIGRAINE

Neurobiological, genetic and advanced brain imaging studies have provided new insights into migraine pathogenesis. Basic concepts of migraine pathophysiology include: (i) an altered neuronal excitability during the interictal phase; (ii) biochemical abnormalities outside attacks involving serotonergic, dopaminergic, and noradrenergic systems; (iii) cortical spreading depression as the basis of aura; (iv) trigeminovascular activation that accounts for the headache phase; and (v) activation of the periacqueductal gray matter (PAG) as a putative "migraine generator or modulator", which may explain some aspects of central sensitization or change in phenotypic expression of the disorder from an episodic to a chronic form [Sanchez-Del-Rio et al, 2006; Cutrer & Huerter, 2007; Silberstein, 2004; Dodick & Silberstein, 2006; Diener & May, 1996; May & Matharu, 2007].

In the last few years a great body of experimental evidence has supported the occurrence of a peripheral sensitization of trigeminal nociceptive afferents innervating the intracranial blood vessels and meninges as the first event that accounts for the pulsating/throbbing head pain during a migraine attack and the associated increase in intracranial sensitivity [Malick & Burstein, 2000]. This results in the aggravation of pain by mechanical stimuli such as those produced by the small increases in intracranial pressure due to coughing or bending.

Peripheral sensitization of trigeminal nociceptive neurons may be induced by the release of algogenic or inflammatory substances from various sources, including these same neurons after their activation. Some of these substances, such as IL-1β, IL-6, TNF-α and PAF can contribute to NGF release from peripheral trigeminal endings [Burstein, 2001]. This inflammatory component associated with trigeminovascular activation is supported by the demonstration of an upregulation of proinflammatory cytokines, such as IL-1β, IL-6 and TNF-α, which have been recognized to play a pivotal role in the experimental model of neurogenic inflammation [DeLeo & Yezierski, 2001].

Experimental data also suggest that NGF activates mast cells through the collaborative interaction with lysophosphatidyl serine expressed on the membrane of activated platelets. This could be of relevance for neurogenic inflammation subsequent to trigeminovascular system activation [Levy et al, 2006; Sugiyama et al, 1985; Kawamoto et al, 2002; Bonini et al, 2003].

Furthermore, the development of a delayed inflammatory response in the context of neurogenic inflammation consequent to trigeminal activation has been emphasized in a rat model of meningeal inflammation due to a brief nitroglycerin infusion, by the findings of a dose-dependent Type II inducible NO synthase (iNOS) mRNA up-regulation in dura mater macrophages and the increase in the corresponding protein expression at 4, 6 and 10 hours after infusion. Consistent with development of a delayed inflammatory response, IL-1β in dura mater and IL-6 in dural macrophages and CSF were detected [Reuter et al, 2001]. In a more recent study, carried out in the same model, the increase in iNOS expression was preceded by a significant increase in nuclear factor-kappaB (NF-κB) activity, due to the reduction in the inhibitory protein-κ-Bα (IκBα) and the upregulation of NF-κB [Reuter et al,

2002]. In agreement with these experimental data, the transitory increase in levels of the proinflammatory cytokines IL-1β, IL-6 and TNF-α was demonstrated by our group in the internal jugular blood of migraine without aura patients assessed in the early phase of the attack. These elevated levels were associated with an increase in intracellular adhesion molecule 1 (ICAM-1) and a significant increase in NF-κB activity preceding the increase in iNOS expression in mononuclear cells [Sarchielli et al, 2006a, 2006b]. A transient increase in PAF levels was also detected in the internal jugular blood of migraine patients during attacks [Sarchielli et al, 2004b].

All the above substances are part of the "inflammatory soup" that can activate meningeal trigeminal nociceptive neurons. In this context, NGF induced by proinflammatory cytokines and PAF can directly sensitize nociceptive neurons through rapid modulation of heat/vanilloid receptors or by up-regulating ion channels, with particular regard to those involved in TTX-s and TTX-r Na^+ currents [Cummins et al, 2007; Padilla et al, 2007; Fabbretti et al, 2006].

The repetitive activation of trigeminal endings induces an activity- or use-dependent neuronal plasticity of trigeminal nociceptive neurons. This modifies their subsequent performance, enabling normally innocuous inputs to activate them (allodynia) and determining exaggerated or prolonged responses to noxious inputs (hyperalgesia) [Sarchielli et al, 2007a]. This central neuronal activation results in temporal summation of nociceptive responses. More specifically, the activity-dependent plasticity is the consequence of the summation of C-fiber-evoked slow synaptic currents produced by glutamate acting on NMDA and metabotropic glutamate receptors (mGluRs), and neuropeptides, such as CGRP and SP, as well as by the recruitment of postsynaptic voltage-gated Ca^{2+} plateau currents and release of BDNF. Increased synaptic and voltage-gated currents contribute to a use-dependent facilitation (*wind-up*) of trigeminal nociceptors during repetitive C-fiber inputs by producing nonlinear cumulative depolarizations lasting tens of seconds [Vikelis & Mitsikostas, 2007; Dodick & Silberstein, 2006]. These events are responsible for a progressive increase in the amount of pain experienced and underlie the early stages of central sensitization, which are interfaced with a later stage in which a cascade of intracellular biochemical events leads to longer lasting changes in the properties of trigeminal nociceptive neurons.

PATHOPHYSIOLOGY OF FIBROMYALGIA

Fibromyalgia syndrome (FM) is a chronic pain syndrome defined as a widespread pain for more than 3 months and the presence of >11 of 18 tender points [Wolfe et al, 1990]. It represents the extreme of a wide spectrum of musculo-skeletal pain syndromes, which mostly affects women with a ratio of 9:1 to men [Wolfe et al, 1990]. The majority of studies concerning FM patients have shown abnormalities of pain sensitivity using different methods of sensory testing. A generalized lowering of pressure pain thresholds in FM patients and a mechanical pain hypersensitivity (allodynia) of FM patients not limited to tender points but diffusely widespread are common features [Petzke et al, 2003].

Pain in FM is consistently felt in the musculature and has been related to the sensitization of both peripheral and CNS pain pathways [Price & Staud, 2005; Staud & Rodriguez, 2006].

Accumulating evidence suggests that peripheral impulse input from deep tissues and joints plays an important role in FM patients. The most significant findings point to relevant alterations in skin and muscles. They include the presence of ragged red fibers and moth-eaten fibers, inflammatory infiltrates and DNA fragmentation in affected muscles, muscle perfusion deficits confirmed by the reduction in pH (related to ischemia), reduction in phosphorylation potential, and in total oxidative capacity [Averill et al, 1986; Jacobsen et al, 1991; Kalyan-Raman et al, 1984; Bengtsson et al, 1986]. An increase in inflammatory mediators and proinflammatory cytokines as well as advanced glycation end products has also been demonstrated [Hoheisel et al, 1998; Wallace et al, 2001; Giovengo et al, 1999]. All these biochemical mediators induce changes in the properties of muscle primary nociceptive afferents underlying their peripheral sensitization. The consequent maintained tonic impulse input from muscles contributes to the development of central sensitization [Arendt-Nielsen et al, 2003; Sorensen et al, 1998]. In this condition, the long-term neuroplastic changes in DH neurons exceed the antinociceptive capabilities of affected patients, resulting in ever-increasing pain sensitivity and spontaneous pain. Nociceptive activity in the peripheral tissues of FM patients, however, does not necessarily have to be extensive, because central sensitization requires little sustained input to maintain the sensitized state and chronic pain.

From a clinical point of view, allodynia for cutaneous/muscle mechanical stimulation and hyperalgesia for electrical as well as thermal stimulation, support the occurrence of central sensitization in FM patients [Price et al, 2002; Staud et al, 2003]. They have also been shown to display enhanced temporal summation of heat-evoked second pain compared to pain-free controls [Price et al, 2002; Staud et al, 2003]. Recent research clearly confirmed in these patients enhanced pain sensitivity, with lower stimulus intensities needed to establish *wind-up* and lower stimulus frequencies to maintain it than in controls [Staud et al, 2001, 2004]. In addition, *wind-up* after-sensations are greatly prolonged, indirectly suggesting that *wind-up* is abnormally maintained in FM [Staud et al, 2001, 2004]. Abnormal *wind-up*, prolonged after-sensations, and mechanical allodynia are, therefore, the result of abnormal nociceptive input from the periphery and abnormal central pain mechanisms relative to controls. They represent peculiar features of central sensitization that have been found to be relevant predictors of clinical pain in FM [Price & Staud, 2005; Staud & Rodriguez, 2006]. Pain-related negative affects have been shown to significantly contribute to pain in these pathological conditions [Price & Staud, 2005; Staud & Rodriguez, 2006].

INVOLVEMENT OF NGF IN THE PATHOPHYSIOLOGICAL MECHANISMS OF MIGRAINE AND FIBROMYALGIA

Localization studies outlined the pivotal contribution of NGF signaling to nociception of trigeminal neurons. NGF has been demonstrated in these neurons to be effective in enhancing capsaicin and K^+ evoked CGRP release [Price et al, 2005]. This action, together with activation of the ERK pathway, is mediated by stimulation of the CGRP promoter. NGF has also been demonstrated to increase functional activities of the $TRPA_1$ channel and P2X3 receptors. This leads to plastic changes in the trigeminal nociceptive pathways mediating head pain [Price et al, 2005; Diogenes et al, 2007; Simonetti et al, 2006], which is counteracted by anti-NGF treatment [D'Arco et al, 2007].

These observations outlined the relevance of NGF in trigeminal pain and prompted research aimed at determining its levels in migraine. The more relevant pathophysiological mechanisms of migraine involving NGF are shown in Figure 2.

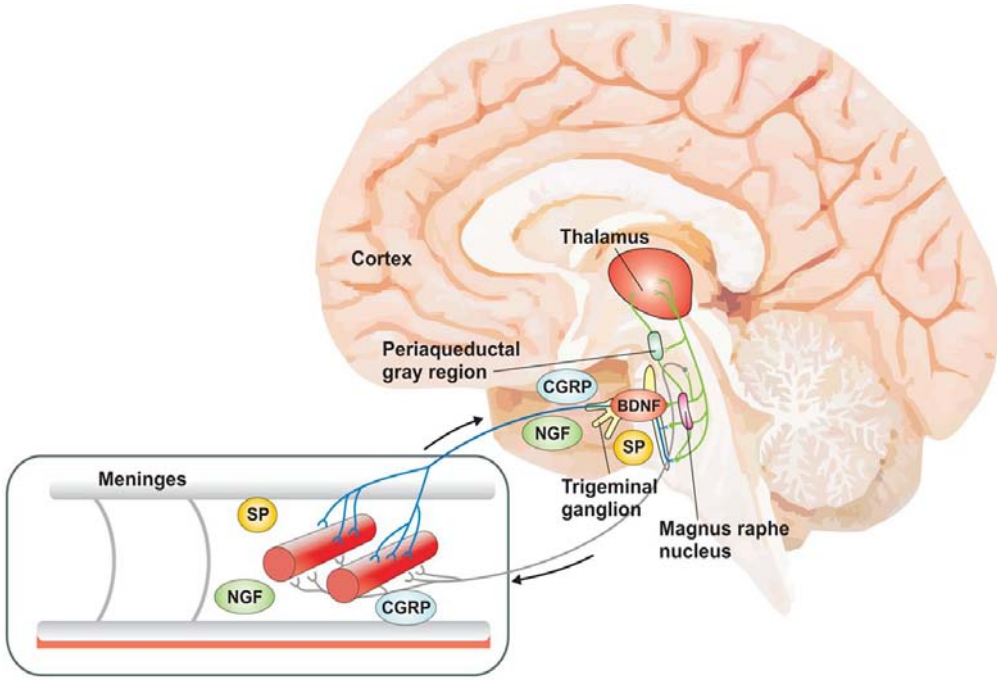

Figure 2. Relevance of NGF in the pathophysiological mechanisms of migraine. Activation of nociceptive afferent fibers of trigeminal ganglion (TG) neurons innervating the meningeal blood vessels and the subsequent activation of second-order dorsal horn neurons in the trigeminal nucleus caudalis (TNC) is the main mechanism underlying migraine headache. In the meninges, NGF released by activated mastcells, is responsible of the release of vasoactive neuropeptides CGRP and NKA, by interacting with own high affinity receptor TrkA on trigeminal terminals. There are also other molecules contributing to the release of these peptides, belonging to the so called inflammatory soup (K+, PAF, bradikinin, 5HT, some proinflammatory cytokines) which contributes to neurogenic vasodilation of meningeal vessels. Sensory neuropeptides are also anterogradely transported and released in the DH by the central terminals of the first-order nociceptors interacting with second-order dorsal horn neurons. NGF also induce the de novo synthesis of sensory neuropeptides and up-regulate P2X3 and TRPV1 receptors. It also induce the synthesis of BDNF in the TGN form which this latter neurotrophin is anterogradely transported and released from the central terminals of trigeminal nociceptors in the TNC. Through interaction with own receptor TrkB, BDNF increases the excitability of TNC neurons, also by stimulate glutamatergic transmission via enhancement of NMDA receptor functioning. Through the same post-synaptic mechanisms described for neuropathic pain it intervenes in central sensitization which is involved in head pain maintenance and chronification. TG: trigeminal ganglion, TNC: trigeminal nucleus caudalis, NGF: Nerve Growth Factor, TrkA: Tropomyosin-Related Kinase A, CGRP:Calcitonin Gene Related Peptide, NKA: Neurokinin-A, PAF: Platelet Activating Factor, 5HT: 5-hydroxytryptamine, DH: Dorsal Horn, P2X3: Purinergic receptor P2X, ligand-gated ion channel, 3, BDNF: Brain-derived neurotrophic factor, TRPV1: transient potential receptor vanilloid 1, NMDA: N-methyl-D-aspartic acid.

NGF measurements in the peripheral blood of CM patients showed reduced levels that were attributed to altered platelet turnover of this NT [Blandini et al, 2005]. Further research, however, carried out on patients with chronic daily headache (CDH) with a previous history of episodic migraine without aura and, more recently, on patients suffering from CM diagnosed according to the current International Classification Headache Disorders (ICHD-II) criteria [Subcommittee of the International Headache Society, 2004], suggests the implication of NGF in the maintenance of chronic head pain.

The first of these studies, carried out by our group, investigated NGF levels in the CSF of a group of CDH patients comparing them with those of an age- and sex-matched control group. This latter group included subjects for whom CSF and blood examinations as well as adequate instrumental investigations excluded CNS or systemic diseases. Values of SP and CGRP were also determined and were significantly correlated with NGF levels in the CSF of both CDH patients and controls. Significantly higher CSF levels of NGF, SP and CGRP were detected without significant differences between patients with symptomatic drug overuse and those without. A significant correlation also emerged in the same study between NGF levels and the duration of chronic headache and number of days with headache per month [Sarchielli et al, 2001].

If the evidence of increased CSF levels of NGF confirmed the pivotal role of this NT in CDH patients, the concomitant increase in the levels of sensory neuropeptides of trigeminal origin, SP and CGRP, supported the intervention of NGF in enhancing their synthesis, transport, content and release from trigeminal nociceptive neurons in the same patients, as shown in animal pain models [Sarchielli et al, 2001].

In a subsequent study, levels of NGF and those of another NGF-dependent NT, BDNF, as well as glutamate were determined in the CSF of a further group of patients affected by CDH with a previous history of episodic migraine [Sarchielli et al, 2002]. Significantly higher levels of both NGF and BDNF were found in CDH patients compared with controls. Glutamate levels were also significantly higher than those of controls. Levels of the two NTs were significantly correlated with each other and with levels of glutamate in the patient group. As in the previous study, no significant differences were found in BDNF, NGF and glutamate levels between CDH patients with analgesic abuse and those without.

These findings concur with those obtained in experimental pain models, which demonstrated that the nociceptive effect of NGF seems to be exerted through the upregulation of BDNF mRNA and protein in TrkA nociceptive neurons. In contrast, BDNF release is responsible for increased synaptic activity through the potentiation of glutamatergic transmission, as suggested by the increase in glutamate levels in the CSF of our CDH patients. The increase in glutamatergic transmission is believed to underlie central sensitization, thus playing a pivotal role in the maintenance of head pain in these patients.

Based on the above results, it can be hypothesized that similar mechanisms could be implicated in other chronic painful conditions, such as FM. The most relevant pathophysiological mechanisms involving NGF are displayed in Figure 3. Based on this assumption, it is therefore assessed NGF and BDNF levels in the CSF of patients with primary FM syndrome and compared results obtained in the CSF with those of patients with CM, diagnosed according to the current ICHD-II criteria, and those of control subjects [Sarchielli et al, 2007b]. The relationship between these levels and the duration of chronic pain and quantitative pain measures in both patient groups was also assessed. As in our previous research, significantly higher levels of NGF, BDNF and glutamate were found in

patients with CM. The same levels were also increased in patients with primary FM syndrome. The findings of an increase in NGF levels in the CSF concurs, in particular, with results of a previous study from Giovengo et al [1999], demonstrating increased concentration of this NT compared to controls although with a greater variability.

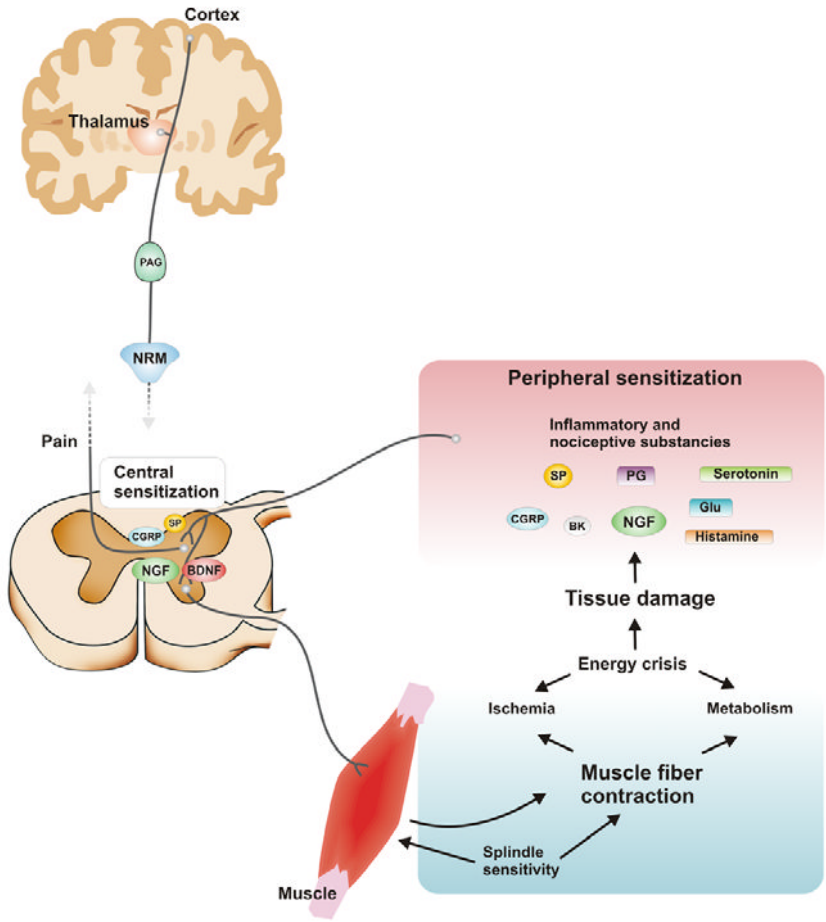

Figure 3. Potential mechanisms of NGF in the pathophysiological events underlying fibromyalgia. Abnormalities in skin and muscles have been demonstrated in fibromyalgia which include an increase of proinflammatory cytokines and excitatory amino acids, a release of SP in muscle tissue, DNA fragmentation within muscle cells, and muscle perfusion deficits. In this condition NGF can be released by inflammatory cells, Schwann cells and keratinocytes and interacts with own high affinity receptors TRkA on nociceptive neurons. By this way it stimulates the release and synthesis of the sensory neuropeptides SP and CGRP both in periphery and in DH, increasing excitability of first and second-order neurons. Like in the case of neuropathic pain, NGF also induces BDNF expression in DRG through TrkA and intracellular ERK-MAPK pathway activation. BDNF is transported and released in the DH where interacts with its on receptor TRkB on second-order neurons contributing to central sensitization by enhancing glutamatergic transmission, underlying widespread pain sensation in fibromyalgia. NGF: nerve growth factor; SP: Substance P; CGRP: calcitonin-gene related peptide; TrkA: Tropomyosin-Related Kinase A, TrkB: Tropomyosin-Related Kinase B, BK: bradykinin; PAG: periaqueductal gray region; NRM: magnus raphae nucleus, BDNF Brain-Derived Neurotrophic Factor NMDA: N-methyl-D-asparate receptor; DH: Dorsal Horn, ERK: Extracellular Signal-Regulated kinases, MAPK: Mitogen-Activated Protein Kinase.

Although accumulating evidence suggests that pain is maintained by tonic impulse input from deep tissues, such as muscle and joints in combination with central sensitization mechanisms in FM [Saragovi et al, 2000], no correlation was found between quantitative sensory testing measures and glutamate as well as BDNF and NGF levels in the CSF of our FM patients. This was also true for patients with CM.

The involvement of NGF in both chronic pathological conditions leads to the hypothesis that its increase is not a specific feature of CM, since it could be detected in other chronic painful conditions intervening in common mechanisms underlying the pathogenic basis of pain perpetuation. Furthermore, the rise in NGF levels seems to be related to pain *per se* because it is not influenced by analgesic or anti-inflammatory drug abuse.

POTENTIAL OF NGF TARGETING STRATEGIES IN NEUROPATHIC PAIN, CHRONIC MIGRAINE AND FIBROMYALGIA

A growing body of evidence suggests the anti-hyperalgesic effect of pharmacological interference with NGF-TrkA interactions in inflammatory and neuropathic pain [Saragovi et al, 2000; Hefti et al, 2006; Gwak et al, 2003; Salter, 2005; Koltzenburg et al, 1999; Wild et al, 2007; Ro et al, 1999; Christiansen & Hulschboch, 1997; Halvorson et al, 2005; Chudler et al, 1997; Jimenez-Andrade et al, 2007].

NGF-neutralizing antibodies were also able to attenuate mechanical and thermal hyperalgesia following spinal cord injury or CCI sciatic nerve [Ro et al, 1999], which corresponds, at a molecular level, to a decrease in CGRP density in the spinal cord (laminae I to IV) [Christiansen & Hulschboch, 1997]. In addition, the subcutaneous infusion of a synthetic TrkA–IgG fusion protein, which binds NGF with high-affinity capacity, produced a slow reduction in CGRP and $TRPV_1$ expression by sensory neurons [Xue et al, 2007]. Furthermore, autoimmunization of rodents with NGF reduced Nav 1.8 mRNA expression and TTX-r Na^+ currents in appropriate sensory neurons [Chudler et al, 1997].

NGF-neutralizing antibodies have been demonstrated to exert an analgesic effect greater than that achieved with acute administration of 10 or 30 mg/kg morphine in an experimental model of bone-cancer pain [Halvorson et al, 2005]. This therapy also reduced some neurochemical changes associated with peripheral and central sensitization in DRG and spinal cord, without influencing disease progression or markers of sensory and sympathetic innervation in the skin or bone [Sevcik et al, 2005].

Recently monoclonal antibodies against NGF have been used as novel NGF sequestering therapies [Cattaneo et al, 1999]. They were tested in a skeletal pain model in mouse due to fracture where pain was reversed by 10mg/kg morphine [Jimenez-Andrade et al, 2007]. In this model, monoclonal anti-NGF antibodies reduced fracture-induced pain-related behaviors by over 50%. Treatment with anti-NGF antibodies also decreased *c-fos* and dynorphin upregulation in the spinal cord at day 2 post-fracture but not p-ERK and *c-fos* expression at 20 and 90 min, respectively, suggesting the involvement of this NT in the maintenance but not in the acute generation of such type of pain. While anti-NGF antibodies do not significantly cross the blood-brain barrier, it can be hypothesized that their anti-hyperalgesic effect is due to the blockade of the activation and/or sensitization of the CGRP/TrkA positive fibers that normally represent the majority of sensory fibers innervating bone.

Anti-NGF antibodies have been recently demonstrated to reduce some, but not all, signs of the complex regional pain syndrome (CRPS) I-like in a rat fracture model. They reduced neuropeptide levels in sciatic nerve and nociceptive sensitization in the anti-NGF treated animals. Conversely, anti-NGF antibodies did not decrease hindpaw edema, warmth or cytokine production, suggesting the complexity of the mechanism of CRPS pathogenesis in which only some aspects seem to be related to NGF up-regulation and signaling [Sabsovich et al, 2007].

Functional neutralization of the TrkA receptor using NGF-sequestering molecules is another approach to antagonize NGF action targeting the signaling transduction pathways depending on receptor activation. One such molecule, MNAC13, a monoclonal antibody, has been demonstrated to display a high affinity and specificity for the NGF receptor TrkA, and a neutralizing activity toward the NGF/TrkA interaction [Cattaneo et al, 1999; Covaceuszach, 2001, 2005]. Treatment with the MNAC13 anti-TrkA antibody impairs TrkA signaling, resulting in the inhibition of SHC-mediated as well as phospholipase Cγ (PLCγ) pathways [Ugolini et al, 2007]. This monoclonal antibody has been tested in the formalin-evoked pain licking responses in mice, where it produced a significant antiallodynic effect. The same antiallodynic effect and a remarkable functional recovery were observed in mice subjected to sciatic nerve ligation, with effects persisting after administration [Ugolini et al, 2007].

Non peptidergic molecules with antagonistic properties, such as compound PD90780 [Colquhoun et al, 2004; Spiegel et al, 1995] and kynurenic acid derivatives [Jarvis et al, 1997] have been shown to prevent the binding of NGF on low-affinity p75 receptors, but their use has not been assessed in animal pain models.

More recently, a novel nonpeptidic molecule, ALE-0540, has been demonstrated not to prevent the binding of NGF to the p75 receptor but to specifically inhibit the binding of NGF to TrkA. By preventing TrkA phosphorylation, ALE-0540 not only antagonizes the signal transduction pathway and biological responses mediated by this receptor but also blocks both neuropathic and thermally-induced inflammatory pain. Furthermore, in the L5/L6 ligation model of neuropathic pain in rats, both systemic and intrathecal administration of ALE-0540 produced an antiallodynic effect [Owolabi et al, 1999]. This promising nonpeptidic small molecule opens, therefore, new perspectives in the development of agents for the treatment of pain. The pharmacological and safety profile of this first non peptidergic NGF receptor antagonist molecule revealed the lack of interaction with known analgesic targets, including histamine 1, endothelin A, 5-HT_2, cannabinoids and opioid (μ, δ, and k) receptors reflecting the lack of central side effects of these compounds.

Further NGF mimetics with antagonistic action were produced with potential clinical applications. In particular, small monomeric cyclic analogs miming the β-turn regions of NGF were designed and synthesized [LeSauteur et al, 1995]. Among them, potent competitive antagonists were derived from the NGF β-turn C-D, which inhibits NGF binding to TrkA receptors and neurite outgrowth in PC12 cells. Until now, these analogues were used to study the biological and receptor binding properties of NGF but they have never been tested in animal pain models. A small peptide named C (92-96) that blocks NGF-TrkA interactions, in particular, was studied for it effects on cortex cholinergic synapses and has been demonstrated to induce a significant decrease in the size of vesicular acetylcholine transporter-immunoreactive sites [Debeir et al, 1999]. The use of this approach for modulating and reducing molecular and neuronal events underlying chronic pain can be

hypothesized from a theoretical point of view. However, it opens the question of the dependency on NGF of correct functioning of the CNS and the potential impact of this treatment on the correct maintenance of synaptic contacts and functioning in the adult CNS, which may not be limited to cholinergic neurons [Hulsebosch et al, 1988; Garofalo et al, 1992].

Studies in animal pain models in which TrkA immunoadhesins are administered have shown that sequestering of endogenous NGF is able to block the hyperalgesia associated with inflammation [Katsura et al, 2006]. However, these large molecules are unlikely to be clinically useful. Therefore, efforts have been recently directed to identify small molecule agonists/antagonists based on crystal structure of the NGF-TrkAd5 complex, taking into account the biochemical evidence supporting the involvement of domain 5 of the TrkA receptor in the binding of NGF. TrkAd5 has been shown to be efficacious in preclinical models of inflammatory pain by the sequestration of excess levels of endogenous NGF, and therefore, represents a novel therapeutic agent for chronic pain conditions in which an inflammatory component can be recognized [Dawbarn et al, 2006].

In the last few years, there has also been a growing interest in new targets for pain therapy among NGF intracellular signaling pathways. Activation of PKC could be one of these potential targets even in NGF hyperalgesia [Khasar et al, 1999]. A PKC ε selective inhibitor peptide or related compounds have been proposed for the treatment of chronic pain states, but need to be tested in animals before use in human painful conditions.

Despite the promising strategies against NGF, it is currently difficult to define the potential role of anti-NGF strategies in neuropathic conditions. As already shown in some experimental models, NGF was helpful in favoring the regeneration of damaged sensory neurons. This furnished the rationale for the use of this NF in the treatment of diabetic or HIV-induced neuropathies, which failed, however, to demonstrate any significant clinical efficacy.

Neotrofin, an interesting molecule that enhances endogenous NGF levels, has been recently developed to prevent phenotypic, functional and structural changes occurring in diabetic neuropathy, by antagonizing the depletion of NGF protein. This effect was accompanied by the maintenance of normal nerve levels of the sensory neuropeptides SP and CGRP. Thermal hypoalgesia and conduction slowing of large sensory fibers in diabetic rats were ameliorated by neotrofin treatment, which had no effect on conduction slowing in large motor fibers or on reduced myelinated fiber axonal caliber. Therefore, the use of small molecules able to enhance endogenous production of NGF may be a promising alternative to either exogenous treatment with neurotrophic factors or gene therapy as a therapeutic approach to treat some neuropathies characterized by sensory loss, in particular diabetic neuropathy. Future trials on these small molecules are warranted [Calcutt et al, 2006].

Despite the above observations, the majority of studies on NGF antagonism in animal models of neuropathic pain have focused only on the prevention of hyperalgesia and allodynia after injury. In a recent study, in particular, the effects of anti-NGF antibodies in models of neuropathic and inflammatory pain inflammation, spinal nerve ligation and STZ-induced neuropathic pain models were assessed from approximately day 3 to day 7 after treatment. A significant effect on thermal hyperalgesia was observed only in the spinal nerve ligation model. In the mouse CCI model, monoclonal anti-NGF antibodies were able to decrease tactile allodynia when administered 2 weeks after surgery. Repeated administration of this antibody to CCI in mice for 3 weeks produced a sustained reversal (from day 4 to day

21) of tactile allodynia that was restored 5 days after the end of administration. Based on the above results, NGF antagonists, such as fully human monoclonal anti-NGF antibodies, can be hypothesized to have a potential therapeutic utility, at least in some human neuropathic pain conditions, with no development of tolerance to antagonism [Wild et al, 2007].

The only anti-TrkA monoclonal antibody with functional neutralizing properties, MNAC13, has been clearly shown to induce analgesia not only in inflammatory pain but also in neuropathic pain models (formalin-evoked pain-licking responses and sciatic nerve ligation, respectively), with a surprisingly long-lasting effect in the latter. Furthermore, a clear synergistic effect was observed when MNAC13 was administered in combination with opioids at doses that were not efficacious *per se*. These findings provide, therefore, a direct demonstration that strategies based on neutralizing antibodies directed against the TrkA receptor may display potent analgesic effects that should be tested in future research [Ugolini et al, 2007].

In a recent approach, NGF-mimetic peptides were used that were based on chemical modification of amino acid residues at loop 1, loop 4, and the N-terminal region of the NGF molecule as the most relevant for its biological activity. One of these peptides with the highest *in vitro* activity (L1L4), demonstrated by induction of DRG differentiation and tyrosine phosphorylation of TrkA receptor stimulation, has been shown to reduce neuropathic behavior and restore neuronal function in a rat model of peripheral neuropathic pain, thereby suggesting a putative therapeutic role [Colangelo et al, 2008].

Evidence of the involvement of NGF in the pathophysiology of chronic pain and the effort in the development of molecules targeting this NT open, therefore, new insight into the identification of novel therapeutic strategies for the treatment of chronic painful conditions, including inflammatory and neuropathic pain. Much research, however, is needed for evaluating their real application in humans in these pathological conditions.

CONCLUSIONS

The potential usefulness of anti-NGF treatment in migraine despite recent findings outlining the relevant contribution of NGF signaling to trigeminal nociceptive neurons still remains to be established. Blockade of NGF can be hypothesized as a novel, analgesic symptomatic approach, whereas the use of anti-NGF strategies as prophylactic treatment is more difficult to postulate due to potential CNS side effects, with the only exception of the chronic form of migraine, refractory to any other prophylactic treatment. In this condition, central sensitization is also sustained by an increase in BDNF induced by NGF and related increase in glutamatergic transmission [Kerr et al, 1999; Bennett, 2001]. Because BDNF may function as a modulator of central sensitization, strategies against this NT can be hypothesized as a novel treatment of persistent pain, including neuropathic pain and resistant migraine. The same is true for FM in which central sensitization is the major pathophysiological mechanism.

In contrast with findings available for NGF targeting treatments, limited data are available concerning BDNF. The sequestration of endogenous BDNF reduced pain-related behavior in two models of inflammatory pain [Kerr et al, 1999] and also in neuropathic pain [Yajima et al, 2002, 2005], which resulted in a depression of spinal ERK activation by 40%.

BDNF sequestration, however, did not affect mechanical hyperalgesia induced by peripheral capsaicin administration [Mannion et al, 1999]. In addition, in BDNF knockout mice, this NT has been shown to be required for normal pain sensitivity in the hot plate and formalin tests, which suggests the difficulty involved in identifying antagonizing molecules that are selectively directed against the pathological pain mechanism and with a relevant effect on the treatment of pain [MacQueen et al, 2001].

Therapeutic strategies targeting BDNF (anti-BDNF antibodies or TrkB targeting molecules) should, however, be considered with caution. BDNF, in fact, exerts a dual effect by intervening not only in enhancing glutamate transmission, playing a pivotal role in central sensitization, but also in stimulating GABA release from DH interneurons [Pezet et al, 2002], therefore exerting a potential compensatory mechanism in chronic pain. The two opposite effects of BDNF should be investigated in animal pain models before developing novel strategies targeting this NT, with potential application to inflammatory and neuropathic pain as well as CM and FM.

REFERENCES

Amann, R; Schuligoi, R; Herzeg, G; Donnerer, J. *Intraplantar injection of nerve growth factor into the rat hind paw: Local edema and effects on thermal nociceptive threshold*. Pain, 1996 64, 323–329.

Amaya, F; Shimosato, G; Nagano, M; Ueda, M; Hashimoto, S; Tanaka, Y; Suzuki, H; Tanaka, M. *NGF and GDNF differentially regulate TRPV1 expression that contributes to development of inflammatory thermal hyperalgesia*. Eur J Neurosci, 2004 20, 2303-2310.

Amaya, F; Wang, H; Costigan, M; Allchorne, AJ; Hatcher, JP; Egerton, J; Stean, T; Morisset, V; Grose, D; Gunthorpe, MJ; Chessell, IP; Tate, S; Green, PJ; Woolf, CJ. *The voltage-gated sodium channel Na(v)1.9 is an effector of peripheral inflammatory pain hypersensitivity*. J Neurosci, 2006 26, 12852-12860.

Anand, P. *Neurotrophic factors and their receptors in human sensory neuropathies.* Prog Brain Res, 2004 146, 477-492.

Anand, U; Otto, WR; Casula, MA; Day, NC; Davis, JB; Bountra, C; Birch, R; Anand, P. *The effect of neurotrophic factors on morphology, TRPV1 expression and capsaicin responses of cultured human DRG sensory neurons.* Neurosci Lett, 2006 399, 51-56.

Apfel, SC. *Nerve growth factor for the treatment of diabetic neuropathy: what went wrong, what went right, and what does the future hold?* Int Rev Neurobiol, 2002 50, 393-413.

Apfel, SC. *Neurotrophic factors and diabetic peripheral neuropathy*. Eur Neurol, 1999 41, 27-34.

Apfel, SC; Wright, DE; Wiideman, AM; Dormia, C; Snider, WD; Kessler, JA. *Nerve growth factor regulates the expression of brain-derived neurotrophic factor mRNA in the peripheral nervous system.* Mol Cell Neurosci, 1996 7, 134−142.

Arendt-Nielsen, L; Graven-Nielsen, T. *Central sensitization in fibromyalgia and other musculoskeletal disorders.* Curr Pain Headache Rep, 2003 7, 355-361.

Arnett, MG; Ryals, JM; Wright, DE. *Pro-NGF, sortilin, and p75NTR: potential mediators of injury-induced apoptosis in the mouse dorsal root ganglion*. Brain Res, 2007 1183, 32-42.

Averill, S; Michael, GJ; Shortland, PJ; Leavesley, RC; King, VR; Bradbury, EJ; McMahon, Bartels EM; Danneskiold-Samsoe, B. *Histological abnormalities in muscle from patients with certain types of fibrositis.* Lancet, 1986 1, 755–757.

Averill, S; Michael, GJ; Shortland, PJ; Leavesley, RC; King, VR; Bradbury, EJ; McMahon, SB; Priestley, JV. *NGF and GDNF ameliorate the increase in ATF3 expression which occurs in dorsal root ganglion cells in response to peripheral nerve injury*. Eur J Neurosci, 2004 19, 1437-1445.

Bengtsson, A; Henriksson, KG; Larsson, J. *Reduced high-energy phosphate levels in the painful muscles of patients with primary fibromyalgia*. Arthritis Rheum, 1986 29, 817–821.

Bennett, DL. *Neurotrophic factors: important regulators of nociceptive function*. Neuroscientist, 2001 7, 13-17.

Bienenstock, J; Tomioka, M; Matsuda, H; Stead, RH; Quinonez, G; Simon, GT; Coughlin, MD; Denburg, JA. *The role of mast cells in inflammatory processes: evidence for nerve/mast cell interactions.* Int Arch Allergy Appl Immunol, 1987 82, 238-243.

Blandini, F; Rinaldi, L; Tassorelli, C; Sances, G; Motta, M; Samuele, A; Fancellu, R; Nappi, G; Leon, A. *Peripheral levels of BDNF and NGF in primary headaches*. Cephalalgia, 2006 26, 136-142.

Bonini, S; Rasi, G; Bracci-Laudiero, ML; Procoli, A; Aloe, L. *Nerve growth factor: neurotrophin or cytokine?* Int Arch Allergy Immunol, 2003 131, 80-84.

Bowsher, D; Haggett, C. *Paradoxical burning sensation produced by cold stimulation in patients with neuropathic pain*. Pain, 2005 117, 230.

Bracci-Laudiero, L; Aloe, L; Caroleo, MC; Buanne, P; Costa, N; Starace, G; Lundeberg, T. *Endogenous NGF regulates CGRP expression in human monocytes, and affects HLA-DR and CD86 expression and IL-10 production.* Blood, 2005 106, 3507-3514.

Brodie, C. *Platelet activating factor induces nerve growth factor production by rat astrocytes.* Neurosci Lett, 1995 186, 5-8.

Burstein, R. *Deconstructing migraine headache into peripheral and central sensitization*. Pain, 2001 89, 107-110.

Cahill, CM; Dray, A; Coderre, TJ. *Intrathecal nerve growth factor restores opioid effectiveness in an animal model of neuropathic pain*. Neuropharmacology, 2003 45, 543-552.

Calcutt, NA; Freshwater, JD; Hauptmann, N; Taylor, EM; Mizisin, AP. *Protection of sensory function in diabetic rats by Neotrofin.* Eur J Pharmacol, 2006 534, 187-193.

Cattaneo, A; Capsoni, S; Margotti, E; Righi, M;, Kontsekova, E; Pavlik, P; Filipcik, P; Novak, M. *Functional blockade of tyrosine kinase A in the rat basal forebrain by a novel antagonistic anti-receptor monoclonal antibody*. J Neurosci, 1999 19, 9687-9697.

Chao, MV; Rajagopal, R; Lee, FS. *Neurotrophin signalling in health and disease*. Clin Sci (Lond), 2006 110, 167-173.

Chen, Y; Li, GW; Wang, C; Gu, Y; Huang, LY. *Mechanisms underlying enhanced P2X receptor-mediated responses in the neuropathic pain state*. Pain, 2005 119, 38-48.

Chien, CC; Fu, WM; Huang, HI; Lai, YH; Tsai, YF; Guo, SL; Wu, TJ; Ling, QD. *Expression of neurotrophic factors in neonatal rats after peripheral inflammation*. J Pain, 2007 8, 161-167.

Christensen, MD; Hulsebosch, CE. *Spinal cord injury and anti-NGF treatment results in changes in CGRP density and distribution in the dorsal horn in the rat.* Exp Neurol, 1997 147, 463-475.

Christianson, JA; Ryals, JM; Johnson, MS; Dobrowsky, RT; Wright, DE. *Neurotrophic modulation of myelinated cutaneous innervation and mechanical sensory loss in diabetic mice.* Neuroscience, 2007 145, 303-313.

Christianson, JA; Ryals, JM; McCarson, KE; Wright, DE. *Beneficial actions of neurotrophin treatment on diabetes-induced hypoalgesia in mice.* J Pain, 2003 4, 493-504.

Chudler, EH; Anderson, LC; Byers, MR. *Nerve growth factor depletion by autoimmunization produces thermal hypoalgesia in adult rats*. Brain Res, 1997 765, 327-330.

Colangelo, AM; Bianco, MR; Vitagliano, L; Cavaliere, C; Cirillo, G; De Gioia, L; Diana, D; Colombo, D; Redaelli, C; Zaccaro, L; Morelli, G; Papa, M; Sarmientos, P; Alberghina, L; Martegani, E. *A new nerve growth factor-mimetic peptide active on neuropathic pain in rats.* J Neurosci, 2008 28, 2698-2709.

Colquhoun, A; Lawrance, GM; Shamovsky, IL; Riopelle, RJ; Ross, GM. *Differential activity of the nerve growth factor (NGF) antagonist PD90780 [7-(benzolylamino)-4,9-dihydro-4-methyl-9-oxo-pyrazolo[5,1-b]quinazoline-2-carboxylic acid] suggests altered NGF-p75NTR interactions in the presence of TrkA.* J Pharmacol Exp Ther, 2004 310, 505-511.

Cortright, DN; Szallasi, *A. Biochemical pharmacology of the vanilloid receptor TRPV1. An update.* Eur J Biochem, 2004 271, 1814-1819.

Coutaux, A; Adam, F; Willer, JC; Le Bars, D. *Hyperalgesia and allodynia: peripheral mechanisms.* Joint Bone Spine, 2005 72, 359-371.

Covaceuszach, S; Cattaneo, A; Lamba, D. *Neutralization of NGF-TrkA receptor interaction by the novel antagonistic anti-TrkA monoclonal antibody MNAC13: a structural insight.* Proteins, 2005 58, 717-727.

Covaceuszach, S; Cattaneo, A; Lamba, D. *Purification, crystallization and preliminary X-ray analysis of the Fab fragment from MNAC13, a novel antagonistic anti-tyrosine kinase A receptor monoclonal antibody.* Acta Crystall D Biol Cruystallog, 2001 57, 1307-1309.

Créange, A; Barlovatz-Meimon, G; Gherardi, RK. *Cytokines and peripheral nerve disorders.* Eur Cytokine Netw, 1997 8, 145-151.

Cruise, BA; Xu, P; Hall, AK. *Wounds increase activin in skin and a vasoactive neuropeptide in sensory ganglia.* Dev Biol, 2004 271, 1–10.

Cui, M; Honore, P; Zhong, C; Gauvin, D; Mikusa, J; Hernandez, G; Chandran, P; Gomtsyan, A; Brown, B; Bayburt, EK; Marsh, K; Bianchi, B; McDonald, H; Niforatos, W; Neelands, TR; Moreland, RB; Decker, MW; Lee, CH; Sullivan, JP; Faltynek, *CR. TRPV1 receptors in the CNS play a key role in broad-spectrum analgesia of TRPV1 antagonists.* J Neurosci, 2006 26, 9385-9393.

Cummins, TR; Aglieco, F; Renganathan, M; Herzog, RI; Dib-Hajj, SD; Waxman, SG. *Nav1.3 sodium channels: rapid repriming and slow closed-state inactivation display quantitative differences after expression in a mammalian cell line and in spinal sensory neurons.* J Neurosci, 2001 21, 5952–5961.

Cummins, TR; Sheets, PL; Waxman, SG. *The roles of sodium channels in nociception: Implications for mechanisms of pain.* Pain, 2007 131, 243-257.

Cummins, TR; Waxman, SG. *Downregulation of tetrodotoxin-resistant sodium currents and upregulation of a rapidly repriming tetrodotoxin-sensitive sodium current in small spinal sensory neurons after nerve injury*. J Neurosci, 1997 17, 3503–3514.

Cutrer, FM; Huerter, K. *Migraine aura*. Neurologist, 2007 13, 118-125.

D'Arco, M; Giniatullin, R; Simonetti, M; Fabbro, A; Nair, A; Nistri, A; Fabbretti, E. *Neutralization of nerve growth factor induces plasticity of ATP-sensitive P2X3 receptors of nociceptive trigeminal ganglion neurons.* J Neurosci, 2007 27, 8190-8201.

Dawbarn, D; Fahey, M; Watson, J; Tyler, S; Shoemark, D; Sessions, R; Zhang, R; Brady, L; Willis, C; Allen, SJ. *NGF receptor TrkAd5: therapeutic agent and drug design target.* Biochem Soc Trans, 2006 34, 587-590.

Debeir, T; Saragovi, HU; Cuello, AC. *A nerve growth factor mimetic TrkA antagonist causes withdrawal of cortical cholinergic boutons in the adult rat.* Proc Natl Acad Sci U S A, 1999 96, 4067-4072.

DeLeo, JA; Yezierski, RP. *The role of neuroinflammation and neuroimmune activation in persistent pain*. Pain, 2001 90, 1-6.

Della Seta, D; de Acetis, L; Aloe, L; Alleva, E. *NGF effects on hot plate behaviors in mice*. Pharmacol Biochem Behav, 1994 49, 701–705.

Diamond, J; Foerster, A; Holmes, M; Coughlin, M. *Sensory nerves in adult rats regenerate and restore sensory function to the skin independently of endogenous NGF*. J Neurosci, 1992 12, 1467-1476.

Diener, HC; May, A. *New aspects of migraine pathophysiology: lessons learned from positron emission tomography*. Curr Opin Neurol, 1996 9, 199-201.

Di Marco, E; Mathor, M; Bondanza, S; Cutuli, N; Marchisio, PC; Cancedda, R; De Luca M. *Nerve growth factor binds to normal human keratinocytes through high and low affinity receptors and stimulates their growth by a novel autocrine loop.* J Biol Chem, 1993 268, 22838-22846.

Diogenes, A; Akopian, AN; Hargreaves, *KM. NGF up-regulates TRPA1: implications for orofacial pain.* J Dent Res, 2007 86, 550-555.

Djouhri, L; Dawbarn, D; Robertson, A; Newton, R; Lawson, SN. *Time course and nerve growth factor dependence of inflammation-induced alterations in electrophysiological membrane properties in nociceptive primaryafferent neurons.* Neurosci, 2001 21, 8722-8733.

Dodick, D; Silberstein, S. *Central sensitization theory of migraine: clinical implications* Headache, 2006 46, S182-S191.

Donnelly-Roberts, D; McGaraughty, S; Shieh, CC; Honore, P; Jarvis, MF. *Painful purinergic receptors.* J Pharmacol Exp Ther, 2008 324, 409-415.

Donnerer, J; Liebmann, I; Schicho, R. *ERK and STAT3 phosphorylation in sensory neurons during capsaicin –induced impairment and nerve growth factor treatment*. Pharmacology, 2005 75, 116-121.

Fabbretti, E; D'Arco, M; Fabbro, A; Simonetti, M; Nistri, A; Giniatullin, R. *Delayed upregulation of ATP P2X3 receptors of trigeminal sensory neurons by calcitonin gene-related peptide.* J Neurosci, 2006 26, 6163-6171.

Facer, P; Casula, MA; Smith, GD; Benham, CD; Chessell, IP; Bountra, C; Sinisi, M; Birch, R; Anand, P. *Differential expression of the capsaicin receptor TRPV1 and related novel receptors TRPV3, TRPV4 and TRPM8 in normal human tissues and changes in traumatic and diabetic neuropathy*. BMC Neurol, 2007 7, 11.

Fitzgerald, M; Wall, PD; Goedert, M; Emson, PC. *Nerve growth factor counteracts the neurophysiological and neurochemical effects of chronic sciatic nerve section*. Brain Res, 1985 332, 131-41.

Fjell, J; Cummins, TR; Fried, K; Black, JA; Waxman, SG. *In vivo NGF deprivation reduces SNS expression and TTX-R sodium currents in IB4-negative DRG neurons*. J Neurophysiol, 1999 81, 803-810.

Garcia-Larrea, L; Magnin, M. *Pathophysiology of neuropathic pain: review of experimental models and proposed mechanisms*. Presse Med, 2008 37, 315-340.

Gardiner, NJ; Fernyhough, P; Tomlinson, DR; Mayer, U; von der Mark, H; Streuli, CH. *Alpha7 integrin mediates neurite outgrowth of distinct populations of adult sensory neurons*. Mol Cell Neurosci, 2005 28, 229-240.

Garofalo, L; Ribeiro-da-Silva, A; Cuello, AC. *Nerve growth factor-induced synaptogenesis and hypertrophy of cortical cholinergic terminals*. Proc Natl Acad Sci U S A, 1992 89, 2639-2643.

Giovengo, SL; Russell, IJ; Larson, AA. *Increased concentrations of nerve growth factor in cerebrospinal fluid of patients with fibromyalgia*. J Rheumatol, 1999 26, 1564-1569.

Gould, HJ 3rd; Gould, TN; England, JD; Paul, D; Liu, ZP; Levinson, SR. *A possible role for nerve growth factor in the augmentation of sodium channels in models of chronic pain*. Brain Res, 2000 854, 19-29.

Govrin-Lippmann, R; Devor, M. *Ongoing activity in severed nerves: Source and variation with time*. Brain Res, 1978 159, 406–410.

Gwak, YS; Nam, TS; Paik, KS; Hulsebosch, CE; Leem, JW. *Attenuation of mechanical hyperalgesia following spinal cord injury by administration of antibodies to nerve growth factor in the rat*. Neurosci Lett, 2003 336, 117-120.

Halvorson, KG; Kubota, K; Sevcik, MA; Lindsay, TH; Sotillo, JE; Ghilardi, JR; Rosol, TJ; Boustany, L; Shelton, DL; Mantyh, PW. *A blocking antibody to nerve growth factor attenuates skeletal pain induced by prostate tumor cells growing in bone*. Cancer Res, 2005 65, 9426-9435.

Hao, J; Ebendal, T; Xu, X; Wiesenfeld–Hallin, Z; Eriksdottter Jonhagen, M. *Intracerebroventricular infusion of nerve growth factor induces pain-like response in rats*. Neurosci Lett, 2000 286, 208-212.

Hathway, GJ; Fitzgerald, M. *Time course and dose-dependence of nerve growth factor-induced secondary hyperalgesia in the mouse*. J Pain, 2006 7, 57-61.

Headache Classification Subcommittee of the International Headache Society. *The International Classification of Headache Disorders:* 2nd edition. Cephalalgia, 2004 24, 9-160.

Hefti, FF; Rosenthal, A; Walicke, PA; Wyatt, S; Vergara, G; Shelton, DL; Davies, AM. *Novel class of pain drugs based on antagonism of NGF*. Trends Pharmacol Sci, 2006 27, 85-91.

Helyes, Z; Elekes, K; Németh, J; Pozsgai, G; Sándor, K; Kereskai, L; Börzsei, R; Pintér, E; Szabó, A; Szolcsányi, J. *Role of transient receptor potential vanilloid 1 receptors in endotoxin-induced airway inflammation in the mouse*. Am J Physiol Lung Cell Mol Physiol, 2007 292, L1173-L1181.

Hoheisel, U; Kaske, A; Mense, S. *Relationship between neuronal activity and substance P-immunoreactivity in the rat spinal cord during acute and persistent myositis*. Neurosci Lett, 1998 257, 21–24.

Huang, J; Zhang, X; McNaughton, PA. *Inflammatory pain: the cellular basis of heat hyperalgesia*. Curr Neuropharmacol. 2006 4:197-206.

Hulsebosch, CE; Coggeshall, RE. *Intraspinal sprouting after administration of nerve growth factor antibodies to neonatal rats.* Brain Res, 1988 461, 322-327.

Ichikawa H; Yabuuchi T; Jin HW; Terayama, R; Yamaai, T; Deguchi, T; Kamioka, H; Takano-Yamamoto, T; Sugimoto, T. *Brain-derived neurotrophic factor-immunoreactive primary sensory neurons in the rat trigeminal ganglion and trigeminal sensory nuclei.* Brain Res, 2006 1081, 113-118.

Jacobsen, S; Wildschiodtz, G; Danneskiold-Samsoe, B. *Isokinetic and isometric muscle strength combined with transcutaneous electrical muscle stimulation in primary fibromyalgia syndrome*. J Rheumatol, 1991 18, 1390–1393.

Jarvis, CR; Xiong, ZG; Plant, JR; Churchill, D; Lu, WY; MacVicar, BA; MacDonald, JF. *NT modulation of NMDA receptors in cultured murine and isolated rat neurons.* J Neurophysiol, 1997 78, 2363-2371.

Jimenez-Andrade, JM; Martin, CD; Koewler, NJ; Freeman, KT; Sullivan, LJ; Halvorson, KG; Barthold, CM; Peters, CM; Buus, RJ; Ghilardi, JR; Lewis, JL; Kuskowski, MA; Mantyh, PW. *Nerve growth factor sequestering therapy attenuates non-malignant skeletal pain following fracture*. Pain, 2007 133, 183-196.

Kalyan-Raman, U; Kalyan-Raman, K; Yunus, M; Masi, A. *Muscle pathology in primary fibromyalgia syndrome: a light microscopic, histochemical and ultrastructural study*. J Rheumatol, 1984 11, 808–813.

Kanaan, SA; Poole, S; Saade, NE; Jabbur, S; Safieh-Garabedian, B. *Interleukin-10 reduces the endotoxin-induced hyperalgesia in mice.* J Neuroimmunol, 1998 86, 142–150.

Kassel, O; de Blay, F; Duvernelle, C; Olgart, C; Israel-Biet, D; Krieger, P; Moreau, L; Muller, C; Pauli, G; Frossard, N. *Local increase in the number of mast cells and expression of nerve growth factor in the bronchus of asthmatic patients after repeated inhalation of allergen at low-dose.* Clin Exp Allergy, 2001 31, 1432-1440.

Katsura, H; Obata, K; Mizushima, T; Yamanaka, H; Kobayashi, K; Dai, Y; Fukuoka, T; Tokunaga, A; Sakagami, M; Noguchi, K. *Antisense knock down of TRPA1, but not TRPM8, alleviates cold hyperalgesia after spinal nerve ligation in rats*. Exp Neurol, 2006 200, 112-123.

Katz, WA; Rothenberg, R. Section 3: *The nature of pain: pathophysiology*. J Clin Rheumatol, 2005 11, S11-S15.

Kawamoto, K; Aoki, J; Tanaka, A; Itakura, A; Hosono, H; Arai, H; Kiso, Y; Matsuda, H. *Nerve growth factor activates mast cells through the collaborative interaction with lysophosphatidylserine expressed on the membrane surface of activated platelets*. J Immunol, 2002 168, 6412-6419.

Kerr, BJ; Bradbury, EJ; Bennett, DL; Trivedi, PM; Dassan, P; French, J; Shelton, DB; McMahon, SB; Thompson, SW. *Brain-derived neurotrophic factor modulates nociceptive sensory inputs and NMDA-evoked responses in the rat spinal cord*. J Neurosci, 1999 19, 5138-5148.

Kerr, BJ; Souslova, V; McMahon, SB; Wood, JN. *A role for the TTX-resistant sodium channel Nav 1.8 in NGF-induced hyperalgesia, but not neuropathic pain*. Neuroreport, 2001 12, 3077-3080.

Khan, GM; Chen, SR; Pan, HL. *Role of primary afferent nerves in allodynia caused by diabetic neuropathy in rats.* Neuroscience, 2002 114, 291-299.

Khasar, SG; Lia, YH; Martin, A; Dadgar, J; McMahon, T; Wang, D; Hundle, B; Aley, KO; Isemberg, W; McKarter, G; Green, PC; Hodge, CW; Levine JD; Messing, RO. *A novel*

nociceptor signaling pathway revealed in protein kinase C epsilon mutant mice. Neuron, 1999 24, 253-260.

Kitamura, N; Konno, A; Kuwahara, T; Komagiri, Y. *Nerve growth factor-induced hyperexcitability of rat sensory neuron in culture*. Biomed Res, 2005 26, 123-130.

Koltzenburg, M; Bennett, DL; Shelton, DL; McMahon, SB. *Neutralization of endogenous NGF prevents the sensitization of nociceptors supplying inflamed skin*. Eur J Neurosci, 1999 11, 1698-1704.

Krenz, NR; Weaver, LC. *Nerve growth factor in glia and inflammatory cells of the injured rat spinal cord.* J Neurochem, 2000 74, 730–739.

Larkin, M. *Nerve growth factor promising in diabetic neuropathy...and in HIV-1-related neuropathy*. Lancet, 1998 352, 1039.

Lee, YJ; Zachrisson, O; Tonge, DA; McNaughton, PA. *Upregulation of bradykinin B2 receptor expression by neurotrophic factors and nerve injury in mouse sensory neurons*. Mol Cell Neurosci, 2002 19, 186-200.

Leffler, A; Cummins, TR; Dib-Hajj, SD; Hormuzdiar, WN; Black, JA; Waxman, SG. *GDNF and NGF reverse changes in repriming of TTX-sensitive Na(+) currents following axotomy of dorsal root ganglion neurons*. J Neurophysiol, 2002 88, 650-658.

Leon, A; Buriani, A; Dal Toso, R; Fabris, M; Romanello, S; Aloe, L; Levi-Montalcini, R. *Mast cells synthesize, store, and release nerve growth factor.* Proc Natl Acad Sci U S A, 1994 91, 3739-3743.

LeSauteur, L; Wei, L; Gibbs, BF; Saragovi, HU. *Small peptide mimics of nerve growth factor bind TrkA receptors and affect biological responses*. J Biol Chem, 1995 270, 6564-6569.

Levi-Montalcini, R; Skaper, SD; Dal Toso, R; Petrelli, L; Leon, A. *Nerve growth factor: From neurotrophin to neurokine.* Trends Neurosci, 1996 19, 514–520.

Levine, JD; Alessandri-Haber, N. *TRP channels: targets for the relief of pain*. Biochim Biophys Acta, 2007 1772, 989-1003.

Levy, D; Burstein, R; Strassman, AM. *Mast cell involvement in the pathophysiology of migraine headache:* A hypothesis. Headache, 2006 46, S13-S18.

Lewin, GR; Rueff, A; Mendell, LM. *Peripheral and central mechanisms of NGF-induced hyperalgesia.* Eur J Neurosci, 1994 6, 1903-1912.

Lindholm, D; Hengerer, B; Heumann, R; Carroll, P; Thoenen, H. *Glucocorticoid hormones negatively regulate nerve growth factor expression in vivo and in cultured rat fibroblasts.* Eur J Neurosci, 1990 2, 795-801.

Lu, VB; Ballanyi, K; Colmers, WF; Smith, PA. *Neuron type-specific effects of brain-derived neurotrophic factor in rat superficial dorsal horn and their relevance to 'central sensitization'.* J Physiol, 2007 584, 543-563.

Ma, QP; Woolf, CJ. *The progressive tactile hyperalgesia induced by peripheral inflammation is nerve growth factor dependent*. Neuroreport, 1997 8, 807-810.

MacQueen, GM; Ramakrishnan, K; Croll, SD; Siuciak, JA; Yu, G; Young, LT; Fahnestock, M. *Performance of heterozygous brain-derived neurotrophic factor knockout mice on behavioral analogues of anxiety, nociception, and depression*. Behav Neurosci, 2001 115, 1145-1153.

Malcangio, M; Garrett, NE; Tomlinson, DR. *Nerve growth factor treatment increases stimulus-evoked release of sensory neuropeptides in the rat spinal cord*. Eur J Neurosci, 1997 9, 1101-1104.

Malcangio, M; Ramer, MS; Boucher, TJ; McMahon, SB. *Intrathecally injected neurotrophins and the release of substance P from the rat isolated spinal cord*. Eur J Neurosci, 2000 12, 139-144.

Malick, A; Burstein, R. *Peripheral and central sensitization during migraine*. Funct Neurol, 2000 15, 28-35.

Malik-Hall, M; Dina, OA; Levine, JD. *Primary afferent nociceptor mechanisms mediating NGF-induced mechanical hyperalgesia*. Eur J Neurosci, 2005 21, 3387-3394.

Mamet, J; Lazdunski, M; Voilley, N. *How nerve growth factor drives physiological and inflammatory expressions of acid-sensing ion channel 3 in sensory* neurons. Biol Chem, 2003 278, 48907-48913.

Mannion, RJ; Costigan, M; Decosterd, I; Amaya, F; Ma, QP; Holstege, JC; Ji, RR; Acheson, A; Lindsay, RM; Wilkinson, GA; Woolf, CJ. *Neurotrophins: peripherally and centrally acting modulators of tactile stimulus-induced inflammatory pain hypersensitivity*. Proc Natl Acad Sci U S A, 1999 96, 9385-9390.

Marcol, W; Kotulska, K; Larysz-Brysz, M; Kowalik, JL. *BDNF contributes to animal model neuropathic pain after peripheral nerve transection.* Neurosurg Rev, 2007 30, 235-243.

Matzner, O; Devor, M. *Hyperexcitability at sites of nerve injury depends on voltage-sensitive Na^+ channels.* J Neurophysiol, 1994 72, 349–359.

May, A; Matharu, M. *New insights into migraine: application of functional and structural imaging*. Curr Opin Neurol, 2007 20, 306-309.

McArthur, JC; Yiannoutsos, C; Simpson, DM; Adornato, BT; Singer, EJ; Hollander, H; Marra, C; Rubin, M; Cohen, BA; Tucker, T; Navia, BA; Schifitto, G; Katzenstein, D; Rask, C; Zaborski, L; Smith, ME; Shriver, S; Millar, L; Clifford, DB; Karalnik, IJ. *A phase II trial of nerve growth factor for sensory neuropathy associated with HIV infection. AIDS Clinical Trials Group Team 291*. Neurology, 2000 54, 1080-1088.

McMahon, SB. *NGF as a mediator of inflammatory pain*. Philos Trans R Soc Lond B Biol Sci, 1996 351, 431-440.

Mendell, LM; Albers, KM; Davis, BM. *Neurotrophins, nociceptors, and pain*. Microsc Res Tech, 1999 45, 252-261.

Meuser, T; Pietruck, C; Gabriel, A; Xie, GX; Lim, KJ; Pierce Palmer, P. *5-HT7 receptors are involved in mediating 5-HT-induced activation of rat primary afferent neurons*. Life Sci, 2002 71, 2279-2289.

Michael, GJ; Averill, S; Nitkunan, A; Rattray, M; Bennett, DL; Yan, Q; Priestley, JV. *Nerve growth factor treatment increases brain-derived neurotrophic factor selectively in TrkA-expressing dorsal root ganglion cells and in their central terminations within the spinal cord.* J Neurosci, 1997 17, 8476–8490.

Mizisin, AP; Shelton, GD; Wagner, S; Rusbridge, C; Powell, HC. *Myelin splitting, Schwann cell injury and demyelination in feline diabetic neuropathy.* Acta Neuropathol, 1998 95, 171-174.

Molliver, DC; Lindsay, J; Albers, KM; Davis, BM. *Overexpression of NGF or GDNF alters transcriptional plasticity evoked by inflammation*. Pain, 2005 113, 277-284.

Mousa, SA; Cheppudira, BP; Shaqura, M; Fischer, O; Hofmann, J; Hellweg, R; Schäfer, M. *Nerve growth factor governs the enhanced ability of opioids to suppress inflammatory pain.* Brain, 2007 130, 502-513.

Nicol, GD; Vasko, MR. *Unraveling the story of NGF-mediated sensitization of nociceptive sensory neurons*: ON or OFF the Trks? Mol Interv, 2007 7, 26-41.

Noguchi, K; Obata, K; Dai, Y. *Changes in DRG neurons and spinal excitability in neuropathy*. Novartis Found Symp, 2004 261, 103-110.

Numazaki, M; Tominaga, M. *Nociception and TRP Channels. Curr Drug Targets CNS* Neurol Disord, 2004 3, 479-485.

Oaklander, AL. *Mechanisms of pain and itch caused by herpes zoster (shingles).* J Pain, 2008 9, S10-S18.

Obata, K; Katsura, H; Mizushima, T; Yamanaka, H; Kobayashi, K; Dai, Y; Fukuoka, T; Tokunaga, A; Tominaga, M; Noguchi, K. *TRPA1 induced in sensory neurons contributes to cold hyperalgesia after inflammation and nerve injury*. J Clin Invest, 2005 115, 2393-2401.

Obata, K; Yamanaka, H; Dai, Y; Mizushima, T; Fukuoka, T. *Contribution of degeneration of motor and sensory fibers to pain behavior and the changes in neurotrophic factors in rat dorsal root ganglion*. Exp Neurol, 2004a 188, 149–160.

Obata, K; Yamanaka, H; Dai, Y; Mizushima, T; Fukuoka, T; Tokunaga, A; Noguchi, K. *Differential activation of MAPK in injured and uninjured DRG neurons following chronic constriction injury of the sciatic nerve in rats.* Eur J Neurosci, 2004b 20, 2881-2895.

Okuse, K; Chaplan, SR; McMahon, SB; Luo, ZD; Calcutt, NA; Scott, BP; Akopian, AN; Wood, JN. *Regulation of expression of the sensory neuron-specific sodium channel SNS in inflammatory and neuropathic pain.* Mol Cell Neurosci, 1997 10, 196-207.

Omoigui, S. *The biochemical origin of pain: the origin of all pain is inflammation and the inflammatory response, Part 2 of 3 – inflammatory profile of pain syndromes.* Med Hypotheses, 2007 69, 1169-1178.

Owolabi, JB; Rizkalla, G; Tehim, A; Ross, GM; Riopelle, RJ; Kamboj, R; Ossipov, M; Bian, D; Wegert, S; Porreca, F; Lee, DK. *Characterization of antiallodynic actions of ALE-0540, a novel nerve growth factor receptor antagonist, in the rat*. J Pharmacol Exp Ther, 1999 289, 1271-1276.

Padilla, F; Couble, ML; Coste, B; Maingret, F; Clerc, N; Crest, M; Ritter, AM; Magloire, H; Delmas, P. *Expression and localization of the Nav1.9 sodium channel in enteric neurons and in trigeminal sensory endings: implication for intestinal reflex function and orofacial pain.* Mol Cell Neurosci, 2007 35, 138-152.

Palazzo, E; Rossi, F; Maione, S. *Role of TRPV1 receptors in descending modulation of pain.* Mol Cell Endocrinol, 2008 286, S79-S83.

Park, SY; Choi, JY; Kim, RU; Lee, YS; Cho, HJ; Kim, DS. *Downregulation of voltage-gated potassium channel alpha gene expression by axotomy and neurotrophins in rat dorsal root ganglia.* Mol Cells, 2003 16, 256-259.

Petersen, KL; Rowbotham, MC. *Will ion-channel blockers be useful for management of nonneuropathic pain?* J Pain, 2000 1, 26-34.

Petrenko, AB; Yamakura, T; Baba, H; Shimoji, K. *The role of N-methyl-D-aspartate (NMDA) receptors in pain: a review*. Anesth Analg, 2003 97, 1108-1116.

Petzke, F; Clauw, DJ; Ambrose, K; Khine, A; Gracely, RH. *Increased pain sensitivity in fibromyalgia: effects of stimulus type and mode of presentation*. Pain, 2003 105, 403-413.

Pezet, S; McMahon, SB. NTs: *Mediators and modulators of pain.* Annu. Rev Neurosci, 2006 29, 507–538.

Pezet, S; Cunningham, J; Patel, J; Grist, J; Gavazzi, I; Lever, IJ; Malcangio, M. *BDNF modulates sensory neuron synaptic activity by a facilitation of GABA transmission in the dorsal horn.* Mol Cell Neurosci, 2002 21, 51-62.

Pezet, S; Onténiente, B; Grannec, G; Calvino, B. *Chronic pain is associated with increased TrkA immunoreactivity in spinoreticular neurons.* Neurosci, 1999 19, 5482-5492.

Price, DD; Staud, R; Robinson, ME; Mauderli, AP; Cannon, R; Vierck, CJ. *Enhanced temporal summation of second pain and its central modulation in fibromyalgia patients.* Pain, 2002 99, 49-59.

Price, DD; Staud, R. *Neurobiology of fibromyalgia syndrome*. J Rheumatol Suppl, 2005 75, 22-28.

Price, TJ; Louria, MD; Candelario-Soto, D; Dussor, GO; Jeske, NA; Patwardhan, AM; Diogenes, A; Trott, AA; Hargreaves, KM; Flores, CM. *Treatment of trigeminal ganglion neurons in vitro with NGF, GDNF or BDNF: effects on neuronal survival, neurochemical properties and TRPV1-mediated neuropeptide secretion*. BMC Neurosci, 2005 6, 4.

Quintão, NL; Santos, AR; Campos, MM; Calixto, JB. *The role of neurotrophic factors in genesis and maintenance of mechanical hypernociception after brachial plexus avulsion in mice.* Pain, 2008 136, 125-133.

Ramer, MS; French, GD; Bisby, MA. *Wallerian degeneration is required for both neuropathic pain and sympathetic sprouting into the DRG*. Pain, 1997 72, 71-78.

Ramer, MS; Kawaja, MD; Henderson, JT; Roder, JC; Bisby, MA. *Glial overexpression of NGF enhances neuropathic pain and adrenergic sprouting into DRG following chronic sciatic constriction in mice*. Neurosci Lett, 1998 251, 53-56.

Ramer, MS; Bradbury, EJ; McMahon, SB. Nerve growth factor induces P2X(3) expression in sensory neurons. J Neurochem, 2001 77, 864-875.

Reuter, U; Bolay, H; Jansen-Olesen, I; Chiarugi, A; Sanchez del Rio, M; Letourneau, R; Theoharides, TC; Waeber, C; Moskowitz, MA. *Delayed inflammation in rat meninges: implications for migraine pathophysiology*. Brain, 2001 124, 2490-2502.

Reuter, U; Chiarugi, A; Bolay, H; Moskowitz, MA. *Nuclear factor-kappaB as a molecular target for migraine therapy.* Ann Neurol, 2002 51, 507-516.

Ritter, AM; Lewin, GR; Kremer, NE; Mendell, LM. *Requirement for nerve growth factor in the development of myelinated nociceptors in vivo*. Nature, 1991 350, 500–502.

Ro, LS; Chang, KH. *Neuropathic pain: mechanisms and treatments*. Chang Gung Med J, 2005 28, 597-605.

Ro, LS; Chen, ST; Tang, LM; Jacobs, JM. *Effect of NGF and anti-NGF on neuropathic pain in rats following chronic constriction injury of the sciatic nerve*. Pain, 1999 79, 265-274.

Roberts, LA; Connor, M. *TRPV1 antagonists as a potential treatment for hyperalgesia*. Recent Patents CNS Drug Discov, 2006 1, 65-76.

Romero, MI; Rangappa, N; Garry, MG; Smith, GM. *Functional regeneration of chronically injured sensory afferents into adult spinal cord after neurotrophin gene therapy*. Neurosci, 2001 21, 8408-8416.

Ruiz, G; Baños, JE. *The effect of endoneurial nerve growth factor on calcitonin gene-related peptide expression in primary sensory neurons*. Brain Res, 2005 1042, 44-52.

Saban, MR; Nguyen, NB; Hammond, TG; Saban, R. *Gene expression profiling of mouse bladder inflammatory responses to LPS, substance P, and antigen-stimulation.* Am J Pathol, 2002 160, 2095–2110.

Sabsovich, I; Wei, T; Guo, TZ; Zhao, R; Shi, X; Li, X; Yeomans, DC; Klyukinov, M; Kingery, WS; Clark, JD. *Effect of anti-NGF antibodies in a rat tibia fracture model of complex regional pain syndrome type I.* Pain, 2008 138, 47-60.

Safieh-Garabedian, B; Dardenne, M; Pleau, JM; Saade, NE. *Potent analgesic and anti-inflammatory actions of a novel thymulin-related peptide in the rat*. Br J Pharmacol, 2002 136, 947–955.

Salter, MW. *Cellular signalling pathways of spinal pain neuroplasticity as targets for analgesic development.* Curr Top Med Chem, 2005 5, 557-567.

Sanchez-Del-Rio, M; Reuter, U; Moskowitz, MA. *New insights into migraine pathophysiology*. Curr Opin Neurol, 2006 19, 294-298.

Saragovi, HU; Gehring, K. *Development of pharmacological agents for targeting NTs and their receptors.* Trends Pharmacol Sci, 2000 21, 93-98.

Sarchielli, P; Alberti, A; Baldi, A; Coppola, F; Rossi, C; Pierguidi, L; Floridi, A; Calabresi, P. *Proinflammatory cytokines, adhesion molecules, and lymphocyte integrin expression in the internal jugular blood of migraine patients without aura assessed ictally*. Headache, 2006a 46, 200-207.

Sarchielli, P; Alberti, A; Coppola, F; Baldi, A; Gallai, B; Floridi, A; Floridi, A; Capocchi, G; Gallai, V. *Platelet-activating factor (PAF) in internal jugular venous blood of migraine without aura patients assessed during migraine attacks*. Cephalalgia, 2004b 24, 623-630.

Sarchielli, P; Alberti, A; Floridi, A; Gallai, V. *Levels of nerve growth factor in cerebrospinal fluid of chronic daily headache patient*s. Neurology, 2001 57, 132-134.

Sarchielli, P; Alberti, A; Gallai, B; Coppola, F; Baldi, A; Floridi, A; Gallai, V. *Brain-derived neurotrophic factor in cerebrospinal fluid of patients with chronic daily headache: relationship with nerve growth factor and glutamate levels.* J Headache Pain, 2002 3, 129-135.

Sarchielli, P; Di Filippo, M; Nardi, K; Calabresi, P. *Sensitization, glutamate, and the link between migraine and fibromyalgia.* Curr Pain Headache Rep, 2007a 11, 343-351.

Sarchielli, P; Mancini, ML; Floridi, A; Coppola, F; Rossi, C; Nardi, K; Acciarresi, M; Pini, LA; Calabresi, P. *Increased levels of neurotrophins are not specific for chronic migraine: evidence from primary fibromyalgia syndrome*. J Pain, 2007b 8, 737-745.

Sarchielli, P; Floridi, A; Mancini, ML; Rossi, C; Coppola, F; Baldi, A; Pini, LA; Calabresi, P. *NF-kappaB activity and iNOS expression in monocytes from internal Jugular blood of migraine without aura patients during attacks*. Cephalalgia, 2006b 26, 1071-1079.

Sarchielli, P; Gallai, V. *Nerve growth factor and chronic daily headache: a potential implication for therapy.* Expert Rev Neurother, 2004a 4, 115-127.

Schifitto, G; Yiannoutsos, C; Simpson, DM; Adornato, BT; Singer, EJ; Hollander, H; Marra, CM; Rubin, M; Cohen, BA; Tucker, T; Koralnik, IJ; Katzenstein, D; Haidich, B; Smith, ME; Shriver, S; Millar, L; Clifford, DB; McArthur, JC; *AIDS Clinical Trials Group Team 291. Long-term treatment with recombinant nerve growth factor for HIV-associated sensory neuropathy*. Neurology, 2001 57, 1313-1316.

Scholz, J; Woolf, CJ. *Can we conquer pain?* Nat Neurosci, 2002 5, 1062–1067.

Schwartz, F; Brodie, C; Appel, E; Kazimirsky, G; Shainberg, *A. Effect of helium/neon laser irradiation on nerve growth factor synthesis and secretion in skeletal muscle cultures.* Photochem Photobiol B, 2002 66, 195-200.

Sevcik, MA; Ghilardi, JR; Peters, CM; Lindsay, TH; Halvorson, KG; Jonas, BM; Kubota, K; Kuskowski, MA; Boustany, L; Shelton, DL; Mantyh, PW. *Anti-NGF therapy profoundly*

reduces bone cancer pain and the accompanying increase in markers of peripheral and central sensitization. Pain, 2005 115, 128-141.

Shu, XQ; Mendell, LM. *Neurotrophins and hyperalgesia*. Proc Natl Acad Sci U S A, 1999 96, 7693-7696.

Siddall, PJ; Cousins, MJ. *Spinal pain mechanisms.* Spine, 1997 22, 98–104.

Silberstein, SD. *Migraine pathophysiology and its clinical implications Cephalalgia*, 2004 24, 2-7.

Simonetti, M; Fabbro, A; D'Arco, M; Zweyer, M; Nistri, A; Giniatullin, R; Fabbretti, E. *Comparison of P2X and TRPV1 receptors in ganglia or primary culture of trigeminal neurons and their modulation by NGF or serotonin*. Mol Pain, 2006 2, 11.

Skaper, SD; Pollock, M; Facci, L. *Mast cells differentially express and release active high molecular weight neurotrophins.* Brain Res Mol Brain Res, 2001 97, 177-185.

Soderling, TR; Derkach, VA. *Postsynaptic protein phosphorylation and LTP*. Trends Neurosci, 2000 23, 75-80.

Sorensen, J; Graven-Nielsen, T; Henriksson, KG; Bengtsson, M; Arendt-Nielsen, L. *Hyperexcitability in fibromyalgia*. J Rheumatol, 1998 25, 152-155.

Spiegel, K; Agrafiotis, D; Caprathe, B; Davis, RE; Dickerson, MR; Fergus, JH; Hepburn, TW; Marks, JS; Van Dorf, M; Wieland, DM; et al. *PD 90780, a non peptide inhibitor of nerve growth factor's binding to the P75 NGF receptor*. Biochem Biophys Res Commun, 1995 217, 488-494.

Staud, R; Cannon, RC; Mauderli, AP; Robinson, ME; Price, DD; Vierck, CJ. *Temporal summation of pain from mechanical stimulation of muscle tissue in normal controls and subjects with fibromyalgia syndrome*. Pain, 2003 102, 87-95.

Staud, R; Price, DD; Robinson, ME; Mauderli, AP; Vierck, CJ. *Maintenance of windup of second pain requires less frequent stimulation in fibromyalgia patients compared to normal controls.* Pain, 2004 110, 689-696.

Staud, R; Rodriguez, ME. *Mechanisms of disease: pain in fibromyalgia syndrome*. Nat Clin Pract Rheumatol, 2006 2, 90-98.

Staud, R; Vierck, CJ; Cannon, RL; Mauderli, AP; Price, DD. *Abnormal sensitization and temporal summation of second pain (wind-up) in patients with fibromyalgia syndrome*. Pain, 2001 91, 165–175.

Steiner, P; Pfeilschifter, J; Boeckh, C; Radeke, H; Otten, U. *Interleukin-1 beta and tumor necrosis factor-alpha synergistically stimulate nerve growth factor synthesis in rat mesangial cells.* Am J Physiol, 1991 261, F792-F798.

Sugiyama, K; Suzuki, Y; Furuta, H. *Histamine-release induced by 7S nerve-growth factor of mouse submandibular salivary glands.* Arch Oral Biol, 1985 30, 93-95.

Summer, GJ; Puntillo, KA; Miaskowski, C; Dina, OA; Green, PG; Levine, JD. *TrkA and PKC-epsilon in thermal burn-induced mechanical hyperalgesia in the rat.* J Pain, 2006 7, 884-891.

Sutherland, SP; Cook, SP; McCleskey, EW. *Chemical mediators of pain due to tissue damage and ischemia.* Prog Brain Res, 2000 129, 21-38.

Svensson, P; Cairns, BE; Wang, K; Arendt-Nielsen, L. *Injection of nerve growth factor into human masseter muscle evokes long-lasting mechanical allodynia and hyperalgesia*. Pain, 2003 104, 241–247.

Talhouk, RS; Saade, NE; Mouneimne, G; Masaad, CA; Safieh-Garabedian, B. *Growth hormone releasing hormone reverses endotoxon-induced localized inflammatory*

hyperalgesia without reducing the upregulated cytokines, nerve growth factor and gelatinase activity. Prog Neuropsychopharmacol Biol Psychiatry, 2004 28, 625-631.

Toma, H; Winston, J; Micci, MA; Shenoy, M; Pasricha, PJ. *Nerve growth factor expression is up-regulated in the rat model of L-arginine-induced acute pancreatitis*. Gastroenterology, 2000 119, 1373-1381.

Torcia, M; Bracci-Laudiero, L; Lucibello, M; Nencioni, L; Labardi, D; Rubartelli, A; Cozzolino, F; Aloe, L; Garaci, E. *Nerve growth factor is an autocrine survival factor for memory* B lymphocytes. Cell, 1996 85, 345-356.

Ueda, M; Hirose, M; Takei, N; Ibuki, T; Naruse, Y; Amaya, F; Ibata, Y; Tanaka, M. *Nerve growth factor induces systemic hyperalgesia after thoracic burn injury in the rat.* Neurosci Lett, 2002 328, 97–100.

Ueyama, T; Hamada, M; Hano, T; Nishio, I; Masuyama, Y; Furukawa, S. *Production of nerve growth factor by cultured vascular smooth muscle cells from spontaneously hypertensive and Wistar-Kyoto rats*. J Hypertens, 1993 11, 1061-1065.

Ugolini, G; Marinelli, S; Covaceuszach, S; Cattaneo, A; Pavone, F. *The function neutralizing anti-TrkA antibody MNAC13 reduces inflammatory and neuropathic pain*. PNAS, 2007 104, 2985-2990.

Vedder, H; Affolter, HU; Otten, U. *Nerve growth factor (NGF) regulates tachykinin gene expression and biosynthesis in rat sensory neurons during early postnatal development.* Neuropeptides, 1993 24, 351-357.

Verge, VM; Merlio, JP; Grondin, J; Ernfors, P; Persson, H; Riopelle, RJ; Hökfelt, T; Richardson, PM. *Colocalization of NGF binding sites, trk mRNA, and low-affinity NGF receptor mRNA in primary sensory neurons: responses to injury and infusion of NGF*. J Neurosci, 1992 12, 4011-4022.

Vikelis, M; Mitsikostas, DD. *The role of glutamate and its receptors in migraine*. CNS Neurol Disord Drug Targets, 2007 6, 251-257.

Wall, PD; Gutnick, M. Ongoing activity in peripheral nerves: *The physiology and pharmacology of impulses originating from a neuroma.* Exp Neurol, 1974 43, 580–593.

Wallace, D; Kinker-Israeli, M; Hallegua, D; Silverman, S; Silver, DM. *Cytokines play an aetiopathogenetic role in fibromyalgia: a hypothesis and pilot study.* Rheumatology, 2001 40, 743–749.

Wan, L; Luo, A; Yu, H; Tian, Y. *Effect of touch-stimulus on the expression of C-fos and TrkA in spinal cord following chronic constriction injury of the sciatic nerve in rats*. J Huazhong Univ Sci Technolog Med Sci, 2005 25, 219-222.

Wanigasekara, Y; Keast, JR. *Nerve growth factor, glial cell line-derived neurotrophic factor and neurturin prevent semaphorin 3A-mediated growth cone collapse in adult sensory neurons.* Neuroscience, 2006 142, 369-379.

Wehrman, T; He, X; Raab, B; Dukipatti, A; Blau, H; Garcia, KC. *Structural and mechanistic insights into nerve growth factor interactions with the TrkA and p75 receptors*. Neuron, 2007 53, 25-38.

Weis, J; Dimpfel, W; Schröder, JM. *Nerve conduction changes and fine structural alterations of extra- and intrafusal muscle and nerve fibers in streptozotocin diabetic rats*. Muscle Nerve, 1995 8, 175-184.

Wiesmann, C; de Vos, AM. *Nerve growth factor: structure and function*. Cell Mol Life Sci, 2001 58, 748-759.

Wild, KD; Bian, D; Zhu, D; Davis, J; Bannon, AW; Zhang, TJ; Louis, JC. *Antibodies to nerve growth factor reverse established tactile allodynia in rodent models of neuropathic pain without tolerance*. J Pharmacol Exp Ther, 2007 322, 282-287.

Wilson-Gerwing, TD; Verge, VM. *Neurotrophin-3 attenuates galanin expression in the chronic constriction injury model of neuropathic pain.* Neuroscience, 2006 141, 2075-2085.

Wolfe, F; Smythe, HA; Yunus, MB; Bennett, RM; Bombardier, C; Goldenberg, DL; Tugwell, P; Campbell, SM; Abeles, M; Clark, P; et al. *The American College of Rheumatology 1990 Criteria for the Classification of Fibromyalgia. Report of the Multicenter Criteria Committee*. Arthritis Rheum, 1990 33, 160–172.

Woodall, AJ; Richards, MA; Turner, DJ; Fitzgerald, EM. *Growth factors differentially regulate neuronal Ca(v) channels via ERK-dependent signalling*. Cell Calcium, 2008 43, 562-575.

Woolf, CJ; Allchorne, A; Safieh-Garabedian, B; Poole, S. *Cytokines, nerve growth factor and inflammatory hyperalgesia: the contribution of tumour necrosis factor alpha*. J Pharmacol, 1997 121, 417-424.

Woolf, CJ; Chong, MS. *Preemptive analgesia: Treating postoperative pain by preventing the establishment of central sensitization*. Anesth Analg, 1993 77, 362–379.

Woolf, CJ; Ma, QP; Allchorne, A; Poole, S. *Peripheral cell types contributing to the hyperalgesic action of nerve growth factor in inflammation*. J Neurosci, 1996 16, 2716-2723.

Wu, C; Boustany, L; Liang, H; Brennan, TJ. *Nerve growth factor expression after plantar incision in the rat.* Anesthesiology, 2007 107, 128-135.

Xu, P; Hall, AK. *Activin acts with nerve growth factor to regulate calcitonin gene-related peptide mRNA in sensory neurons.* Neuroscience, 2007 150, 665-674.

Xue, Q; Jong, B; Chen, T; Schumacher, MA. *Transcription of rat TRPV1 utilizes a dual promoter system that is positively regulated by nerve growth factor*. Neurochem, 2007 101, 212-222.

Yajima, Y; Narita, M; Narita, M; Matsumoto, N; Suzuki, T. *Involvement of a spinal brain-derived neurotrophic factor/full-length TrkB pathway in the development of nerve injury-induced thermal hyperalgesia in mice*. Brain Res 2002 958, 338-346.

Yajima, Y; Narita, M; Usui, A; Kaneko, C; Miyatake, M; Narita, M; Yamaguchi, T; Tamaki, H; Wachi, H; Seyama, Y; Suzuki, T. *Direct evidence for the involvement of brain-derived neurotrophic factor in the development of a neuropathic pain-like state in mice*. J Neurochem 2005 93, 584-594.

Zahn, PK; Subieta, A; Park, SS; Brennan, TJ. *Effect of blockade of nerve growth factor and tumor necrosis factor on pain behaviors after plantar incision*. J Pain, 2004 5, 157–163.

Zhang, X; Huang, J; McNaughton, PA. *NGF rapidly increases membrane expression of TRPV1 heat-gated ion channels.* EMBO J, 2005 24, 4211-4223.

Zhu, W; Oxford, GS. *Phosphoinositide-3-kinase and mitogen activated protein kinase signaling pathways mediate acute NGF sensitization of TRPV1*. Mol Cell Neurosci, 2007 34, 689-700.

Zhuang, ZY; Xu, H; Clapham, DE; Ji, RR. *Phosphatidylinositol 3-kinase activates ERK in primary sensory neurons and mediates inflammatory heat hyperalgesia through TRPV1 sensitization.* J Neurosci, 2004 24, 8300-8309.

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Chapter 3

HOMEOSTATIC ROLE OF THE PARASYMPATHETIC NERVOUS SYSTEM IN HUMAN BEHAVIOR

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ABSTRACT

It was recently proposed that respiratory sinus arrhythmia (RSA) reflects the ability of the organism to integrate behavioral and metabolic demands, improving its homeostasis efficiency. Since the various anatomical and functional levels of the vagus nerve provide the conceptual basis of this allostatic model, it was designed under the name of the polyvagal theory. Therefore, altered RSA responses to various challenges could help to detect some dysfunctional states. We review here the putative homeostatic roles of this vagal loop, i.e., afferent and efferent pathways, in the domain of psychological and behavioral homeostasis. Evaluation of the autonomic activity was issued from the temporal and frequency domain analyses of heart rate variability (HRV). HRV analysis is an elegant noninvasive way to assess autonomic activity via the sympathovagal balance, and is more and more widely used in clinical and fundamental experiments. However, one must be cautious in the interpretation of the various HRV indices to preclude any erroneous conclusion. Keeping this in mind, there is actually a body of evidence arguing for a robust association between changes in vagal activity and (1) fatigue and mood changes during training adaptation and (2) some eating behaviors such as cephalic endocrine and exocrine secretions. They suggest that normal adaptation to psychological or physical loads and energy challenges requires the integrity of the parasympathetic nervous system and an equilibrate sympathovagal balance. Any impaired function of the parasympathetic component of the autonomous nervous system may lead to severe deleterious consequences, either psychological (depression and chronic fatigue) or metabolic (postprandial hyperglycemia). For the purpose of preventing overtraining, we propose a heuristic sequential psychological and sympathovagal evolution that we called the "Multistage Psycho-Autonomic Model of Adaptation to Training" (MPAMAT). In conclusion, these results are consistent with an

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allostatic role of the parasympathetic nervous system in a wide variety of functions, and confirm HRV analyses as promising for improving the detection and prevention of several psychological and metabolic altered states.

1. INTRODUCTION

The polyvagal theory [1] links autonomic function to psychological and behavioral processes. Its purpose is to provide an integrative model associating neurophysiology with psychology and behavior in a phylogenetic perspective [2]. Although criticized [3], this psychophysiological approach involving the autonomic nervous system (ANS) and, more specifically, vagal activity is historically important. It may help to improve our understanding of the way neural activity influences some altered mood states, or contributes to the mechanism of non-cognitive anticipation [4, 5]. In brief, it leads to the consideration of the ANS not only as responding to a psychological or environmental stimulus (the classic Stimulus-Response model), but also as involved in every step of the system, from the afferent to the efferent pathways of the loop. More than initially designed by early psycho-physiologists [6], the objective is not only to translate a mental process in a measurable physiological variable but also to understand how the different components of the ANS contribute to assess and interpret the environment (e.g., safe or dangerous) and produce adapted behavioral and physiological responses [2]. However, it must be noticed that psychophysiology was sometimes opposed to physiological explanations of behavior. Both involving a complex intrication of neuroendocrine factors, this distinction will not be made in the present paper.

The polyvagal theory is primarily based on the determination of ANS activity using heart rate variability (HRV). HRV describes the variations between successive heartbeats and is regulated by sympathetic and parasympathetic modulations. Spectral analysis of HRV was proposed more than two decades ago as a noninvasive tool for studying cardiac autonomic control [7], and several validity procedures have been conducted by different teams [8-10]. In brief, this technique consists of the analysis of the variability between R to R intervals after normalisation (N to N), using time and frequency domains [11-13]. Results from frequency domain analysis provide a quantification of the low (LF) and high (HF) frequency oscillations of NN intervals, reflecting the autonomic modulation of the sinoatrial node. The LF band corresponds mainly to sympathetic and partially to parasympathetic modulations, whereas the HF band represents only parasympathetic modulations [7]. The LF and HF bands are expressed as absolute or normalized values [10, 14], the latter allowing the discrimination between the absolute activity and the proportional part of sympathetic and parasympathetic modulation in the global ANS activity. Even if the validity of HRV spectral analysis to assess autonomic control is still discussed [15, 16], it was found to be clinically relevant to identify disturbances of the ANS in some pathological states such as heart failure [17], chronic obstructive pulmonary disease [18], bronchial hyperresponsiveness [19] or asthma [20]. Moreover, a reduced HRV is associated with increased mortality after myocardial infarction [21, 22], proving the power of this analysis as a prognosis marker. One of the strengths of HRV is that it is noninvasive and adapted to everyday life conditions or laboratory-controlled tasks (Figure 1).

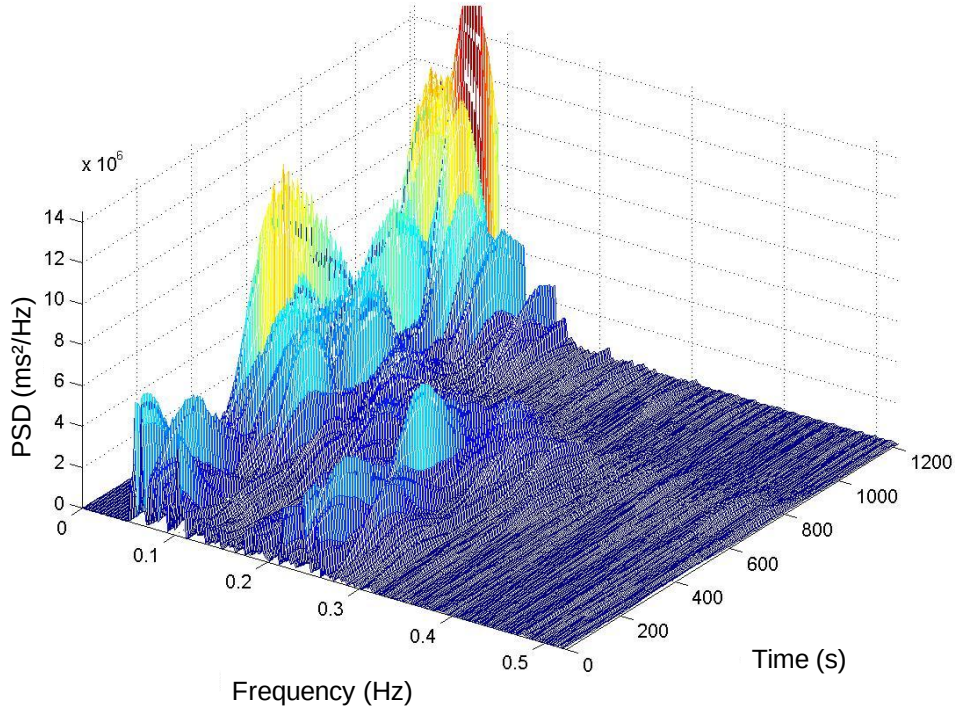

Figure 1. Example of HRV analyses during a head up tilt test. The power spectral density (PSD) differs between the components: Very Low Frequency (VLF), Low Frequency (LF) and High Frequency (HF). The HF decreases during standing whereas the LF power increases, suggesting a balance from PNS modulation to an SNS modulation during an orthostatic test.

ANS is modified during episodes in which the homeostasis is challenged. The polyvagal theory is actually defined by their authors as an adaptative reaction to challenges [2]. These challenges could be for example acute phases of fatigue or overtraining due to an intense and prolonged psychological or physical workload, such as reported in students or in athletes. Energy needs also represent a homeostatic body challenge since it must trigger a behavioral sequence (arousal, quest for food and initiation of eating), physiological reflexes (hepatic production of glucose until food is actually available, anticipatory secretions improving absorption and metabolism of nutrients) and affective factors (pleasure attributed to the sensory properties of food, reward). As proposed by Pagani and Lucini [23], HRV may represent a quantifiable measure of the "interaction between subject's effort and environmental demands." Indeed, it has been proposed that HRV could be linked to the same functional network in the brain which would goal-directed behavior and adaptability in relation with emotion [24-26]. Moreover, it could provide an objective and concrete assessment independently from subjective components, e.g., psychometric questionnaires. In the present paper, we will discuss the putative role of homeostatic ANS changes in relation with behavior and psychology. Recent data obtained by our team with HRV spectral analysis on the relationships between changes in parasympathetic activity and (1) fatigue and mood states and (2) eating behavior will be briefly presented. Moreover, we will propose for the

first time a model linking psychological factors to parasympathetic activity in the domain of training: the "Multistage Psychoautonomic Model of Adaptation to Training."

2. PARASYMPATHETIC ACTIVITY AND FATIGUE

2.1. Parasympathetic activity and chronic fatigue syndrome

2.1.1. Chronic fatigue syndrome and autonomic imbalance

Chronic fatigue syndrome (CFS) is an interesting model for studying the relations between ANS variations and psychopathological processes since it has been the subject of a large body of work in the recent years. The diagnosis of CFS is derived from self-reported symptoms not caused by other known medical origins. Symptoms include clinically evaluated, unexplained, persistent or relapsing fatigue, lasting more than 6 months, not substantially alleviated by rest, and resulting in a substantial reduction in activity level. This fatigue is associated with various symptoms [27] but muscle weakness, pain in multiple joints, postexertional malaise, impaired memory-concentration and unrefreshing sleep are apparently the best for discriminating this syndrome from major depressive disorders [28, 29]. The exact aetiology of CFS is still unknown, and a multifactorial pathogenesis is likely. Initial infection severity and decreased immunity [30, 31] seem the main factors presently suspected, but an autonomic imbalance could be involved [32], the latter being the possible consequence of the formers.

Details of this autonomic imbalance have been reported by several authors in various conditions: rest, deep respiration, tilt test, postural change. In brief, subjects classified as CFS showed increased heart rate (HR) at rest and during a voluntary orthostatic test [33, 34]. This is in favor of an increased SNS activity [35] and a reduced adaptation to postural challenge. The HRV analysis was also specifically used in some studies and was proposed as a technique to differentiate CFS from healthy individuals during a mild orthostatic test [36, 37].

Interestingly, some authors combined a psychological stressor and the assessment of autonomic tone or baroreflex [38, 39]. LaManca et al. [38] observed that cognitive task could be associated in some CFS patients with a diminished cardiovascular response. Peckerman et al. [39] showed that the cardiovascular response to a stressful speech task predicts severity of CFS, suggesting abnormalities in peripheral and/or central mechanisms of cardiovascular stress responses in CFS patients, depending on their profile.

This autonomic imbalance was also found recently during nocturnal recordings, with increased HR and reduced LF, very low frequency (VLF), and total power (TP) of HRV observed in individuals diagnosed as CFS compared to control subjects [40]. From these HRV results and the associated increase in plasma norepinephrine, the authors suggested that CFS subjects experienced greater physiologic effort and a SNS predominance at rest during sleep. However, in this study, this SNS predominance was not confirmed by a change in HRV modulation towards sympathetic stimulation, e.g., no change in the LF/HF ratio, which is one of the major indices of SNS modulation.

The HR response to exercise in CFS is usually consistent with an autonomic dysfunction. Despite normal resting cardiac function, reduced HR acceleration during a graded exercise test [41] and at maximal levels [42] has been reported, whereas HR was increased at

submaximal levels [43]. Moreover, in this population, a reduced parasympathetic activity, as evaluated by HRV analysis, was found either in the sitting or the standing posture [44], during walking or recovery [45], despite no differences in mean HR, tidal volume, minute ventilation, respiratory rate, oxygen consumption or total spectrum power of HRV.

One hypothesis is that impaired parasympathetic activity could be involved in the decrease of exercise capacities and submaximal HR observed in CFS patients. These patients would have a decrease in exercise tolerance due to the existence of an abnormal sense of efforts and/or reluctance to exercise due to an unexpressed fear of relapse [46]. This would be in agreement with one important feature of the polyvagal theory considering the vagal response as inhibiting SNS-induced fear or danger feeling [2]. It was actually proposed that the disrupted vagal response to an energy demand situation could explain, at least in part, some of the fatigue reported in CFS. The parasympathetic component of the ANS is classically considered as the energy-conserving component. Impaired parasympathetic response in periods of high demand may lead to excess energy expenditure, and the consequence a disproportionate fatigue relative to the effort.

The link between subjective and objective dimensions of autonomic dysfunction in CFS individuals has been spectacularly reinforced recently with a questionnaire called the Composite Autonomic Symptom Scale (COMPASS) and showing a strong predictive power of autonomic dysfunctions [47].

These results are consistent with a homeostatic role for the parasympathetic nervous system in a wide variety of functions. The decrease of parasympathetic activity during CFS could be the support of most of associated symptoms. The return to reference level in using exercise training for example could have large long-term benefits.

2.1.2. Psychometric assessment of fatigue in CFS and relation with autonomic imbalance

Another way to assess noninvasively fatigue-induced changes in behavior is to use adapted questionnaires such as the Profile of Mood States (POMS). The POMS consists of 65 items that address six components of mood with various subscales: Tension, Depression, Anger, Vigor, Fatigue, and Confusion [48]. Subjects are asked to describe their feelings over the previous week using a 5-point responses scale ranging from 0 (not at all) to 4 (extremely) for each item. An overall measure of Total Mood Disturbance (TMD) is calculated from all six subscales. To this day, few studies have assessed results on the POMS in CFS [49-51]. Compared to control subjects, CFS patients were actually found to display higher scores for Tension, Fatigue, Depression and Confusion sub-scales and lower Vigor score resulting in a higher total TMD score and greater disturbance of mood [49]. Moreover, patients with CFS exhibited an abnormally reduced seasonal variation in mood and behavior according to the Seasonal Pattern Assessment Questionnaire and the POMS [51].

The influence of exercise on mood alterations in CFS is still largely unknown. After maximal exercise, CFS patients have been found to display more fatigue and less vigor than control subjects [52] and a persistent vigor decrease 4 days later. However with lower intensities, some studies suggest that exercise may represent a beneficial and even a therapeutic approach. Thus, after a 30 minute isometric exercise, scores of Fatigue, Depression and Confusion decreased, arguing for an exercise-induced mood improvement [50]. After 12 weeks of graded exercise, similar results were reported, in particular lower perceived exertion scores and an increased work capacity, interpreted by authors as a decrease

in avoidance behavior [53]. Unfortunately, no estimation of parasympathetic activity was done.

It may seem paradoxical to treat fatigue and altered mood states with an increase of physical activity, but it was shown that in subjects practicing moderate exercise, negative mood scores and more specifically depressive symptoms, occurred as soon as 1 week after cessation of exercise [54, 55]. Still more strikingly, a low initial parasympathetic activity was predictive of negative mood states [55, 56]. One hypothesis would be that a reduction in exercise load, and more generally in daily activity, are involved in CFS-induced mood alterations or even in CFS *per se*, mediated by a decrease in parasympathetic activity.

Inconsistently, a recent large prospective longitudinal study revealed that increased levels of exercise throughout childhood and early adult life was associated with an increased risk to later develop CFS, and all the more if exercise was continued following the onset of fatigue [57]. An intensity threshold is probably necessary since a simple increase in daily walking has been reported to worsen mood and fatigue symptoms [58] but not 30 min of intermittent walking [58, 59]. Possibly, spontaneous physical activity without any control in acute phase of fatigue may lead to an exacerbation of the perceived exhaustion and to mood disturbances. On the opposite, a relevant physical exercise program could allow the reestablishment of basal ANS parameters and restore mood. Research is needed to assess the optimal training program to improve fitness and symptoms in CFS patients.

2.2. Parasympathetic activity and overtraining

2.2.1. Overtraining syndrome and autonomic imbalance

Overtraining syndrome has been initially described in athletes but is a poorly defined entity. Its diagnosis requires at least a severe fatigue associated with a decrease in performances [60]. Overtraining is suspected to be the consequence of excessive training intensity or too little recovery time [61], often combined with other stressors linked or not to training. Therefore, overtraining can be considered to result from an imbalance between the overall strain of training and the individual's tolerance [62]. The common symptoms are sleep disorders, changes in blood hormones [60, 62, 63], and in HRV indices [64]. Before overtraining occurs, this imbalance leads to transitory phases of overreaching which are reversible with a short resting period. This short-term overreaching is often more or less voluntary experienced by athletes during a training program to allow overcompensation and to improve performances.

The hypothesis of an underlying autonomic imbalance in overtraining is old [65, 66] and now proposed as its major cause [67]. Inadequate physical workload leading to acute or chronic fatigue has been shown to shift autonomic regulation from a parasympathetic to a sympathetic predominance [68-70], and more specifically to an abnormal sympathetic response to orthostatic stimuli [71]. Moreover, it has been reported that athletes diagnosed as overtrained display a weaker orthostatic response to a head-up tilt test as compared to trained athletes [64] or control subjects [72, 73]. However, in a young athlete with well established overtraining, a shift of HRV toward an increased HF power indicated an autonomic imbalance toward a higher parasympathetic modulation [74], showing that the relation

between overtraining and HRV is not straightforward. To reconcile these results, it can be postulated that in later phases of ANS imbalance, the parasympathetic modulation in overtraining may mimic the values of normal subject, consistent with the bell shape concept of Iwasaki et al. [75].

These autonomic imbalance data were reinforced by those from neuroendocrine changes. During heavy endurance training or overreaching periods, a reduced adrenal responsiveness to adrenocorticotropic hormone (ACTH) was reported, partially compensated by an increased pituitary ACTH release[67, 76-78]. In an advanced stage of overtraining, there is additional evidence for decreased catecholamine excretion and sensitivity of target organs [79-84].

All these results suggest that an imbalance between training and recovery in athletes or in subjects with a high physical load, may lead to an ANS dysregulation towards an increase in sympathetic activity and a decrease in basal parasympathetic activity. In the final stage of overtraining, sympathetic hyperactivity seems to withdraw and shift again towards a relative parasympathetic predominance.

2.2.2. Mood alterations and relation with autonomic imbalance

Overtraining is not only characterized by a sport-specific or task-specific decrease in performance, but also disturbances in mood state [60, 85]. Thus, competitive swimmers followed during nine training seasons showed mood disturbances that increased in a training dose-response manner [86] and returned to baseline levels during relative recovery or tapering [86, 87]. When the POMS is used to assess mood states, normal training is associated with decreased scores of Tension, Depression, Anger, Fatigue and Confusion and an increased score of Vigor [87, 88]. Interestingly, performances during the whole-season championship were actually associated with changes in mood states and more specifically to the decrease in Vigor score [89, 90], showing that psychometric profiles and adaptative processes to training load are closely linked and have relevant consequences on physical capacities. Unfortunately, in the domain of adaptation to training loads, there is to our knowledge no study correlating mood and ANS characteristics.

Hitherto, relations between psychological and autonomic indices of HRV have led to discrepant results but argue for the importance of parasympathetic activity in some psychological traits. If some authors failed to detect any correlation [91] most reported significant relations. Thus, among patients at risk of developing cardiovascular diseases, those with depressive and anxiety-related symptoms had reduced HRV and its parasympathetic component relative to controls [92-94]. Moreover, patients with symptoms related to chronic psychosocial stress displayed decreased parasympathetic activity and impaired orthostatic ANS response [95]. Importantly, these autonomic changes were correlated to stress perception scores and allowed to accurately discriminate control subjects and patients. Consistently, an association between blunted parasympathetic modulation of HRV and perceived stress [96], anxiety [97-100] and depression [100, 101] among men and women was repeatedly observed. Parasympathetic activity was also negatively associated with hopelessness [102]. Moreover, depressive subjects showing improvement of mood during the day had increased parasympathetic activity in the evening compared with the morning [103]. Interestingly, the HF power returned to the level of control subjects when depression was treated with cognitive therapy [104] or acupuncture [105], and HRV biofeedback increased the parasympathetic activity and decreases depression scores [106, 107]. Lastly,

parasympathic modulation was recently reported to account for the relation between sadness and anterior cingulate cortex function evaluated by error-related event potentials [108].

Several groups of population such as workers or students, facing high physical load, are at risk of developing extreme acute fatigue, and even overreaching or overtraining equivalents. If autonomic imbalance is linked to some altered mood states, it would be interesting to assess sympathovagal balance in the general population in relation with different psychometric profiles. This might help to explore the role of ANS in some mood disorders and represent an objective evaluation of mental dysfunction. A decrease in parasympathetic activity and a shift towards sympathetic activation were reported among "stressed" first year medical students [109]. Recently we investigated students in Physical Education (PE) who cumulated a heavy physical workload and university psychological stress and therefore that we suspected to display some autonomic imbalance and altered mood state such as described in overtraining. We used the POMS questionnaire for evaluating mood state, and HRV to assess sympathovagal balance during an orthostatic test, followed by correlations between each POMS subscale and each HRV indice [110]. Two groups were distinguished using repeated POMS questionnaires in a step-by-step procedure, only subjects displaying highest or lowest scores being selected. Subjects in the upper or lower score category over the selection procedure were classified as potentially overtrained (POT) and control subjects (CTR) respectively. Compared to the CTR group, POT subjects showed a greater decrease of two temporal indices of HRV reflecting mainly parasympathetic activity during the orthostatic test. This suggests that the adaptative process to postural change was impaired in individuals with stable and persistent negative mood states. Correlations showed a contrasted picture: in POT group, the score on the Vigor subscale was positively correlated with global ANS activity in the supine position, whereas the score on the Depression subscale was negatively correlated with percentages of change of parasympathetic activity during the orthostatic test (**Figure 2**). In brief, individuals displaying potential overtraining equivalent showed an impaired parasympathetic response to orthostatism; the weaker was this response, the higher were their scores on the Depression subscale. We then conducted a longitudinal prospective study in a similar population, tracking the changes in mood state and autonomic response to an orthostatic test at three periods of the university year i.e. October, January and June (unpublished data). Results showed a spectacular decrease in whole ANS activity and in Vigor score as soon as the second period. Interestingly, in this sample of heterogeneous subjects, the changes in parasympathetic activity between the first and second periods were actually correlated with changes in Depression scores, arguing for the robustness of this relation.

Some authors proposed that negative effects may be a unifying and potentially "toxic" element linking individual trait negative emotions to ANS dysregulation. These results are in agreement with those obtained by our team and suggest a close relationship between mood disturbance and parasympathetic activity. For Thayer & Lane [25], a decreased parasympathetic modulation of cardiac function [111], impairs the adaptation to rapid changes in environment and appropriate responses.

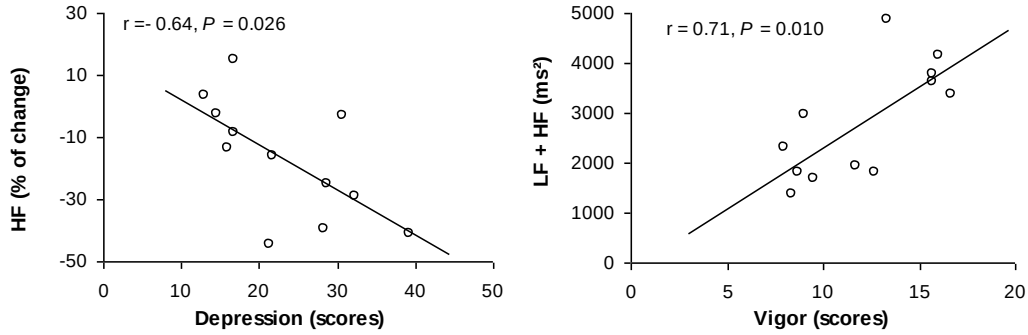

Figure 2. Correlations between (A) depression subscale of the POMS questionnaire and changes in the HF band of HRV indices during the head-up tilt test for the potentially OT subjects, (B) vigor subscale of the POMS questionnaire and the total spectral power (LF+HF, ms²) measure in supine position for the potentially OT subjects.

2.3. The multistage psychoautonomic model of adaptation to training

Given the present data gathered in various studies, we think that a heuristic modelization of fatigue, altered mood states and impaired autonomic imbalance in the field of training can be proposed. Called the "Multistage Psychoautonomic Model of Adaptation to Training," it describes various stages of mental and autonomic responses to training load, from normal to overtraining and chronic fatigue, *via* transitional fatigue and overreaching. This model defines three main stages, but intermediate situations might be added in the future (Figure 3).

Stage 1: Normal training adaptation

During this stage, the increase in sympathetic activity when stimulated is down-regulated and the peripheral sensitivity increased, which leads by a retronegative loop to a secondary reduction in global SNS activity. This is potentialized by training-induced improvement of physical capacities. In a homeostatic perspective, this is in favor of a more accurate restoration of energy stores and mental well-being (e.g., appetite, quality of sleep, pleasure taken to practice the discipline). Therefore, mood state is globally improved compared to pretraining state, specially vigor, fatigue, anxiety and depression, all the more potently that total and HF powers of HRV are elevated.

Stage 2: Overreaching / Fatigue

Here, a dramatic increase in sympathetic activity without significant changes in parasympathetic activity is observed, leading to a relative drop of the parasympathetic contribution to total autonomic activity. Training load transitory exceeds the upper limits of individual adaptive capacities, leading to fatigue but without a degradation of performances after short recovery. At this stage, the homeostatic process still operates and, under certain

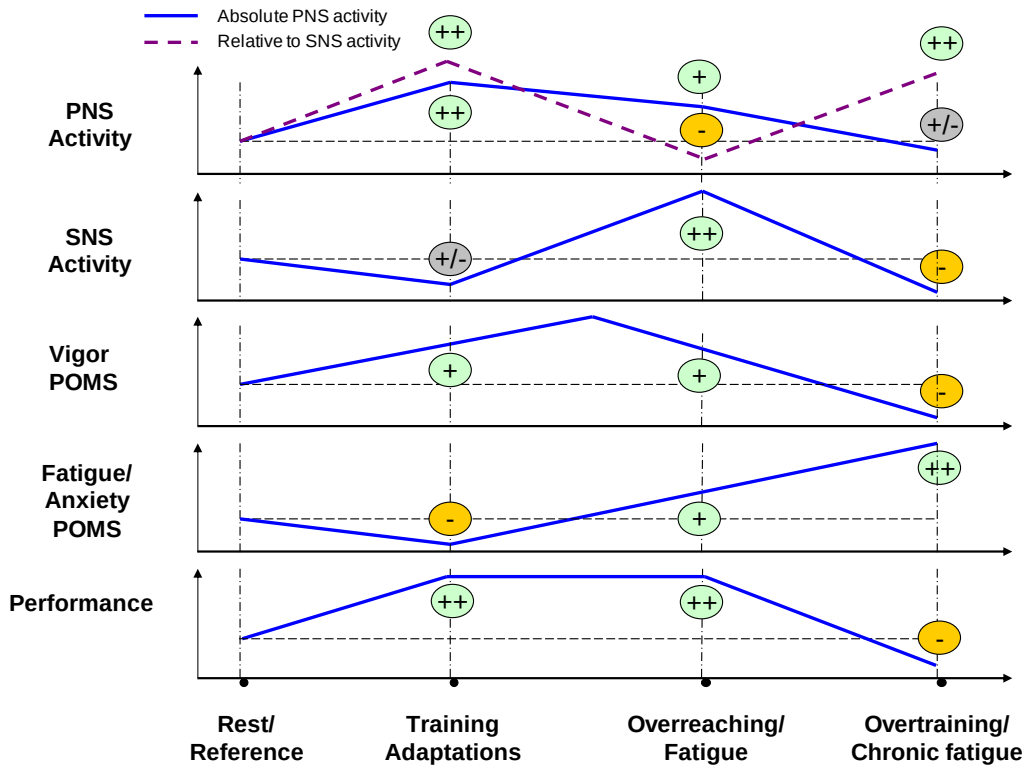

Figure 3. The Multistage Psychoautonomic Model of Adaptation to Training. A schematic representation of the changes during the adaptation to training process for the parasympathetic nervous system (PNS) activity expressed in absolute and reactive values, the sympathetic nervous system (SNS) activity, Vigor, Fatigue and Anxiety sub-scores assessed by the Profil of Mood States (POMS) questionnaire, and performance.

conditions (temporary decrease of the workload), can return to the previous normal training adaptative stage, resulting in improved performances. However, insufficient recovery will lead to a decrease in the reconditioning abilities and impaired homeostasis during rest. In parallel, the imbalance of ANS towards sympathetic activity during a long period may progressively inhibit the level of parasympathetic input to organs. Mood is here characterized by a decrease in vigor with a progressive increase in fatigue and anxiety, again correlated with parasympathetic activity.

Stage 3: Overtraining / Chronic fatigue

This third stage is observed following a long period of inadequate balance between sustained workload and time spent for recovery. Progressively, after an excessive and prolonged activation of the sympathetic activity, negative hormonal feedback and desensitization of organ receptors will result in complete withdrawal of sympathetic activity. The major autonomic event will be a dramatic fall of sympathetic activity associated with a

decrease, but of smaller magnitude, of its parasympathetic counterpart. At this final stage, vigor reaches its nadir, associated with a rise in fatigue, anxiety and even depression.

This model might represent a potentially useful tool to track the effect of training or recovery on autonomic balance and mood state normalization, notably during rehabilitation program in subjects with ANS dysregulation. Moreover, it may allow a precocious insight for anticipating inadequate physical and psychological load (training, work, treatment, readaptation, etc.).

However, further research is needed to validate each stage of this model, in particular interindividual variability and composite profiles i.e., mood and ANS characteristics of various stages. This would help to understand large interindividual variations, some athletes tolerating psycho-physiological loads exceeding their upper limits without reaching the next stage.

In conclusion, there is now a body of evidence that a decrease in global ANS activity, and more specifically in parasympathetic activity, is tightly linked to mood state alterations and other fatigue symptoms. Overtraining is particular adapted to explore these relations. Based on the literature and on our own experience, we think the model that we called the "Multistage Psychoautonomic Model of Adaptation to Training" may represent a heuristic tool for categorizing main stages of the adaptative processes to training. We hope that it will provide a basis of discussion between searchers on this topic. Even if psychological and autonomic parameters are of primary importance in the study of homeostasis during training, endocrine, immunological and metabolical parameters are greatly needed to complete this model.

3. PARASYMPATHETIC NERVOUS SYSTEM AND EATING BEHAVIOR

3.1. HRV as a method of assessing postprandial sympathovagal balance

The body is usually considered to be in 3 possible nutritional states: postingestive, postabsorptive or fasting. Autonomic activity in relation with eating behavior has been primary assessed to (1) compare postprandial and fasting state, (2) compare effects of different macronutrients on sympathovagal balance and (3) explore cephalic phase reflexes (CPR) at the onset of meal initiation. In the postprandial state, the sympathetic nervous system was usually the studied variable because of its role in thermogenesis and its potential importance in energy balance [112]. Parasympathetic activity has been mainly studied in the preprandial period due to its contribution to cephalic phases [113]. For estimating the relation between sympathovagal balance and eating behavior, there were some recent interesting attempts to substitute HRV to microneurography or norepinephrine turnover for sympathetic activity. Being noninvasive, this method is actually particularly adapted to the study of spontaneous behavior. Importantly, contrary to some recommendations, the quality of the HRV reproducibility requires that subjects do not control their breathing either this is in relation with food intake [114] or not [115].

The demonstration of a progressive shift to a prominent sympathetic activity (increase LF/HF indice) after glucose [116] and insulin [117] infusions, associated with consistent

increased blood norepinephrine levels infusion, demonstrated the sensitivity of the procedure, being in agreement with previous studies conducted with traditional methods [118, 119]. In more realistic eating conditions i.e., after meals, this sympathovagal shift toward sympathetic activity was found to be primary due to decreased parasympathetic activity [120]. This was confirmed after a high carbohydrate meal, whereas no change was observed after the high-fat meal [121]. Moreover, obese subjects did not display any postprandial change in HRV whatever was the macronutrient composition of the meal, showing a possible defect in the food-induced sympathetic activation. Since this postprandial sympathetic prominence is mediated by insulin [117], this result suggests that HRV may contribute to assess postprandial insulin resistance at a precocious state. If parasympathetic values only are considered, data show that total activity as assessed by the HF power in absolute value (ms^2) decreased 2 and 3 hours after a high carbohydrate meal [121] or infusion of glucose [116] or free fatty acids [122], illustrating that this change in sympathovagal balance is not only a sympathetic activation but also a decreased in parasympathetic activity.

In a recent study, we recorded HRV continuously from breakfast until 3 hours after lunch meal, requested spontaneously [123]. Analyses in the frequency domain were for the first time considered for each 256 NN intervals, allowing an average ANS evaluation every 5 min. Between 10:30 and 11:30, subjects stayed quietly at rest (control condition) or exercised on an ergocycle at 70% VO_{2max} (exercise condition) or were exposed to a simulated altitude of 4300 m via a hypoxic normobaric system (hypoxia condition). Results showed that in the control condition, there was a rapid and profound decrease in the HF bands, either in absolute or in normalized units, with a nadir reached 10 to 15 min after the start of consumption and followed by a very slow increase over the next 180 min. Since the LF power also decreased, the increase in LF/HF was not significant, and argue for the conclusions of Lu et al. [120] about the primary role of parasympathetic withdrawal in the postprandial changes in autonomic activity. Importantly, exercise but not hypoxia, amplified this parasympathetic decrease. This was associated with a greater glucose response to the meal and a sustained fat oxidation. Given the role of the vagus nerve in increasing the glucose-induced insulin secretion, and the inhibition of fat oxidation by insulin, this may explain why exercise before a meal maintains its fat oxidation rate even in the postprandial period, as it was demonstrated in a classic experiment 25 years ago [124].

In preadolescents [125], the postprandial shift towards sympathetic activity was confirmed following a breakfast meal, whereas continuing overnight fast led to an increase in parasympathetic activity during the morning, this being more prominent in male than female subjects [126]. It must be noted that some failed to find such modification [127]. In conclusion, HRV appears a major tool to assess the consequence of eating on sympathovagal balance and might contribute to the understanding of the mechanism of glucose intolerance in individuals at risk of type 2 diabetes, and the benefit of exercise.

3.2. Prandial pattern and the problem of meal definition

Eating behavior can be modelized as a simplistic sequence of eating episodes separated by non-eating periods. Each eating episode is initiated after the perception of a hunger signal by the central nervous system and is interrupted when a satiation signal occurs. The intra-meal structure is somewhat more complex since each successive food item of the meal gives

birth to a satiation signal conditioned on the sensory characteristics of this item, but for convenience, we will only consider the satiation signal interrupting the meal. Thus, we see that the classification according to nutritional states is not relevant to the domain of eating behavior. We have proposed [128] that an hunger signal and a glucose decline should be necessary to consider an eating occasion as a meal whereas any other intake not driven by homeostatic should be called snacks. Actually, a discrete blood glucose decline just prior to spontaneous meal onset has been repeatedly found in animals [129] and in humans [130], even in conditions mimicking everyday life [128] or using an ambulatory apparatus recording continuously interstitial glucose [131]. This preprandial glucose decline is considered as a peripheral sign of central glucopenia acting on orexigenic neuropeptides secreted in glucosensitive hypothalamic cells, and triggering food seeking [132]. Since in most studies authors rarely specify whether subjects were or not in a hunger state when the test meals were provided, to classify these eating occasions as real meals is impossible. This is an important limitation since the role of ANS and more specifically of the parasympathetic activity may, according to the polyvagal theory, primary concern biobehavioral mechanism linking energy homeostasis to spontaneous eating, and therefore may disappear in fixed eating or in snacking conditions. To this day, the involvement of parasympathetic in eating behavior has been mainly demonstrated in two mechanisms: cephalic phase reflexes and lipoprivic feeding.

3.3. Cephalic phase reflexes

3.3.1. Cephalic phases reflexes: roles and mechanisms

The demonstration of a “psychic secretion” by Pavlov [133] triggered by the presence of food in the oral cavity paved the way for the exploration of the complete neural connection between sensory cues and endocrine or exocrine secretions, and gave birth to the sciences of conditioning. Renamed cephalic phase reflexes (CPR) or preabsorptive secretions, they were found to react to sensory cues of foods and to modulate activity at various sites: salivary glands, gastrointestinal tract, pancreas (enzyme secretion and hormone release), thermogenic, cardiovascular and renal system [134]. The CPR can be triggered by all sensory stimuli (sight, smell, taste, texture), but also, surprisingly, by the mere thought of a palatable food [135], suggesting that the sensory loop is not a necessary condition for its onset. Uncoupling sensory from nutrient stimulation showed that CPR were primary dependant of sensory cues and were considered as a mean to prepare digestion but also metabolical pathways of nutrients. The latter was more specifically linked to the secretion of insulin after a brief visual, olfactory or gustative stimulus of a food, and called cephalic phase of insulin release (CPIR). However, other cephalic responses have been described in humans either for triacylglycerol [136] or for hormones also involved in eating behavior such as glucagon [137] and ghrelin [138] but will not be treated here.

3.3.2. Cephalic phase of insulin release

Due to the small magnitude and duration of the phenomenon, CPIR is quite delicate to observe in human subjects but has finally been well-documented [113]. The role of vagal nerves in CPIR is supported by functional arguments. Receptors in the oropharyngeal cavity send orosensory messages to the nucleus of the solitary tract. After integration, this yields an

activation of the efferent vagal fibers from the dorsal motor nucleus of the vagus (DMNV). The endocrine pancreas is actually innervated by parasympathetic fibers travelling in the vagus nerve, and its activation releases acetylcholine within the islets leading to secretion of insulin, glucagon and pancreatic polypeptide (PP). Interestingly, the secretion of PP is under almost exclusive dependence of vagal stimulation [139], allowing an indirect exploration of its activity. For example a muscarinic antagonist such as atropine totally abolishes its secretion. Even if the vagal contribution to CPIR has been demonstrated using atropine [140], the fact that trimethaphan, a parasympathetic and sympathetic ganglionic blocker, impairs CPIR more than atropine alone, argues for a non cholinergic participation [141]. Although of small magnitude, CPIR was found to have a potent effect in the postprandial glucose concentrations. Thus oral sensory stimulation prevents elevated levels of glucose and insulin [141, 142] and its inhibition led to an increase in blood glucose concentrations [141].

Interestingly, this difference in glucose response was correlated with CPIR, arguing for the crucial role of this vagal loop. Presently, this reflex phenomenon is considered as having potentially important consequences in terms of glucose tolerance and prevention of diabetes. Moreover, it is in line with the polyvagal theory, involving afferent and efferent fibres improving bodily responses to the energy challenge via adaptation to sensory cues provided by orosensory factors [143]. To this day, HRV has rarely been used for exploration of CPIR and the only change observed in the study published was a slight decrease that authors do not explain [144]. It is clear that to find subtle variations of parasympathetic activity revealing a cephalic phase, analyzing HRV recording on a large amount of successive short intervals is required (e.g. at least 5 min in order to have 256 RR intervals) and not, as usually done, sporadic or cumulative measures.

3.4. Lipoprivic feeding

Fatty acid oxidation inhibitors such as methyl-palmoxirate [145] and mercaptoacetate [146] have been shown to stimulate eating, suggesting that oxidation of non-esterified fatty acids (NEFA) contributes to satiety, leading to the concept of lipoprivic feeding. Consistently, an impaired NEFA oxidation was shown to be a predictive factor of diet-induced obesity in animals [147] and humans [148]. This eating-stimulatory effect of mercaptoacetate was primary thought to challenge the glucostatic theory. Glucose would not be the only link between energy status and behavior but any substrate providing ATP may share this role. However it was found that this mechanism was not mediated by a direct shortage of NEFA in the brain but by abdominal vagal afferents [149]. More recently, the same team demonstrated that neither vagal afferents or hepatic fatty acid oxidation were involved in the effect on feeding behavior of a highly selective beta(3)-adrenergic receptor agonist [150], questioning the importance of peripheral NEFA oxidation in the lipoprivic feeding. Interpretations of the reported results in terms of consequences on glucose disposal and energy homeostasis for the glucosensitive neuronal cells in the hypothalamus areas could help to propose an integrative model.

3.5. Putative role of the parasympathetic nervous system in eating behavior: The lessons of subdiaphragmatic vagal deafferentation

In rats, subdiaphragmatic vagal deafferentation (SDA) has been extensively used in the last 30 years to determine the role of vagal tone in eating behavior. As expected, this surgical procedure was followed by a total absence of CPIR [151] with four consequences on glucose variations relative to meals: no preabsorptive decline, higher postprandial peak, accelerated return to baseline and a late secondary drop under basal level (i.e., hypoglycemia). These data were predictive of the findings on the benefit of CPIR on postprandial glucose tolerance [141].

SDA was found to suppress food intake in animals but this effect was small and primarily attributed to visceral malaise (delayed gastric emptying) causing conditioned taste aversion, and not specifically to an internal neuroendocrine loop [152]. However, SDA was potent enough to reverse hyperphagia and obesity of rats made obese by lesion of the ventro-median hypothalamus area [153]. SDA was also reported to abolish any rise in blood insulin level after stimulation of the DMNV [154]. It is to note that under normal conditions, central nervous system exerts a constant inhibitory control on insulin secretion alleviated by reduction or activation of SNS and PNS activities respectively.

Recently, the contribution of vagal afferents to eating behavior was quite challenged. Thus, lean Zucker rats gained similar weight whether they had sham or actually SDA [155]. Ghrelin, a potent stimulator of food intake in part vagally mediated, had similar stimulating effects on eating behavior when animals were vagally deafferented [156]. When leptinemia was lowered by 80% in subjects kept fasted during 72h and supplemented or not with r-metHuLeptin, reduced cardiac vagal tone measured using HRV was not altered by leptin replacement [157]. Moreover, the reduced-intake power of hydroxycitrate, an inhibitor of neolipogenesis that may improve NEFA oxidation, was not changed by vagal deafferentation [158]. The importance of vagal nerves is however clear in the satiety effect induced by cholecystokinin [159]. Interestingly, these authors have shown that this action necessitates the contribution of vagal efferents to completely operate, arguing for the validity of the polyvagal theory involving vagal pathways in both the afferent and efferent directions to fully satisfy adaptation to homeostatic challenges. Moreover, the vagus nerve was recently found to be involved in the intestine-brain axis that reduces glucose production when long chain fatty acids (LCFA) are present in the upper intestine [160] but not in the eating response since vagotomy did not change the usual observed reduction in food intake [161]. In definitive, the influence of vagal afferents on eating seems mainly to concern intrameal satiation with increased meal size [162]. All these results suggest that at best, vagal afferents are involved in the progressive decline in hunger during or after a meal, but are not as crucial as initially thought or still defended by some authors [163], even for the effect of several peripheral agents modulating eating behavior.

3.6. Portal glucose receptors, vagal afferents and eating behavior

The presence of glucoreceptors, localised in the portal circulation, detecting glucose decline, and sending messages of depletion *via* vagal afferents in order to initiate food intake, was proposed by Russek 45 years ago [164]. Further experiments actually showed that

discharges of the hepatic branch of the vagus nerve varied in the opposed manner of portal glucose levels [165]. The consequence on spontaneous eating behavior was consistent; eating initiation was actually demonstrated when decreased portal glucose activated vagal afferents [166]. This was associated with a stimulation of the lateral hypothalamus [167], a brain area still considered as the main feeding centre, even after the large amount of knowledge accumulated these late years on the central control of eating behavior [168, 169], and even in other behaviors than eating such as spontaneous physical activity [170]. More than glucoreceptors *per se*, portal receptors should be considered as glucose-sensitive portal receptors (GSPR) since they are also responsive to glucose metabolites such as pyruvate [171].

The fact that the stimulation of the GSPR by glucose decline triggers eating behavior raises the hypothesis that this phenomenon is part of the biological preprandial sequence. However, its effect on insulin secretion is opposite to those of cephalic phases, with an inhibition of the vagal pancreatic efferents and therefore a decreased insulin secretion [172]. These authors concluded that vagal afferent fibers inhibited the brainstem centers of the vagal efferents pathways to the pancreas. This balance between afferent and efferent vagal nerves were confirmed in later experiments [173]. Importantly, this increased vagal tone of afferents fibers may lead to increased hepatic glucose production (HGP) since blockade of vagal efferents was reported to dramatically reduce outflow of hepatic glucose [174]. Interestingly, very recent findings lead to consider the DMNV not only as a relay area but as an integrative center of mutiple afferent information (including vagal subdiaphragmatic fibers) such as leptin, glucose and cholecystokinin [175]. This raises new hypotheses on its actual role in the central organization of eating behavior, one being a transitory production of glucose from the liver when a portal glucose decline is detected. Moreover, hypothalamic ATP-sensitive potassium channels (and more specifically in the arcuate nucleus), activated by leptin, insulin and long-chain fatty acylCoAs, all intake-reducing stimuli, were found to decrease endogenous glucose production, a mechanism involving the DMNV and the integrity of the efferent hepatic vagal branch [176-178]. This relation between a signal of nutrient disposal for energy and HGP mediated by the vague is a promising field of research and strongly in favor of the parasympathetic nervous system as a homeostatic actor of eating behavior.

Less explored but of interest in the future is the possible role of vagal tone in macronutrient selection, since SDA reduces carbohydrate intake in the rat, more specifically of liquid diet [153], whereas in humans vagotomy undertaken for clinical purposes is often followed by reduction of carbohydrate intake [179] and of pleasure provided by sweet taste [180]. This field of research has been strangely quite absent from scientific focus these later years. Since it has been reported that saturated fat-induced inhibition of carbohydrate intake was mediated by vagal afferent fibers from the liver [181], it would be interesting to conduct new experiments on this putative role of the parasympathetic component in macronutrient choice.

4. CONCLUSION

All of these results are in favor of one important feature of the polyvagal theory [2]: the role of the vagal loop, i.e., afferent and efferent pathways in the domain of psychological and behavioral homeostasis. In this chapter, we reported results showing a tight association between changes in PNS activity as assessed by the temporal and frequency domain analyses of the variability of heart rate and (1) fatigue or mood changes during training adaptation and (2) eating behavior either with or without sensory stimulation. They suggest that normal adaptation to working load and energy challenges both yield to a potent parasympathetic activation maintaining the integrity of the body, considered a homeostatic system, whereas impaired sympathovagal responses are associated with deleterious consequences. Many research studies are still needed, and HRV technique must be used with caution to avoid any mistaken inference from a noninvasive but delicate way to explore the autonomous nervous system. Some authors have addressed most of these concerns, and their recommendations need to be carefully followed [3]. Moreover, the actual contribution of the afferent and efferent vagal fibers will require specific studies.

REFERENCES

[1] Porges, SW. Orienting in a defensive world: mammalian modifications of our evolutionary heritage. A Polyvagal Theory. *Psychophysiology* 1995;32(4):301-18.

[2] Porges, SW. The polyvagal perspective. *Biol Psychol* 2007;74(2):116-43.

[3] Grossman, P, Taylor EW. Toward understanding respiratory sinus arrhythmia: relations to cardiac vagal tone, evolution and biobehavioral functions. *Biol Psychol* 2007;74(2):263-85.

[4] Booth, DA, Grinker JA. Learned control of meal size in spontaneously obese and nonobese bonnet macaque monkeys. *Physiol Behav* 1993;53(1):51-7.

[5] Shaffer, SE, Tepper BJ. Effects of learned flavor cues on single meal and daily food intake in humans. *Physiol Behav* 1994;55(6):979-86.

[6] Razran, G. The observable unconscious and the inferable conscious in current Soviet psychophysiology: interoceptive conditioning, semantic conditioning, and the orienting reflex. *Psychol Rev* 1961;68:1-147.

[7] Akselrod, S, Gordon D, Ubel FA, Shannon DC, Berger AC, Cohen RJ. Power spectrum analysis of heart rate fluctuation: a quantitative probe of beat-to-beat cardiovascular control. *Science* 1981;213(4504):220-2.

[8] Akselrod, S, Gordon D, Madwed JB, Snidman NC, Shannon DC, Cohen RJ. Hemodynamic regulation: investigation by spectral analysis. *Am J Physiol* 1985;249(4 Pt 2):H867-75.

[9] Pomeranz, B, Macaulay RJ, Caudill MA, Kutz I, Adam D, Gordon D, et al. Assessment of autonomic function in humans by heart rate spectral analysis. *Am J Physiol* 1985;248(1 Pt 2):H151-3.

[10] Pagani, M, Lombardi F, Guzzetti S, Rimoldi O, Furlan R, Pizzinelli P, et al. Power spectral analysis of heart rate and arterial pressure variabilities as a marker of sympatho-vagal interaction in man and conscious dog. *Circ Res* 1986;59(2):178-93.

[11] Souza Neto, EP, Neidecker J, Lehot JJ. [To understand blood pressure and heart rate variability]. *Ann Fr Anesth Reanim* 2003;22(5):425-52.

[12] Pichon, A, Roulaud M, Antoine-Jonville S, de Bisschop C, Denjean A. Spectral analysis of heart rate variability: interchangeability between autoregressive analysis and fast Fourier transform. *J Electrocardiol* 2006;39(1):31-7.

[13] Montano, N, Porta A, Cogliati C, Costantino G, Tobaldini E, Casali KR, et al. Heart rate variability explored in the frequency domain: a tool to investigate the link between heart and behavior. *Neurosci Biobehav Rev* 2009;33(2):71-80.

[14] Montano, N, Ruscone TG, Porta A, Lombardi F, Pagani M, Malliani A. Power spectrum analysis of heart rate variability to assess the changes in sympathovagal balance during graded orthostatic tilt. *Circulation* 1994;90(4):1826-31.

[15] Parati, G, Mancia G, Di Rienzo M, Castiglioni P. Point: cardiovascular variability is/is not an index of autonomic control of circulation. *J Appl Physiol* 2006;101(2):676-8; discussion 681-2.

[16] Taylor, JA, Studinger P. Counterpoint: cardiovascular variability is not an index of autonomic control of the circulation. *J Appl Physiol* 2006;101(2):678-81; discussion 681.

[17] Saul, JP, Arai Y, Berger RD, Lilly LS, Colucci WS, Cohen RJ. Assessment of autonomic regulation in chronic congestive heart failure by heart rate spectral analysis. *Am J Cardiol* 1988;61(15):1292-9.

[18] Volterrani, M, Scalvini S, Mazzuero G, Lanfranchi P, Colombo R, Clark AL, et al. Decreased heart rate variability in patients with chronic obstructive pulmonary disease. *Chest* 1994;106(5):1432-7.

[19] Pichon, A, de Bisschop C, Diaz V, Denjean A. Parasympathetic airway response and heart rate variability before and at the end of methacholine challenge. *Chest* 2005;127(1):23-9.

[20] Fujii, H, Fukutomi O, Inoue R, Shinoda S, Okammoto H, Teramoto T, et al. Autonomic regulation after exercise evidenced by spectral analysis of heart rate variability in asthmatic children. *Ann Allergy Asthma Immunol* 2000;85(3):233-7.

[21] La Rovere, MT, Bigger JT, Jr., Marcus FI, Mortara A, Schwartz PJ. Baroreflex sensitivity and heart-rate variability in prediction of total cardiac mortality after myocardial infarction. ATRAMI (Autonomic Tone and Reflexes After Myocardial Infarction) Investigators. *Lancet* 1998;351(9101):478-84.

[22] Kleiger, RE, Miller JP, Bigger JT, Jr., Moss AJ. Decreased heart rate variability and its association with increased mortality after acute myocardial infarction. *Am J Cardiol* 1987;59(4):256-62.

[23] Pagani, M, Lucini D. Chronic fatigue syndrome: a hypothesis focusing on the autonomic nervous system. *Clin Sci (Lond)* 1999;96(1):117-25.

[24] Benarroch, EE. The central autonomic network: functional organization, dysfunction, and perspective. *Mayo Clin Proc* 1993;68(10):988-1001.

[25] Thayer, JF, Lane RD. A model of neurovisceral integration in emotion regulation and dysregulation. *J Affect Disord* 2000;61(3):201-16.

[26] Devinsky, O, Morrell MJ, Vogt BA. Contributions of anterior cingulate cortex to behaviour. *Brain* 1995;118 (Pt 1):279-306.

[27] Fukuda, K, Straus SE, Hickie I, Sharpe MC, Dobbins JG, Komaroff A. The chronic fatigue syndrome: a comprehensive approach to its definition and study. International Chronic Fatigue Syndrome Study Group. *Ann Intern Med* 1994;121(12):953-9.

[28] King, C, Jason LA. Improving the diagnostic criteria and procedures for chronic fatigue syndrome. *Biol Psychol* 2005;68(2):87-106.

[29] Hawk, C, Jason LA, Torres-Harding S. Differential diagnosis of chronic fatigue syndrome and major depressive disorder. *Int J Behav Med* 2006;13(3):244-51.

[30] Klimas, NG, Koneru AO. Chronic fatigue syndrome: inflammation, immune function, and neuroendocrine interactions. *Curr Rheumatol Rep* 2007;9(6):482-7.

[31] Appel, S, Chapman J, Shoenfeld Y. Infection and vaccination in chronic fatigue syndrome: myth or reality? *Autoimmunity* 2007;40(1):48-53.

[32] Freeman, R, Komaroff AL. Does the chronic fatigue syndrome involve the autonomic nervous system? *Am J Med* 1997;102(4):357-64.

[33] Galland, BC, Jackson PM, Sayers RM, Taylor BJ. A matched case control study of orthostatic intolerance in children/adolescents with chronic fatigue syndrome. *Pediatr Res* 2008;63(2):196-202.

[34] Schondorf, R, Benoit J, Wein T, Phaneuf D. Orthostatic intolerance in the chronic fatigue syndrome. *J Auton Nerv Syst* 1999;75(2-3):192-201.

[35] Wyller, VB, Saul JP, Walloe L, Thaulow E. Sympathetic cardiovascular control during orthostatic stress and isometric exercise in adolescent chronic fatigue syndrome. *Eur J Appl Physiol* 2008;102(6):623-32.

[36] Yamamoto, Y, LaManca JJ, Natelson BH. A measure of heart rate variability is sensitive to orthostatic challenge in women with chronic fatigue syndrome. *Exp Biol Med (Maywood)* 2003;228(2):167-74.

[37] Yoshiuchi, K, Quigley KS, Ohashi K, Yamamoto Y, Natelson BH. Use of time-frequency analysis to investigate temporal patterns of cardiac autonomic response during head-up tilt in chronic fatigue syndrome. *Auton Neurosci* 2004;113(1-2):55-62.

[38] LaManca, JJ, Peckerman A, Sisto SA, DeLuca J, Cook S, Natelson BH. Cardiovascular responses of women with chronic fatigue syndrome to stressful cognitive testing before and after strenuous exercise. *Psychosom Med* 2001;63(5):756-64.

[39] Peckerman, A, LaManca JJ, Qureishi B, Dahl KA, Golfetti R, Yamamoto Y, et al. Baroreceptor reflex and integrative stress responses in chronic fatigue syndrome. *Psychosom Med* 2003;65(5):889-95.

[40] Boneva, RS, Decker MJ, Maloney EM, Lin JM, Jones JF, Helgason HG, et al. Higher heart rate and reduced heart rate variability persist during sleep in chronic fatigue syndrome: a population-based study. *Auton Neurosci* 2007;137(1-2):94-101.

[41] Montague, TJ, Marrie TJ, Klassen GA, Bewick DJ, Horacek BM. Cardiac function at rest and with exercise in the chronic fatigue syndrome. *Chest* 1989;95(4):779-84.

[42] Sargent, C, Scroop GC, Nemeth PM, Burnet RB, Buckley JD. Maximal oxygen uptake and lactate metabolism are normal in chronic fatigue syndrome. *Med Sci Sports Exerc* 2002;34(1):51-6.

[43] Riley, MS, O'Brien CJ, McCluskey DR, Bell NP, Nicholls DP. Aerobic work capacity in patients with chronic fatigue syndrome. *Bmj* 1990;301(6758):953-6.

[44] Sisto, SA, Tapp W, Drastal S, Bergen M, DeMasi I, Cordero D, et al. Vagal tone is reduced during paced breathing in patients with the chronic fatigue syndrome. *Clin Auton Res* 1995;5(3):139-43.

[45] Cordero, DL, Sisto SA, Tapp WN, LaManca JJ, Pareja JG, Natelson BH. Decreased vagal power during treadmill walking in patients with chronic fatigue syndrome. *Clin Auton Res* 1996;6(6):329-33.

[46] Wallman, KE, Morton AR, Goodman C, Grove R. Physiological responses during a submaximal cycle test in chronic fatigue syndrome. *Med Sci Sports Exerc* 2004;36(10):1682-8.

[47] Newton, JL, Okonkwo O, Sutcliffe K, Seth A, Shin J, Jones DE. Symptoms of autonomic dysfunction in chronic fatigue syndrome. *Qjm* 2007;100(8):519-26.

[48] McNair, D, Lorr M, Droppelman L. Manual for the profil of mood states. San Diego: CA: Educationnal and and Industrial Testing Service; 1971.

[49] Tuck, I, Wallace D. Chronic fatigue syndrome: a woman's dilemma. *Health Care Women Int* 2000;21(5):457-66.

[50] Lloyd, A, Gandevia S, Brockman A, Hales J, Wakefield D. Cytokine production and fatigue in patients with chronic fatigue syndrome and healthy control subjects in response to exercise. *Clin Infect Dis* 1994;18 Suppl 1:S142-6.

[51] Garcia-Borreguero, D, Dale JK, Rosenthal NE, Chiara A, O'Fallon A, Bartko JJ, et al. Lack of seasonal variation of symptoms in patients with chronic fatigue syndrome. *Psychiatry Res* 1998;77(2):71-7.

[52] Sisto, SA, LaManca J, Cordero DL, Bergen MT, Ellis SP, Drastal S, et al. Metabolic and cardiovascular effects of a progressive exercise test in patients with chronic fatigue syndrome. *Am J Med* 1996;100(6):634-40.

[53] Wallman, KE, Morton AR, Goodman C, Grove R, Guilfoyle AM. Randomised controlled trial of graded exercise in chronic fatigue syndrome. *Med J Aust* 2004;180(9):444-8.

[54] Berlin, AA, Kop WJ, Deuster PA. Depressive mood symptoms and fatigue after exercise withdrawal: the potential role of decreased fitness. *Psychosom Med* 2006;68(2):224-30.

[55] Weinstein, AA, Deuster PA, Kop WJ. Heart rate variability as a predictor of negative mood symptoms induced by exercise withdrawal. *Med Sci Sports Exerc* 2007;39(4):735-41.

[56] Glass, JM, Lyden AK, Petzke F, Stein P, Whalen G, Ambrose K, et al. The effect of brief exercise cessation on pain, fatigue, and mood symptom development in healthy, fit individuals. *J Psychosom Res* 2004;57(4):391-8.

[57] Harvey, SB, Wadsworth M, Wessely S, Hotopf M. Etiology of Chronic Fatigue Syndrome: Testing Popular Hypotheses Using a National Birth Cohort Study. *Psychosom Med* 2008.

[58] Black, CD, O'Connor P J, McCully KK. Increased daily physical activity and fatigue symptoms in chronic fatigue syndrome. *Dyn Med* 2005;4(1):3.

[59] Clapp, LL, Richardson MT, Smith JF, Wang M, Clapp AJ, Pieroni RE. Acute effects of thirty minutes of light-intensity, intermittent exercise on patients with chronic fatigue syndrome. *Phys Ther* 1999;79(8):749-56.

[60] Urhausen, A, Kindermann W. Diagnosis of overtraining: what tools do we have? *Sports Med* 2002;32(2):95-102.

[61] Fry, RW, Morton AR, Keast D. Overtraining in athletes. An update. *Sports Med* 1991;12(1):32-65.

[62] Urhausen, A, Gabriel H, Kindermann W. Blood hormones as markers of training stress and overtraining. *Sports Med* 1995;20(4):251-76.

[63] Morgan, WP, Costill DL, Flynn MG, Raglin JS, O'Connor PJ. Mood disturbance following increased training in swimmers. *Med Sci Sports Exerc* 1988;20(4):408-14.

[64] Uusitalo, AL, Uusitalo AJ, Rusko HK. Exhaustive endurance training for 6-9 weeks did not induce changes in intrinsic heart rate and cardiac autonomic modulation in female athletes. *Int J Sports Med* 1998;19(8):532-40.

[65] Israel, S. Problems of overtraining from an internal medical and performance physiological standpoint. *Medizin Sport* 1976;16(1):1-12.

[66] Kindermann, W. Overtraining, consequence of autonomic imbalance? *Dtsch Z Sportmed* 1986;34:138-145.

[67] Lehmann, M, Foster C, Dickhuth HH, Gastmann U. Autonomic imbalance hypothesis and overtraining syndrome. *Med Sci Sports Exerc* 1998;30(7):1140-5.

[68] Pichot, V, Roche F, Gaspoz JM, Enjolras F, Antoniadis A, Minini P, et al. Relation between heart rate variability and training load in middle-distance runners. *Med Sci Sports Exerc* 2000;32(10):1729-36.

[69] Pichot, V, Busso T, Roche F, Garet M, Costes F, Duverney D, et al. Autonomic adaptations to intensive and overload training periods: a laboratory study. *Med Sci Sports Exerc* 2002;34(10):1660-6.

[70] Garet, M, Tournaire N, Roche F, Laurent R, Lacour JR, Barthelemy JC, et al. Individual Interdependence between nocturnal ANS activity and performance in swimmers. *Med Sci Sports Exerc* 2004;36(12):2112-8.

[71] Iellamo, F, Legramante JM, Pigozzi F, Spataro A, Norbiato G, Lucini D, et al. Conversion from vagal to sympathetic predominance with strenuous training in high-performance world class athletes. *Circulation* 2002;105(23):2719-24.

[72] Mourot, L, Bouhaddi M, Perrey S, Cappelle S, Henriet MT, Wolf JP, et al. Decrease in heart rate variability with overtraining: assessment by the Poincare plot analysis. *Clin Physiol Funct Imaging* 2004;24(1):10-8.

[73] Hynynen, E, Uusitalo A, Konttinen N, Rusko H. Heart rate variability during night sleep and after awakening in overtrained athletes. *Med Sci Sports Exerc* 2006;38(2):313-7.

[74] Hedelin, R, Wiklund U, Bjerle P, Henriksson-Larsen K. Cardiac autonomic imbalance in an overtrained athlete. *Med Sci Sports Exerc* 2000;32(9):1531-3.

[75] Iwasaki, K, Zhang R, Zuckerman JH, Levine BD. Dose-response relationship of the cardiovascular adaptation to endurance training in healthy adults: how much training for what benefit? *J Appl Physiol* 2003;95(4):1575-83.

[76] Wittert, GA, Livesey JH, Espiner EA, Donald RA. Adaptation of the hypothalamopituitary adrenal axis to chronic exercise stress in humans. *Med Sci Sports Exerc* 1996;28(8):1015-9.

[77] Lehmann, M, Foster C, Keul J. Overtraining in endurance athletes: a brief review. *Med Sci Sports Exerc* 1993;25(7):854-62.

[78] Lehmann, MJ, Lormes W, Opitz-Gress A, Steinacker JM, Netzer N, Foster C, et al. Training and overtraining: an overview and experimental results in endurance sports. *J Sports Med Phys Fitness* 1997;37(1):7-17.

[79] Fry, AC, Schilling BK, Weiss LW, Chiu LZ. beta2-Adrenergic receptor downregulation and performance decrements during high-intensity resistance exercise overtraining. *J Appl Physiol* 2006;101(6):1664-72.

[80] Fry, AC, Kraemer WJ, Van Borselen F, Lynch JM, Triplett NT, Koziris LP, et al. Catecholamine responses to short-term high-intensity resistance exercise overtraining. *J Appl Physiol* 1994;77(2):941-6.

[81] Jost, J, Weiss M, Weicker H. Sympathoadrenergic regulation and the adrenoceptor system. *J Appl Physiol* 1990;68(3):897-904.

[82] Nieto, JL, Diaz-Laviada I, Malpartida JM, Galve-Roperh I, Haro A. Adaptations of the beta-adrenoceptor-adenylyl cyclase system in rat skeletal muscle to endurance physical training. *Pflugers Arch* 1997;434(6):809-14.

[83] Ohman, EM, Butler J, Kelly J, Horgan J, O'Malley K. Beta-adrenoceptor adaptation to endurance training. *J Cardiovasc Pharmacol* 1987;10(6):728-31.

[84] Werle, EO, Strobel G, Weicker H. Decrease in rat cardiac beta 1- and beta 2-adrenoceptors by training and endurance exercise. *Life Sci* 1990;46(1):9-17.

[85] Armstrong, LE, VanHeest JL. The unknown mechanism of the overtraining syndrome: clues from depression and psychoneuroimmunology. *Sports Med* 2002;32(3):185-209.

[86] Morgan, WP, Brown DR, Raglin JS, O'Connor PJ, Ellickson KA. Psychological monitoring of overtraining and staleness. *Br J Sports Med* 1987;21(3):107-14.

[87] Hooper, SL, Mackinnon LT, Ginn EM. Effects of three tapering techniques on the performance, forces and psychometric measures of competitive swimmers. *Eur J Appl Physiol Occup Physiol* 1998;78(3):258-63.

[88] Pierce, EF, Jr. Relationship between training volume and mood states in competitive swimmers during a 24-week season. *Percept Mot Skills* 2002;94(3 Pt 1):1009-12.

[89] Filaire, E, Bernain X, Sagnol M, Lac G. Preliminary results on mood state, salivary testosterone:cortisol ratio and team performance in a professional soccer team. *Eur J Appl Physiol* 2001;86(2):179-84.

[90] Filaire, E, Lac G, Pequignot JM. Biological, hormonal, and psychological parameters in professional soccer players throughout a competitive season. *Percept Mot Skills* 2003;97(3 Pt 2):1061-72.

[91] Virtanen, R, Jula A, Salminen JK, Voipio-Pulkki LM, Helenius H, Kuusela T, et al. Anxiety and hostility are associated with reduced baroreflex sensitivity and increased beat-to-beat blood pressure variability. *Psychosom Med* 2003;65(5):751-6.

[92] Carney, RM, Blumenthal JA, Stein PK, Watkins L, Catellier D, Berkman LF, et al. Depression, heart rate variability, and acute myocardial infarction. *Circulation* 2001;104(17):2024-8.

[93] Krittayaphong, R, Cascio WE, Light KC, Sheffield D, Golden RN, Finkel JB, et al. Heart rate variability in patients with coronary artery disease: differences in patients with higher and lower depression scores. *Psychosom Med* 1997;59(3):231-5.

[94] Pitzalis, MV, Iacoviello M, Todarello O, Fioretti A, Guida P, Massari F, et al. Depression but not anxiety influences the autonomic control of heart rate after myocardial infarction. *Am Heart J* 2001;141(5):765-71.

[95] Lucini, D, Di Fede G, Parati G, Pagani M. Impact of chronic psychosocial stress on autonomic cardiovascular regulation in otherwise healthy subjects. *Hypertension* 2005;46(5):1201-6.

[96] Dishman, RK, Nakamura Y, Garcia ME, Thompson RW, Dunn AL, Blair SN. Heart rate variability, trait anxiety, and perceived stress among physically fit men and women. *Int J Psychophysiol* 2000;37(2):121-33.

[97] Thayer, JF, Friedman BH, Borkovec TD. Autonomic characteristics of generalized anxiety disorder and worry. *Biol Psychiatry* 1996;39(4):255-66.

[98] Watkins, LL, Grossman P, Krishnan R, Sherwood A. Anxiety and vagal control of heart rate. *Psychosom Med* 1998;60(4):498-502.

[99] Fuller, BF. The effects of stress-anxiety and coping styles on heart rate variability. *Int J Psychophysiol* 1992;12(1):81-6.

[100] Bleil, ME, Gianaros PJ, Jennings JR, Flory JD, Manuck SB. Trait negative affect: toward an integrated model of understanding psychological risk for impairment in cardiac autonomic function. *Psychosom Med* 2008;70(3):328-37.

[101] Thayer, JF, Friedman BH, Borkovec TD, Johnsen BH, Molina S. Phasic heart period reactions to cued threat and nonthreat stimuli in generalized anxiety disorder. *Psychophysiology* 2000;37(3):361-8.

[102] Schwarz, AM, Schachinger H, Adler RH, Goetz SM. Hopelessness is associated with decreased heart rate variability during championship chess games. *Psychosom Med* 2003;65(4):658-61.

[103] Rechlin, T, Weis M, Kaschka WP. Is diurnal variation of mood associated with parasympathetic activity? *J Affect Disord* 1995;34(3):249-55.

[104] Carney, RM, Freedland KE, Stein PK, Skala JA, Hoffman P, Jaffe AS. Change in heart rate and heart rate variability during treatment for depression in patients with coronary heart disease. *Psychosom Med* 2000;62(5):639-47.

[105] Chambers, AS, Allen JJ. Vagal tone as an indicator of treatment response in major depression. *Psychophysiology* 2002;39(6):861-4.

[106] Nolan, RP, Kamath MV, Floras JS, Stanley J, Pang C, Picton P, et al. Heart rate variability biofeedback as a behavioral neurocardiac intervention to enhance vagal heart rate control. *Am Heart J* 2005;149(6):1137.

[107] Siepmann, M, Aykac V, Unterdorfer J, Petrowski K, Mueck-Weymann M. A Pilot Study on the Effects of Heart Rate Variability Biofeedback in Patients with Depression and in Healthy Subjects. *Appl Psychophysiol Biofeedback* 2008.

[108] Dywan, J, Mathewson KJ, Choma BL, Rosenfeld B, Segalowitz SJ. Autonomic and electrophysiological correlates of emotional intensity in older and younger adults. *Psychophysiology* 2008;45(3):389-97.

[109] Srinivasan, K, Vaz M, Sucharita S. A study of stress and autonomic nervous function in first year undergraduate medical students. *Indian J Physiol Pharmacol* 2006;50(3):257-64.

[110] Nuissier, F, Chapelot D, Vallet C, Pichon A. Relations between psychometric profiles and cardiovascular autonomic regulation in physical education students. *Eur J Appl Physiol* 2007;99(6):615-22.

[111] Saul, JP, Rea RF, Eckberg DL, Berger RD, Cohen RJ. Heart rate and muscle sympathetic nerve variability during reflex changes of autonomic activity. *Am J Physiol* 1990;258(3 Pt 2):H713-21.

[112] Tataranni, PA. From physiology to neuroendocrinology: a reappraisal of risk factors of body weight gain in humans. *Diabetes Metab* 1998;24(2):108-15.

[113] Teff, K. Nutritional implications of the cephalic-phase reflexes: endocrine responses. *Appetite* 2000;34(2):206-13.

[114] Dionne, IJ, White MD, Tremblay A. The reproducibility of power spectrum analysis of heart rate variability before and after a standardized meal. *Physiol Behav* 2002;75(3):267-70.

[115] Pitzalis, MV, Mastropasqua F, Massari F, Forleo C, Di Maggio M, Passantino A, et al. Short- and long-term reproducibility of time and frequency domain heart rate variability measurements in normal subjects. *Cardiovasc Res* 1996;32(2):226-33.

[116] Paolisso, G, Manzella D, Ferrara N, Gambardella A, Abete P, Tagliamonte MR, et al. Glucose ingestion affects cardiac ANS in healthy subjects with different amounts of body fat. *Am J Physiol* 1997;273(3 Pt 1):E471-8.

[117] Paolisso, G, Manzella D, Rizzo MR, Barbieri M, Varricchio G, Gambardella A, et al. Effects of insulin on the cardiac autonomic nervous system in insulin-resistant states. *Clin Sci (Lond)* 2000;98(2):129-36.

[118] Rowe, JW, Young JB, Minaker KL, Stevens AL, Pallotta J, Landsberg L. Effect of insulin and glucose infusions on sympathetic nervous system activity in normal man. *Diabetes* 1981;30(3):219-25.

[119] Berne, C, Fagius J, Pollare T, Hjemdahl P. The sympathetic response to euglycaemic hyperinsulinaemia. Evidence from microelectrode nerve recordings in healthy subjects. *Diabetologia* 1992;35(9):873-9.

[120] Lu, CL, Zou X, Orr WC, Chen JD. Postprandial changes of sympathovagal balance measured by heart rate variability. *Dig Dis Sci* 1999;44(4):857-61.

[121] Tentolouris, N, Tsigos C, Perea D, Koukou E, Kyriaki D, Kitsou E, et al. Differential effects of high-fat and high-carbohydrate isoenergetic meals on cardiac autonomic nervous system activity in lean and obese women. *Metabolism* 2003;52(11):1426-32.

[122] Paolisso, G, Manzella D, Rizzo MR, Ragno E, Barbieri M, Varricchio G, et al. Elevated plasma fatty acid concentrations stimulate the cardiac autonomic nervous system in healthy subjects. *Am J Clin Nutr* 2000;72(3):723-30.

[123] Charlot, K, Pichon A, Chapelot D. Prior acute exercise and hypoxia alter postprandial glucose metabolism. *Obesity Facts* 2009;2(suppl 2):177-8.

[124] Bielinski, R, Schutz Y, Jequier E. Energy metabolism during the postexercise recovery in man. *Am J Clin Nutr* 1985;42(1):69-82.

[125] Pivik, RT, Dykman RA. Cardiovascular effects of morning nutrition in preadolescents. *Physiol Behav* 2004;82(2-3):295-302.

[126] Pivik, RT, Dykman RA, Tennal K, Gu Y. Skipping breakfast: gender effects on resting heart rate measures in preadolescents. *Physiol Behav* 2006;89(2):270-80.

[127] Ambarish, V, Barde P, Vyas A, Deepak KK. Comparison between pre-prandial and post-prandial heart rate variability (HRV). *Indian J Physiol Pharmacol* 2005;49(4):436-42.

[128] Chapelot, D, Marmonier C, Aubert R, Gausseres N, Louis-Sylvestre J. A role for glucose and insulin preprandial profiles to differentiate meals and snacks. *Physiol Behav* 2004;80(5):721-31.

[129] Louis-Sylvestre, J, Le Magnen J. Fall in blood glucose level precedes meal onset in free-feeding rats. *Neurosci Biobehav Rev* 1980;4 Suppl 1:13-5.

[130] Melanson, KJ, Westerterp-Plantenga MS, Saris WH, Smith FJ, Campfield LA. Blood glucose patterns and appetite in time-blinded humans: carbohydrate versus fat. *Am J Physiol* 1999;277(2 Pt 2):R337-45.

[131] Pittas, AG, Hariharan R, Stark PC, Hajduk CL, Greenberg AS, Roberts SB. Interstitial glucose level is a significant predictor of energy intake in free-living women with healthy body weight. *J Nutr* 2005;135(5):1070-4.

[132] Levin, BE. Metabolic sensing neurons and the control of energy homeostasis. *Physiol Behav* 2006;89(4):486-9.

[133] Pavlov, I. The work of the digestive glands. London: Charles Griffin; 1902.

[134] Mattes, RD. Physiologic responses to sensory stimulation by food: nutritional implications. *J Am Diet Assoc* 1997;97(4):406-13.

[135] Goldfine, ID, Abraira C, Gruenewald D, Goldstein MS. Plasma insulin levels during imaginary food ingestion under hypnosis. *Proc Soc Exp Biol Med* 1970;133(1):274-6.

[136] Mattes, RD. Oral fat exposure alters postprandial lipid metabolism in humans. *Am J Clin Nutr* 1996;63(6):911-7.

[137] Secchi, A, Caldara R, Caumo A, Monti LD, Bonfatti D, Di Carlo V, et al. Cephalic-phase insulin and glucagon release in normal subjects and in patients receiving pancreas transplantation. *Metabolism* 1995;44(9):1153-8.

[138] Arosio, M, Ronchi CL, Beck-Peccoz P, Gebbia C, Giavoli C, Cappiello V, et al. Effects of modified sham feeding on ghrelin levels in healthy human subjects. *J Clin Endocrinol Metab* 2004;89(10):5101-4.

[139] Schwartz, TW. Pancreatic polypeptide: a unique model for vagal control of endocrine systems. *J Auton Nerv Syst* 1983;9(1):99-111.

[140] Teff, KL, Townsend RR. Early phase insulin infusion and muscarinic blockade in obese and lean subjects. *Am J Physiol* 1999;277(1 Pt 2):R198-208.

[141] Ahren, B, Holst JJ. The cephalic insulin response to meal ingestion in humans is dependent on both cholinergic and noncholinergic mechanisms and is important for postprandial glycemia. *Diabetes* 2001;50(5):1030-8.

[142] Teff, KL, Engelman K. Oral sensory stimulation improves glucose tolerance in humans: effects on insulin, C-peptide, and glucagon. *Am J Physiol* 1996;270(6 Pt 2):R1371-9.

[143] Chapelot, D, Louis-Sylvestre J. The role of orosensory factors in eating behavior as observed in humans. In: Harris RBS, Mattes RD, editors. *Appetite and food intake: Behavioral and physiological considerations*. New York: CRC Press; 2008. p. 133-61.

[144] Nederkoorn, C, Smulders FT, Jansen A. Cephalic phase responses, craving and food intake in normal subjects. *Appetite* 2000;35(1):45-55.

[145] Friedman, MI, Tordoff MG. Fatty acid oxidation and glucose utilization interact to control food intake in rats. *Am J Physiol* 1986;251(5 Pt 2):R840-5.

[146] Langhans, W, Scharrer E. Role of fatty acid oxidation in control of meal pattern. *Behav Neural Biol* 1987;47(1):7-16.

[147] Ji, H, Friedman MI. Reduced capacity for fatty acid oxidation in rats with inherited susceptibility to diet-induced obesity. *Metabolism* 2007;56(8):1124-30.

[148] Zurlo, F, Lillioja S, Esposito-Del Puente A, Nyomba BL, Raz I, Saad MF, et al. Low ratio of fat to carbohydrate oxidation as predictor of weight gain: study of 24-h RQ. *Am J Physiol* 1990;259(5 Pt 1):E650-7.

[149] Langhans, W, Scharrer E. Evidence for a vagally mediated satiety signal derived from hepatic fatty acid oxidation. *J Auton Nerv Syst* 1987;18(1):13-8.

[150] Brandt, K, Arnold M, Geary N, Langhans W, Leonhardt M. Vagal afferents mediate the feeding response to mercaptoacetate but not to the beta (3) adrenergic receptor agonist CL 316,243. *Neurosci Lett* 2007;411(2):104-7.

[151] Campfield, LA, Smith FJ, LeMagnen J. Altered endocrine pancreatic function following vagotomy: possible behavioral and metabolic bases for assessing completeness of vagotomy. *J Auton Nerv Syst* 1983;9(1):283-300.

[152] Sclafani, A, Kramer TH. Aversive effects of vagotomy in the rat: a conditioned taste aversion analysis. *Physiol Behav* 1985;34(5):721-5.

[153] Sclafani, A, Aravich PF, Landman M. Vagotomy blocks hypothalamic hyperphagia in rats on a chow diet and sucrose solution, but not on a palatable mixed diet. *J Comp Physiol Psychol* 1981;95(5):720-34.

[154] Ionescu, E, Rohner-Jeanrenaud F, Berthoud HR, Jeanrenaud B. Increases in plasma insulin levels in response to electrical stimulation of the dorsal motor nucleus of the vagus nerve. *Endocrinology* 1983;112(3):904-10.

[155] Ferrari, B, Arnold M, Carr RD, Langhans W, Pacini G, Bodvarsdottir TB, et al. Subdiaphragmatic vagal deafferentation affects body weight gain and glucose metabolism in obese male Zucker (fa/fa) rats. *Am J Physiol Regul Integr Comp Physiol* 2005;289(4):R1027-34.

[156] Arnold, M, Mura A, Langhans W, Geary N. Gut vagal afferents are not necessary for the eating-stimulatory effect of intraperitoneally injected ghrelin in the rat. *J Neurosci* 2006;26(43):11052-60.

[157] Chan, JL, Mietus JE, Raciti PM, Goldberger AL, Mantzoros CS. Short-term fasting-induced autonomic activation and changes in catecholamine levels are not mediated by changes in leptin levels in healthy humans. *Clin Endocrinol (Oxf)* 2007;66(1):49-57.

[158] Leonhardt, M, Hrupka BJ, Langhans W. Subdiaphragmatic vagal deafferentation fails to block the anorectic effect of hydroxycitrate. *Physiol Behav* 2004;82(2-3):263-8.

[159] Moran, TH, Baldessarini AR, Salorio CF, Lowery T, Schwartz GJ. Vagal afferent and efferent contributions to the inhibition of food intake by cholecystokinin. *Am J Physiol* 1997;272(4 Pt 2):R1245-51.

[160] Wang, PY, Caspi L, Lam CK, Chari M, Li X, Light PE, et al. Upper intestinal lipids trigger a gut-brain-liver axis to regulate glucose production. *Nature* 2008;452(7190):1012-6.

[161] Ogawa, N, Yamaguchi H, Shimbara T, Toshinai K, Kakutani M, Yonemori F, et al. The vagal afferent pathway does not play a major role in the induction of satiety by intestinal fatty acid in rats. *Neurosci Lett* 2008;433(1):38-42.

[162] Schwartz, GJ, Salorio CF, Skoglund C, Moran TH. Gut vagal afferent lesions increase meal size but do not block gastric preload-induced feeding suppression. *Am J Physiol* 1999;276(6 Pt 2):R1623-9.

[163] Friedman, MI. Obesity and the hepatic control of feeding behavior. *Drug News Perspect* 2007;20(9):573-8.

[164] Russek, M. Participation of hepatic glucoreceptors in the control of intake of food. *Nature* 1963;197:79-80.

[165] Niijima, A. Afferent impulse discharges from glucoreceptors in the liver of the guinea pig. *Ann N Y Acad Sci* 1969;157(2):690-700.

[166] Novin, D, VanderWeele DA, Rezek M. Infusion of 2-deoxy-D-glucose into the hepatic-portal system causes eating: evidence for peripheral glucoreceptors. *Science* 1973;181(102):858-60.

[167] Shimizu, N, Oomura Y, Novin D, Grijalva CV, Cooper PH. Functional correlations between lateral hypothalamic glucose-sensitive neurons and hepatic portal glucose-sensitive units in rat. *Brain Res* 1983;265(1):49-54.

[168] Adan, RA, Tiesjema B, Hillebrand JJ, la Fleur SE, Kas MJ, de Krom M. The MC4 receptor and control of appetite. *Br J Pharmacol* 2006;149(7):815-27.

[169] Petrovich, GD, Gallagher M. Control of food consumption by learned cues: a forebrain-hypothalamic network. *Physiol Behav* 2007;91(4):397-403.

[170] Kotz, CM. Integration of feeding and spontaneous physical activity: role for orexin. *Physiol Behav* 2006;88(3):294-301.

[171] Niijima, A. Glucose-sensitive afferent nerve fibres in the hepatic branch of the vagus nerve in the guinea-pig. *J Physiol* 1982;332:315-23.

[172] Lee, KC, Miller RE. The hepatic vagus nerve and the neural regulation of insulin secretion. *Endocrinology* 1985;117(1):307-14.

[173] Nagase, H, Inoue S, Tanaka K, Takamura Y, Niijima A. Hepatic glucose-sensitive unit regulation of glucose-induced insulin secretion in rats. *Physiol Behav* 1993;53(1):139-43.

[174] Cardin, S, Walmsley K, Neal DW, Williams PE, Cherrington AD. Involvement of the vagus nerves in the regulation of basal hepatic glucose production in conscious dogs. *Am J Physiol Endocrinol Metab* 2002;283(5):E958-64.

[175] Zhu, JN, Guo CL, Li HZ, Wang JJ. Dorsomedial hypothalamic nucleus neurons integrate important peripheral feeding-related signals in rats. *J Neurosci Res* 2007;85(14):3193-204.

[176] Obici, S, Feng Z, Arduini A, Conti R, Rossetti L. Inhibition of hypothalamic carnitine palmitoyltransferase-1 decreases food intake and glucose production. *Nat Med* 2003;9(6):756-61.

[177] Pocai, A, Lam TK, Gutierrez-Juarez R, Obici S, Schwartz GJ, Bryan J, et al. Hypothalamic K(ATP) channels control hepatic glucose production. *Nature* 2005;434(7036):1026-31.

[178] Pocai, A, Obici S, Schwartz GJ, Rossetti L. A brain-liver circuit regulates glucose homeostasis. *Cell Metab* 2005;1(1):53-61.

[179] Faxen, A, Rossander L, Kewenter J. The effect of parietal cell vagotomy and selective vagotomy with pyloroplasty on body weight and dietary habits. A prospective randomized study. *Scand J Gastroenterol* 1979;14(1):7-11.

[180] Kral, JG. Vagal mechanisms in appetite regulation. *Int J Obes* 1981;5(5):481-9.

[181] Grossman, BM, White BD, Edwards GL, Martin RJ. Vagotomy and mercaptoacetate influence the effect of dietary fat on macronutrient selection by rats. *J Nutr* 1994;124(6):804-9.

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Chapter 4

In memory of Professor Andrey V. Popov,
the outstanding neuroethologist

LIMK1: THE KEY ENZYME OF ACTIN REMODELING BRIDGES SPATIAL ORGANIZATION OF NUCLEUS AND NEURAL TRANSMISSION: FROM HETEROCHROMATIN VIA NON-CODING RN AS TO COMPLEX BEHAVIOR

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ABSTRACT

According to present knowledge, systemic realization of genetic activity in the dynamic spatial organization of the genome in the nucleus provides such a level of plasticity of complex biological systems that allows them to adequately respond to environmental stimuli or signals during the development, modulate and shift the balance of contacting chromatin components and dimensions of their interactions, resulting in structural rearrangements. The chromosome positions within the nucleus determine both normal development and progression of genomic diseases, i.e., changes according to the environmental requirements, current needs of the organism, and its individual experience. At the same time, the striking output of the evolution of higher organisms, largely ignored to date, is that only 1.2% of the mammalian genome encodes proteins and the vast majority of the expressed information is in RNA. There are hundreds of thousands of non-coding (nc) RNAs, as well as many other yet-to-be-discovered small regulatory RNAs. A new paradigm envisions the interactions between these two worlds, the one of

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protein and the other of RNA, as providing a dynamic link between the transcriptome and the environment and, therefore, the progressive maturation and functional plasticity of the nervous system in health and disease. Also, a wide repertoire of ncRNAs plays an important role in chromatin organization, gene expression, and disease etiology via a signal cascade of actin remodeling (LIMK1, cofilin, actin). The activity of the protein kinase LIMK1 that controls spine development, local dendritic translation at postsynaptic sites and ionotropic glutamate receptor trafficking is regulated by a brain-specific miRNA miR-134. This miRNA is localized to the synapto-dendritic compartment of rat hippocampal neurons and negatively regulates the size of dendritic spines - postsynaptic sites of excitatory synaptic transmission. Moreover, LIMK1 hemizygosity is considered to cause cognitive defects in a genome disorder Williams syndrome. *Drosophila* is a helpful model organism to determine the sequence of events in this system of hierarchical relationships. Drosophila LIMK1 gene (*agnostic*) with a specific chromosome architecture around the gene capable of generating miRNAs, recapitulates many features both of Williams syndrome and of neurodegenerative disorders. Mutants in the gene have increased expression of LIMK1 and cofilin, modified chromosome packaging and homologous and nonhomologous pairing, implemented in different rates of unequal recombination. Also, they display congofilic inclusions both in the adult brain and larval tissues presumably leading to severe defects in learning and memory during courtship conditioning

INTRODUCTION

Recent findings both in neurobiology and genetics promote an outbreak in our traditional notion of neural transmission. Nowadays in our understanding of synaptic plasticity, long term potentiation (LTP) and long term depression (LTD) presumed to comprise a fine cellular basis for learning and memory we have to address the whole spectrum of purely genetic topics of neuron–specific transcription, epigenetic chromatin remodeling, trafficking of mRNAs from soma to the remote sites of their local translation in axons and dendrites. Our pursuit of unraveling the etiology of neural diseases posed a problem of a multilevel organization of the genetic material in the nucleus of a nerve cell (van Driel et al., 2003). The first level is a linear arrangement of the sequence in the chromosome. The second is belonging of a sequence to a particular structural–functional chromosome block. The third is the spatial association of these blocks in the nucleus and their belonging to a particular nuclear domain. Therefore, the notion of gene activity *per se* is meaningless, since it is the result of a network of genetic and biological relationships. Consequently, the view on systemic realization of genetic activity, whose critical aspect is the spatial organization of the genome in the nucleus, became crucial. This dynamic nuclear medium emphasizes the significance of the role of self-organization in the formation of its structure, when the chromatin domains located far apart in the linear DNA, as well as chromosome arms, can have physical contacts or terminate them. This provides such level of plasticity of complex biological systems that allows them to adequately respond to environmental stimuli or signals during the development, modulate and shift the balance of contacting components and dimensions of their interactions, resulting in structural rearrangements. The chromosome positions within the nucleus determine both normal development and progression of genomic diseases (O'Brien et al., 2003), i.e., changes according to the environmental requirements, current needs of the organism, and its individual experience.

The role of the main factor of bridging all of the genomic levels is fulfilled by nuclear actin, which is capable to: (1) regulate transcription by activating all three classes of RNA polymerases; (2) participate in chromatin remodeling, interacting with numerous proteins; and (3) line the nuclear membrane, determining the sites of chromosome attachment and the formation of nuclear pores, regulating export from the nucleus (Olave et al., 2002; Pederson and Aebi, 2005; Sjolinder et al., 2005; Grummt, 2006; Percipalle and Visa, 2006; Chen and Shen, 2007; Percipalle et al., 2003; 2009). Actin is not only a major cytoskeletal component in all eukaryotic cells but also a nuclear protein that accompanies the mRNA through the entire RNA biogenesis pathway, from gene to polysomes. To this point, Percipalle (2009) rises three important questions: 1) if actin is associated with all eukaryotic RNA polymerases, actin is also likely to be located at gene promoter; 2) how does actin mediate polymerase assembly at the promoter and 3) is this function independent from the observation that actin is also in chromatin remodeling complexes? Indeed, chromatin immunoprecipitation experiments confirmed the presence of actin at rDNA promoter, promoters of inducible and constitutively expressed RNA polymerase II genes, as well as its association with the promoter of the RNA polymerase III U6 snRNA gene and also demonstrated that actin is present at the coding region of constitutively active genes, coupled to elongating RNA polymerases I and II (Percipalle, 2009 and ref therein). These findings put forward an intriguing possibility that actin performs a chaperone function in the molecular interplay between RNA polymerase and the machines involved in chromatin reorganization at the gene promoter to facilitate the establishment of transcription-competent RNA polymerases (Louvet and Percipalle, 2009). This is in accord with ideas in a new and rapidly evolving field - an assembly of a neuron-specific chromatin remodeling complexes (Olave et al., 2002; Aigner et al., 2007; Schleicher and Jockusch, 2008; de la Torre-Ubieta and Bonni, 2008) which appeared to be linked to the role of epigenetic promoter remodeling of actin cytoskeleton proteins like Reelin and GABAergic promoter hypermethylation in schizophrenia (Niu et al., 2008; Gregyrio et al., 2009; Costa et al., 2009).

The next important step in RNA biogenesis is mRNA transport and its localization in a certain cell compartment for a proper function. For this, immediately upon transcription, pre-messenger RNA molecules become associated with hnRNPs to form RNP complexes. hnRNPs influence RNA stability, cytoplasmic localization and mRNA translation. As shown in Diptera *C.tentans*, actin is incorporated in nascent pre-mRNPs, is associated with hnRNP proteins (Percipalle et al., 2001; 2009). The *D. melanogaster* hnRNP A1-like Squid protein (hrp40) governs the specific localization of the grk mRNA to the dorsoanterior corner of the oocyte during mid-oogenesis (Neuman-Silberberg et al., 1993; Matunis et al., 1994). These early findings have paved a road for a newly-emerged field of local translation in axons and dendrites (Lin and Holt, 2007) which, according to present notion, is regulated by ever growing number of noncoding (nc) RNAs (Mattick, 2007; Savvateeva-Popova et al., 2008).

Since both the neuron-specific chromatin remodeling and local translation in neurons shed a new light on the mechanisms of action of actin in modern studies on neural transmission, let's first introduce the main players in actin remodeling and second – follow up the *Drosophila* model which enables a journey along the way from a gene in the cascade of actin remodeling to complex behavior.

THE MAIN PLAYERS IN THE SIGNAL CASCADE OF ACTIN REMODELING

The signal cascade of actin remodeling: receptors of neurotransmitters – small Rho GTPases (RhoA, Cdc42 and Rac1) – LIM kinase 1 (LIMK1) – cofilin – actin is believed to play the main role in dendrite- and synaptogenesis. LIMK1 being the key enzyme of actin remodeling (da Silva and Dotti, 2002; Miller and Kaplan, 2003), phosphorylates cofilin on a conserved serine residue, Ser3. Thereby LIMK1 inactivates ADF/cofilin, whereas phosphatases such as Slingshot (SSH) can activate ADF/ cofilin by dephosphorylating Ser3. LIMK1 contains two LIM domains, a PDZ domain, and a protein kinase domain, as well as a domain for binding to SRP-α_N (Signal Recognition Particle). Inactivation of the second LIM domain by site-specific mutagenesis or deletion of the PDZ domain increase the activity of LIMK1 in vivo (Birkenfeld et al., 2001). Since the role of the PDZ domains is to organize supramolecular complexes of signal transduction, they are crucial for functioning of many receptors, such as NMDA NR2/D, AMPA, GluR2, mGluR5, beta-AR, melatonin and for ion channels Shaker K^+, voltage-gated Na^+, N-type Ca^{2+} (te Velhuis and Bagowski, 2007). Cofilin, a ligand for both monomeric and polymeric actin, contains a nuclear location sequence, and in its dephosphorylated state can transport actin into the nucleus. When bound to actin polymers, it distorts their conformation such that these filaments do not bind the diagnostic stain for actin, RHODAMIN- phalloidin anymore. Among other nuclear proteins which form complexes with actin and interfere with the formation of conventional actin filaments is profilin which binds to monomeric actin. Nuclear profilin is apparently involved in the regulation of the level of nuclear actin, as profilin –actin complexes are recognized and exported from mammalian nuclei by a specific exportin, while actin free of profilin can apparently be exported by a different exportin, due to its nuclear export sequences. Thus, these proteins might be critical in creating forms specific for nuclear actin, as detected by specific antibodies (reviewed in Lee-Hoeflich et al., 2004; Schleicher and Jockusch, 2008). The “actin cascade” is particularly important in dendrites, since LIMK1 null mice display defects in the formation of actin-based dendritic spines in hippocampal neurons and decreased levels of cofilin phosphorylation (Meng et al., 2002). Moreover, the hemizigosity of LIMK1 contributes to Williams syndrome, a genome disorder characterized by a strong cognitive defect in visuo-spatial cognition (Donnai and Karmiloff-Smith, 2000). Another LIMK1-dependent signaling pathway is involved in synaptogenesis which is crucial for normal learning acquisition and memory formation. The tail region of BMPR-II (bone morphogenetic protein receptor II) was isolated during a search for LIMK1-interacting proteins and this finding highlighted the dual roles of the BMP signaling (Foletta et al., 2003). BMPs are involved in axon pathfinding, morphological differentiation of dendrites and many other cellular processes. Their signals are transduced by the kinase receptors BMPR-I and BMPR-II, leading to Smad transcription factor activation via BMPR-I. A second, parallel pathway, involves a two-step mechanism: binding of LIMK1 to BMPRII relieves an autoinhibitory effect of LIM and PDZ domains on the catalytic domain of LIMK1, thereafter BMP-dependent activation of Cdc42 results in phosphorylation of the activation loop Thr residue, thereby increasing LIMK1 catalytic activity (Lee-Hoeflich et al., 2004). In *Drosophila*, BMP-like molecules regulate neuromuscular synapse morphology and neuropeptide cell identity via the BMPRII-like receptor, *wishful thinking* (*Wit*), required for synapse stabilization (Marqués

et al., 2002; Eaton and Davis, 2005). Moreover, a BMP7 gradient elicits bidirectional turning responses from nerve growth cones by acting through LIMK1 and SSH phosphatase to regulate actin-depolymerizing factor (ADF)/cofilin-mediated actin dynamics (Wen et al., 2007). Interestigly, manipulation of LIMK1 activity failed to alter dendrite growth in Xenopus retinal ganglion cells, but was critical for axon extension (Hocking et al., 2009). This brings us to a problem of directional steering in axons and dendrites which appears to be closely intermingled with a new topic of local translation in these nerve cell extensions. But first we have to address more traditional problem of transcriptional regulation in soms of the nerve cells.

FROM CHROMATIN REMODELING TO NEUROGENESIS

The packaging of genomic DNA into chromatin is crucial step in the regulation of gene expression. Chromatin remodelling complexes which alter local chromatin structure operate as large, multiunit machines in mammals, insects, yeast and plants to reorganize the genetic material by unravelling nucleosomes and converting the genetic material into a form suitable for transcription. Though the details of organization of such machines are far from clear, it is already evident that actin and nuclear actin-related proteins (Arps) are involved in chromatin remodeling. Actin was identified in complex with specific subunits of most ATPases (Gangaraju and Bartholomew, 2007) together with four nuclear Arps (4, 5, 6 and 8) in all organisms, with the exception of yeast (Olave et al., 2002a; Percipalle and Visa 2006; Schleicher and Jockusch, 2008). Another fascinating finding was the identification of a polymorphic, neuron-specific chromatin remodeling complex (Olave et al., 2002b). This was done in assumption that "the expected characteristics of such a chromatin remodeling complex would be that it be expressed in all neuronal cell types, not be expressed outside the nervous system and that it be activated at or near the time of neuronal subtype differentiation" (Olave et al., 2002b). As shown, vertebrate neurons have a specialized chromatin remodeling complex, bBAF, specifically containing the actin-related protein, BAF53b, which is first expressed in postmitotic neurons at. BAF53b is combinatorially assembled into polymorphic complexes with ubiquitous subunits including the two ATPases BRG1 and BRM. Brahma-related gene-1 (BRG1), the central catalytic subunit of the SWI/SNF chromatin-modifying enzymatic complexes, shows neural-enriched expression (Seo et al., 2005), uses the energy derived from ATP-hydrolysis to disrupt the chromatin architecture of target promoters and is believed to be a major coregulator of transcription (Trotter and Archer, 2008). Pretty soon it has been shown, that actin-related proteins at chromatin level not only ubiquitously control of the cell cycle and developmental transitions (Meagher et al., 2007), but neural development itself is based on a switch in subunit composition of a chromatin remodeling complex (Lessard, 2007). For example, global chromatin changes accompany the transition from proliferating mammalian neural stem cells to committed neuronal lineages. While proliferating neural stem and progenitor cells express complexes in which BAF45a, a Krüppel/PHD domain protein and the actin-related protein BAF53a are quantitatively associated with the SWI2/SNF2-like ATPases, Brg and Brm, the neuronal differentiation requires the replacement of these subunits by the homologous BAF45b, BAF45c, and BAF53b. However, combinatorial assembly appears to be unique to vertebrates, because *Drosophila* and *C. elegans* have only one gene encoding each subunit including BAF53 and

there is yet no evidence of combinatorial assembly of the *Drosophila* complex (Olave et al., 2002b). Nevertheless, this has brought to awareness, that combinatorial assembly of subunits in SWI/SNF-like complexes in vertebrates is necessary to achieve biological specificity in generation and refinement of dendrites during normal brain development and neural plasticity in response to neuronal activity (Wu et al., 2007; de la Torre-Ubieta and Bonni, 2008). For instance, a novel element which constitutes the SWI/SNF complex, is activity-dependent neuroprotective protein (ADNP), a heterochromatin 1-binding protein, and its complete deficiency leads to dramatic changes in gene expression, neural tube closure defects, and death at gestation day 9 in mice (Mandel and Gozes, 2007). Thus, the statements "From chromatin to dendrites" (de la Torre-Ubieta and Bonni, 2008), "A novel model for an older remodeler" (Aigner et al., 2007) and the question "How many remodelers does it take to make a brain?" (Brown et al., 2007) shed a new light on the old and purely genetic issue of chromatin organization. Moreover, epigenetic mechanisms have been implicated in different aspects of brain development, such as neuronal differentiation and plasticity (Hsieh and Gage, 2005).

REGULATION OF GENE ACTIVITY AT POSTTRANSLATION LEVEL

Protein synthesis underlying synaptic plasticity mediated by activity or experience and memory is controlled at the level of mRNA translation. About 1-4% of the neuron transcriptome is found in RNA granules and the characterization of bound mRNAs reveal that they encode proteins of the cytoskeleton, the translation machinery, vesicle trafficking, and/or proteins involved in synaptic plasticity. ncRNAs and microRNAs (miRNAs) are also found in dendrites and likely regulate RNA translation (Sánchez-Carbente-Mdel and Desgroseillers, 2008). Also, axons and their growth cones are specialized neuronal sub-compartments that possess translation machinery and have distinct mRNAs (Yoon et al., 2009). Therefore, the axonal pathfinding and activity-dependent synaptic plasticity utilize the similar mechanisms of regulating local translation.

Lin and Holt (2007) raise an interesting question - why regulate protein activity by translation rather than posttranslational modifications? This question is especially important because quite unexpectedly the topics of chromosome structure and of regulation of epigenetic processes by ncRNAs have appeared to be intimately related. Therefore, the authors give a number of very reasonable answers.

1) From a theoretical standpoint, cells have limited volume, and further crowding with macromolecules might slow diffusion or alter reaction rates unacceptably;
2) Since an mRNA can be a template for theoretically unlimited translation, it may be more efficient in the face of this biophysical limit to store mRNA rather than inactive proteins;
3) A constant turnover of proteins that tightly regulates the levels of specific proteins may occur in synaptic plasticity;
4) Regulation of proteins by mRNA translation rather than protein modification provides more flexibility, because the activity of a protein can be regulated by arbitrary mRNA sequences rather than constituent domains of the protein;
5) Proteins do not always contain the information necessary for their localization;

6) Axonal mRNA splicing might provide an additional layer of regulation for axonally translated proteins.

Different axonal guidance cues induce rapid translation of cytoskeletal proteins or regulators based on whether they are attractive or repulsive: proteins induced by attractive cues build up the cytoskeleton, whereas proteins induced by repulsive cues break it down. For example, an attractive gradient of netrin-1 or BDNF induces asymmetrical translation of β-actin in axonal growth cones within 5 min. Contrary to attractive cues, the repellent Slit2 induces a protein synthesis-dependent increase in growth cone cofilin within 5 min. Another repellent, Semaphorin3A (Sema3A), induces axonal synthesis of the small GTPase RhoA, which is required for Sema3A-induced growth cone collapse. RhoA mediates neurite retraction through regulation of the actin cytoskeleton (reviewd in Lin and Holt, 2007). Moreover, evidence from the *Drosophila* midline axon guidance system suggests that the F-actin-microtubule cross-linker Short stop (Shot) might link the translation machinery to the cytoskeleton in the growth cone (Van Horck and Holt, 2008). mRNA transport and translation are coupled and regulated by RNA-binding proteins, which transport mRNAs in "granules", large ribonucleoprotein (RNP) complexes that hold mRNAs repressed at the initiation or elongation stage (Bramham, 2008). Interestingly, RNA-binding protein Fragile X Mental Retardation Protein (FMRP) is reqired both for axonal growth cones (Antar et al., 2006) and for regulation of local translation in dendrites (Zalfa et al., 2006). Moreover, the 3'UTR of RhoA mRNA contains a possible binding site for FMRP (Wu et al., 2005). Therefore, Long-term potentiation and depression (LTP and LTD) might be considered analogous to attractive and repulsive turning (Lin and Holt, 2007).

What proteins ate locally translated? Using proteomics methodologies as a novel means to catalog axonally synthesized proteins from injury-conditioned adult rat dorsal root ganglion (DRG) neurons, it was possible to demonstrate that microtubule, intermediate filament and microfilament proteins, several heat shock proteins (HSPs) and heat shock-like proteins, endoplasmic reticulum (ER)-resident chaperone proteins, proteins linked to neurodegenerative disorders including those with proteolytic functions, and metabolic proteins were locally synthesized in axons (Willis et al., 2005). By RT-PCR was possible to detect peripherin, vimentin, and cofilin mRNAs in DRG axonal preparations. Also, the stress-response proteins B crystallin, HSP27, HSP60, HSP70, HSP90, grp75 and grp78/BiP are synthesized in the DRG axons. Among mRNAs whose translation modulates the ability of the dendrite to receive and integrate presynaptic information are those encoding CamKIIalpha, NMDA receptor subunits, and the postsynaptic density (PSD) scaffolding protein Homer2. Local translation of these previously dormant mRNAs may be inhibited until neurons are exposed to appropriate extracellular stimuli such as a neurotrophic factor (for example, brain-derived neurotrophic factor (BDNF) or neurotransmitter release at the synapse (Schratt et al, 2004). Three of the BDNF-regulated mRNAs discs large homologue 2 (DLG2), Neurod2, LIMK1were found to contain conserved 3'UTR sequence elements that were partially complementary to mouse miR-134. Presumably, the association of LIMK1 mRNA with miR-134 keeps the LIMK1 mRNA in a dormant state while it is being transported within dendrites to synaptic sites. In the absence of synaptic activity, miR-134 may recruit a silencing complex that has a key role in repressing LIMK1 mRNA translation. This then limits the synthesis of new LIMK1 protein and restricts the growth of dendritic spines. At the same time miR-134 was proposed to be not the single miRNA capable to regulate LIMK1 expression. (Schratt et

al, 2006). As to *Drosophila*, dme-miR-210 is predicted to bind with LIMK1 gene mRNA transcripts (miRBASE Targets database, Fig. 1).

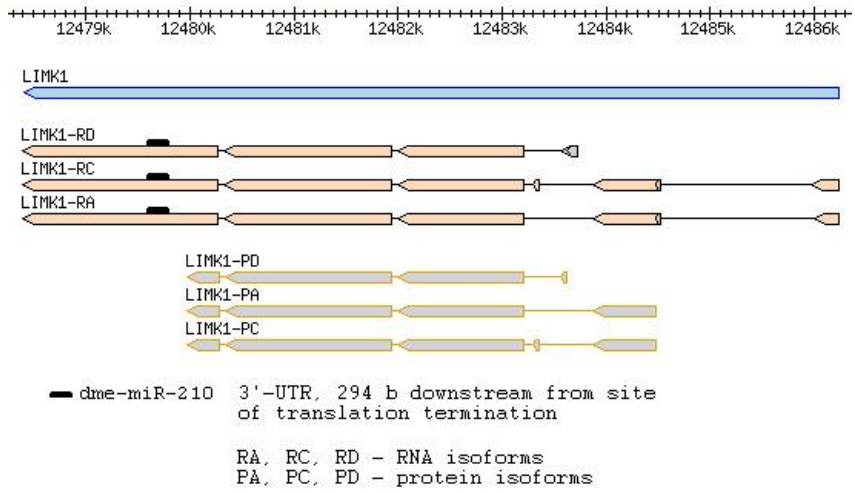


Figure 1. *Drosophila melanogaster* LIMK1 gene transcripts in complex with micro RNA dme-miR-210.

CHROMATIN STRUCTURE: STUDIES IN DROSOPHILA

What was long ago learned from studies on the *Drosophila* polytene chromosomes, became evident only after the completion of Genome projects in different species. *Drosophila melanogaster* offers a unique system in which one can study the whole scope of aformentioned phenomena. The design of recombination maps of the *Drosophila g*enes in its 4 chromosomes started as early, as 1913 (Surtevant, 1913). Due to the fact that the giant polytene chromosomes can be found in larval tissues, especially in salivary glands, a high proportion of the genes have also been located cytogenetically taking the advantage of numerous chromosome aberrations. In the detailed map of the polytene chromosomes established by Bridges (1935) each of approximately 5000 polytene bands is assigned a unique code, which identifies each band within one of 102 numbered chromosomal divisions, each with up to six lettered subdivisions. This has been one of the decisive starting steps to the *Drosophila* Genome project which finally helped to create detailed and highly correlated genetic, cytogenetic and molecular maps, down to the nucleotide level (Kafatos et al., 1991).

In general, *Drosophila* chromosomes are homologous associated in the early stages of development in addition to their association observed during meiosis. The giant polytene chromosomes exhibit a close synapsis of homologues along their entire lengths and a given locus occupies a discrete subregion within the nucleus (Marshall et al., 1996). Therefore, homologous pairing is believed to be crucial for the establishment of 3-D architecture of the nucleus. This organization stems from a combination of an overall centromere–telomere spatial organization (the classical “Rabl” configuration), as well as more specific patterning (Fung et al., 1998). What is important, this homologous chromosome pairing proceeds through multiple independent initiations. When the chromosome synapsis is distorted due to

either specific chromosome architecture or to the response to any type of stressful conditions, different chromosome aberrations, deletions, duplications and inversions arise in the *Drosophila* polytene chromosomes. When the results of the Human genomic projects have started to be thoroughly analyzed, this phenomenon known for a long time from the *Drosophil*a studies, appeared to be quite common to the human genome. It has turned out that chromosome-specific low-copy repeats, or duplicons, occur in multiple regions of the human genome. Homologous recombination between different duplicon copies leads to chromosomal rearrangements, such as deletions, duplications, inversions, and inverted duplications, depending on the orientation of the recombining duplicons (Purandare and Patel, 1997; Ji et al., 2000). When such rearrangements cause dosage imbalance of a developmentally important gene(s), this results in genetic diseases now termed genomic disorders which arise at a frequency exceeding this of single gene mutations (Lupski, 1998; 2009; Stankiewicz and Lupski, 2006). Among such syndromes with multiple manifestations are Prader-Willi syndrome at 15q11–q13, Di-George syndrome at 22q11, Charcot-Marie-Tooth syndrome type 1A(CMT1A) at 7p12, Smith-Magenis syndrome (SMS) at 17p11.2 which is a mental retardation/multiple congenital anomalies syndrome and Williams syndrome (WS) due to deletion at 7q11.23. WS, due to a contiguous 1.5 Mb gene deletion at 7q11.23, is associated with a distinctive facial appearance, cardiac abnormalities, infantile hypercalcemia, and growth and developmental retardation. More than 17 genes are uncovered by the deletion and two adjacent, one for elastin and the other for LIMK1 are believed to have a major impact on WS manifestations (Donnai and Karmiloff-Smith, 2000). Thus, elastin hemizygosity is associated with supravalvular aortic stenosis and other vascular stenoses and LIMK1 hemizygosity may contribute to the characteristic cognitive profile – defects in visual-spatial processing. The WS deletion is flanked by large repeats containing genes and pseudogenes. The deletions arise spontaneously by inter- or intrachromosomal crossover events within misaligned duplicated regions of high sequence identity that flank the typical deletion (Francke, 1999). Also, the WS region can generate duplications: the 7q11.23 duplication could be involved in complex clinical phenotypes, ranging from developmental or language delay to mental retardation and autism (Depienne et al., 2007).

We have designed a model for the Williams syndrome, using spontaneous and mutant variants of the *Drosophila* locus *agnostic* containing the *CG1848*gene for the LIMK1 located on the X-chromosome in region 11 AB. Alleles of the *agnostic* locus differently determine (1) the structure of LIMK1 gene; (2) the chromosome architecture in the region of the locus location; (3) chromosome packaging; (4) features of homologous and nonhomologous pairing, implemented in different rates of unequal recombination; (5) activities of the components of cascade LIMK1 – cofilin – actin; (6) the appearance of cytoplasmic amyloid-like inclusions; and (7) the capability of learning acquisition and preserving memory (Medvedeva et al., 2008). Locus *agnostic* was found by screening for temperature-sensitive mutations induced by ethyl methane sulphonate (EMS) on the background of strain Canton-S (CS), which could impair the activity of enzymes for cAMP synthesis and degradation (Savvateeva et al., 1978). A mutant of this locus, agn^{ts3}, exhibits extremely high activity of Ca^{2+} /CaM phosphodiesterase, elevated ability of females for classical olfactory learning with negative reinforcement at 25°C and inability to learn at 29°C (Savvateeva and Kamyshev, 1981). Immunofluorescent staining of the adult brain sections with antibodies to LIMK1 reveals its predominant localization in the central complex and optic lobes in normal flies. The agn^{ts3} mutants demonstrate a drastic increase of anti-LIMK1 in all brain structures which

can be seen in normal flies only after their exposure at 29^0 C (Fig. 2). Similarly to WS, the hemizygosity for the gene leads to a loss of LIMK1 temperature dependency and its predominant localization in the visual system. PCR analysis of the LIMK1 gene detected polymorphism both in strains carrying mutations in the *agnostic* locus, and in the wild-type strains. However, the polymorphic sites are unevenly distributed over the gene, occurring more often at the 3' end of the region examined. As to agn^{ts3}, it shows a putative insertion of DNA fragment, possibly a transposon, within the LIMK1 sequence downstream to the last exon region (Fig. 3). The site of insertion is close to A/T rich hairpin, detected using Vector NTI 9.1 software (Invitrogene, USA) (Fig. 4, b). The insertion might also result from specific chromatin architecture in the region where approximately 6 kb from the end of LIMK1 gene starts a 20kb-long stretch of AT-rich repeats (Fig. 4, a). Therefore, it is not surprising, that standard genetic mapping procedures reveal 3-fold map expansion around the agn^{ts3} mutation (Savvateeva-Popova et al., 2002).

Using MicroInspector software we detect a great number of miRNAs capable of binding to *D. melanogaster* LIMK1 mRNA with different specificity and free binding energy. Some miRNAs also bind to the region downstream from the mRNA last exon which may possibly encode a part of LIMK1 or transposon RNA (Fig. 4, c). One of miRNAs dme-miR-9b binds 5bp upstream from the beginning of A/T-rich hairpin. Thus, insertion may cause an impairment of miRNA binding site leading to desregulation of LIMK1 gene expression.

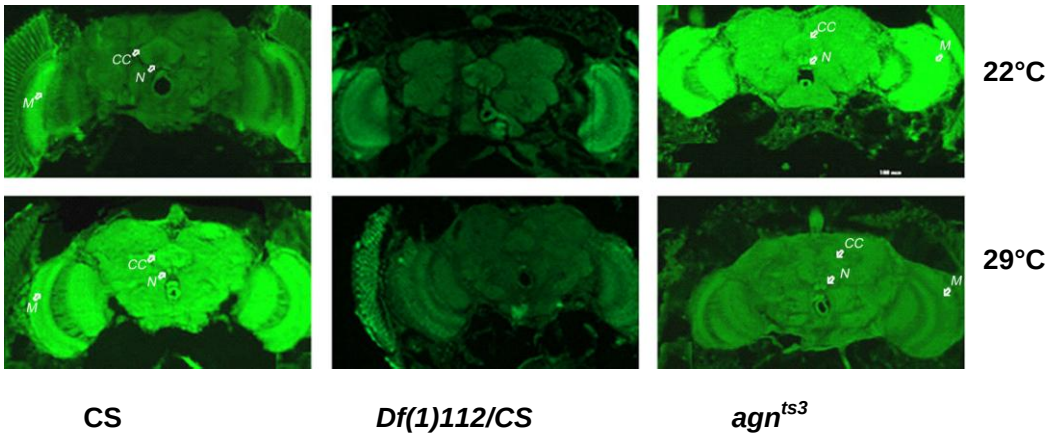


Figure 2. Expression of LIMK1 in the imaginal brain as revealed by immunofluorescence. Paraffin sections of the brain of adult homozygous females the wild-type Canton-S, agn^{ts3} and hemizygous *Df(1)112/CS* were stained with antibodies against LIMK1 (dilution 1:500) and secondary FITC-conjugated (dilution 1:400) antibodies. (goat LIMK1, donkey-anti-goat IgG-FITC, donkey serum, Santa Cruz). Flies were kept at a permissive temperature of 22°C or exposed at 29°C for 2 h. Brain structures: CC, central complex; N, noduli; M, medulla.

The aforementioned similarities with WS are in accord with the recent trend to identify *Drosophila* genes related to human disease genes in assumption that cross-genomic analysis of human disease genes is very promising since modern *Drosophila* databases combine the data of classic and molecular genetics (Reiter et al., 2001). Undoubtedly, such an approach refers only to considering the first level of organization of the genetic material, i.e. a linear arrangement of the sequence in the chromosome. However, the demonstration of high frequency of occurrence of genomic disorders exceeding that of single gene mutations

requires considering the second level – belonging of a certain gene sequence to a particular structural–functional chromosome block.

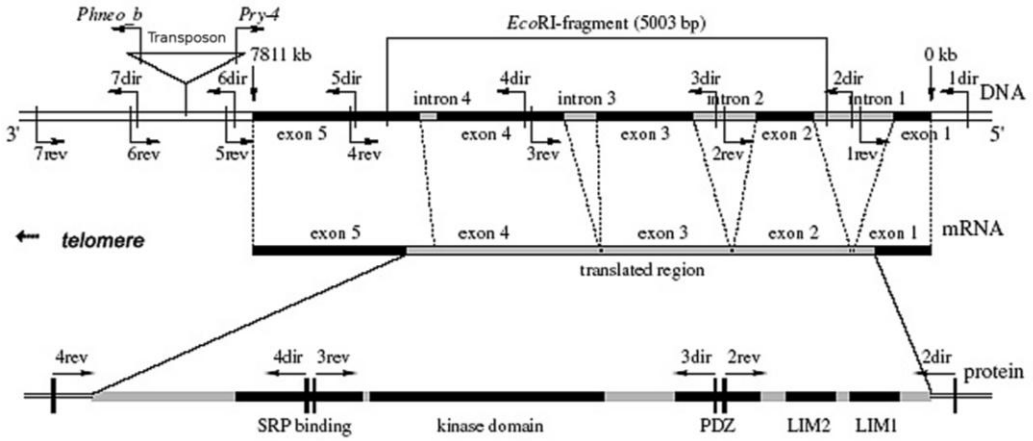


Figure 3. Insertion of genetic material in 3' UTR of LIMK1 gene *agn*^{ts3} sites of primer binding are indicated relative to DNA, RNA and protein sequences.

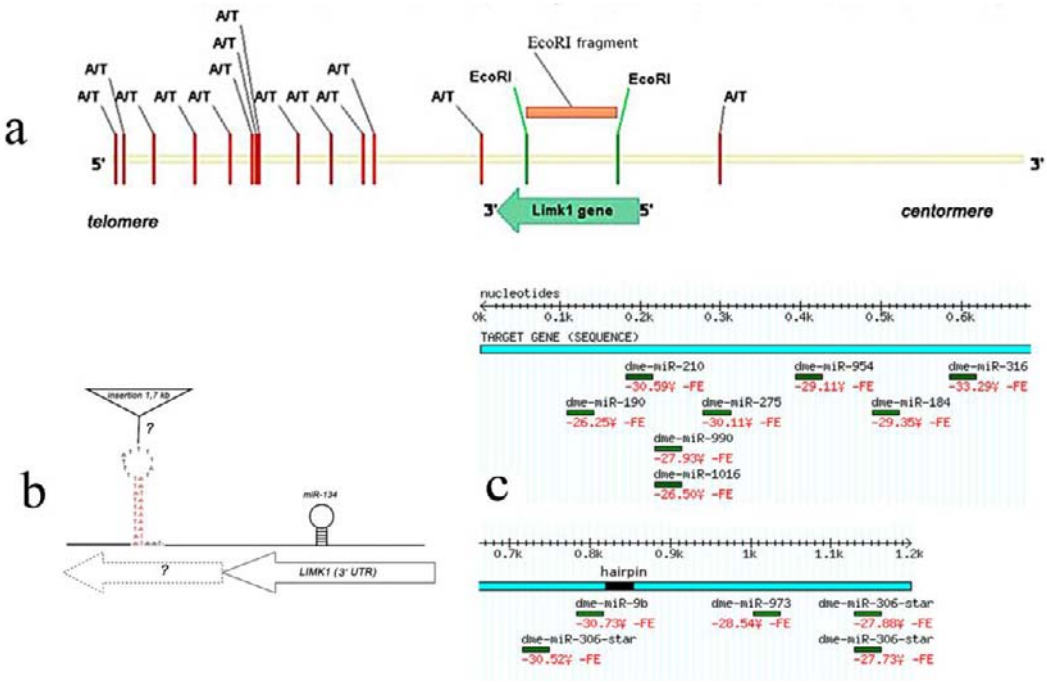


Figure 4. Architecture of the region in vicinity of *Drosophila* LIMK1 gene. (a): A/T-rich regions in vicinity of the *Drosophila* LIMK1 gene (The National Centre for Biotechnology Information, NCBI); (b): Possible changes near 3'UTR of the *Drosophila* LIMK1 gene; (c): MicroInspector prediction of miRNAs binding to LIMK1 gene sequence downstream from the polyadenylation site. Free energies of binding are shown in red.

The hairpin itself might generate miRNAs. Using miRBase software TargetscanFly we revealed a moderate homology between the hairpin sequence and precursors of two miRNAs dme-miR-973 and dme-miR-1014. dme-miR-973 binds to 25 conserved targets, with a total of 27 conserved sites and 11 poorly conserved sites. Among the functions of these target genes are: signal transduction, microtubule cytoskeleton organization, actin filament-based process. dme-miR-1014 has 388 conserved targets, with a total of 419 conserved sites and 102 poorly conserved sites. Among its well-known target genes are: *dCrebA*, *adenylyl cyclase 78C*, *dunce*, calmodulin-binding transcription activator *Camta*, *phosphodiesterase 1c*, *Su(var)3-9*. The functions of target genes are: calmodulin binding, GTP binding, small GTPase mediated signal transduction, Rho protein signal transduction and axon guidance. These genes also participate in epigenetic processes, chromatin architecture modulation, olfactory behavior, learning and memory. Though the hairpin sequence is too short to form the mature miRNAs, the transposon insertion may donate its sequence to produce a complete pre-miRNA hairpin structure.

SECOND LEVEL OF GENOME ORGANIZATION: STRUCTURAL ORGANIZATION OF A CHROMOSOME, EUCHROMATIC AND HETEROCHROMATIC REGIONS OF THE DROSOPHILA POLYTENE CHROMOSOMES

The specificity of genome architecture is providing substrates for homologous recombination between nonhomologous regions of chromosomes which harbor different types of repetitive sequences, segmental duplications with a certain level of homology (Prokofyeva-Belgovskaya and Khvosotova, 1939; Shaw and Lupski, 2004). Since in humans this can result in DNA rearrangements that cause disease, the problem of chromosome organization deserves a special attention. The *Drosophila melanogaster* genome consists of four chromosomes that contain 165 Mb of DNA, 120 Mb of which are euchromatic. It is possible to differentiate in the polytene chromosomes their euchromatin from heterochromatin: banding patterns, distribution of satellite DNAs and location of the rDNA. In polytene chromosomes, condensed (bands), decondensed (interbands), genetically active (puffs), and silent regions (pericentric and intercalary heterochromatin (IH) as well as areas subjected to position effect variegation (PEV) were found long ago and their features were described in detail (Bridges, 1935; Prokofyeva-Belgovskaya and Khvosotova, 1939; Prokofyeva-Belgovskaya, 1945, 1986; Khesin and Leibovitch, 1976; reviwed in Zhimulev et al., 2004). Long thought to be inert, heterochromatin is now recognized to give rise to small ncRNAs, which, by means of RNA interference, direct the modification of proteins and DNA in heterochromatic repeats and transposable elements (Lippman and Martienssen, 2004). Heterochromatin has thus emerged as a key factor in epigenetic regulation of gene expression, chromosome behavior and evolution.

IH consists of extended chromosomal domains which are interspersed throughout the euchromatin and contain silent genetic material. These domains comprise either clusters of functionally unrelated genes or tandem gene duplications and stretches of noncoding sequences. Also, IH harbors homeotic genes. Repeats of various kinds have been localized in the IH, such as transposable elements or the tRNA genes; and tandem repeats, such as histone or the ribosomal RNA genes, satellite DNA, and oligonucleotide tracts (Wimber and

Steffensen, 1973). Strong repression of genetic activity means that IH displays properties that are normally attributable to classic pericentric heterochromatin: high compaction, late replication and underreplication in polytene chromosomes, and the presence of heterochromatin-specific proteins (Zhimulev, 1998; Grewal et al., 2002; Huisinga et al, 2006; Belyaeva et al., 2008). Moreover, IH regions are often considered to be the so called weak spots (Zhimulev et al., 1982). Low temperature considerably promotes the expression of chromosome "fragility" in the weak spots. Decrease in heterochromatin amount (removal of the Y chromosome) in the nucleus produces a sharp increase in break frequencies. There is circumstantial evidence concerning the action of genetic modifiers of PEV. A comparison of strains contaning En-var(3)2 and Su-var(3)9 releaved higher break frequencies in larvae having an enhancer of PEV. This allows concluding that treatments increasing PEV also increase the heterochromatin fragility. Indeed, peaks of the highest frequencies of spontaneous and induced aberrations are observed mainly in the IH regions. For the X-chromosome these are regions 1F, 3C, 4E, 7B, 9A, 11A, 12D, 12E, 16F, 19E (Kaufmann, 1946; Ilyinskaya et al., 1988). Interestingly, some of these regions coincide with recombinational boundaries found in the X-chromosome: 3C4–6/7, 7A–7E, 11A, and a region proximal of 18C (Hawley, 1980). The most unusual is region 11A involved in Kosikov duplication, which is characterized by homology between 11A and 12D and between 11B and 12E (Kosikov, 1936). Region 11A is a hot spot of chromosome breaks, ectopic contacts, underreplication, and recombination, which take place on exposure to common chemical mutagen ethyl methanesulfonate (EMS) in particular (Xamena et al., 1985). Owing to these properties, the region may be used as a marker of intercalary heterochromatin or as a test system suitable for analyzing various cytological phenomena in *D. melanogaster* (Belyaeva et al., 1998). 11A is adjacent to the region that contains the *agnostic* locus harboring a gene for LIMK1. Note again that WS results from chromosome aberrations arising because of the specific chromosome architecture; namely, the relevant region contains numerous complex duplicons, which allow unequal meiotic crossing over. Likewise, many repeats are characteristic of region 11A–11B9 of the *D. melanogaster* X-chromosome on evidence of Southern hybridization analysis of P1 phages containing *D. melanogaster* genome material (Savvateeva-Popova et al., 2002; 2004).

GENE SILENCING

Soon after its discovery 75 years ago, heterochromatin was found to silence genes. Zhimulev and Belyaeva (2003) envision IH regions as comprising stable inactivated genes, whose silencing is developmentally programmed. Moreover, post-translational modification of histones and the specific nonhistone protein complexes participate in the establishment and maintenance of silencing for all heterochromatin types (Grewal and Rice, 2004). Studies in the fission yeast have begun to highlight the genetic pathways critical for the assembly and epigenetic maintenance of heterochromatin, including key roles played by the RNAi machinery, H3 lysine 9 methylation and heterochromatin protein 1 (HP1) (Horn and Peterson, 2006). Although it is known that chromatin architecture is altered by methylation of the DNA and by various types of modifications to histones (the so-called 'histone code'), including compound patterns of methylation, acetylation, phosphorylation, ubiquitinylation,

sumoylation, ADP-ribosylation, carbonylation, deimination and proline isomerization at various residues, a rapidly emerging notion that "epigenetic memory" is mainly based on the fuction of different types of ncRNAs, such as miRNA, siRNA, piRNA (Moazed et al., 2006; Kutter and Svoboda, 2008; Scott and Li, 2008; Obbard et al., 2009). They are genome-encoded, endogenous negative regulators of translation and mRNA stability originating from long primary transcripts with local hairpin structures. RNAi is triggered by the processing of long double-stranded RNA (dsRNA) into small interfering RNAs (siRNAs), which mediate sequence-specific cleavage of nascent mRNAs. The third common class of repressive small RNAs, PIWI-associated RNAs (piRNAs), is produced in a Dicer-independent manner. Current data suggest that piRNAs protect the germline from mobile genome invaders such as transposons. A small RNA involved in RNA silencing associates with proteins in an effector ribonucleoprotein complex usually referred to as RNA-Induced Silencing Complex (RISC). Key components of RISC complexes are proteins of the Argonaute family, which determine RISC functions. Argonaute-2 (ago-2) is required for proper nuclear migration, pole cell formation, and cellularization during the early stages of embryonic development in *Drosophila* (Deshpande et al., 2005). Why this newly-emerging preference of ncRNAs-regulated silencing pathways is so promising? The most reasonable explanation is as follows (Mattick et al., 2009). There are only a limited number of enzymes (DNA methyltransferases, histone methyltransferases, acetylases, deacetylases etc.) and repressive and permissive (Polycomb-group and Trithorax-group) chromatin-modifying complexes involved, very few of which are known to have affinity for particular DNA sequences. Also, it is known that RNA is an integral component of chromatin and that many of the proteins involved in chromatin modifications, as well as transcription factors have the capacity to bind RNA or complexes containing RNA. These include DNA methyltransferases and methyl DNA binding domain proteins, heterochromatin protein 1 (HP1), the multi-KH domain protein DPP1 which suppresses heterochromatin-mediated silencing in *Drosophila*, and domains commonly found in chromatin remodelling enzymes and effector proteins such as SET domains, tudor domains and chromodomains. Moreover, signal-induced ncRNAs can act as selective ligands to modulate histone acetyltransferase activity at specific genomic positions (for references see Mattick et al., 2009). The nuclear organization of chromatin insulators and chromatin domains is also affected by the RNAi machinery and recent deep sequencing studies have shown that double-stranded RNAs formed by sense-antisense transcript pairs originating from inverted repeats, bidirectional/antisense transcripts from retrotransposons, pseudogenes and mRNAs in mouse oocytes and *Drosophila* somatic cells are processed into large numbers of small RNAs that may have regulatory functions in epigenetic pathway (Watanabe et al., 2008; Tam et al., 2008; Ghildiyal et al., 2008). However, though endogenous *Drosophila* siRNAs have not yet been identified, siRNAs can be derived from long hairpin RNA genes (hpRNAs). The *Drosophila* hpRNA pathway is a hybrid mechanism that combines canonical RNA interference factors (Dicer-2, Hen1 known as CG12367 and Argonaute 2) with a canonical miRNA factor (Loquacious) to generate approximately 21-nucleotide siRNAs. These novel regulatory RNAs reveal unexpected complexity in the sorting of small RNAs, and open a window onto the biological usage of endogenous RNA interference in *Drosophila* (Okamura et al., 2008). This is in accord with our finding of a hairpin structure which simultaneously serves as a site for integrating of transposons in vicinity of the *agnostic* gene (Medvedeva et al., 2008). It is possible that the impairment of the agn^{ts3} gene 3'UTR might affect miRNA – dependent post-translational regulation of the

agnostic gene due to mRNA-miRNA complementation defects. Normally, miRNA binding to mRNA 3'UTR prevents from translational termination leading to a transcript degradation (Mathieu and Bender, 2004). In the agn^{ts3} mutants this putative complementation defect might lead to an increase in number of LIMK1 transcripts which, in comparison to the wild type, would much more successfully pass through translation.

How these ncRNA regulatory pathways can control LIMK1 gene expression at the level of chromatin organization? As detected by immunofluorescence techniques in larval salivary glands the extremely high activity of LIMK1 and p-cofilin in agn^{ts3}, normally exceeding those of the wild type, decrease only after HS. In wild type HS results in an increase of the LIMK1 and p-cofilin levels (Medvedeva et al., 2008). How does this potent change in the activity of both the LIMK1 gene product and p-cofilin affect homologous and nonhomologous chromosome pairing?

Table 1 presents the data on revealing a sensitive period of forming non-homolog contacts. In the wild type these are the first 4.5 hrs after egg laying when temperature treatment modifys frequency of ectopic contacts (FEC). Following fertilization, the *Drosophila* embryo progresses through 13 synchronous mitotic cycles to create a syncitial blastoderm with thousands of nuclei arrayed just below the surface of the outer membrane (Foe et al. 1993). These mitotic divisions are initially just a few minutes long and then slow down during cycles 11–13 before finally pausing for at least 60 min during interphase 14, at which time the syncitial blastoderm cellularizes (Foe et al., 1993). The last few syncitial divisions are of particular interest, since the homolog pairing is first observed during this time, progressing to appreciable but locus-specific levels of pairing during the long interphase of cycle 14 (Hiraoka et al., 1993; Fung et al., 1998; Gemkowet al., 1998).

Interestingly, the onset of homolog pairing during the late syncitial mitotic cycles coincides with the critical period of embryogenesis when many aspects of the developmental program switch from maternal to zygotic control, known as the maternal-to zygotic transition (MZT, Bateman and Wu, 2008). At the moment of beginning of the zygotic gene transcription occurs the formation of the heterochromatic regions which might be influenced by temperature treatments (Lippman and Martienssen, 2004; 2006). This is in accord with our data that temperature treatment administered during second half of embryogenesis (Table 1) does not affect FEC.

Table 1. Frequency of ectopic contacts between regions of intercalary heterochromatin in the 2L arm of the polytene chromosome 2 in wild type Canton-S (CS) and agn^{ts3} following low and high temperature treatments.

	CS	***agn^{ts3}***
intact	***0,48±0,033***	***0,71±0,022***
15^0 C, 24 hr of embryonic development	***0,702±0,042****	***0,78±0,049***
29^0 C, 24 hr of embryonic development	***0,67±0,035****	***0,62±0,027****
37^0 C, first 2,5 hrs of embryonic development	***0,72±0,044****	***0,74±0,064***
37^0 C, 2,5 hrs beginning from 8th hr of embryonic development	***0,55±0,033***	***0,65±0,08***
15^0 C, 24 hr I instar larva	***0,48±0,024***	***0,65±0,018****
15^0 C, 24 hr II instar larva	***0,66±0,036****	***0,58±0,028****

* P < 0,05 differences from the intact animals from each strain, Student's t criterion

Interestingly, *agn*^{ts3} mutants show a maternal effect on FEC in 2L (Fig. 5). Presumably, mRNAs for LIMK1 are maternally transmitted to the zygote. Therefore, the absence of temperature response results from prolonged survival of the mutant mRNA, since the degradation of maternal mRNAs is directed by miRNAs (Schier, 2007). This might be the aforementioned consequence of altered regulatory interaction between miRNAs and LIMK1 mRNA in *agn*^{ts3}.

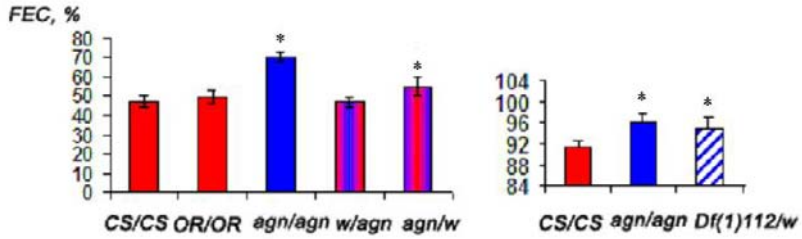


Figure 5. Frequency of ectopic contacts in 2L-arm.

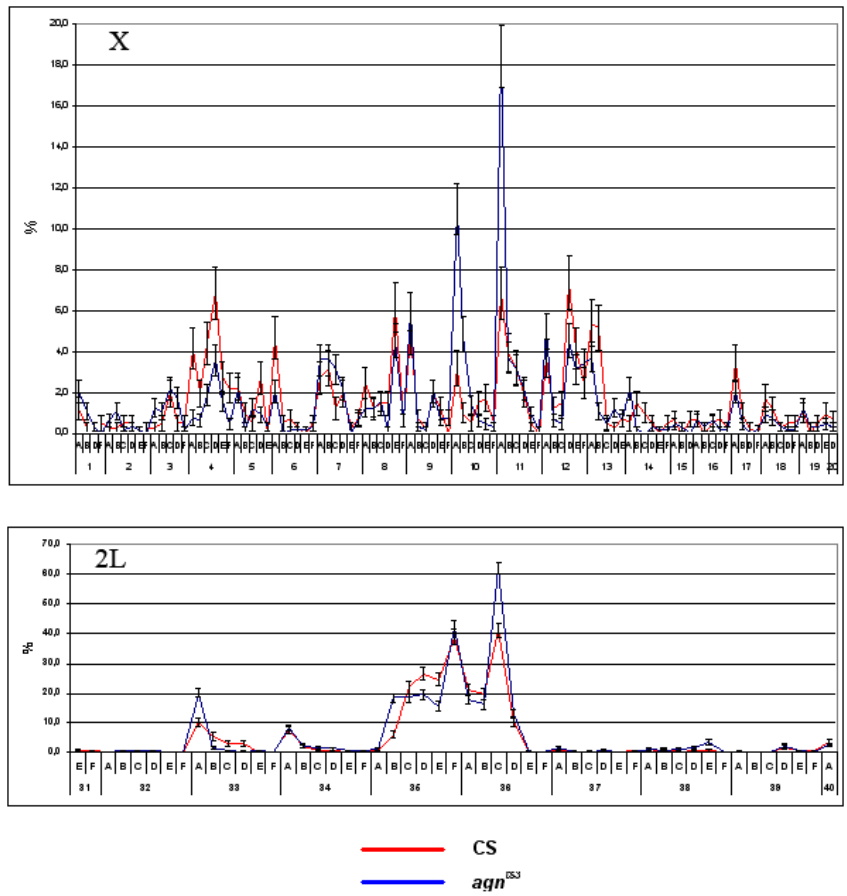


Figure 6. Diagrams of the frequency of ectopic contacts.

Our results on forming of non-homolog contacts at the blastoderm stage are in accord with findings that temperature treatments during first 3 hrs of embryogenesis enhance PEV-dependent silencing (Vlassova et al., 1991). The coincidence of the onset of these two phenomena points to both their interrelations and to an increase of heterochromatic silencing in response to temperature treatment. Therefore, we can conclude that:

1) similarly to PEV, the frequency of ectopic contacts can indicate the rate of gene silencing
2) the *agnostic* gene is involved into repressive mechanisms leading to gene silencing

The distribution of FECs (Fig 6) shows that certain IH blocks demonstrate the most sharp differences in agn^{ts3} mutants. The insertion in AT-rich region adjacent to LIMK1 gene might be the source of small ncRNAs which can stimulate RNA-directed methylation of heterochromatic regions known to contain sequences complementary to those of anti-viral defense (Mathieu and Bender, 2004). The increase in the repressive properties of these chromatin regions might lead to an increase in FEC.

THIRD LEVEL O GENOME ORGANIZATION: SPATIAL ORGANIZATION OF CHROMOSOMES IN THE NUCLEUS

The long-lasting awareness derived from findings on fixed preparations is that chromosomes in the nucleus are motionless, finally begun to expire. First, functional studies of chromosome behavior suggested that many essential processes, such as recombination, require interphase chromosomes to move around within the nucleus (Belmont et al., 1997; Vasquez et al., 2001; Marshall, 2002). Since chromosomes occupy discrete territories within the nucleus, individual loci have to undergo limited movement to reach a suitable environment for gene regulation (Heard and Bickmore 2007). Further studies from diverse organisms have shown that distinct interchromosomal interactions are associated with many developmental events, since stable interchromosomal contacts must be formed between maternal and paternal homologous chromosomes (Bateman and Wu, 2008). Although communication between chromosomes was first postulated long ago in *Drosophila* and other dipteran insects (Stevens, 1908; Lewis, 1954), the importance of the three-dimensional organization of the genome has started to draw attention only recently. Somatic pairing in *Drosophila*, especially in the giant polytene chromosomes, provides an excellent model for understanding interchromosomal interactions and their effect on gene expression. Importantly, somatic pairing or synapsis is initiated by multiple independent associations rather than by ‘‘zippering’’ the chromosomes from a discrete pairing initiation site (Fung et al., 1998). At present, at least two mechanisms that bring homologous sequences together within the nucleus are considered: those that act between dispersed homologous sequences and those that act to align and pair homologous chromosomes (Blumenstil et al., 2008). The most profound study of such mechanisms in so called non-homologous, ectopic pairing comes from the Zhimulev’s lab. Ectopic contacts may arise simultaneously in several regions of different chromosomes. Zhimulev and co-workers have classified the centers of ectopic pairing of polytene chromosomes in the early 1980s. According to our data, the ectopic

contact matrix constructed for the wild-type X-chromosome confirmed this early observation and in the case of the mutant X-chromosome, a dramatic increase was also established for FECs of regions 11A and 11B with regions 9A, 10A, 10B, 12A, 12D–F, 13AB, and 14A (Savvateeva-Popova et al., 2004). Interestingly, such X-chromosomal regions contain genes for components of signal transduction and ionic channels (Adams et al., 2000). Like LIMK1, many of these proteins have PDZ domains, which mediate multiple protein–protein interactions in supramolecular complexes involved in signal transduction. It is probably regions of ectopic chromosome pairing that harbor these genes and brings them close together to ensure their efficient and rapid transcription. For instance, region 10B7-8 contains the *Disc large* gene, whose product first allowed a discovery of the PDZ domain. Region 10B4-5 contains the *disheveled* gene, whose product is involved in the Wnt–frizzled signaling cascade. It is noteworthy that deletion causing WS involves several genes, including a homolog of the *D. melanogaster frizzled* gene. Such regions are scattered all over *Drosophila* chromosomes. For instance, Rossi et al., (2007) report that 70% of heterochromatic gene models of chromosome 2 encode putative proteins sharing significant similarity with human proteins, such as specific RNA pol II transcription factors, voltage-gated potassium channel, MAP-kinase and different protein kinases, serine/threonine phosphatase and proteins of RNA biogenesis.

Also, there is strong positive correlation between increased Suppressor of Underreplication (*SuUR*) gene expression extent, amount of DNA truncation, and formation of ectopic contacts in IH regions (Belyaeva et al., 2006). Only when induced during early stages of chromosome polytenization, *SuUR* overexpression results in the formation of partial chromosomal aberrations whose breakpoints map exclusively to the regions of intercalary and pericentric heterochromatin. IH underreplication in polytene chromosomes results in free double-stranded ends of DNA molecules; ligation of these free ends is the most likely mechanism for ectopic pairing between IH and pericentric heterochromatic regions.

A strong SUUR interactor is HP1, the well-studied heterochromatin protein, and the C-terminal part of HP1, which contains the hinge and chromoshadow domains interact with the central region of SUUR. In addition, recruitment of SUUR to ectopic HP1 sites on chromosomes provides evidence for their association in vivo (Pindyurin et al., 2008). Notably, the SuUR protein, which is bound in regions of ectopic pairing of IH, has the N-terminal region homologous to the N-terminal domain of the SW12/SNF2 family proteins (Ambach et al., 2000).

Since actin plays a dual role as a component of complexes of remodeling and pre-mRNA-binding proteins, any change in the actin dynamics, resulting from a mutational lesion of LIMK1 activity, should affect the properties of heterochromatin and the spatial features of the organization of the whole chromosome. This can be characterized by asynapsis of polytene chromosomes of salivary glands. Furthermore, physical interactions between homologous sequences have been either directly observed or implicated in many epigenetic phenomena, including transvection, paramutation in plants and in mice, repeat induced point mutation and methylation induced premeiotically, meiotic silencing of unpaired DNA, meiotic sex chromosome inactivation and X inactivation (reviewed in Bateman and Wu, 2008).

Moreover, homologous chromosome pairing is essential for creating prerequisites for unequal crossing over. The IH itself provides association with the nuclear membrane by filaments of ectopic contacts in sites referred to as terminal asynapsis points (Mathog et al., 1984). Consequently, a change in the distribution of asynaptic regions along the agn^{ts3}

chromosome may show change in nuclear localization of the corresponding chromosome region. As follows from (Fig. 7), strong interstrain differences in asynapsis characteristics were found between chromosomes of strains CS and *agn*^{ts3}.

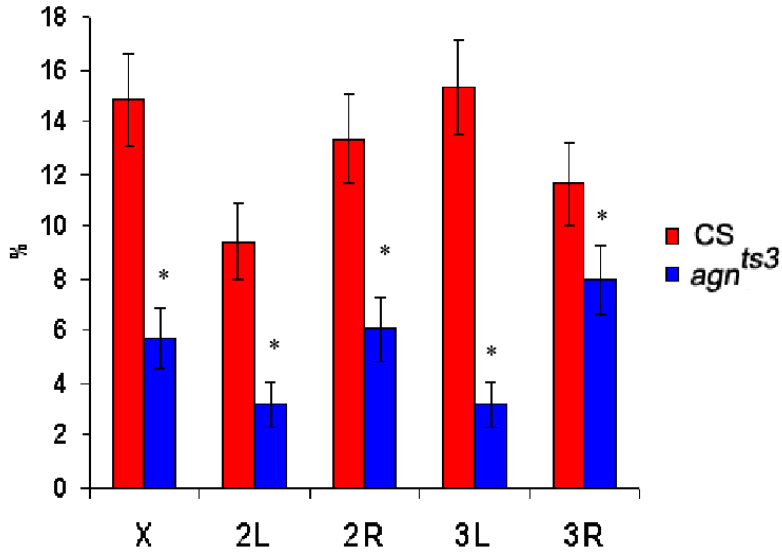
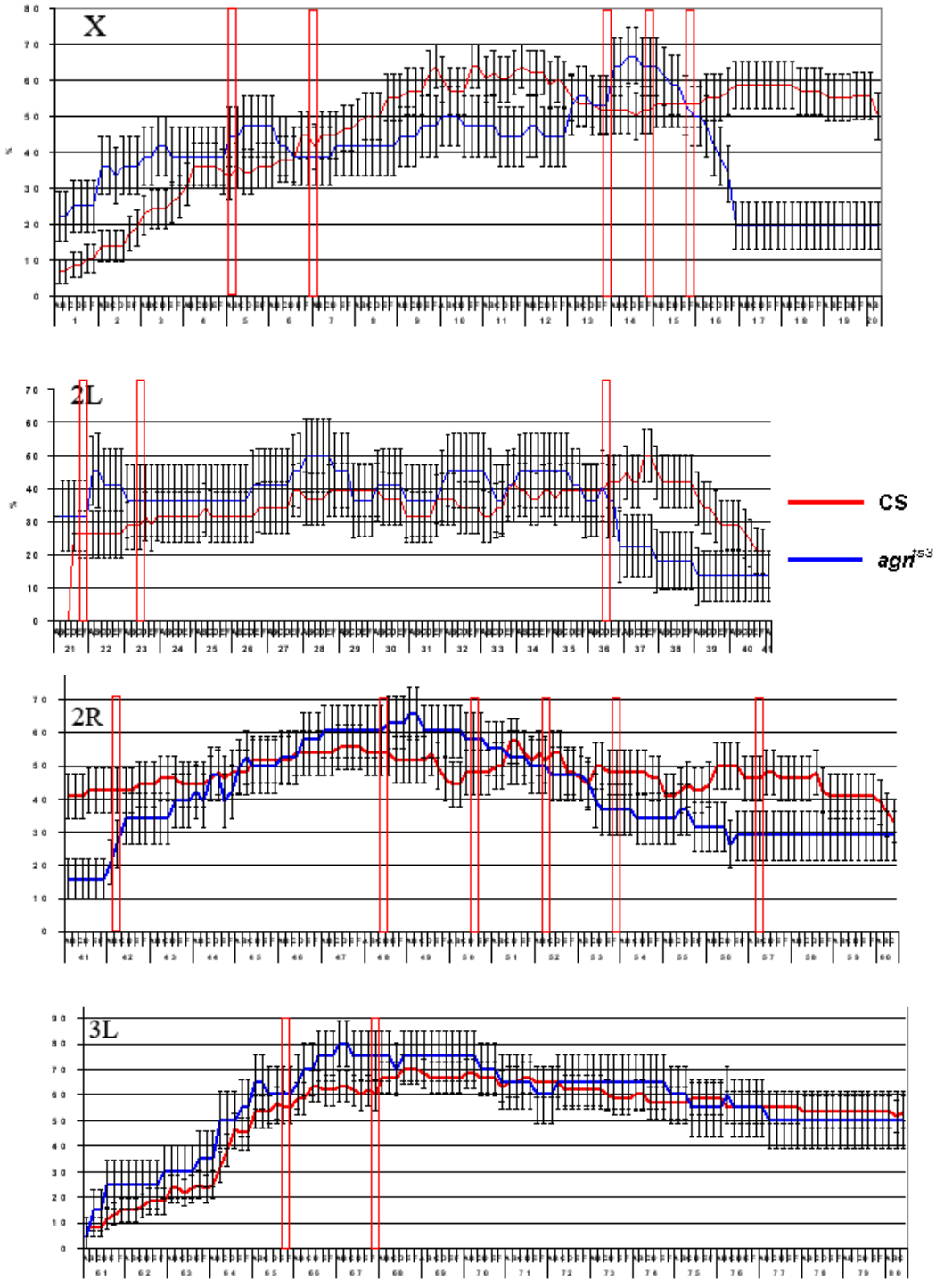


Figure 7. Interstrain differences in asynapsis frequencies in the *Drosophila* chromosomes.

The frequency of asynapsis occuring in *agn*^{ts3} mutants is nearly threefold lower than that in CS flies. As *agn*^{ts3} flies are characterized by higher frequency of ectopic contacts, this suggests association between the characters examined, the involvement of IH in homologous pairing, and participation of the *agnostic* gene in these processes. Our data on the distribution of the asynaptic regions along chromosome arms (Fig. 8) allow to suppose their localization within the nucleus. Assuming that some nuclear compartments favor homologos pairing while others do not, we can arbitrary consider the frequency of asynapsis occurrence equal to 40%±5 as high asynaptic level and assign «+». Consequently, the low frequency of assynapsis may be assigned as «-». Then, the frequencies of asynapsis in proximal regions of the polytene chromosome is as follows: in the X - CS«+», *agn*«-»; in 2R - CS«+», *agn*«-»; in 3L - CS«+», *agn*«+»; in 3R - CS«+», *agn*«-»; in 2L- CS«-», *agn*«-». Therefore, we can suggest that in wild type proximal regions of the X, 2R, 3R and 3L are localized in a compartment disfavoring homologous pairing. As for *agn*^{ts3} the proximal regions of the X, 2R and 3R occupy the same compartment, as 2L in CS.

The frequencies of asynapsis in distal regions might be the following: in 2R - CS«+», *agn*«+»; in X - CS«-», *agn*«-»; in 2L- CS«-», *agn*«+»; in 3L - CS«-», *agn*«-»; in 3R - CS«-», *agn*«-». In this case, only 2L-arm in *agn*^{ts3} shows different localization than in wild type. The possibility of occurrence of two alternative values of frequencies of asynapsis in neighboring regions of the same chromosome might indicate that a certain region can occupy different nuclear compartments.


X
2L
2R
3L
%
CS
agn^ts3

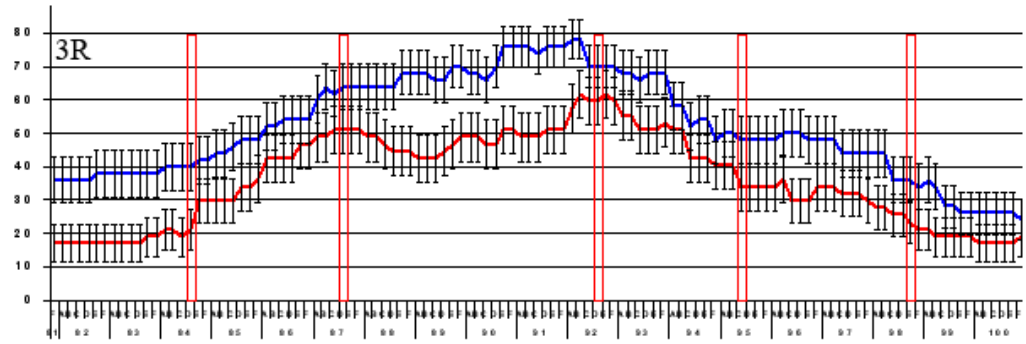


Figure 8. Asynaptic profile of *Drosophila* chromosomes. Red bars mark the boundaries of regions demonstrating alternative interstrain asynaptic values.

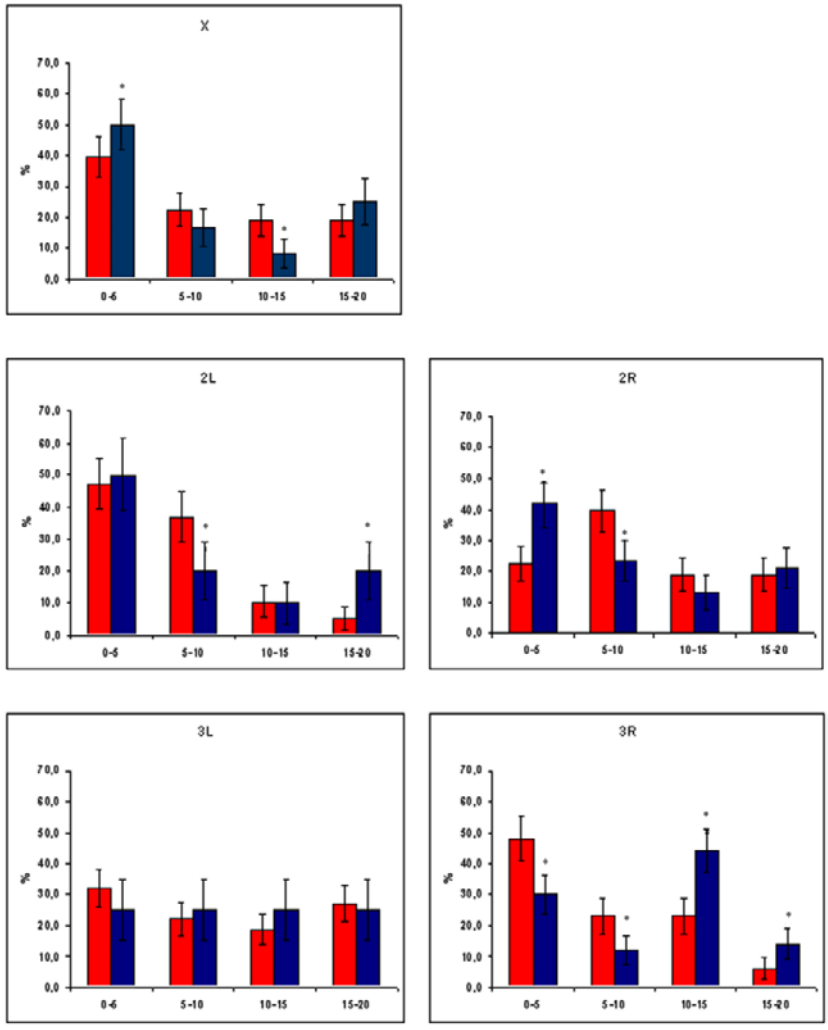


Figure 9. Interstrain differences in asynapsis lengths in *Drosophila* chromosomes. Ordinate – frequency of asynapsis, per cent. Abscissa – number of asynaptic sections. ■ – *CS*, ■ – agn^{ts3}

Several types of distribution of asynaptic regions along the chromosome can be distinguished (Fig 9). We classify these distributions into type 1 and type 2. Type 1 is characterized by prevalence of short asynapses with a gradual decrease in frequency of each subsequent gradation. This type includes the X-chromosome, 2L in CS and agn^{ts3}, as well as 2R in $agn^{ts3}$and 3R in CS. In type 2 distribution, the frequency of long asynapses is enhanced. The examples of this type are 2R in CS, 3L in CS and agn^{ts3}, and especially 3R in agn^{ts3}.

In view of the above, one can assume that modification of asynaptic state constitutes two mutually dependent actors: (1) if the distributions of long and short asynapses are similar, interstrain differences in asynapsis frequencies would depend on the nuclear compartment, into which falls the interstitial region flanked by terminal asynapsis points; the nucleus may have advantageous and disadvantageous conditions for homologous synapsis; (2) if the distributions of asynapsis lengths are different, the heterochromatin state and the degree of its attachment to the membrane and nuclear matrix are of importance. Our results demonstrate association between ectopic and homologous pairing. In $agn^{ts3}$with high FEC, far closer homologous synapsis is observed, which confirms the role of mutational LIMK1 impairment in the organization of genetic apparatus.

EPIGENETIC MECHANISMS IN MEMORY FORMATION

Long-term potentiation (LTP) of synaptic transmission is a primary experimental model of memory formation in neuronal circuits (Lynch, 2004; Raymond, 2008). Although the research on the molecular basis of memory is very intensive, it still focuses mainly on proteins despite of the fact that ncRNAs are predominantly enriched in the brain where they can direct epigenetic modifications (Mercer et al., 2008).

Models of synaptic plasticity comprise at least three sequential but mechanistically distinct components which involve different compartments of a nerve cell and different levels of regulation (Raymond 2007). The first or early phase, which lasts up to 3 hours and is manifested in synapse, is dependent on modifications of existing proteins. This early phase is thought to relate to the formation of short–term memories and is unaffected by protein-synthesis inhibitors (Lynch 2004). The intermediate phase, lasting 2 to 8 hours, is dependent on local translation in dendrites from pre-existing RNA, but independent of gene transcription. The final or late phase that produces a sustained response is dependent on gene transcription in soma of the nerve cell in addition to protein synthesis. These three phases have been identified in both vertebrates and invertebrates and are believed to represent a general feature of synaptic plasticity.

Epigenetic changes including chromatin modifications and DNA methylation play important roles in regulating networks of gene expression underlying memory formation and maintenance (Levenson and Sweatt, 2006). Histones associated with genes involved in synaptic plasticity are dynamically acetylated in response to L-LTP induction and memory formation is also blocked by the inhibition of DNA methyltransferase. These observations show that epigenetic changes are integral to memory formation and indeed the processes of acetylation and methylation seem to function in a combined and coordinated manner. Chromatin modifications are additionally coordinated with the transcriptional cascades induced by changes in synaptic plasticity. For example, the CREB binding protein (CBP) is a

transcriptional co-activator that may also act as a histone acetyltransferase, providing a direct link between chromatin modification and CREB-dependent pathways. ncRNAs may provide an additional link between transcriptional networks such as CREB-dependent pathways and epigenetic modifications.

We tested the learning acquisition and memory formation in 5 day-old wild type and *agn*^{ts3} males in conditioned courtship suppression paradigm (Kamyshev et al., 1999). Earlier, we have developed a design of heat shock treatment of adult flies and during their development to affect them in the period of the formation of brain structures responsible for learning (HS1, the late embryonic–early larval stage, the time of formation of mushroom bodies) and the memory formation (HS2, prepupal stage, the formation of the central complex), as well as in adults (HS) (Nikitina et al., 2003).

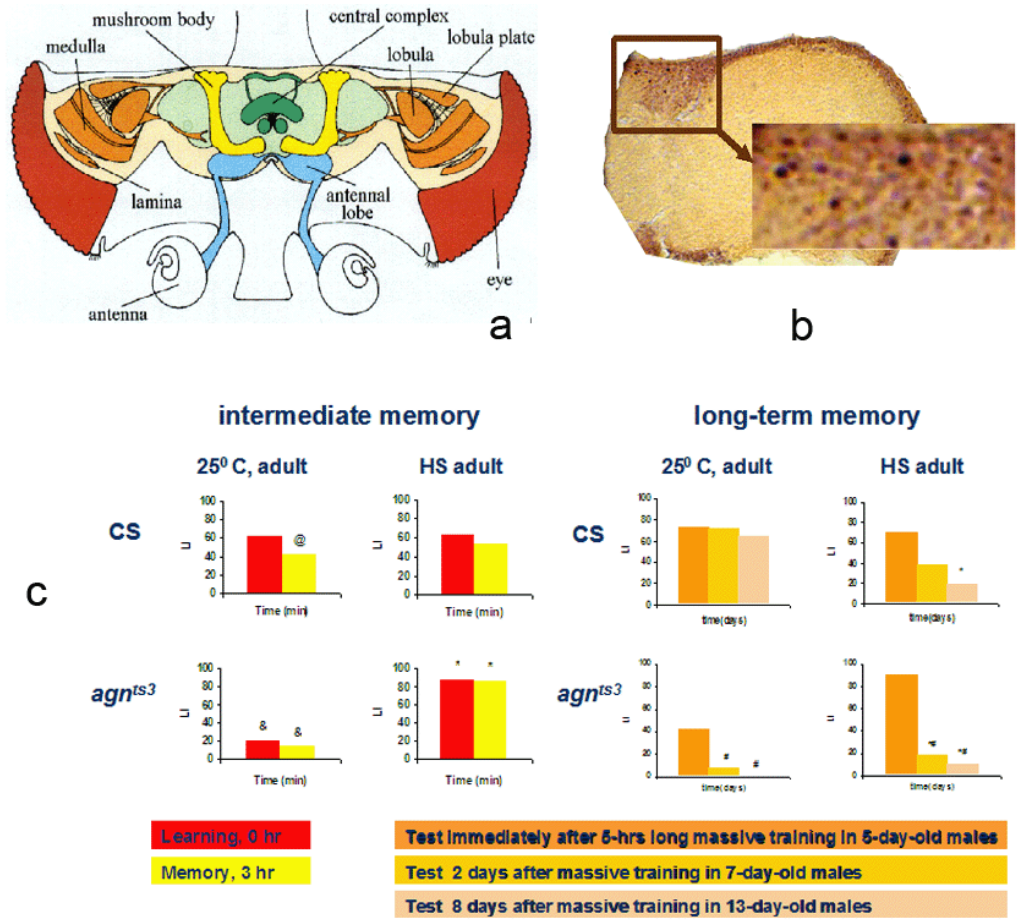


Figure 10. Learning acquisition and memory retention (conditioned courtship suppression paradigm) and Congo Red positive amyloid-like inclusions I the brain. (a): a scheme of the *Drosophila* brain; (b): Congophilic inclusions in the brain nerve cells; (c): effects of adult heat shock (HS) on intermediate and long-term memory. Learning Index (LI); * - LI after heat shock significantly differ from LI at 25^{0}C; & - LI significantly lower than that of Canton-S strain under similar conditions; @ - LI significantly differ from LI 0 hours after training; # -LI significantly differ from Canton-S strain under similar conditions in tests for long term memory formation

The results were surprising and paradoxical: intact *agn*^{ts3} mutants showed 3-h (intermediate) memory and learning ability that were threefold lower than those in Canton S flies. The *agn*^{ts3} mutants treated with heat shock during the mushroom body formation exhibited even more drastic, six -fold, reduction in these parameters. However, after a heat shock treatment of adult *agn*^{ts3}or a heat shock at the prepupal stage, during the formation of the brain central complex, the memory and learning indices were not only the same as in CS flies, but showed a trend for increase. As to long-term memory, when it is tested 2 and 8 days after 5 hrs training at day 5, it is also severely suppressed in intact *agn*^{ts3} compared to wild type. Again, heat shock administration to adult males somehow improves the poor performance of *agn*^{ts3}, and heat shock at the stage of formation of the central complex of the brain (HS2) brings the long-term memory practically to the wild type level (Nikitina et al., 2009, unpublished, Fig. 10). The studies aimed to correlate these findings with CREB level are underway but as we have already shown (Medvedeva et al., 2008), the altered intermediate memory depends on the rate of formation of amyloid-like inclusions in the brain. In case of *agn*^{ts3} mutational change of each of the analyzed levels disrupts systemic regulation of genetic and cytogenetic processes, which results in the formation of amyloid-like inclusions. It is believed (Lee at al., 2004) that the cytoplasmic aggregates block axonal transport, contributing to neurophysiological dysfunction presumably due to synaptic pathology which is also common to human AD brain (Masliah et al., 1989).

In the wild-type strain, heat shock decreases the spontaneous level of amyloid-like inclusions in larvae and does not lead to the formation of inclusions in the adult brain, which is diagnostic for many neurodegenerative diseases. By contrast, in the *agn*^{ts3} mutant, the amount of inclusions in all larvae examined at normal temperature significantly decreases after heat shock (Medvedeva et al., 2008).

Such factors as actin and cofilin have been shown to be involved in the formation of inclusions (mostly cytoplasmic, less frequently, nuclear) in many neurodegenerative diseases, including all varieties of prionic conditions (Minamide et al., 2000; Maselli et al., 2003). Granules of the actin–cofilin complex are preferentially found in postmortem sections of patients with the Alzheimer disease; in them, all amyloid-like inclusions are surrounded by actin-cofilin complex, but not all inclusions of these complexes contain amyloid aggregates. In the case of the Alzheimer disease, the earliest events occur on the cell membrane, where multiprotein complexes are formed with participation of integrins and LIMK1. Its activation by neurotoxic fragments of the amyloid protein and the subsequent cofilin phosphorylation result in rearrangement of the actin cytoskeleton and the formation of actin filaments (Heredia et al., 2006). In terms of genetics, upon analysis of mutant and spontaneous variants of the *agnostic* locus, this series of events is as follows: the LIMK gene structure—disruption of LIMK1 and cofilin expression—the formation of Congophilic (amyloid-like) inclusions surrounded by the actin–cofilin complex—the resultant cognitive level.

CONCLUSION

Recent combined efforts of neurobiology and genetics has brought to a new notion that epigenetic changes leading to chromatin remodeling play important roles in regulating networks of gene expression during development of the nervous system, when they determine the switch from nerve stem cells to dendrite development and axon path finding, and in the

adult brain where they underlie memory formation and maintenance (Lin and Holt, 2007). Though the detailed mechanisms are still far from clear, it is already possible to conceive that directional steering in axons and dendrites based on attractive and repulsive turning is analogous to long-term potentiation and depression (LTP and LTD). Chromatin modifications in soma of the nerve cell are coordinated with the transcriptional cascades induced by changes in synaptic plasticity which can also promote local translation in dendrites and axons of dormant mRNAs for neurotransmitter receptors and components of actin cytoskeleton. At the protein level actin and actin related proteins appear to be the main players in the game, at the level of RNA its flexibility and a vast number of non-coding RNAs predominantly enriched in the brain enable the RNA machinery to set the rules of this game. Both non-coding RNAs and actin-dependent processes orchestrate the responses of an organism to environmental requirements, current needs of the organism, and its individual experience. The critical aspect of this response is the spatial organization of the genome in the nucleus when the chromatin domains located far apart in the linear DNA, as well as chromosome arms, can have physical contacts thereby predisposing to unequal recombination which results in structural rearrangements. The chromosome positions within the nucleus determine both normal development and progression of genomic diseases. For example, hemizygosity for LIMK1, the key enzyme of actin remodeling (small GTPases of Rho family – LIMK1 – cofilin – actin) leads to cognitive pathology of the Williams syndrome and the mostly studied details of the crosstalk of non-coding RNAs and signaling cascades refer to LIMK1 (Schratt et al., 2006). The signaling cascade of actin remodeling acts downstream of different receptors including those for neurotransmitters and is tightly involved in the feedback regulation of different receptors and ion channels. Notably, many human and *Drosophila* genes coding for neurotransmitter receptors, ion channels and components of different signaling cascades map to heterochromatic regions. Our findings on structural impairments of the *agnostic* locus harboring the *Drosophila* gene for LIMK1, its belonging to intercalary heterochromatic regions, its role in heterochromatin formation, spatial organization of the chromosomes in the nucleus, and cognitive process allow the extrapolation of these peculiarities on aforementioned genes mapping to heterochromatin. Probably, the genes from signaling cascades which are localized in heterochromatin like LIMK1 gene can regulate gene expression during memory formation not only via activation of effector molecules, but also via chromatin remodeling due to specificity of their localization. The heterochromatic genes containing repeats in 3'UTRs similarly to LIMK1 gene might serve as a source of non-coding RNAs. This could lead both to gene silencing and to formation of ectopic contacts of the heterochromatic regions complementary to sequences of these non-coding RNAs. It seems plausible that LIMK1 itself can act as a regulatory factor in bridging three hierarchic levels of genome organization, the first being the linear organization of transcriptionally active and regulatory DNA sequences; the second being the chromatin level mediating switching between different functional states of transcriptional activity or repression of gene clusters and the third being the nuclear level, a dynamic, three-dimensional spatial organization of the chromosomes, epigenetically regulating gene clusters of different chromosomes.

REFERENCES

Adams, M. D.; Celniker, S. E.; Holt, R. A.; et al. (2000). *The genome sequence of Drosophila melanogaster.* Science, 287, 2185–2195.

Aigner, S.; Denli, A. M.; Gage, F. H. (2007). *A novel model for an older remodeler: The BAF swap in neurogenesis.* Neuron, 55, 171–173.

Ambach, A.; Saunus, J.; Konstandin, M.; et al. (2000). *The serine phosphatases PP1 and PP2A associate with and activate the actin-binding protein cofilin in human T lymphocytes*. Eur. J. Immunol., 30, 3422–3431.

Antar, L. N.; Li, C.; Zhang, H.; Carroll, R. C.; Bassell, G. J. (2006). *Local functions for FMRP in axon growth cone motility and activity-dependent regulation of filopodia and spine synapses.* Mol. Cell Neurosci., 32, 37–48.

Aravin, A. A.; Lagos-Quintant, M.; Yalcin, A.; Zavolan, M.; Marks, D.; et al. (2003). *The small RNA profile during Drosophila melanogaster development.* Dev. Cell, 5, 337–350.

Bateman, J. R. & Wu, C.-t. (2008). *A genomewide survey argues that every zygotic gene product is dispensable for the initiation of somatic homolog pairing in Drosophila.* Genetics, 180, 1329–1342.

Belyaeva, E. S.; Andreyeva, E. N.; Belyakin, S. N.; Volkova, E. I.; Zhimulev, I. F. (2008). *Intercalary heterochromatin in polytene chromosomes of Drosophila melanogaster.* Chromosoma, 117, 411–418.

Belyaeva, E. S.; Demakov, S. A.; Pokholkova, G. V.; Alekseyenko, A. A.; Kolesnikova, T. D.; Zhimulev, I. F. (2006). *DNA underreplication in intercalary heterochromatin regions in polytene chromosomes of Drosophila melanogaster correlates with the formation of partial chromosomal aberrations and ectopic pairing*. Chromosoma, 115, 355–366.

Belyaeva, E. S.; Zhimulev, I. F.; Volkova, E. I.; et al. (1998). Su(UR)ES: a *gene suppressing DNA underreplication in intercalary and pericentric heterochromatin of Drosophila melanogaster polytene chromosomes*. Proc. Natl. Acad. Sci. USA, 95, 7532–7537.

Birkenfeld, J.; Betz, H.; Roth, D. (2001). *Inhibition of neurite extension by overexpression of individual domains of LIM kinase1*. J. Neurochem., 78, 924–927.

Blumenstiel, J. P.; Fu, R.; Theurkauf, W. E.; Hawley, R. S. (2008). *Components of the RNAi machinery that mediate long-distance chromosomal associations are dispensable for meiotic and early somatic homolog pairing in Drosophila melanogaster.* Genetics, 180, 1355–1365.

Blumenstiel, J. P.; Fu, R .; Theurkauf, W. E.; Hawley, R. S.; Hiraoka, Y.; Dernburg, A. F.; Parmelee, S. J.; Rykowski, M. C.; Agard, D. A.; et al. (1993). *The onset of homologous chromosome pairing during Drosophila melanogaster embryogenesis*. J. Cell Biol., 120, 591–600.

Bramham, C. R. (2008). *Local protein synthesis, actin dynamics, and LTP consolidation*. Curr. Opin. Neurobiol., 18, 524–531.

Bridges, C. B. (1935). *Salivary chromosome maps with a key to the banding of the chromosomes of Drosophila melanogaster*. J. Hered., 26, 60–64.

Brown, E.; Malakar, S.; Krebs, J. E. (2007). *How many remodelers does it take to make a brain? Diverse and cooperative roles of ATP-dependent chromatin-remodeling complexes in development.* Biochem. Cell Biol., 85, 444–462.

Chen, M. & Shen, X. (2007). *Nuclear actin and actin-related proteins in chromatin dynamics*. Curr. Opin. Cell Biol.;19, 326–30.

Costa, E.; Chen, Y.; Dong, E.; Grayson, D. R.; Kundakovic, M.; Maloku, E.; Ruzicka, W.; Satta, R.; Veldic, M.; Zhubi, A.; Guidotti, A. (2009). *GABAergic promoter hypermethylation as a model to study the neurochemistry of schizophrenia vulnerability*. Expert. Rev. Neurother., 9, 87–98.

da Silva, J. S. & Dotti, C. G. (2002). *Breaking the neuronal sphere: regulation of the actin cytoskeleton in neuritogenesis*. Nat. Rev. Neurosci., 3, 694–704.

de la Torre-Ubieta, L. & Bonni, A. (2008). *Combinatorial assembly of neurons: from chromatin to dendrites.* Trends in Cell Biology, 18, 48–51.

Depienne, C.; Heron, D.; Betancur, C.; Benyahia, B.; Trouillard, O.; Bouteiller, D.; Verloes, A.; LeGuern, E.; Leboyer, M.; Brice, A. (2007). *Autism, language delay and mental retardation in a patient with 7q11 duplication.* Journal of Medical Genetics; 44, 452–458.

Deshpande, G.; Calhoun, G.; Schedl, P. (2005). *Drosophila argonaute-2 is required early in embryogenesis for the assembly of centric/centromeric heterochromatin, nuclear division, nuclear migration, and germ-cell formation*. Genes Dev., 19, 1680–1685.

Donnai, D., Karmiloff-Smith, A. (2000). *Williams syndrome: from genotype through to the cognitive phenotype.* Am. J. Med. Genet., 97, 64–71.

Eaton, B. A. & Davis, G. W. (2005). *LIM kinase1 controls synaptic stability downstream of the type II BMP receptor*. Neuron, 47, 695–708.

Foe, V. E.; Odell, G. M.; Edgar, B. A. (1993). *Mitosis and morphogenesis in the Drosophila embryo: point and counterpoint pp. 149–300 in The Development of Drosophila melanogaster*. Cold Spring Harbor, Cold Spring Harbor Laboratory Press, NY

Foletta, V. C.; Lim, M. A.; Soosairajah, J.; Kelly, A. P.; Stanley, E. G.; Shannon, M.; He, W.; Das, S.; Massague, J.; Bernard, O. (2003). *Direct signaling by the BMP type II receptor via the cytoskeletal regulator LIMK1*. J. Cell Biol., 162, 1089–1098.

Francke, U. (1999). *Williams-Beuren syndrome: genes and mechanisms*. Hum. Mol. Genet., 8, 1947–1954.

Fung, J. C.; Marshall, W. F.; Dernburg, A.; Agard, D. A.; Sedat, J. W. (1998). *Homologous chromosome pairing in Drosophila melanogaster proceeds through multiple independent initiations*. J. Cell Biol., 141, 5–20.

Gangaraju, V. K. & Bartholomew, B. (2007). *Mechanisms of ATP dependent chromatin remodeling*. Mutat Res., 618, 3–17.

Gemkow, M. J.; Verveer, P. J.; Arndt-Jovin, D. J. (1998). *Homologous association of the Bithorax complex during embryogenesis: consequences for transvection in Drosophila melanogaster*. Development, 125, 4541–4552.

Ghildiyal, M.; Seitz, H.; Horwich, M. D.; Li, C.; Du, T.; et al. (2008). *Endogenous siRNAs derived from transposons and mRNAs in Drosophila somatic cells.* Science, 320, 1077–1081.

Gregyrio, S. P.; Sallet, P. C.; Do, K. A.; Lin, E.; Gattaz ,W. F.; Dias-Neto, E. (2009). *Polymorphisms in genes involved in neurodevelopment may be associated with altered brain morphology in schizophrenia: preliminary evidence*. Psychiatry Res., 165, 1–9.

Grewal, S. I. S. & Elgin, S. C. R. (2002). *Heterochromatin: new posibitis for the inheritance of structure.* Curr. Opin. Cell. Genet. Devel., 12, 171-187.

Grewal, S. I. & Rice, J. C. (2004). *Regulation of heterochromatin by histone methylation and small RNAs*. Curr. Opin. Cell Biol., 16, 230–238.

Grummt, I. (2006). *Actin and myosin as transcription factors*. Curr. Opin. Genet. Dev., 16, 191–196.

Hawley, R. S. (1980). *Chromosomal sites necessary for normal levels of meiotic recombination in Drosophila melanogaster*: I. Evidence for and mapping of the sites. Genetics, 94, 625–646.

Heard, E. & Bickmore, W. (2007). *The ins and outs of gene regulation and chromosome territory organisation.* Curr. Opin. Cell Biol., 19, 311–316.

Heredia, L.; Helguera, P.; de Olmos, S.; et al. (2006). *Phosphorylation of actin-depolymerizing factor/cofilin by LIM-kinase mediates amyloid beta-induced degeneration: a potential mechanism of neuronal dystrophy in Alzheimer's disease*. J. Neurosci., 26, 6533–6542.

Hocking, J. C.; Hehr, C. L.; Bertolesi, G.; Funakoshi, H.; Nakamura, T.; McFarlane, S. (2009). *LIMK1 acts downstream of BMP signaling in developing retinal ganglion cell axons but not dendrites*. Dev Biol., [Epub ahead of print]

Horn, P. J. & Peterson, C. L. (2006). *Heterochromatin assembly: a new twist on an old model.* Chromosome Res., 14, 83–94.

Hsieh, J. & Gage, F.H. (2005*). Chromatin remodeling in neural development and plasticity.* Curr. Opin. Cell Biol., 17, 664–671.

Huisinga, K. L.; Brower-Toland, B.; Elgin, S. (2006). *The contradictory definitions of heterochromatin: transcription and silencing.* Chromosoma, 115, 110–122.

Ilyinskaya, N. B.; Petrova, N. A.; Demin, S. Yu. (1988). *Seasonal dynamics of chromosomal polymorphism in Chironomus plumosus L. (Diptera. Chironomidae).* Genetika, 24, 1393–1401. [In Russian].

Ji, Y.; Eichler, E. E.; Schwartz, S.; Nicholls, R. D. (2000). *Structure of chromosomal duplicons and their role in mediating human genomic disorders.* Genome Res., 10, 597–610.

Kafatos, F. C.; Louis, C.; Savakis, C.; Glover, D. M.; Ashburner, M.; Link, A. J.; Sidĭn-Kiamos, I.; Saunders, R. D. (1991). *Integrated maps of the Drosophila genome: progress and prospects.* Trends Genet., 7, 155–161.

Kamyshev, N. G.; Iliadi, K. G.; Bragina, J. V. (1999). *Drosophila conditioned courtship: two ways of testing memory*. Learn. Mem., 6, 1–20.

Kaufmann, B. P. (1946). *Organization of chromosome. 1. Break distribution and chromosome recombination in Drosophila melanogaster.* J. Exp. Zool., 102, 293–320.

Khesin, R. B. & Leibovitch, B. A. (1976). *Structure of chromosomes, histones and gene activity in Drosophila.* Molecular Biol., 10, 3–34. [In Russian].

Kosikov, K. V. (1936). *A new duplication in the Drosophila melanogaster X chromosome and its evolutionary significance.* Dokl. Akad. Nauk SSSR, 3, 297–300.

Kutter, C. & Svoboda, P. (2008). *miRNA, siRNA, piRNA: Knowns of the unknown*. RNA Biol., 5, 181–188.

Lee, W.; Yoshihara, M.; Littleton, J. T. (2004). *Cytoplasmic aggregates trap polyglutamine-containing proteins and block axonal transport in a Drosophila model of Huntington's disease.* Proc. Nat. Acad. Sci. USA., 101, 3224–3229.

Lee-Hoeflich, S. T.; Causing, C. G.; Podkowa, M.; Zhao, X.; Wrana, J. L.; Attisano, L. (2004). *Activation of LIMK1 by binding to the BMP receptor, BMPRII, regulates BMP-dependent dendritogenesis*. EMBO J., 23, 4792–4801.

Lessard, J.; Wu, J. I.; Ranish, J. A.; Wan, M.; Winslow, M. M.; Staahl, B. T.; Wu, H.; Aebersold, R.; Graef, I. A.; Crabtree, G. R. (2007). *An essential switch in subunit*

composition of a chromatin remodeling complex during neural development. Neuron, 55, 201–215.

Levenson, J. M.; Qiu, S.; Weeber, E. J. (2008). *The role of reelin in adult synaptic function and the genetic and epigenetic regulation of the reelin gene*. Biochim. Biophys. Acta., 1779, 422–431.

Levenson, J. M. & Sweatt, J. D. (2006). *Epigenetic mechanisms: a common theme in vertebrate and invertebrate memory formation*. Cell Mol. Life Sci., 63, 1009–1016.

Lin, A. C. & Holt, C. E. (2007). *Local translation and directional steering in axons*. EMBO J., 26, 3729–3736.

Lippman, Z. & Martienssen, R. (2004). *The role of RNA interference in heterochromatic silencing*. Nature, 431, 364–370.

Louvet, E. & Percipalle, P. (2009). *Transcriptional control of gene expression by actin and myosin.* Int. Rev. Cell Mol. Biol., 272, 107–147.

Lupski, J. R. (2009). *Genomic disorders ten years on*. Genome Med., 1, 42.

Lupski, J. R. (1998). *Genomic disorders: structural features of the genome can lead to DNA rearrangements and human disease traits*. Trends Genet., 14, 417–422.

Lynch, M. A. (2004). *Long-term potentiation and memory*. Physiol. Rev., 84, 87–136.

Mande, S. & Gozes, I. (2007). *Activity-dependent neuroprotective protein constitutes a novel element in the SWI/SNF chromatin remodeling complex*. J. Biol. Chem., 282, 34448–34456.

Marqués, G.; Bao, H.; Haerry, T. E.; Shimell, M. J.; Duchek, P.; Zhang, B.; O'Connor, M. B. (2002). *The Drosophila BMP type II receptor Wishful Thinking regulates neuromuscular synapse morphology and function*. Neuron, 33, 529–543.

Marshall, W. F.; Straight, A.; Marko, J. F.; Swedlow, A. F.; Dernburg, B. A.; Belmont, A. W.; Murray, D. A.; Sedat, J. W. (1997). *Interphase chromosomes undergo constrained diffusional motion in living cells.* Curr. Biol., 7, 930–939.

Marshall, W. F. (2002). *Order and disorder in the nucleus*. Review Current Biology, 12, 724-728.

Marshall, W. F.; Straight, A.; Marko, J. F.; Swedlow, J.; Dernburg, A.; Belmont, A.; Murray, A. W.; Agard, D. A.; Sedat, J. W. (1997). *Interphase chromosomes undergo constrained diffusional motion in living cells.* Current Biology, 7, 930–939.

Maselli, A.; Furukawa, R.; Thomson, S. A.; et al. (2003). *Formation of Hirano bodies induced by expression of an actin cross-linking protein with a gain-of-function mutation.* Eukaryot. Cell, 2, 778–787.

Masliah, E.; Terry, R. D.; DeTeresa, R. M.; Hansen, L. A. (1989). *Immunohistochemical quantification of the synapse-related protein synaptophysin in Alzheimer disease*. Neurosci. Lett., 103, 234–239.

Mathieu, O. & Bender, J. (2004). *RNA-directed DNA methylation.* J. Cell Sci., 117, 4881–4888.

Mathog, D.; Hochstrasser, M.; Gruenbaum, Y.; et al. (1984). *Characteristic folding pattern of polytene chromosomes in Drosophila salivary gland nuclei.* Nature, 308, 414–421.

Matsumoto, M.; Setou, M.; Inokuchi, K. (2007). *Transcriptome analysis reveals the population of dendritic RNAs and their redistribution by neural activity.* Neurosci. Res., 57, 411–423.

Mattick, J. S. (2007*). A new paradigm for developmental biology*. Exp. Biol., 210, 1526–1547.

Mattick, J. S.; Amaral, P. P.; Dinger, M. E.; Mercer, T. R.; Mehler, M. F. (2009). *RNA regulation of epigenetic processes.* Bioessays, 31, 51–59.

Matunis, E. L.; Kelley, R. L.; Dreyfuss, G. (1994). *Essential role for a heterogeneous ribonucleoprotein (hnRNP) in oogenesis: hrp40 is absent form the germ line in the dorso-ventral mutant squid.* Proc. Natl. Acad. Sci. USA, 91, 2781–2784.

Meagher, R. B.; Kandasamy, M. K.; Deal, R. B.; McKinney, E. C. (2007). *Actin-related proteins in chromatin-level control of the cell cycle and developmental transitions.* Trends Cell Biol., 17, 325–332.

Medvedeva, A. V.; Molotkov, D. A.; Nikitina, E. A.; Popov, A. V.; Karagodin, D. A.; Baricheva, E. M.; Savvateeva-Popova, E. V. (2008*). Systemic regulation of genetic and cytogenetic processes by a signal cascade of actin remodeling: locus agnostic in Drosophila*. Russian Journal of Genetics, 44, 669–681.

Meng, Y.; Zhang, Y.; Tregoubov, V.; Janus, C.; Cruz, L.; Jackson, M.; Lu, W. Y.; MacDonald, J. F.; Wang, J. Y.; Falls, D. L.; Jia, Z. (2002). *Abnormal spine morphology and enhanced LTP in LIMK-1 knockout mice.* Neuron, 35, 121–133.

Mercer, T. R.; Dinger, M. E.; Mariani, J.; Kosik, K. S.; Mehler, M. F.; Mattick, J. S. (2008). *Noncoding RNAs in long-term memory formation.* Neuroscientist, 14, 434–445.

Miller, F. D. & Kaplan, D. R. (2003) *Signaling mechanisms underlying dendrite formation*. Curr. Opin. Neurobiol., 13, 391–398.

Minamide, L. S.; Striegl, A. M.; Boyle, J. A.; et al. (2000). *Neurodegenerative stimuli induce persistent ADF/cofilin–actin rods that disrupt distal neurite function.* Nature Cell Biol., 2, 628–636.

Moazed, D.; Вьhler, M.; Buker, S. M.; Colmenares, S. U.; Gerace, E. L.; Gerber, S. A.; Hong, E. J.; Motamedi, M. R.; Verdel, A.; Villйn, J.; Gygi, S. P. (2006). *Studies on the mechanism of RNAi-dependent heterochromatin assembly*. Cold Spring Harb. Symp. Quant. Biol., 71, 461–471.

Muotri, A. R. & Gage, F. H. (2006). *Generation of neuronal variability and complexity*. Nature, 441, 1087–1093.

Nagata, K.; Ohashi, K.; Yang, N.; Mizuno, K. (1999). *The N-terminal LIM domain negatively regulates the kinase activity of LIM-kinase* 1. Biochem J., 343, 99–105.

Neuman-Silberberg, F. S. & Schupbach, T. (1993) *The Drosophila dorsoventral patterning gene gurken produces a dorsally localized RNA and encodes a TGFa-like protein*. Cell, 75, 165.

Nikitina, E. A.; Tokmacheva, E. V.; Savvateeva-Popova, E. V. (2003). *Heat shock during the develorment of central structures of the Drosophila brain: memory formation in the l(1)ts403 mutant of Drosophila melanogaster*. Russ. J. Genet., 39, 25–31.

Niu, S.; Yabut, O.; D'Arcangelo, G. (2008). *The Reelin signaling pathway promotes dendritic spine development in hippocampal neurons.* J. Neurosci., 28, 10339–10348.

Obbard1, D. J.; Gordon, K. H. J.; Buck, A. H.; Jiggins, F. M. (2009). *The evolution of RNAi as a defence against viruses and transposable elements.* Phil. Trans. R. Soc., 364, 99–115.

O'Brien, T. P.; Bult, C. J.; Cremer, C.; et al. (2003). *Genome function and nuclear architecture: from gene expression to nanoscience*. Genome Res., 13, 1029–1041.

Okamura, K.; Chung, W. J.; Ruby, J. G.; Guo, H.; Bartel, D. P.; Lai, E. C. (2008). *The Drosophila hairpin RNA pathway generates endogenous short interfering RNAs.* Nature, 453, 803–806.

Olave, I. A.; Reck-Peterson, S.; Crabtree, G. R. (2002a). *Nuclear actin and actin-related proteins in chromatin remodeling*. Annu. Rev. Biochem., 71, 755–781.

Olave, I.; Wang, W.; Xue, Y.; Kuo, A.; Crabtree, G. R. (2002b). *Identification of a polymorphic, neuron-specific chromatin remodeling complex.* Genes Dev., 16, 2509–2517.

Pederson, T. & Aebi, U. (2005). *Nuclear actin extends, with no contraction in sight*. Mol. Biol. Cell, 16, 5055–5060.

Percipalle, P. & Visa, N. (2006). *Molecular functions of nuclear actin in transcription*. J. Cell Biol., 172, 967–971.

Percipalle, P.; Jonsson, A.; Nashchekin, U. (2003). *Actin in the nucleus: what form and what for?* J. Struct. Biol., 140, 3–9.

Percipalle, P.; Raju, C. S.; Fukuda, N. (2009). *Actin-associated hnRNP proteins as transacting factors in the control of mRNA transport and localization.* RNA Biol., 6, [Epub ahead of print]

Percipalle, P. (2009*). The long journey of actin and actin-associated proteins from genes to polysomes.* Cell Mol. Life Sci., 66, 2151-2165.

Percipalle, P.; Zhao, J.; Pope, B.; Weeds, A.; Lindberg, U.; Daneholt, B. (2001). *Actin bound to the heterogeneous nuclear ribonucleoprotein hrp36 is associated with Balbiani ring mRNA from the gene to polysomes*. J. Cell Biol., 153, 229–236.

Pindyurin, A. V.; Boldyreva, L. V.; Shloma, V. V.; Kolesnikova. T. D.; Pokholkova, G. V.; Andreyeva, E. N.; Kozhevnikova, E. N.; Ivanoschuk, I. G.; Zarutskaya, E. A.; Demakov, S. A.; Gorchakov, A. A.; Belyaeva, E. S.; Zhimulev, I. F. (2008). *Interaction between the Drosophila heterochromatin proteins SUUR and HP1*. J. Cell Sci., 121, 1693–1703.

Prokofyeva-Belgovskaya, A. A. (1986). *Heterochromatic regions of chromosomes*. Moscow., Nauka. [In Russian].

Prokofyeva-Belgovskaya, A. A. (1945). *Heterochromatinization as a change in chromosome cycle.* Zhurnal Obshch. Biologii, 6, 93–124. [In Russian].

Prokofyeva-Belgovskaya, A. A. & Khvosotova, V. V. (1939). *Distribution of chromosome rearrangement breaks in Drosophila melanogaster X chromosome*. Doklady Acad. Nauk SSSR, 23, 269–271. [In Russian].

Purandare, S. M. & Patel, P. I. (1997). *Recombination hot spots and human disease*. Genome Res., 7, 773–786.

Rando, O. J.; Zhao, K.; Janmey, P.; Crabtree, G. R. (2002). *Phosphatidylinositol-dependent actin filament binding by the SWI/SNF-like BAF chromatin remodeling complex*. Proc. Natl. Acad. Sci. USA, 99, 2824–2829.

Raymond, C. R. (2007). *LTP forms 1, 2 and 3: different mechanisms for the "long" in long term potentiation.* Trends Neurosci., 30, 167–175.

Raymond, C. R. (2008). *Different requirements for action potentials in the induction of different forms of long-term potentiation*. The Journal of Physiology, 586, 1859–65.

Reiter, L. T.; Potocki, L.; Chien, S.; Gribskov, M.; Bier, E. (2001). *A systematic analysis of human disease-associated gene sequences in Drosophila melanogaster*. Genome Res., 11, 1114–1125.

Rusinov, V.; Baev, V.; Minkov, I. N.; Tabler, M. (2005). *MicroInspector: a web tool for detection of miRNA binding sites in an RNA sequence*. Nucleic Acids Res., 33, (Web Server issue):W696–700.

Sбnchez-Carbente, M. R. & Desgroseillers, L. (2008). *Understanding the importance of mRNA transport in memory.* Prog. Brain Res., 16, 41–58.

Savvateeva-Popova, E.; Medvedeva, A.; Popov, A.; Evgen'ev, M. (2008). *Role of non-coding RNAs in neurodegeneration and stress response in Drosophila.* Biotechnol. J., 3, 1010–1021.

Savvateeva-Popova, E. V.; Peresleni, A. I.; Sharagina, L. M.; et al. (2002*). Complex study of Drosophila mutants in the agnostic locus: a model for connecting chromosomal architecture and cognitive functions.* Zh. Evolyuts. Biokhim. Fiziol., 38, 557–577.

Savvateeva, E. V. & Kamyshev, N. G. (1981). *Behavioral effects of temperature sensitive mutations affecting metabolism of cAMP in D. melanogaster*. Pharm. Biochem. Behav., 14, 603–611.

Savvateeva, E. V.; Kamyshev, N. G.; Rosenblyum, S. R. (1978). *Isolation of temperature-sensitive mutations that impair metabolism of cyclic-3.5.-adenosine monophosphate in D. melanogaster.* Dokl. Akad. Nauk SSSR, 240, 1443–1445.

Schier, A. F. (2007). *The maternal-zygotic transition: death and birth of RNAs.* Science, 316, 406–407.

Schleicher, M. & Jockusch, B. M. (2008). *Actin: its cumbersome pilgrimage through cellular compartments.* Histochem. Cell Bio., 129, 695–704.

Schratt, G. M.; Tuebing, F.; Nigh, E. A.; Kane, C. G.; Sabatini, M. E.; Kiebler, M.; Greenberg, M. E. (2006). *A brain-specific microRNA regulates dendritic spine development.* Nature, 439, 283–289.

Schratt, G. M.; Nigh, E. A.; Chen, W. G.; Hu, L.; Greenberg, M. E. (2004). *BDNF regulates the translation of a select group of mRNAs by a mammalian target of rapamycin-phosphatidylinositol 3-kinase-dependent pathway during neuronal development*. J. Neurosci., 24, 7366–7377.

Scott, M. J. & Li, F. (2008). *How do ncRNAs guide chromatin-modifying complexes to specific locations within the nucleus?* RNA Biol., 5, 13–16.

Seo, S.; Richardson, G. A.; Kroll, K. L. (2005). *The SWI/SNF chromatin remodeling protein Brg1 is required for vertebrate neurogenesis and mediates transactivation of Ngn and NeuroD.* Development, 132, 105–115.

Shaw, C. J.; Lupski, J. R. (2004). *Implications of genome architecture for rearrangement-based disorders: the genomic basis of disease*. Human Mol. Gen., 13, 57–64.

Sjolinder, M.; Bjork, P.; Soderberg, E.; et al. (2005). *The growing pre-mRNA recruits actin and chromatin-modifying factors to transcriptionally active genes*. Genes Dev., 19, 1871–1884.

Stankiewicz, P. & Lupski, J. R. (2006). *The genomic basis of disease, mechanisms and assays for genomic disorders.* Genome Dyn., 1, 1–16.

Stevens, N. M. (1908). *A study of the germ cells of certain Diptera with reference to the heterochromosomes and the phenomena of synapsis*. J. Exp. Zool., 5, 359–374.

Sturtevant, A. H. (1913). *The linear arrangement of six sex-linked factors in Drosophila, as shown by their mode of association*. Jour. Exp. Zool.,14, 43–59.

Tam, O. H.; Aravin, A. A.; Stein, P.; Girard, A.; Murchison, E. P., et al. (2008). *Pseudogene-derived small interfering RNAs regulate gene expression in mouse oocytes.* Nature, 453, 534–538.

Trotter, K. W. & Archer, T. K. (2008). *The BRG1 transcriptional coregulator*. Nucl. Recept. Signal., 6, e004.

Van Driel, R.; Fransz, P. F.; Verschure, P. J. (2003). *The eukaryotic genome: a system regulated at different hierarchical levels.* J. Cell Sci., 116, 4067–4075.

Van Horck, F. P. & Holt, C. E. (2008). *A cytoskeletal platform for local translation in axons*. Sci. Signal., 1, 11.

Vazquez, J.; Belmont, A. S.; Sedat, J. W. (2001). *Multiple regimes of constrained chromosome motion are regulated in the interphase Drosophila nucleus.* Current Biology, 11, 1227–1239.

Velthuis, A. J. & Bagowski, C. P. (2007). *PDZ and LIM domain-encoding genes: molecular interactions and their role in development*. Scientific World Journal, 7, 1470–1492.

Vlassova, I .E.; Graphodatsky, A. S.; Belyaeva, E. S.; Zhimulev, I. F. (1991). *Constitutive heterochromatin in early embryogenesis of Drosophila melanogaster*. Mol. Gen. Genet., 229, 316–318.

Watanabe, T.; Totoki, Y.; Toyoda, A.; Kaneda, M.; Kuramochi-Miyagawa, S.; et al. (2008). *Endogenous siRNAs from naturally formed dsRNAs regulate transcripts in mouse oocytes.* Nature, 453, 539–543.

Wen, Z.; Han, L.; Bamburg, J. R.; Shim, S.; Ming, G. L.; Zheng, J. Q. (2007). *BMP gradients steer nerve growth cones by a balancing act of LIM kinase and Slingshot phosphatase on ADF/cofilin*. J. Cell Biol., 178, 107–119.

Willis, D.; Li, K. W.; Zheng, J.-Q.; Chang, J. H.; Smit, A.; Kelly, T.; Merianda, T. T.; Sylvester, J.; van Minnen, J.; Twiss, J. L. (2005). *Differential transport and local translation of cytoskeletal, injury-response, and neurodegeneration protein mRNAs in axons.* J. Neurosci., 25, 778–791.

Wimber, D. E. & Steffensen, D. M. (1973). *Localization of gene function.* Annu. Rev. Genet., 7, 205–223.

Wu, J. I.; Lessard, J.; Olave, I. A.; Qiu, Z.; Ghosh, A.; Graef, I. A.; Crabtree, G.R. (2007). *Regulation of dendritic development by neuron-specific chromatin remodeling complexes.* Neuron., 56, 94–108.

Wu, K. Y.; Hengst, U.; Cox, L. J.; Macosko, E. Z.; Jeromin, A.; Urquhart, E. R.; Jaffrey, S. R. (2005). *Local translation of RhoA regulates growth cone collapse*. Nature, 436, 1020–1024.

Xamena, N.; Creus, A.; Macros, R., (1985). *Effect of intercalating mutagens on crossing over in Drosophila melanogaster females*. Experientia, 41, 1078–1081.

Yoon, B. C.; Zivraj, K. H.; Holt, C. E. (2009). *Local translation and mRNA trafficking in axon pathfinding*. Results Probl. Cell Differ., [Epub ahead of print]

Zalfa, F.; Achsel, T.; Bagni, C. (2006). *mRNPs, polysomes or granules: FMRP in neuronal protein synthesis.* Curr. Opin. Neurobiol., 16, 265–269.

Zhimulev, I. F.; Belyaeva, E. S.; Semeshin, V. F.; Koryakov, D. E.; Demakov, S. A.; Demakova, O. V.; Pokholkova, G. V.; Andreyeva, E. N. (2004). *Polytene chromosomes: 70 years of genetic research.* Int. Rev. Cytol., 241, 203–275.

Zhimulev, I. F. & Belyaeva, E. S. (2003). *Intercalary heterochromatin and genetic silencing.* Bioessays, 25, 1040–1051.

Zhimulev, I. F. (1998). *Polytene chromosomes, heterochromatin, and position effect variegation.* Advances in Genetics, 37, 566.

Zhimulev, I. F.; Semeshin, V. F.; Kulichkov, V. A.; Belyaeva, E. S. (1982). *Intercalary heterochromatin in Drosophila: localization and general characteristics.* Chromosoma, 87, 197–228.

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Chapter 5

"GLIOPATHY" MAINTAINS PERSISTENT HYPEREXCITABILITY OF SPINAL DORSAL HORN NEURONS AFTER SPINAL CORD INJURY: SUBSTRATE OF CENTRAL NEUROPATHIC PAIN

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The principle mechanism underlying enhanced pain sensitivity is a persistent hyperexcitability of spinal dorsal horn neurons. In the central nervous system, the somatosensory information is modulated by the balance between endogenous excitatory and inhibitory circuits, which are modified by glial cells. However, injury to the spinal cord or the peripheral nerve induces maladaptive changes of neuronal circuits in the spinal cord, which result in hyperexcitability of spinal dorsal horn neurons. Once hyperexcitability is developed in the central nervous system following neural injury, the maladaptive neuronal properties produce abnormal pain sensations in response to non-noxious stimuli (called allodynia) as well as enhanced pain sensations in response to noxious stimuli (called hyperalgesia). Thus, maintained hyperexcitability is a fundamental neuronal mechanism representing the persistent pain syndromes, such as neuropathic pain and causalgia following neural injury. Recent literature shows that glial cells are very important players in modulating synaptic circuits for somatosensation. Following neural injury, astrocytic and microglial activation are actively involved in maladaptive modulation of neuronal excitability via release of glutamate, other neurotransmitters and proinflammatory cytokines. We term this abnormal glial function, "gliopathy". In this chapter, we describe cellular mechanisms for the maintained hyperexcitability of spinal dorsal horn neurons mediated by neuronal-glial interactions following spinal cord injury and key neuronal intracellular signaling cascades that provide the feed forward cycle that perpetuates maintained hyperexcitaibitly which is persistent pain.

INTRODUCTION

Traumatic spinal cord injury (SCI) produces abnormal synaptic circuits that transmit maladapted somatosensory information to supraspinal centers in the central nervous system. These abnormal somatosensory transmissions often lead to spontaneous pain as well as enhanced evoked pain, when applied on peripheral receptive fields. One of the clinically related pain symptoms induced by SCI is causalgia (also called complex regional pain syndromes type II), which is described by "burning pain, allodynia, and hyperpathia usually in the hand or foot after partial injury of a nerve or one of its major branch" (Merskey and Bogduk, 1994). The original definition of causalgia is primarily based on a report of injured solders after the American Civil War in 1864, which described the burning and stabbing pain and exquisite sensitivity of the skin that occurred after nerve lesions (Mitchell et al., 1864).

SCI database reports that about 10 % of SCI patients experience causalgia (Gallien et al., 1995). Causalgia, which is thought to be maintained by abnormal sympathetic function, has common features with neuropathic pain and/or inflammatory pain and is hard to separate. Behaviorally, causalgia shares similar outcomes with neuropathic pain syndromes such as mechanical allodynia (non-noxious become noxious) and hyperalgesia (noxious become more noxious) as well as spontaneous pain (pain perceived without evoked stimuli). Electrophysiolocally, they share changed neuronal properties such as spontaneous activity, enhanced evoked activity and prolonged after discharges. Additionally, both behavioral and electrophysiological changes don't develop acutely but rather occur several months later after injury. However, it is hard to characterize causalgia without understanding neuropathic pain, which is induced by traumatic injuries of the nervous system. Central neuropathic pain occurs in as high as 70% of people with SCI. Thus, it is important to study.

It is certain that both syndromes, causalgia and neuropathic pain, show very similar clinical observations and mechanisms. In addition, pharmacological treatments can not separate the two pain syndromes. This chapter will focus on mechanisms for the maladaptive synaptic circuit, which result in neuronal hyperexcitability in the spinal dorsal horn after SCI. We are certain that hyperexcitability of spinal dorsal horn neurons is a key component in spontaneous and enhanced evoked pains under pathophysiological conditions, such as SCI, peripheral nerve injury and inflammation.

ASCENDING PAIN PATHWAY

Pain has been considered as the outcome of unpleasant sensory input by nociceptive stimuli. The term "nociceptive" means the stimulus can potentially cause tissue damage if prolonged, such as pinch and heat. In normal physiological conditions, the spinal cord receives non-noxious and noxious inputs that are applied to the peripheral receptive fields. The information is sent to specific brain areas via separate ascending pathways with well organized somatotopic terminations. Anatomically non-noxious stimuli ascend from the spinal cord to specific brain regions via the dorsal column medial lemniscal pathway whereas noxious or nociceptive input ascend via specific nociceptive ascending pathways such as spinothalamic tract, spinoreticular, spinocervical and postsynaptic dorsal column tracts (Willis and Westlund, 1997). The thalamus in the brain stem is a relay site in the

somatosensory transmission between the spinal cord and the cortex of the brain. The majority of pain perception (nociception) from the spinal cord is mediated by the spinothalamic tract (STT). However, neural injuries including peripheral nerve injury and SCI induce enhanced sensitivity in response to any kind of stimuli and reorganization of synaptic circuits in the spinal dorsal horn. These anatomical and physiological changes induce neuronal hyperexcitability, expansion of receptive field, abnormal sprouting of primary afferent fibers, upregulation of excitatory amino acids receptors and ion channel expression as well as glia activation, all of which participate in the development and maintenance of central sensitization in the spinal dorsal horn (Christensen and Hulsebosch, 1997; Chu et al., 2004; Drew et al., 2001; Gwak et al., 2006; Hains et al., 2004; Watkins and Maier, 2003).

In behavioral and electrophysiological studies, central sensitization correlates well with pain-like behavioral outcomes, such as allodynia and hyperalgesia following SCI (Ma et al., 2006; Hains et al., 2003b; Zhang et al., 2005). Allodynia and hyperalgesia are behavioral changes that can be measured in pain studies. Mechanical allodynia is divided into static and dynamic allodynia. Static allodynia is an abrupt withdrawal behavior in response to weak mechanical stimuli in the skin whereas dynamic allodynia is an abrupt withdrawal behavior in response to lightly brushing the skin. It is known that primary Aβ fiber mediates dynamic allodynia whereas primary Aδ fibers mediate static allodynia (Field et al., 1999). Hyperalgesia is divided into mechanical, cold and thermal hyperalgesia in response to punctate mechanical pressure, cold stimuli and heat stimuli, respectively.

HYPEREXCITABILITY OF SPINAL DORSAL HORN NEURONS

Dorsal horn neurons in the spinal cord are second-order neurons in the transmission of somatosensory information. Discrimination of somatosensory information, mediated by rapid shifts of neurochemical changes at the peripheral receptive field by any kind of stimuli, such as light touch, vibration, noxious stimuli and inflammation, are sent to higher nervous systems by dorsal horn neurons. Perception of somatosensory input, especially nociceptive information, in the spinal cord is regulated by the balance between excitatory and inhibitory circuits. The perception of nociceptive input, i.e. causes pain with potential tissue damage, is beneficial to the human body because it alerts the body of potential damage and prevents severe damage. Thus, pain perception plays a critical role in the physiological condition for body protection. However, when the nervous system is injured, such as SCI, regulation of nociceptive input is lost. Under these conditions, the nervous system loses the ability to encode somatosensory input. Specifically discrimination of the somatosensory input to recognize stimuli is abnormal such that non-noxious stimuli become noxious stimuli or noxious stimuli become more noxious stimuli compared to normal sensitivity, which results in a "hyperexcitable state" of spinal dorsal horn neurons. Generally in the hyperexcitable state, spinal dorsal horn neurons show excessively enhanced and long lasting electrophysiological activity in response to non-noxious and noxious stimuli, which is called hyperexcitability of dorsal horn neurons (Drew et al., 2001; Gwak et al., 2003).

The classic study of neuronal hyperexcitability of spinothalamic tract (STT) neuron in the dorsal horn used intradermal capsaicin treatment (Dougherty and Willis, 1992). Capsaicin is an active component of chili pepper and an irritant to human skin. Following intradermal

injection of capsaicin, the STT neurons show enhanced evoked activity, spontaneous activity and a prolonged afterdischarge activity via activation of glutamate and neurokinin receptors in the spinal dorsal horn (Dougherty et al., 1994; Neugebauer et al., 2000; Zou et al., 2002). Activation of glutamate and neuropeptide receptors by capsaicin treatment triggers massive influx of calcium ions into intracellular compartments. There are two different pathways that lead to increased intracellular calcium concentrations. The first is an influx of calcium ions from the extracellular space, which contain 1000-10000 folds higher calcium concentration than the intracellular calcium concentration. Activation of NMDA receptors and calcium channels play important roles in calcium influx under capsaicin treatment. However, NMDA receptors and calcium channels are voltage dependent. In normal physiological states, the NMDA receptor is blocked by insertion of Mg^{2+} inside the receptor channel. Noxious stimuli or neuronal injury induces membrane depolarization by activation of sodium channels or AMPA receptor activation, the Mg^{2+} ion is removed from the NMDA receptor channel and the influx of calcium ions triggers calcium dependent activation of intracellular downstream events. The second pathway is the release the calcium ions from internal calcium stores. This pathway is predominantly mediated by activation of metabotropic receptors. Activation of metabotropic receptors triggers activation of phospholipase C followed by activation of diacylglycerol that triggers the release of calcium ions from internal calcium stores, such as the endoplasmic reticulum. Elevated intracellular concentrations of calcium ions then initiate activation of protein kinase A, protein kinase C, nitric oxide/cGMP pathways, Calcium/Calmodulin-dependent pathway and MAP Kinase pathways. These intracellular pathways trigger activation of transcriptional events that maintain neuronal hyperexcitability.

After SCI, neuronal hyperexcitability is mediated by an intense discharge barrage by primary afferent fibers and significantly increased excitatory amino acids (such as glutamate) and neuropeptides (such as substance P and CGRP). For example, the predominant biochemical events in the dorsal horn immediately after SCI are rapid increases of extracellular glutamate, neuropeptides and increased production of proinflammatory cytokines, such as TNFα, IL-1 and IL-6, from neurons and activated glia cells (McAdoo et al., 1999; Yang et al., 2004). These components are in high concentrations and enough to activate neuronal receptors and ion channels to initiate excitation and enable increased sensitivity to non-noxious, as well as noxious stimuli. Thus, rapid biochemical changes produce increased ion channel and receptor activation, that stimulates a variety of neuronal intracellular cascades and leads to maladaptive and persistent changes of synaptic circuits, specifically activation of postsynaptic glutamate receptors (ionotropic and metabotropic receptors) and ion channels (such as $NaV_{1.3}$ and $NaV_{1.7}$) (Gwak and Hulsebosch, 2005; Hains et al., 2003a; Waxman, 2007). Ion channels and receptors located in plasma membrane triggers the initiation of intracellular events, that eventually result in cytoplasmic phosphorylation of both receptor and ion channels in the plasma membrane of postsynaptic neurons, providing continued activation. Following activation of plasma membrane components, massive calcium ion concentrations continue to move from extracellular regions into intracellular compartments, trigger activation of several protein kinases (such as MAP Kinase family) and initiate posttranslational processes through activation of transcriptional factors, such as NKκB and CREB (see below). The changes in transcriptional pathways result in changes of target gene expression that feed forward and result in phosphorylation of neuronal receptors and ion channels that maintain the long-lasting excitable state of neurons (Crown et al., 2006; Yu and Yezierski, 2005). Thus, persistent excitability of spinal dorsal

horn neurons is maintained and provides a substrate for abnormal responses (allodynia, hyperalgesia) to external stimuli, such as electrical, chemical and mechanical.

MAP KINASE-CREB PATHWAYS

Intracellular events are key factors in maintaining the long-lasting hyperexcitable states of dorsal horn neurons. While several downstream pathways activate neuronal intracellular events following SCI, some leading to apoptosis, we focus on the mitogen activated protein kinase (MAP Kinase)-transcriptional factor pathway, involved in hippocampal hyperexcitabitly (Izumi et al., 2008) and in dorsal horn hyperexcitability, thus nociceptive transmission. There are several lines of evidence that MAP Kinase-transcriptional events are sequential. First, extracellular glutamate produces activation of ionotropic glutamate receptor and calcium channels that cause massive influx of calcium ions into intracellular spaces. High concentrations of calcium ions triggers activation of PKC, PKA and CaMKII pathways, followed by activation of Ras→ Raf-1 → MEK → activation of ERK/MAPK. Additionally, activation of glutamate metabotropic receptors induces activation of protein kinase C, followed by activation of Ras, Raf-1 and MEK pathways. It should be noted that activation of neurotrophin receptors, such as Trk, initiates activation of Ras without calcium influx (Ji and Woolf, 2001, Mao et al., 1999). However, they both share the MEK → MAPK pathways in nociceptive transmission. It is well known that activation of the MAP Kinase family triggers activation of transcription factors, such as cyclic adenosine 3', 5'-monophosphate response element-binding protein (CREB). The transcriptional pathway, MAPK → CREB, is a major downstream pathway in intracellular cascades that modulates cell growth, proliferation, and modulation of gene transcription, translation and protein expression. In neuronal cells, these pathways can lead to neuronal apoptosis or if internal Ca^{2+} concentrations are subleathal, to increased neuronal hyperexcitability. MAP Kinase is a 42-kDa serine-threonine protein kinase, which is phosphorylated on both threonine and tyrosine sites and purified from epidermal growth factor-treated 3T3-L1 fibroblasts (Ray and Sturgill, 1988; Sturgill et al., 1991). Phosphorylation or activation can be produced by cytokines, ultraviolation irradiation, osmotic shock, heat shock and lipopolysaccharide (Raingeaud et al., 1995; Whitmarsh and Davis, 1996; Widmann et al., 1997). The MAPK family has three members: the extracellular signal-regulated kinase (ERK), c-JUN N-terminal kinase (JNK) and p38 MAPK. Each will be considered in turn.

P38 MAPK

Recently, several lines of evidence demonstrate that activation by phosphorylation of the protein, p38 MAPK, is an important component in neuronal hyperexcitability under pathophysiological conditions. Animal models of traumatic neural injuries or inflammation models demonstrate activated p38 MAPK expression in spinal dorsal horn neurons and in neurons in dorsal root ganglia, at a time when mechanical allodynia is present (Crown et al., 2006; Ji et al., 2002; Zhuang et al., 2006). The blockade of p38 MAPK activation with intrathecal treatment of SB203580, which inhibits phosphorylation of p38 MAPK, attenuated

neuronal hyperexcitability following SCI in a dose dependent manner (Crown et al., 2008, in press; Gwak et al., 2009). In addition, western blot experiments demonstrated that treatment of SB203580 significantly inhibited the over expression of p38 MAPK in the spinal dorsal horn and correlated well with attenuation of mechanical allodynia and dorsal horn neuronal hyperexcitability after SCI. Although activated p38 MAPK contributes to hyperexcitability of spinal dorsal horn neurons and pain behaviors, the temporal cellular localization of p38 MAPK activation after SCI appears to change over time. Electrophysiological and immunohistochemical data suggested that activation of p38 MAPK in neurons is predominantly involved in neuronal hyperexcitability following SCI. However, microlgial activation of p38 MAPK is also observed, suggesting that microglial activation is critically important in the maintenance of hyperexcitability. However, astrocytic p38 MAPK activation did not show significant changes after SCI (Gwak et al., 2009). Others reported that activation of microgial p38 MAPK contribute to neuronal hyperexcitability and mechanical allodynia via TNFα and chemokine receptor activation following SCI (Peng et al., 2006). We propose that the role of p38 MAPK changes over time and that early after SCI, activated p38 MAPK is principally involved in cellular apoptotic mechanisms (Nakahara et al., 1999) contributing to "initiation" mechanisms of chronic pain, see below); whereas, days and weeks after SCI, continued activation of p38 MAPK in neuronal populations contribute to maintained hyperexcitability. We have demonstrated activated p38MAPK localization in both microglia and neurons populations in our SCI model of chronic central neuropathic pain (Crown et al., 2006; Gwak et al., 2009). However, the specific roles that these two neuronal vs. microglia populations play in contributing to maintained neuronal hyperexcitability and on persistent pain is unclear (see discussion below).

pERK

It is well documented that activation of ERK contributes to production of pain-related transcriptional pathways that cause long-term changes of nociceptive transmission (Crown et al., 2006). Activated ERK1/2 leads to activation of transcription factors, such as ELK-1 and NFκB and induces upregulation of NR1, NR2A and NK1 receptors in the dorsal horn after SCI (Yu and Yezierski, 2005). Although there is a lack of data on sequential activation of ERK in specific cell types, activation of ERK appears to be predominantly activated in the microglia after SCI. In addition, in several segments below and remote from the segment of SCI, pERK activation was demonstrated in microglial but not neurons or astrocytes in the chronic phase, leading to increased microglial PGE_2 release that is thought to contribute to neuronal hyperexcitability and mechanical allodynia after SCI (Zhao et al., 2007). Similar events also occur in peripheral neuropathic pain models. The induction of ERK activation shows differential temporal cellular localization in the dorsal horn after spinal nerve ligation. ERK activation is primarily in neurons within 10 mins of injury, and declines within 6 hrs, followed by microglia (1-3 days) and astrocytes (10-21 days). This data suggest that activation of ERK in neurons and microglia is involved in development of pain after peripheral nerve injury whereas activation of ERK in astrocytes is involved in maintenance of pain. Additionally, ERK activation occurs first in superficial layers (I, II) and later in deep dorsal horn laminae (Zhuang et al., 2005). However, several lines of evidence demonstrate that microglial activation is involved in the hyperexcitability of spinal dorsal horn neurons in

the chronic, persistent pain state after SCI. Hains and Waxman reported that SCI increased microglia hypertrophy (a classic response of "activation") and neuronal hyperexcitability in spinal regions remote from the injury site, 4 weeks after SCI. In addition, after inhibition of microglial activation by minocycline, a tetracycline related compound, both mechanical allodynia and neuronal hyperexcitability were attenuated compared to vehicle control groups (Hains and Waxman, 2006). While authors concluded that microlgial activation contributes to development and maintenance of pain hypersensitivity via ERK activation following SCI, there were no experiments that used specific ERK inhibitors to test this hypothesis.

pJNK

JNK activity has been implicated in oxidative stress and is a cofactor in the apoptotic process. However, few studies exist on the role of JNK activation (by phosphorylation called pJNK) on the development and maintenance of pain following SCI. Crown et al demonstrated that moderate spinal contusion injury did not induce a significant increase in pJNK expression and there was no significant correlation with pJNK expression and pain behaviors, such as vocalization (Crown et al., 2006). However, peripheral nerve injury studies suggest that activated JNK contributes to the development and maintenance of peripheral neuropathic pain. Additionally Ma and Quirion reported that astroglial pJNK immunoreactivity was observed in spinal dorsal horn and gracile nucleus after partial sciatic nerve injury, an injury that results in neuropathic pain behavior (Ma and Quirion, 2002). Recently, Zhuang et al presented more direct evidence that activation of JNK contributes to development and maintenance of mechanical allodynia after spinal nerve ligation. After L5 spinal nerve ligation, rapid activation of JNK was observed in L5 dorsal root ganglion neurons accompanied by slow and persistent activation of JNK in the same segmental spinal astrocytes. These data also suggested that neuronal and glial activation of JNK contribute to distinct mechanisms for development and maintenance of mechanical allodynia following peripheral nerve injury, not SCI (Zhuang et al., 2006).

pCREB

Cyclic adenosine 3', 5'-monophosphate response element-binding protein (CREB), is a 43kDa nuclear transcription factor that is activated by phosphorylation, pCREB, at the serine 133 site. Phosphorylated CREB binds to genes contain CRE promoters and begins the transcription process for a number of immediate genes (Ji and Woolf, 2001). The activation of MAP Kinases triggers phosphorylation of CREB and it is certain that pCREB is involved in nociceptive transmission through transcription of gene products that result in persistent allodynia. Crown et al demonstrated the first direct evidence that SCI induces an increased number and density of pCREB in STT neurons, 35 days after SCI (Crown et al., 2005). In addition, pCREB expression is correlated with mechanical allodynia in rats given a SCI (Crown et al., 2006). This finding suggests that transcriptional processes mediated by pCREB are critical for pain transmission in the chronic phase because STT neurons are one of the important tracts responsible for nociceptive transmission.

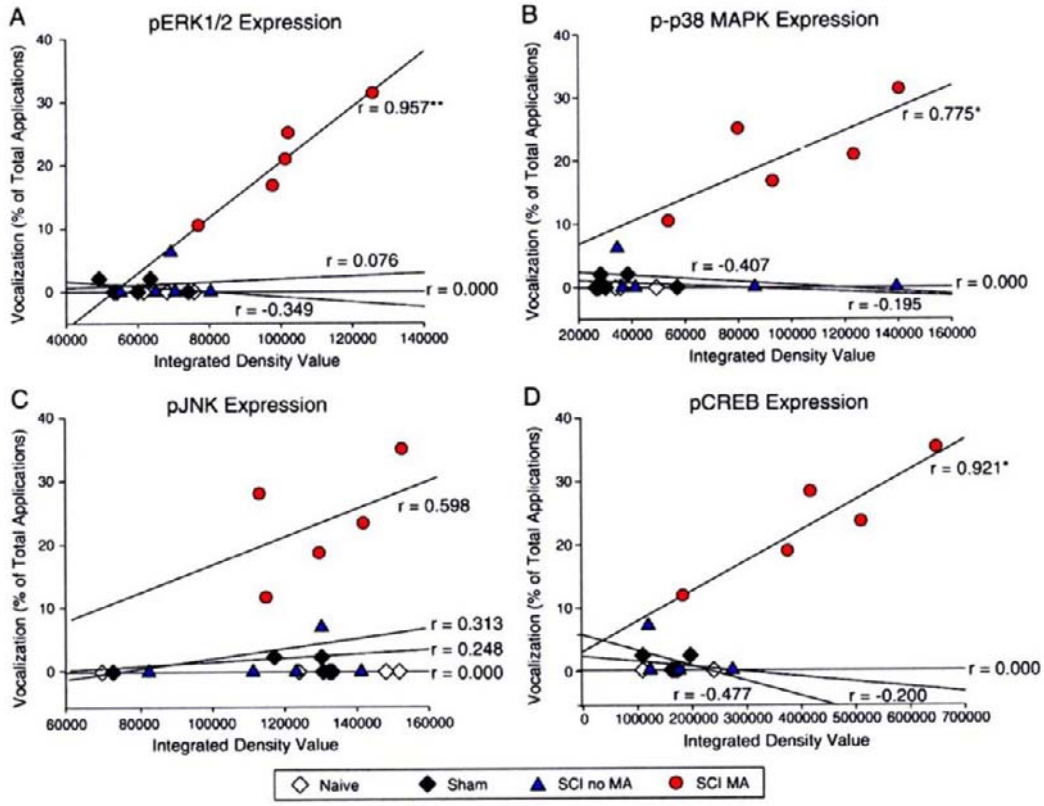

Figure 1. Correlations between vocalization reactivity and activation of pERK1/2, p-p38 MAPK, pJNK and pCREB. (A) The correlations for pERK1/2 expression and vocalization to the 26g force von Frey stimulus determined a significant positive relationship for SCI MA rats (**P<0.01) but not for any of the other three groups (P>0.05). (B) The correlations for p-p38 MAPK expression and vocalization to the 26 g force von Frey stimulus determined a significant positive relationship for SCI MA rats. (*P<0.05) but not for any of the others (P>0.05). (C) The correlations for pJNK expression and vocalization to the 26 g force von Frey stimulus failed to find significant relation ship for any of the 4 groups (P>0.05). (D) The correlations for pCREB expression and vocalization to the 26 g force von Frey stimulus determined a significant positive relationship for SCI MA rats (*P<0.05) but not for any of the other three groups (P>0.05). MA ; mechanical allodynia. Modified from Crown et al., 2006.

However, spinal excitotoxic injury by quisqualic acid, which activates AMPA and metabotrophic glutamate receptors but not NMDA receptors, did not induce activation of CREB (data not shown), but did induce activation of the other transcription factors such as ELK-1, via activation of ERK1/2 (Yu and Yezierski, 2005). These data suggest that CREB activation is mediated via NMDA receptors; whereas AMPA and metabotropic glutamate receptor activation preferential couple to downstream ELK-1 activation. Since spinal cord contusion injuries result in a 37 fold increase in glutamate concentrations extracellularly, which would stimulate indiscriminantly all glutamate receptors, then different SCI models produce mechanical allodynia through specific receptor mediated and distinct transcriptional pathways. However, in common is the activation of the MAPK family in the spinal dorsal horn, which is critically involved in hypersensitivity of nociceptive transmission. These

downstream pathways show activation of distinct pathways in different cell types contribute to the neuronal hyperexcitability by sensitization of CNS neurons.

Taken together, activation of intracellular downstream events via MAPK pathways importantly contribute to the long-lasting hyperexcitability driven, continued receptor/channel activation, and persistent changes in membrane potential that contribute to continued intracellular pathway activation via transcriptional/translational events for modulation of specific genes and target protein expression after SCI.

PHENOTYPES OF SPINAL DORSAL HORN NEURONS

Following SCI, dorsal horn neurons become hyperexcitable; that is, they are easier to be activated and show more enhanced and prolonged electrical activity in response to stimuli presented on the skin compared to normal neurons. Electrophysiological studies demonstrate changes in neuronal properties such as hyperexcitability of spinal dorsal horn neurons; specifically, increased spontaneous and evoked activities, lowered thresholds and prolonged afterdischarge activity following SCI (Drew et al., 2001; Gwak et al., 2006; Hains and Waxman, 2006). One important factor to consider for electrophysiological studies of spinal dorsal horn neurons is a consideration of phenotypical changes following SCI. Spinal dorsal horn neurons are divided into three different types dependent on their activity pattern in response to various intensities of mechanical stimulation: 1) Low threshold (LT) neurons show increased activity in response to non-noxious stimuli (like brush stimuli with camel hair brush); 2) High threshold (HT) neurons show increased activity in response to moderate and noxious mechanical stimuli (noxious means the intensity may produce tissue damage) but show few or no responses to non-noxious stimuli; 3) Wide dynamic range (WDR) neurons show graded activity in response to increasing intensity of stimulation; ie., greater stimulus intensity produces greater response activity (Chung et al., 1986; Dougherty and Willis, 1991). Several lines of electrophysiological studies suggested that WDR neurons mediate the generation of pain more than other types of spinal dorsal horn neurons after injury (Coghill et al., 1993; Maixner et al., 1986). In addition, the spinal cord consists of well organized somatotopic structures. Spinal laminae I– II (called superficial layer of spinal dorsal horn) and V-VI (called deep dorsal horn) receive nociceptive information from C- and Aδ primary afferent fibers whereas laminae III-IV receive non-nociceptive information from myelinated Aβ primary afferent fibers.

One interesting observation determined from the electrophysiological studies is that unilateral SCI produced a higher incidence of WDR neurons proportionally; whereas LT and HT neurons showed decreased proportions compared to sham and naïve controls (Paik et al., 2000; Hains et al., 2003b; Xu et al., 2002). More specifically, using a random search technique, the number of WDR neurons recorded in superficial dorsal horn layers were increased whereas the number of HT (or NS, nociceptive specific) neurons decreased after spinal hemisection injury compared to sham controls. In the deep dorsal horn, the numbers of WDR neurons were increased whereas the number of LT neurons decreased after spinal hemisection. Addionally, these changes in neuronal proportions were observed in both ipsilateral (injured side) and contralateral (uninjured side) sides of spinal dorsal horn despite the unilateral nature of the SCI. This proportional shift is a distinctly different mechanism

compared to peripheral nerve injury since no peripheral nerve injury model produces pain hypersensitivity in the contralateral side. However, unilateral SCI induces bilateral pain hypersensitivity via bilateral neuronal hyperexcitability mediated by plasticity of neuronal populations not only in both of the hindlimbs (below-level pain), but surprisingly in both of the forelimbs as well (above-level pain) (Christensen and Hulsebosch, 1997).

While phenotypic changes of the spinal dorsal horn neurons certainly occur, providing the substrate for the change in electrophysiological response properties, there is little additional evidence and no detailed mechanisms which can account for the changes in second order neurons following SCI. However, recent electrophysiological and immunohistochemical data suggests possible mechanisms. To review, LT neurons, predominantly in the deep dorsal horn, show strong responses (electrophysiological activity) to non-noxious stimuli whereas HT neurons, predominantly distributed in the superficial dorsal horn, show strong activity to noxious stimuli. However, after SCI, LT neurons become more responsive to noxious stimuli whereas HT neurons become more responsive to non-noxious stimuli. Thus, both populations have changed their somatosensory encoding response properties.

A reasonable explanation for the change in response properties is newly formed synaptic circuits, via degeneration and sprouting (of primary afferent, intrinsic and descending systems), that contribute to neuronal phenotypical changes in the spinal dorsal horn. We know that SCI increases the endogenous level of nerve growth factor (Gwak et al., 2003) and promotes the primary afferent fibers sprouting in the dorsal horn (Hulsebosch, 2002). For example, fine primary afferent fibers containing CGRP, an excitatory neuropeptide, is primarily distributed in superficial dorsal horn (laminae I-II). However, SCI induces the sprouting of CGRP containing primary afferent fibers into deep dorsal horn (laminae III-IV). In addition, treatments of anti-NGF prevent the CGRP containing primary afferent fiber sprouting and hyperexcitability of spinal dorsal horn neurons as well as neuropathic pain behaviors. (Ackery et al., 2007; Christensen and Hulsebosch, 1997; Gwak et al., 2003). Thus synaptic reorganization induced by abnormal sprouting of primary afferent fibers suggests a possible mechanism for the changes in the neuronal populations following SCI. Additionally, Kalous et al reported that SCI induces changes in response properties and synaptic reorganization in both spinal and supraspinal regions (Kalous et al., 2007). After peripheral nerve injury, Woolf et al demonstrated that sprouting of Aβ fibers, that convey non-noxious stimuli and normally terminate in the deep dorsal horn (larmina III-V), sprouted into the superficial dorsal horn (larmina I-II) after peripheral axotomy (Woolf et al., 1995). Thus, the newly formed networks enable deep dorsal horn neurons, that normally receive only non-noxious input, to receive primary afferent noxious input (from the CGRP data); whereas superficial dorsal horn neurons that could now receive Aβ fibers after SCI would respond to non-noxious inputs. The other possibility is a loss of encoding abilities of WDR neurons (Palecek et al., 1992). However, this is not reasonable because SCI resulted in a higher proportion of WDR neurons. If WDR neurons lost their encoding properties, the population would be decreased rather than increased. Finally the loss of endogenous inhibitory neurons, such as GABAerigic and opioid inputs, would cause a shift of the neuronal response properties following SCI (see below) and induce hyperexcitablilty of spinal dorsal horn neurons, which is what we observed (Gwak et al., 2006; 2008).

SPINAL GLIA

Physiological and Structural Neuronal Partners

There are two major neural cells in the central nervous system: nerve cells or neurons and glial cells. Glial cells are composed of astrocytes, microglia and oligodendrocytes in the central nervous system. In the peripheral nervous system, the oligodendrocytes are replaced by Schwann cells, and both provide tight layering of membranes around nerve processes, extruding the cytoplasm, such that the result is ionic insulation of the nerve process by wrappings of glia membranes called myelin. Unmyelinated nerve fibers do not have the multiple membrane wrappings but have lose cytoplasmic associations with glia cells which allows continuity with the extracellular space through mesoaxons. Glial cells (or glia) play very important roles in maintaining extracellular glutamate homeostasis and general physiological homeostasis of the extracellular spaces. More recently glia have been described as playing critical roles in the modulation of neuronal excitatory and inhibitory circuits in sensory systems (Anderson and Swanson, 2000; Chesler and Kaila, 1992; Newman, 2003). In the central nervous system, glial processes contribute to synaptic cleft regulation of substances released by glia and neurons thereby enabling modulation of neurotransmission to regulate physiological states via neurotransmitter receptors and ion channels. The neuronal-glial interaction is mediated by positive bidirectional feedback (Araque et al., 1999). It is important to note that astrocytes and microglia outnumber neurons and play important roles in maintaining homeostasis in the central nervous system (Kuffler et al., 1984). In the healthy nervous system, glia closely maintain certain levels of neurotransmitter concentrations, regulate pH, are involved in glutamate uptake and are involved in other regulatory process through their close interactions with neurons. Unfortunately, injury, stress and/or inflammation cause glial "activation" which changes the physiological function of glial cells that results in abnormal and maladaptative alterations of synaptic circuits and secondary neural damage. Activated glia (see below) have different intracellular pathways, secrete different substances, transporters are reversed and are considerably altered such that their role in modulating somatosensorry information is aberrant and produces severely changed somatosensory transformation of peripheral stimuli in which non-noxious stimuli are now perceived as extremely noxious and spontaneous pain syndromes may develop.

Glial Activation Following Spinal Cord Injury

The classic evidence of "activated" or "reactive" astrocytes and microglia is based on morphological changes wherein both astrocytes and microglia commonly show soma hypertrophy after injury. In addition, activated astrocytes exhibit increased density of intermediate filament deposition and have branches that are thickened, as well as elongated processes; whereas activated microglia exhibit proliferation after injury and demonstrate branches that thicken near the soma and appear to retract (Baron et al., 1990; Garrison et al., 1991; Gould and Goshgarian, 1997; however see Davalos et al., 2005). After SCI, a 37 fold increase in extracellular glutamate is present which initiates glutamate uptake by astrocytes.

High affinity glutamate transporters are identified in astrocytes and the accumulation of glutamate in astrocytes triggers uptake of K^+ by Na^+/K^+–ATPase (Bender et al., 1998). The accumulation of K^+ leads to opening anion channel to enhance the passive influx of Cl^-, K^+, HCO_3^- and followed by H_2O accumulation in the astrocytes. With the hypertrophy of glial soma, activated astrocytes and microglia show upregulation of receptors, ion channels and neurotransmitter tranporters that potentiate neuronal-glial interactions. For example, upregulation of glutamate receptors induces increased intracellular calcium concentrations that trigger the activation of intracellular downstream pathways, which induces persistent changes in glial physiological roles. The abnormal physiology, or gliopathy, results in release of putative neurotransmitters and proinflammatory cytokines that alters synaptic physiology.

Functionally activated glia produce proinflammatory cytokines, reactive oxygen species (ROS), ATP, excitatory amino acids (EAAs), nitric oxide (NO) and etc. (Johnstone et al., 1999; Martin, 1992; Piani et al., 1992; Shafer and Murphy, 1997; Tanaka et al., 1994); which are powerful candidates for mediating pain following neural injury. These factors modulate neuronal response properties via changing the properties and/or activation state of receptors, ion channels, protein kinases and transcription factors altering intracellular pathways in both pre- and postsynaptic neurons (Lampert et al., 2006; Lee et al., 2007). Finally, all of the factors are known to produce neuronal hyperexcitability in dorsal horn neurons in the normal spinal cord and are most likely key factors after SCI.

The role of activated glia in neuropathic pain was first investigated using peripheral neuropathy models. Spinal nerve ligation and sciatic nerve injury induced glial cell activation that is characterized by increased immunoreaction product for GFAP (for astrocytes) and CD-11b (OX-42, for microglia) accompanied by soma hypertrophy, thickened processes of astrocytes and ramified processes of microglia. These observed glia changes correlated with allodynia and hyperalgesia (Coyle, 1998; Meller et al., 1994; Watkins et al., 1997). Additionally, blockade of glial activation prevented mechanical allodynia and thermal hyperalgesia. For example, intrathecal or systemic treatment of propentophylline, a putative glial activation inhibitor, inhibited glia hypertrophy in both microglia and astrocytes, attenuated mechanical allodynia in hindlimbs and attenuated the hyperexcitability of dorsal horn neurons in the segments of lumbar enlargement that provide innervation of the hindlimb (Gwak et al., 2008; Sweitzer et al., 2001; Tawfik et al., 2007). Although propentofylline is not a specific glial inhibitor, PPF is a phosphodiesterase inhibitor which suggests that phosphodiesterase inhibitors would be useful in preventing and treating chronic pain after SCI.

The temporal effect of glial activation on nociceptive transmission is not clearly understood. Activated microglia are proposed by one group to be involved in the early phase of peripheral neuropathic pain whereas activated astrocytes are proposed to be involved in the late phase of neuropathic pain following peripheral nerve injury (Zhuang et al., 2005). However, in SCI both activated astrocytes and microglia are involved in the late phase of central neuropathic pain several segments below and at the level of SCI (Gwak et al., 2008; Hains and Waxman, 2006). In addition, several studies demonstrate activation (or reactive) astrocytes and microglia after peripheral nerve injury, presumably providing a substrate for the observed mechanical allodynia and thermal hyperalgesia (Sweitzer et al., 2001; Tawfik et al., 2007). We hypothesize that SCI produces persistent activation of astrocytes and microglia that contributes to neuropathic pain behavior, the development and persistence of hyperexcitability of spinal dorsal horn neurons (which take several weeks to develop in both

people and in rodent models) that persists for life (Christensen and Hulsebosch, 1997; Grossman et at., 2001). It should be remembered that immediately and within days to weeks following SCI, there are numerous factors that would perpetuate glia activation. Initial events immediately after SCI are the surge in extracellular glutamate concentrations, followed hrs to days later by significant increases in levels of proinflammatory cytokines (Peng et al., 2006) and later by overexpresion of excitatory amino acid receptors (Gwak and Hulsebosch, 2005), changes (increases or decreases) in neurotransmitters and transporters (Vera-Portocarrero et al., 2002) and other processes that contribute to alterations in somatosensory processing (see Hulsebosch, 2002, 2005 for reviews). Thus, any intervention may have a delayed response, particularly if the events are cumulative and multi-factorial ie. glial to neuronal to intracellular signaling in glia or neurons, to alterations in transmitter/receptor/transporter expression. Thus "gliopathy", which is characterized by increased release of gliotransmistters, proinflammatory cytokines, upregulation of membrance bounded receptors/ion transporters is a key player in the hyperexcitability of spinal dorsal horn neurons.

Neuronal-Glial Interactions

Glial processes contribute to form part of the regulatory environment of the synaptic cleft with pre- and postsynaptic neurons. We now know that glia have various receptors and ion channels, which were thought to be only on neurons, such as ionotropic and metabotropic glutamate receptors, peptidergic, purinergic, adrenergic, serotonergic, chemokine and GABAergic receptors as well as sodium, calcium and potassium channels (Biber et al., 1999; Fiebich et al., 1996; Gottlieb and Matute, 1997; Kuhn et al., 2004; Lai et al., 2002; Meller et al., 2002; Tsuda et al, 2003). Neurons release neurotransmitters and neuropeptides, which influence glia function. After SCI, high extracellular glutamate concentrations initiate activation of glia via resultant increases in intracellular calcium concentrations, increases in calcium independent pathways and increases in ATP, GTP etc. pathways via activation of membrane receptors or ion channels (Parpura and Haydon, 2000; Wang et al., 2000).

Although specific mechanisms of astrocytic and microglial activation are not known, activation of different pathways in different glial cell types may contribute to neuronal hyperexcitability by downstream pathways and glial production of proinflammatory factors that are known to sensitize CNS neurons (Nesic et al., 2005). Electrophysiological study demonstrated that glial activation contributes the hyperexcitability of dorsal horn neurons. Arriagada et al. reported that IL-1β driven wind-up is inhibited by propentofylline. For example, attenuation of spinal glia activation can mediate C-fiber mediated effects on dorsal horn neurons (Arriagada, et al., 2007). More directly, Gwak et al. demonstrated that inhibition of glial activation attenuated the hyperexcitability of WDR dorsal horn neurons (Gwak et al., 2009b).

Recent literature demonstrates that activated glia modulate intracellular downstream pathways. For example, several segments below the site of spinal contusion injury, pERK activation was demonstrated in microglia, but not in neurons or astrocytes. The increased pERK was hypothesized to lead to increased microglial PGE_2 release, which is known to contribute to neuronal hyperexcitability and mechanical allodynia after SCI (Zhao et al., 2007). By comparison, in another study, several segments below a unilateral thoracic SCI, activated p38 MAPK was expressed in micoglia and neurons, but not in astrocytes. In

addition, pharmacological blocks of activation of p-p38 MAPK resulted in reduced neuronal hyperexcitability and allodynic behavior (Garry et al., 2005; Gwak, et al., 2009a). Taken together, SCI produces differential activation of astrocytic and microglial pathways leading to increased extracellular glial cytokine production, specifically via p-p38 and pERK to pCREB pathways in the spinal dorsal horn (Crown et al., 2006), that render spinal dorsal horn neurons hyperexcitable.

CELLULAR MECHANISMS OF HYPEREXCITABILITY : LOSS OF DESCENDING INHIBITORY PATHWAYS

As described earlier, the balance between excitatory and inhibitory input is a key factor in modulating nociceptive transmission in the spinal cod. The descending inhibitory pathways originated from periaquctal gray (PAG) matter in midbrain, locus ceruleus (LC) in pons and nucleus raphe-magnus (NRM) in the medulla and project to the spinal cord. It is certain that the descending inhibitory tone is decreased by SCI below the level of injury (Gwak et al., 2006), which physically severs the tracts from their projection targets. Additionally, decreased inhibitory tone appears to occur with peripheral nerve injury and inflammation (Mooer et al., 2002). However, the mechanisms that account for the loss of endogenous inhibitory tone is not clear. One speculation is that neuronal-glial interactions may change and the canges modulate descending inhibitory tone after injury. In the following section, the role of glial modulation of endogenous GABAergic and opioidergic inhibitory tone will be considered.

Loss of Endogenous GABAergic Inhibitory Tone

Several lines of electrophysiological and immunohistochemical studies demonstrate that loss of endogenous GABAergic inhibitory tone produces neuronal hyperexcitability and mechanical allodynia following SCI. As background, spinal GABA is a major inhibitory neurotransmitter, synthesized by the rate-limiting enzyme glutamic acid decarboxylase (GAD), that exists as two different isoforms, GAD_{65} and GAD_{67} (Bowery et al., 1987; Erlander et al., 1991; Todd and McKenzie, 1989). GABA occurs in 24-33% of the interneurons in laminae I-IV in the spinal cord dorsal horn (Bowery et al., 1987; Hunt et al., 1981). GABA receptors are widely distributed in the spinal dorsal horn and exert inhibitory effects via two receptor subtypes: $GABA_A$ and $GABA_B$. Although $GABA_C$ receptors have been identified in the central nervous system, the role of $GABA_C$ receptors in nociceptive transmission in the spinal cord is not clear. $GABA_A$ and $GABA_B$ receptors are located at pre- and post-synaptic sites to primary afferents terminals (Malcangio and Bowery, 1996). $GABA_A$ receptors mediate hyperpolarization of postsynaptic neurons by increasing the permeability to anions, such as chloride ions and increases the resting membrane conductance and also short circuits any excitatory current flowing into the cell (Bowery, 1982). $GABA_B$ receptors mediate presynaptic inhibition of the release of neurotransmitters such as excitatory amino acids or neuropeptides by a reduction in calcium entry (Curtis et al., 1981; Dunlap,

1984; Huston et al., 1990). Postsynaptically, $GABA_B$ receptors hyperpolarize the postsynaptic neurons by increased potassium conductance (Howe et al., 1987).

It is well documented that endogenous GABAergic systems modulate nociceptive input in both normal and pathological states (Hao et al., 1992; Hao et al., 1994; Sokal and Chapman, 2001). In normal conditions, blockade of the GABA receptors results in hyperexcitability of spinal dorsal horn neurons (Sorkin et al., 1998). In pathophysiological conditions, several lines of evidence demonstrate the loss of GABAergic inhibitory tone in the spinal dorsal horn. For example, immunohistochemical studies demonstrate that spinal ischemic injury decreases the GABA immunoreactivity in the spinal cord (Zhang et al., 1994). Specifically, in one set of experiments after spinal ischemic injury induced by intravascular reaction with organic photosensitizing dye, GABA immunoreactivity decreased after 2-3 days. However, there was little morphological evidence of neuronal death and the GABA immunoreactivity increased to near normal levels 15 days after injury. Thus, the data suggests that decreased GABA content and not decreased number of GABA cells plays a critical role in pain development after ischemic SCI. Intrathecal and topical application of muscimol ($GABA_A$ receptor agonist) and baclofen ($GABA_B$ receptor agonist) significantly attenuated mechanical allodynia and dorsal horn hyperexcitability following SCI and inflammation (Garcia-Nicas et al., 2006; Gwak et al., 2006). To demonstrate receptor specificity, GABA antagonists (Bicuculline for $GABA_A$ and Phaclofen for $GABA_B$) were paired with the agonists and the inhibition effect of muscimol and baclofen was prevented (Gwak et al., 2006).

It should be noted that SCI and specific inflammation models are not the only models that demonstrate a loss of GABAergic tone in the dorsal horn. With regard to peripheral nerve injury, levels of spinal GABA (Castro-Lopes et al., 1993) and GABA receptors (Castro-Lopes et al., 1995) are reduced. In addition, intrathecal application of muscimol and baclofen attenuated mechanical allodynia following peripheral nerve injury (Hwang and Yaksh, 1997). Additionally, transplantation of neuronal cells bioengineered to synthesize GABA (Eaton, et al., 1999) attenuate mechanical allodynia after nerve injury. Interestingly, this transplantation treatment produced permanent attenuation of mechanical allodynia. Taken collectively, the behavioral, morphological and electrophysiological studies indicate that alterations in both $GABA_A$ and $GABA_B$ receptor mediated pathways, as well as other hypofunction of GABA systems in general, are involved in pathological pain states after neural injury.

However, the detailed mechanisms for the loss of GABAergtic tone in the pathophysiological condition discussed above are not fully understood. Since damage of spinal cord essentially interrupts the descending inhibitory pathways (Antal et al., 1996) for all spinal circuits below the lesion, a reasonable explanation is that the diminished descending inhibition contributes to loss of GABAergic tone. If a general loss of descending inhibitory influence contributes to mechanisms that subserve mechanical allodynia, then intrathecal delivery of any "lost" inhibitory transmitter would return the dorsal horn spinal circuits to control, non-allodynia conditions. The second line involves the selective loss of GABAergic interneurons in response to the well known intense action potential barrages, with concomitant glutamate release in cytotoxicy levels (Rooney et al., 2007) that results from cutting nerves, which certainly occurs with SCI. These barrages would be transmitted both orthodromically and antidromically, would result in a temporary high discharge over the first hour after neurotrauma in populations of neurons several segments away, with a resultant increase in extracellular glutamate concentrations. Since GABA interneurons in laminae II

demonstrate high concentrations of glutamate receptors, then the GABAergic interneurons would demonstrate increased sensitivity and selective loss. Additionally, SCI would be accompanied by an increase in reactive oxygen species (ROS) that could reach the parenchyma directly or via the circulation. Several recent studies have demonstrated that activation of ROS modulates GABAergic inhibitory function on both pre and postsynaptic sites which may result in increased susceptibility to cell death particularly of GABAergic neurons in response to exposure to ROS or ischemia in general (Sah et al., 2002; Sharma and Sjoguist, 2002). Finally, GABAergic tone is modulated by proinflammatory cytokines, which are released from activated glia produced by SCI (Vikman et al., 2007). Thus, there are several mechanisms that may contribute to phenotypic alterations of the GABAergic tone in sensory circuits in the dorsal horn after SCI.

Modulation of Endogenous Inhibitory Circuits: Glial Modulation of GABAergic Tone

Several studies demonstrate that SCI produces a loss of endogenous spinal GABAergic inhibition both near the injury level and several segments remote from the injury (Drew et al., 2004; Gwak et al., 2006; Liu et al., 2004). Anatomically, GABAergic form both pre- and postsynaptic contacts onto neurons and thus can modulate nociceptive transmission in the central nervous system. Most GABAergic neuronal contacts are predominantly axodendritic and axosomatically synapses and only a minor show axoaxonic synapses. However, it should be remembered that synaptic regulation of GABA involves neuronal presynaptic, postsynaptic and extrasynaptic mechanisms in concert with glial interactions.

Two hypothesis are offered to explain the loss of GABAergic tone, which can be mediated by neuronal-glial interactions: 1) a decrease in the number of GABAergic neurons, such as would occur by selective apoptosis or necrosis of GABAergic cells; 2) downregulation of GABAergic tone without a decrease of number of GABAergic neurons but by a phenotypic change in transmitter expression. In the last decade, there has been no direct evidence that GABAergic cell death is mediated by activated glia following SCI. Thus, the hypothesis of a decrease of GABAergic tone by decreased number of GABAergic cells is not consistent with the data. However, there are several lines of evidence that demonstrate that the downregulation of the GABA transmitter is responsible for the loss of GABAergic tone following SCI and that glial modulation play a critical role in this process. The direct evidence for glial modulation of GABAergic tone is demonstrated by the change in GABA synthase enzyme (GAD) study following SCI. Two isoforms of the GAD protein are characterized in the spinal cord. GAD_{65} is a membrane associated protein and produces vesicular GABA released by exocytosis. Thus, GAD_{65} is involved in rapid and focal communication via synaptic mechanisms to specific neuronal circuits. However, the second isoform, GAD_{67}, is cytosolic and thus release is via extrasynaptic mechanisms. Thus, GAD_{67} is involved in paracrine signaling or intracellular metabolites. Recently, gene therapy approaches using viral vectors which consistently produce GAD_{65} and GAD_{67} protein, demonstrate attenuation of mechanical allodynia following SCI (Hao et al., 2005; Liu et al., 2004). These data suggest that the downregulation of both GAD proteins contributes to the mechanical allodynia after SCI.

Recently, Gwak et al demonstrated that neuronal-glial interactions are able to modulate GAD_{65} protein expression following SCI. Using immunocytochemistry, astrocytes and microglia demonstrate "activation" by increased GFAP or OX-42, respectively and results in downregulation of GAD_{65} protein expression after SCI. Early intrathecal administration of propentophylline reduced astrocytic and microglial activation and prevented the loss of GABAergic inhibitory tone, as measured by preserved levels of the GABA synthase enzyme, GAD_{65}, and attenuated mechanical allodynia for weeks after SCI. The downregulation of GAD_{65} expression level is predominantly observed in the superficial dorsal horn. Immunocytochemical data demonstrated high correlation with western blot analysis (Gwak et al., 2008). These data suggest that a tight coupling of glial/neuronal interaction occurs, and that after SCI, "activated" glial cells contribute to loss of GABAergic inhibitory tone. In other word, activated glia leads to mechanisms that produce hyperexcitability of spinal dorsal horn neurons via decrease of GABAergic tone following SCI (Gwak et al., 2008).

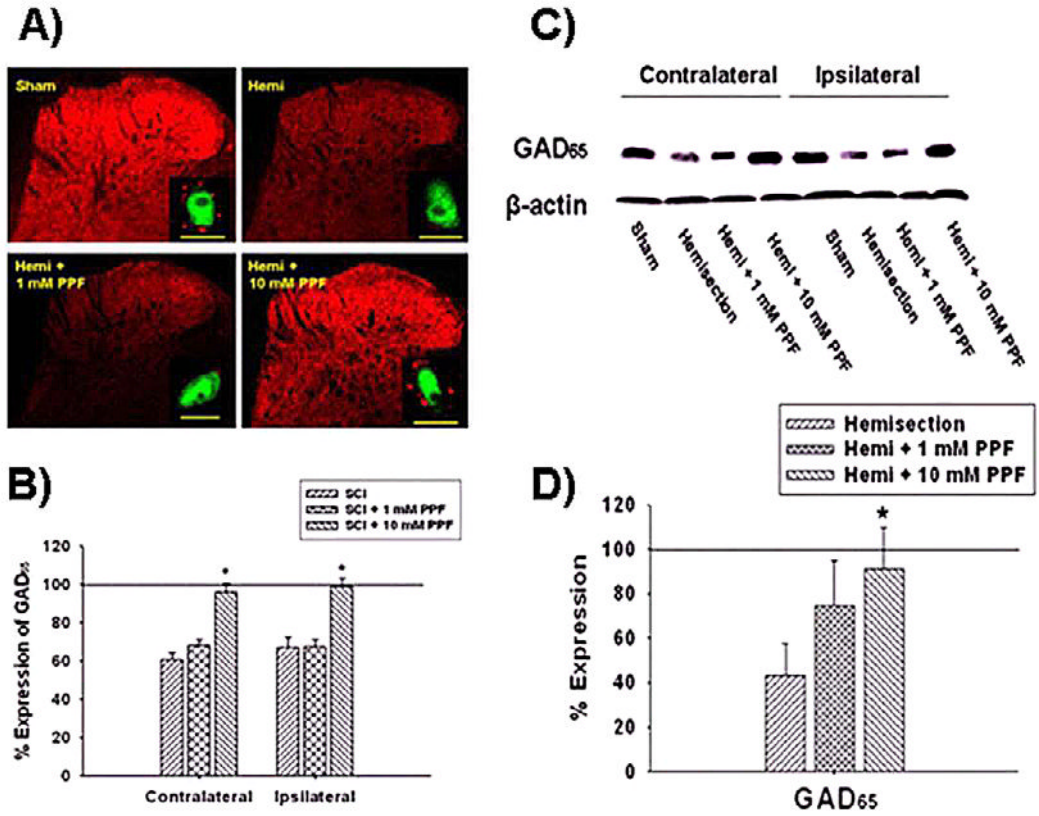

Figure 2. Inhibition of glial activation prevents the downregulation of glutamic acid decarboxylase $(GAD)_{65}$ levels. (A) low magnification shows GAD_{65} expression in the entire dorsal horn and high magnification (scale bar : 20 μm) shows the detail expression of GAD_{65} with neurons (green) and GAD_{65} (red) around neurons in larmina II. (B) Intrathecal treatment with 10 mM PPF significantly attenuated the downregulation of GAD_{65} expression whereas 1 mM PPF did not produce significant changes compared to SCI alone group (*$p<0.05$). (C) Western Blot data shows that spinal cord injury induces decrease of GAD_{65} mRNA protein expression level on lumbar spinal cord compared to the sham controls. (D) Intrathecal treatment with 10 mM PPF significantly prevented the decrease of

GAD_{65} mRNA protein expression level whereas 1 mM PPF did not show any significant changes compared to SCI alone group (*p<0.05). Modified from Gwak et al., 2008.

However, the molecular mechanisms that result in downregulation of GAD protein expression through neuronal-glial interactions after SCI are not clear. One physiological role of glia is to modulate extracellular GABA concentrations by GABA uptake via GABA transporters (Borden, 1996). GABAergic neurons secrete GABA by synaptic and extrasynaptic mechanisms into the extracellular space, where GABA concentrations can become high for milli-seconds. However, GABA is taken up by astrocytes via the high affinity GABA transporters to regulate extracellular GABA concetnrations. Five different GABA transporters have been cloned: a vesicular GABA transporter (VGAT) and four Na^+-dependent transporters in the SLC-6 family (Borden et al., 1992: Guastella et al., 1990). The SLC-6 family is species-dependent, mGAT1-mGAT4 in mouse correspond to rGAT-1, rGAT-2 and rGAT-3 in rat. GAT 1 is widely distributed in neurons and axons (the "neuronal transporter") whereas GAT 2 (BGT-1), GAT 3 and GAT4 are predominantly distributed in astrocytes (the "glial transporters"). GAT-1, GAT-2, GAT-3 are high affinity transporters whereas BGT-1 is a low affinity transporter. These sodium coupled transporters are particularly dense in the synaptic cleft and serve to keep the extracellular neurotransmitter concentrations below neurotoxic level and also serve to terminate the synaptic action of the neurotransmitter (Nelson, 1998). Following GABA uptake, GABA is converted to glutamate by GABA transaminase, after which glutamate is converted by glutamine synthase to glutamine, which serves as a substrate for the production of glutamate and GABA if neurons contain GAD (Schousboe et al., 1993). As we described early, high concentration of glutamate trigger intracellular events that produce altered expression of target proteins. Thus, massive release of GABA from neurons into the extracellular space, and reuptake of extracellular GABA by activated glia is one mechanism (the "GABA-glutamate-glutamine cycle") for the control of GABA synthesis by modulation of GAD expression via concentration dependent feedback mechanisms (Broer et al., 2004; Struzynska and Sulkowski, 2004).

Loss of Endogenous Opioidergic Inhibitory Tone

The opioid peptides are frequently used in pain management and are in two broad classifications: those that are synthesized in the animal (endogenous) or those that are synthesized outside the animals (exogenous). The endogenous opioid groups are divided into three groups: dynorphins, enkephalins and β-endorphin, which are derived from larger translation products, PENK, PDYN and POMC, respectively. Anatomically, the opioid containing neurons are predominantly distributed in the pain processing regions of the CNS, such as the spinal cord, thalamus, periaqueductal grey (PAG), cortex as well as the autonomic nervous system.

Opioid receptors are G-protein coupled receptors and mediate analgesic effects via inhibition of Ca^{2+} channels and activation of G-protein gated inwardly rectifying K^+ (GIRK) channels. Three families of opioid receptors were cloned in 1990's including μ-opioid receptor (MOR1), δ-opioid receptor (DOR1) and κ-opioid receptor (KOR1) (Fukuda et al., 1993; Kieffer et al., 1992; Nishi et al., 1993). Dynorphins, enkephalins and β-endorphin show

high affinity with κ, δ and μ receptors, respectively. Another opioid receptor is the "orphan" opioid-like receptor (ORL1) and its ligand is nociceptin or orphanin FQ (Reinscheid et al., 1995).

Although opioids show strong analgesic effect in several kinds of pain conditions, such as causalgia, neuropathic pain and inflammatory pain, the efficacy of opioid treatment after SCI is controversial. Several animal studies showed that acute morphine treatment attenuates neuropathic pain after SCI. For example, intrathecal treatment of morphine had an antinociceptive effect after ischemic spinal cord (Yu et al., 1997). Additionally, Kim et al reported that intrathecal administration of morphine attenuates mechanical allodynia, and intrathecal treatment produced greater analgesic effects than systemic morphine treatment, with no motor weakness, following SCI (Kim et al., 2003). Although several animal models indicate that morphine has antiallodynia or antihyperalgesic effects after SCI, the patient studies are controversial. Attal et al reported that intravenous morphine treatment attenuates mechanical allodynia in central pain patients (Attal et al., 2002). However, Parisod et al reported that morphine treatment produces allodynia in SCI patients who experience chronic neuropathic pain (Parisod et al., 2003). Furthermore, this case reported that intrathecal low dose and acute, but not high doses, as well as long term, opioid treatment produced allodynia in chronic neuropathic pain after SCI. Surprisingly, some studies suggest that morphine produces allodynia and hyperalgesia, the so called "anti-analgesic effect" that is frequently observed with repeated morphine treatments that result in morphine tolerance. The development of tolerance (greater subsequent doses are needed to achieve the same effect as initial low dose) to morphine or other opioids is one possible mechanism for decreased opioidergic tone.

Modulation of Endogenous Inhibitory Circuits : Glial Modulation of Opioid Effects

It is well documented that opiate therapy is widely used in acute and chronic pain treatments via pain pathway inhibition in the spinal cord. However, high doses and chronic treatment regimens of morphine result in morphine tolerance, so called analgesic tolerance. For example, Christensen and Kayser demonstrated that systemic treatment of morphine attenuated the mechanical and thermal sensitivity in the acute phase, whereas there was no effect in the late phase due to morphine tolerance after peripheral nerve injury (Christensen and Kayser, 2000).

Morphine tolerance is not fully understood, but one speculation is that activated glia, with resultant production of proinflammatory cytokines contributes to morphine tolerance (Raghavendra et al., 2004; Song and Zhao, 2001). The specific mechanism by which glial activation modulates morphine tolerance is not clear, but the glial specific glutamate transporters appear to be important in this mechanism. For example, chronic treatment with morphine produces a down regulation of glial glutamate transporters, increases thermal hyperalgesia, both which are known to occur in hyperalgesia after peripheral nerve injury model (Mao et al., 2002). Glutamate transporters in glia regulate extracellular concentrations of glutamate and prevent persistent and over-accumulation of glutamate concentrations in the synaptic zone, and prevents continued glutamate receptor activation, which would produce continued neuronal hyperexcitability.

Another possible mechanism in the development of morphine tolerance is release of proinflammatory cytokines from activated glia. Proinflammatory cytokines, especially IL-1β are known to modulate the function of the opioid receptor. Intracerebroventricular (i.c.v.) administration of the IL-1 receptor antagonist or pretreatment with IL-1β antiserum diminished the effect of intrathecal dynorphin anti-analgesia whereas the IL-1β agonist produced anti-analgesia (Rady and Fujimoto, 2001). These findings suggest that proinflammatory cytokines increase pain sensitivity, duration and efficacy, via inhibiting endogenous opioid inhibitory circuits (Raghavendra et al., 2004; Shavit et al., 2005). In addition, morphine can activate glia by binding opioid receptors and initiating activation of MAP Kinases, especially ERK1/2, which is known to promote the release of proinflammatory cytokines, such as IL-1, IL-6, TNFα, reactive oxygen species as well as neurotransmitters which are known to enhance pain transmission in the spinal dorsal horn (Johnston et al., 2004; Mouledous et al., 2004). *In vivo* and i*n vitro* studies suggested that repeated morphine treatment results in phosphorylation of MAP Kinases, such as p-p38 MAPK, pERK, and pJNK as well as transcription factors, such as pCREB. Additionally, immunocytochemistry co-localization studies showed that phosphorylated MAP Kinases and transcription factors were co-localized with substance P and calcitonin gene-related peptide, both of which are known to serve as excitatory neuropeptides in pain pathways (Ma et al., 2001). Taken together, these findings suggest that allodynia and hyperalgesia produced by morphine tolerance show similar cellular mechanisms of neuronal hyperexcitability to neuropathic models. Furthermore, these studies suggest that non-neuronal cells, such as astrocytes and microglia, play very important roles in morphine tolerance (Watkins et al., 2005).

CONCLUSION

Neuronal hyperexcitability is the predominant mechanism providing a substrate for abnormal pain symptoms produced by neurotrauma and/or inflammation. The development and maintenance of hyperexcitability is not only precipitated by neuronal events, but also glial cells play a key role in developing and maintaining neuronal hyperexcitability. Neurons and glia appear to stimulate each other through ligand release/ receptor mediated pathways which then trigger activation of membrane bounded protein and intracellular downstream events which then result in continued release via a positive feed forward cycle. While pain mechanisms are not fully understood, and thus effective long term therapies are elusive, particularly for chronic pain syndromes, a broader spectrum of cellular analyses is needed. Normal neuronal-glial interactions are critical for maintenance and survival, but abnormal interactions lead to maintained neuronal hyperexcitability. In particular, glial function, both astrocytes and microglia, are permanently altered after neurotrauma leading to the so called "glial activation." We propose that this abnormal glial state is a diseased state, a "gliopathy." Once mechanisms of gliopathy that lead to maintained neuronal hyperexcitability are understood, therapeutic treatment for chronic pain control will follow.

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REFERENCES

Ackery AD, Norenberg MD, Krassioukov A. *Calcitonin gene-related peptide immunoreactivity in chronic human spinal cord injury.* Spinal Cord 2007;45(10):678-86.

Anderson CM and Swanson RA. *Astrocyte glutamate transport: review of properties, regulation, and physiological functions.* Glia 2000;32:1-14.

Antal, M., Petko, E., Polgar, E., Heizmann, CW., and Storm-Mathisen, J. *Direct evidence of an extensive gabaergic innervation of the spinal dorsal horn by fibers from the rostral ventromedial medullar*. Neuroscience 1996;73:509-518.

Araque A, Parpura V, Sanzgiri RP, Haydon PG. *Tripartite synapses: glia, the unacknowledged partner*. Trends Neurosci. 1999;22(5):208-15.

Arriagada O, Constandil L, Hernandez A, barra R, Soto-Moyano R, Laurido C. *Effects of interleukin-1beta on spinal cord nociceptive transmission in intact and propentofylline-treated rats.* Int. J Neurosci. 2007;117:617-25.

Attal N, Guirimand F, Brasseur L, Gaude V, Chauvin M, Bouhassira D. *Effect of IV morphine in central pain: a randomized placebo-contolled study*. Neurology 2002;58:554-563.

Barron KD, Marciano FF, Amundson R, Mankes R. *Perineuronal glial responses after axotomy of central and peripheral axons. A comparison.* Brain Res. 1990;23;523(2):219-29.

Bender AS, Schousboe A, Reichelt W, Norenberg MD. *Ionic mechanisms in glutamate-induced astrocyte swelling: role of K+ influx.* J Neurosci. Res. 1998;1;52(3):307-21.

Biber K, Laurie DJ, Berthele A, Sommer B, Tölle TR, Gebicke-Härter PJ, van Calker D, Boddeke HW. *Expression and signaling of group I metabotropic glutamate receptors in astrocytes and microglia*. J Neurochem. 1999;72(4):1671-80.

Borden LA. *GABA transporter heterogeneity: pharmacology and cellular localization*. Neurochem. Int. 1996;29:335-56.

Borden LA, Smith KE, Hartig PR, Branchek TA, Weinshank RL. *Molecular heterogeneity of the gamma-aminobutyric acid (GABA) transport system. Cloning of two novel high affinity GABA transporters from rat brain.* J Biol. Chem. 1992;15;267(29):21098-104.

Broer A, Deitmer JW, Broer S. *Astroglial glutamine transport by system N is upregulated by glutamate.* Glia 2004;48:298-310.

Bowery, NG. Baclofen: 10 years on. Trends Pharmacol. Sci. 1982;3:400-403.

Bowery, NG., Hudson, AL., and Price, GW. *$GABA_A$ and $GABA_B$ receptor site distribution in the rat central nervous system*. Neuroscience 1987;20:365-383.

Castro-Lopez JM, Malcangion M, Pan BH, Bowery, NG. *Complex changes of $GABA_A$ and $GABA_B$ receptor binding in the spinal cord dorsal horn following peripheral inflammation or neurectomy.* Brain Res. 1995;679:289-297.

Castro-Lopez JM, Tavaras I, Coimbra A.*GABA decreases in the spinal cord dorsal horn after peripheral neurectomy.* Brain Res. 1993;620:287-291.

Chesler M and Kaila K *Modulation of pH by neuronal activity*. Trends Neurosci. 1992;15:396-402.

Christensen, MD., and Hulsebosch, CE.. *Spinal cord injury and anti-NGF treatment results in changes in CGRP density and distribution in the dorsal horn in the rat*. Exp. Neurol. 1997;147:463-475.

Christensen, MD., and Hulsebosch CE. *Chronic central pain after spinal cord injury*. J Neurotrauma 1997;14:517-537.

Christensen D, Kayser V. *The development of pain-related behaviour and opioid tolerance after neuropathy-inducing surgery and sham surgery*. Pain 2000;1:88(3):231-238.

Chu KL, Faltynek CR, Jarvis MF, McGaraughty S. *Increased WDR spontaneous activity and receptive field size in rats following a neuropathic or inflammatory injury: implications for mechanical sensitivity*. Neurosci. Lett. 2004;372(1-2):123-126.

Chung, JM., Surmeier, DJ., Lee, DJ., Sorkin, LS., Honda, CN., Tsong, Y., and Willis, W.D. *Classification of primate spinothalamic and somatosensory thalamic neurons based on cluster analysis*. J. Neurophysiol. 1986;56:308-327.

Coghill, RC, Mayer DJ, Price DD. *Wide dynamic range but not nociceptive-specific neurons encode multidimemsional features of prolonged repetitive heat pain*. J neurophysiol. 1993;36:703-716,

Coyle DE. *Partial peripheral nerve injury leads to activation of astroglia and microglia which parallels the development of allodynic behavior*. Glia 1998;23(1):75-83.

Crown ED, Ye Z, Johnson KM, Xu GY, McAdoo DJ, Hulsebosch CE. *Increases in the activated forms of ERK 1/2, p38 MAPK, and CREB are correlated with the expression of at-level mechanical allodynia following spinal cord injury*. Exp. Neurol. 2006;199(2):397-407.

Crown ED, Ye Z, Johnson KM, Xu GY, McAdoo DJ, Westlund KN, Hulsebosch CE. *Upregulation of the phosphorylated form of CREB in spinothalamic tract cells following spinal cord injury: relation to central neuropathic pain*. Neurosci. Lett. 2005;384(1-2):139-144.

Crown ED, Gwak YS, Ye Z, Johnson KM, Hulsebosch, CE. *Activation of p38 MAP Kinase is Involved in Central Neuropathic Pain Following Spinal Cord Injury*. Exp. Neurol. (in revision).

Curtis, DR., Lodge, D., Borstein, JC., and Peet, MJ. *Selective effects of (-)-baclofen on spinal synaptic transmission in the cat*. Exp. Brain Res. 1981;42:158-170.

Davalos D, Grutzendler J, Yang G, Kim JV, Zuo Y, Jung S, Littman DR, Dustin ML, Gan WB. *ATP mediates rapid microglial response to local brain injury in vivo*. Nat. Neurosci. 2005;8:752-758.

Dougherty PM, Willis WD. *Enhancement of spinothalamic neuron responses to chemical and mechanical stimuli following combined micro-iontophoretic application of N-methyl-D-aspartic acid and substance* P. Pain 1991;47(1):85-93.

Dougherty PM, Willis WD. *Enhanced responses of spinothalamic tract neurons to excitatory amino acids accompany capsaicin-induced sensitization in the monkey*. J Neurosci. 1992;12:883-984.

Dougherty PM, Palecek J, Palecková V, Willis WD. *Neurokinin 1 and 2 antagonists attenuate the responses and NK1 antagonists prevent the sensitization of primate spinothalamic tract neurons after intradermal capsaicin*. J Neurophysiol. 1994;72(4):1464-1475.

Drew GM, Siddall PJ, Duggan AW. *Responses of spinal neurones to cutaneous and dorsal root stimuli in rats with mechanical allodynia after contusive spinal cord injury*. Brain Res. 2001;2;893(1-2):59-69.

Drew GM, Siddall PJ, Duggan AW. *Mechanical allodynia following contusion injury of the rat spinal cord is associated with loss of GABAergic inhibition in the dorsal horn.* Pain 2004;109(3):379-388.

Dunlap, K. *Functional and pharmacological differences between two types of GABA receptor on embryonic chick sensory neurons*. Neurosci. Lett. 1984;47:265-270.

Eaton, MJ., Martinez, MA., and Karmally, S. *A single intrathecal injection of GABA permanently reverses neuropathic pain after nerve injury*. Brain Res. 1999;835:334-339.

Erlander MG, Tillakaratne NJ, Feldblum S, Patel N, Tobin AJ. *Two genes encode distinct glutamate decarboxylases*. Neuron 1991;7:91-100.

Fiebich BL, Biber K, Gyufko K, Berger M, Bauer J, van Calker D. *Adenosine A2b receptors mediate an increase in interleukin (IL)-6 mRNA and IL-6 protein synthesis in human astroglioma cells.* J Neurochem. 1996;66(4):1426-1431.

Field MJ, Bramwell S, Hughes J, Singh L. *Detection of static and dynamic components of mechanical allodynia in rat models of neuropathic pain: are they signalled by distinct primary sensory neurons ?* Pain 1999;83(2):303-311.

Fukuda K, Kato S, Mori K, Nishi M, Takeshima H. *Primary structures and expression from cDNAs of rat opioid receptor delta- and mu-subtypes.* FEBS Lett. 1993;2:327(3):311-314.

Gallien P, Nicolas B, Robineau S, Lebot MP, Brissot R. *The reflex sympathetic dystrophy syndrome in patients who have had a spinal cord injury.* Paraplegia 1995;33(12):715-720.

Garcia-Nicas E, Laird JM, Cervero F. *GABAA-Receptor blockade reverses the injury-induced sensitization of nociceptor-specific (NS) neurons in the spinal dorsal horn of the rat*. J Neurophysiol. 2006;96(2):661-670.

Garrison CJ, Dougherty PM, Kajander KC, Carlton SM. *Staining of glial fibrillary acidic protein (GFAP) in lumbar spinal cord increases following a sciatic nerve constriction injury.* Brain Res. 1991;22:565(1):1-7.

Garry EM, Delaney A, Blackburn-Munro G, Dickinson T, Moss A, Nakalembe I, Robertson DC, Rosie R, Robberecht P, Mitchell R, Fleetwood-Walker SM. *Activation of p38 and p42/44 MAP kinase in neuropathic pain: involvement of VPAC2 and NK2 receptors and mediation by spinal glia. Mol.* Cell. Neurosci. 2005;30:523-537.

Grossman SD, Rosenberg LJ, Wrathall JR. *Relationship of altered glutamate receptor subunit mRNA expression to acute cell loss after spinal cord contusion.* Exp. Neurol. 2001;168:283-289.

Guastella J, Nelson N, Nelson H, Czyzyk L, Keynan S, Miedel MC, Davidson N, Lester HA, Kanner BI. *Cloning and expression of a rat brain GABA transporter*. Science 1990;14:1303-1306.

Gottlieb M, Matute C. *Expression of ionotropic glutamate receptor subunits in glial cells of the hippocampal CA1 area following transient forebrain ischemia*. J Cereb. Blood Flow Metab. 1997;17(3):290-300.

Gould DJ, Goshgarian HG. *Glial changes in the phrenic nucleus following superimposed cervical spinal cord hemisection and peripheral chronic phrenicotomy injuries in adult rats*. Exp. Neurol. 1997;148(1):1-9.

Gwak YS, Nam TS, Paik KS, Hulsebosch CE, Leem JW. *Attenuation of mechanical hyperalgesia following spinal cord injury by administration of antibodies to nerve growth factor in the rat*. Neurosci. Lett. 2003;336(2):117-120

Gwak YS, Hulsebosch CE. *Upregulation of Group I metabotropic glutamate receptors in neurons and astrocytes in the dorsal horn following spinal cord injury*. Exp. Neurol. 2005;195(1):236-243

Gwak YS, Tan HY, Nam TS, Paik KS, Hulsebosch CE, Leem JW. *Activation of spinal GABA receptors attenuates chronic central neuropathic pain after spinal cord injury*. J Neurotrauma 2006;23(7):1111-1124

Gwak YS, Crown ED, Unabia GC, Hulsebopsch CE. *Propentofylline attenuates allodunia, glial activation and modulates GABAergic tone after spinal cord injury in the rat.* Pain 2008;138: 410-422.

Gwak YS, Unabia GC, Hulsebopsch CE. *Activation of p-38α MAPK contributes to neuronal hyperexcitability in caudal regions remote from spinal cord injury*. Exp. Neurol. 2009a (in press).

Gwak YS, Hulsebosch CE. *Remote Astrocytic and Microglial Activation Modulate Neuronal Hyperexcitability and Below-Level Neuropathic Pain after Spinal Injury in Rat.* Neuroscience 2009b;161:895-903.

Hains BC, Klein JP, Saab CY, Craner MJ, Black JA, Waxman SG. *Upregulation of sodium channel Nav1.3 and functional involvement in neuronal hyperexcitability associated with central neuropathic pain after spinal cord injury*. J Neurosci. 2003a;23(26):8881-8892.

Hains BC, Johnson KM, Eaton MJ, Willis WD, Hulsebosch CE. *Serotonergic neural precursor cell grafts attenuate bilateral hyperexcitability of dorsal horn neurons after spinal hemisection in rat*. Neuroscience 2003b;116(4):1097-1110.

Hains BC, Saab CY, Klein JP, Craner MJ, Waxman SG. *Altered sodium channel expression in second-order spinal sensory neurons contributes to pain after peripheral nerve injury*. J Neurosci. 2004;19;24(20):4832-4839.

Hains BC, Waxman SG. *Activated microglia contribute to the maintenance of chronic pain after spinal cord injury*. J Neurosci. 2006;19:26(16):4308-4317.

Hao, JX., Xu, XJ., and Wiesenfeld-Hallin, Z. *Intrathecal γ-amino-butyric acid$_B$ (GABA$_B$) receptor antagonist CGP 35348 induces hypersensitivity to mechanical stimuli in the rat.* Neurosci. Lett. 1994:182:299-302.

Hao, JX., Xu, XJ., Yu, YX., Seiger, A., and Wiesenfeld-Hallin, Z. *Baclofen reverses the hypersensitivity of dorsal horn wide dynamic range neurons to mechanical stimulation after transient spinal cord ischemia; implications for a tonic GABAergic inhibitory control of myelinated fiber input.* J Neurophysiol. 1992;68:392-396.

Hao S, Mata M, Wolfe D, Huang S, Glorioso JC, Fink DJ. *Gene transfer of glutamic acid decarboxylase reduces neuropathic pain.* Ann. Neurol. 2005;57(6):914-918.

Howe, JR., Sutor, B., and Zieglhansberger, W. (1987). *Baclofen reduces postsynaptic potentials of rat cortical neurons by an action other than its hyperpolarizing action*. J Physiol. 1987;384:539-569.

Hulsebosch CE. R*ecent advances in pathophysiology and treatment of spinal cord injury*. Adv. Physiol. Educ. 2002;26:238-255.

Hulsebosch CE. *From discovery to clinical trials: treatment strategies for central neuropathic pain after spinal cord injury*. Curr. Phar. Des. 2005;11:1411-1420.

Hunt, SP., Kelly, JS., Emson, PC., Kimmel JR., Miller RJ., and Wu, JY. *An immunohistochemical study of neuronal populations containing neuropeptides or γ-aminobutyrate within the superficial layers of the rat dorsal horn*. Neuroscience 1981;6:1883-1898.

Huston, E., Scott, RH., and Dolphin, AC. *A comparision of the effect of calcium channel ligands and GABAB agonists and antagonists on transmitter release and somatic calcium channel currents in cultured neurons.* Neuroscience 1990;38:721-729.

Hwang, JH., and Yaksh, TL. *The effect of spinal GABA receptor agonist on tactile allodynia in a surgically-induced neuropathic pain model in the rat*. Pain 1997;70:15-22.

Izumi Y, Tokuda K, Zorumski CF. *Long-term potentiation inhibition by low-level N-methyl-D-aspartate receptor activation involves calcineurin, nitric oxide, and p38 mitogen-activated protein kinase*. Hippocampus 2008;18(3):258-265.

Johnstone M, Gearing AJ and Miller KM *A central role for astrocytes in the inflammatory response to beta-amyloid; chemokines, cytokines and reactive oxygen species are produced.* J Neuroimmunol. 1999;93:182-193.

Johnston IN, Milligan ED, Wieseler-Frank J, Frank MG, Zapata V, Campisi J, Langer S, Martin D, Green P, Fleshner M, Leinwand L, Maier SF, Watkins LR. *A role for proinflammatory cytokines and fractalkine in analgesia, tolerance, and subsequent pain facilitation induced by chronic intrathecal morphine*. J Neurosci. 2004;24(33):7353-7365.

Ji RR, Woolf CJ. *Neuronal plasticity and signal transduction in nociceptive neurons: implications for the initiation and maintenance of pathological pain*. Neurobiol. Dis. 2001;8(1):1-10.

Ji RR, Samad TA, Jin SX, Schmoll R, Woolf CJ. p38 *MAPK activation by NGF in primary sensory neurons after inflammation increases TRPV1 levels and maintains heat hyperalgesia.* Neuron 2002;36(1):57-68.

Kalous A, Osborne PB, Keast JR. *Acute and chronic changes in dorsal horn innervation by primary afferents and descending supraspinal pathways after spinal cord injury*. J Comp. Neurol. 2007;504(3):238-253.

Kieffer BL, Befort K, Gaveriaux-Ruff C, Hirth CG. *The delta-opioid receptor: isolation of a cDNA by expression cloning and pharmacological characterization*. Proc. Natl. Acad. Sci. 1992;89(24):12048-12052.

Kim J, Jung JI, Na HS, Hong SK, Yoon YW. *Effects of morphine on mechanical allodynia in a rat model of central neuropathic pain.* Neuroreport 2003;14(7):1017-1020.

Kuhn SA, van Landeghem FK, Zacharias R, Färber K, Rappert A, Pavlovic S, Hoffmann A, Nolte C, Kettenmann H. Microglia express *GABA(B) receptors to modulate interleukin release. Mol. Cell*. Neurosci. 2004;25(2):312-322.

Kuffler SW, Nicholls JG and Martin AR (1984) *Physiology of neuroglial cells*. In "From neuron to Bain" pp.323-360. Sinauer, Sunderland, Massachusetts.

Lai JP, Douglas SD, Shaheen F, Pleasure DE, Ho WZ. *Quantification of substance p mRNA in human immune cells by real-time reverse transcriptase PCR assay*. Clin. Diagn. Lab. Immunol. 2002;9(1):138-143.

Lampert A, Hains BC, and Waxman SG *Upregulation of persistent and ramp sodium current in dorsal horn neurons after spinal cord injury*. Exp. Brain Res. 2006;174(4):660-666.

Lee CJ, Mannaioni G, Yuan H, Woo DH, *Gingrich MB and Traynelis SFAstrocytic control of synaptic NMDA receptors*. J Physiol. 2007;581:1057-1081.

Liu J, Wolfe D, Hao S, Huang S, Glorioso JC, Mata M, Fink DJ. *Peripherally delivered glutamic acid decarboxylase gene therapy for spinal cord injury pain.* Mol. Ther. 2004;10(1):57-66.

Ma C, Greenquist KW, Lamotte RH. *Inflammatory mediators enhance the excitability of chronically compressed dorsal root ganglion neurons*. J Neurophysiol. 2006;95(4):2098-2107.

Ma W, Quirion R. *Partial sciatic nerve ligation induces increase in the phosphorylation of extracellular signal-regulated kinase (ERK) and c-Jun N-terminal kinase (JNK) in astrocytes in the lumbar spinal dorsal horn and the gracile nucleus*. Pain 2002;99(1-2):175-184.

Ma W, Zheng WH, Powell K, Jhamandas K, Quirion R. *Chronic morphine exposure increases the phosphorylation of MAP kinases and the transcription factor CREB in dorsal root ganglion neurons: an in vitro and in vivo study.* Eur. J Neurosci. 2001;14(7):1091-1104.

Malcangio, M. and Bowery, NG. *GABA and its receptors in the spinal cord.* Trends Pharmacol. Sci. 1996;17:457-462.

Mao J, Sung B, Ji RR, Lim G. *Chronic morphine induces downregulation of spinal glutamate transporters: implications in morphine tolerance and abnormal pain sensitivity*. J Neurosci. 2002;22(18):8312-23.

Mao Z, Bonni A, Xia F, Nadal-Vicens M, Greenberg ME. *Neuronal activity-dependent cell survival mediated by transcription factor MEF2.* Science 1999;286(5440):785-790.

Martin DD. *Synthesis and release of neuroactive substances by glia cells*. Glia 1992;5:81-94.

Maixner, W, Dubner, R, Bushnell, MC, Kenshalo, DR, Oliveras JL. *Wide-dynamic-range dorsal horn neurons participate in the encoding process by which monkeys perceive the intensity of noxious heat stimuli.* Brain Res. 1986;374:385-388.

McAdoo DJ, Xu GY, Robak G, Hughes MG. *Changes in amino acid concentrations over time and space around an impact injury and their diffusion through the rat spinal cord.* Exp. Neurol. 1999;159(2):538-544.

Meller ST, Dykstra C, Grzybycki D, Murphy S, Gebhart GF. *The possible role of glia in nociceptive processing and hyperalgesia in the spinal cord of the rat*. Neuropharmacology 1994;33(11):1471-1478.

Meller R, Harrison PJ, Elliott JM, Sharp T. *In vitro evidence that 5-hydroxytryptamine increases efflux of glial glutamate via 5-HT(2A) receptor activation*. J Neurosci. Res. 2002;67(3):399-405.

Merskey H and Bogduk N. *Classification of Chronic Pain*. IASP Press, Seattle.

Mitchell SW, Morehouse GE, Keen WK. *Gunshot wounds and other injuries of nerves*. Phildelphia, JB Lippincott, 1864.

Moore KA, Kohno T, Karchewski LA, Scholz J, Baba H, Woolf CJ. *Partial peripheral nerve injury promotes a selective loss of GABAergic inhibition in the superficial dorsal horn of the spinal cord.* J Neurosci. 2002;22(15):6724-6731.

Moulédous L, Diaz MF, Gutstein HB. *Modulation of extracellular signal-regulated kinase (ERK) activity by acute and chronic opioid treatment in neuronal and glial cell lines*. J Neurochem. 2004;90(6):1371-1377.

Nakahara S, Yone K, Sakou T, Wada S, Nagamine T, Niiyama T, Ichijo H. *Induction of apoptosis signal regulating kinase 1 (ASK1) after spinal cord injury in rats: possible*

involvement of ASK1-JNK and -p38 pathways in neuronal apoptosis. J Neuropathol. Exp. Neurol. 1999;58(5):442-450

Nelson N. *The family of Na+/Cl- neurotransmitter transporters*. J Neurochem. 1998;71(5):1785-1803.

Nesic O, Lee J, Johnson KM, Ye Z, Xu GY, Unabia GC, Wood TG, McAdoo DJ, Westlund KN, Hulsebosch CE, Regino Perez-Polo J. *Transcriptional profiling of spinal cord injury-induced central neuropathic pain.* J Neurochem. 2005;95(4):998-1014.

Neugebauer V, Chen PS, Willis WD. *Groups II and III metabotropic glutamate receptors differentially modulate brief and prolonged nociception in primate STT cells*. J Neurophysiol. 2000;84(6):2998-3009.

Newman, E.A. *New roles for astrocytes: regulation of synaptic transmission*. Trends Neurosci. 2003;26(10):536-542.

Nishi M, Takeshima H, Fukuda K, Kato S, Mori K. *cDNA cloning and pharmacological characterization of an opioid receptor with high affinities for kappa-subtype-selective ligands.* FEBS Lett. 1993;6:330(1):77-80.

Paik, KS, Gwak, YS, Yeon, DS, Leem, JW, Nam, TS. *Modulations of response properties of spinal dorsal horn neurons following spinal cord injury in the rat*. Society for Neuroscience 2000

Palecek J, Palecková V, Dougherty PM, Carlton SM, Willis WD. *Responses of spinothalamic tract cells to mechanical and thermal stimulation of skin in rats with experimental peripheral neuropathy*. J Neurophysiol. 1992;67(6):1562-1573.

Parisod E, Siddall PJ, Viney M, McClelland JM, Cousins MJ. *Allodynia after acute intrathecal morphine administration in a patient with neuropathic pain after spinal cord injury*. Anesth. Analg. 2003;97(1):183-186,

Parpura V, Haydon PG. *Physiological astrocytic calcium levels stimulate glutamate release to modulate adjacent neurons*. Proc. Natl. Acad. Sci. 2000;97(15):8629-8634.

Piani D, Spranger M, Frei K, Schaffner A and Fontana A. *Macrophage –induced cytotoxicity of N-methyl-D-aspartate receptor positive neurons involves excitatory amino acids rather than reactive oxygen intermediates and cytokines.* Eur. J Immunol. 1992;22:2429-2439.

Peng XM, Zhou ZG, Glorioso JC, Fink DJ, Mata M. *Tumor necrosis factor-alpha contributes to below-level neuropathic pain after spinal cord injury*. Ann. Neurol. 2006;59(5):843-851.

Rady JJ, Fujimoto JM. *Confluence of antianalgesic action of diverse agents through brain interleukin(1beta) in mice.* J Pharmacol. Exp. Ther. 2001;299(2):659-665.

Raghavendra V, Tanga FY, DeLeo JA. *Attenuation of morphine tolerance, withdrawal-induced hyperalgesia, and associated spinal inflammatory immune responses by propentofylline in rats.* Neuropsychopharmacology. 2004;29(2):327-334.

Raingeaud J, Gupta S, Rogers JS, Dickens M, Han J, Ulevitch RJ, Davis RJ. *Pro-inflammatory cytokines and environmental stress cause p38 mitogen-activated protein kinase activation by dual phosphorylation on tyrosine and threonine*. J Biol. Chem. 1995;270(13):7420-7426.

Ray LB, Sturgill TW. *Characterization of insulin-stimulated microtubule-associated protein kinase. Rapid isolation and stabilization of a novel serine/threonine kinase from 3T3-L1 cells.* J Biol. Chem. 1988;263(25):12721-12727.

Reinscheid RK, Nothacker HP, Bourson A, Ardati A, Henningsen RA, Bunzow JR, Grandy DK, Langen H, Monsma FJ Jr, Civelli O. Orphanin FQ: *a neuropeptide that activates an opioidlike G protein-coupled receptor*. Science 1995;270(5237):792-794.

Rooney BA, Crown ED, Hulsebosch CE, McAdoo DJ. *Preemptive analgesia with lidocaine prevents Failed Back Surgery Syndrome.* Exp. Neurol. 2007;204(2):589-596.

Sah, R., Galeffi, F., Ahrens, R., Jordan, G., and Scwartz-Bloom, RD. *Modulation of the $GABA_A$-gated chloride channel by reactive oxygen species.* J. Neurochem. 2002;80:383-391.

Schousboe A, Westergaard N, Sonnewald U, Petersen SB, Huang R, Peng L, Hertz L. *Glutamate and glutamine metabolism and compartmentation in astrocytes*. Dev. Neurosci. 1993;15:359-366.

Sharma, HS., and Sjoquist, P.O. *A new antioxidant compound H-290/51 modulate glutamate and GABA immunoreactivity in the rat spinal cord following trauma*. Amino Acids 2002;23:261-272.

Shafer RA and Murphy S *Activated astrocytes induce nitric oxide synthase-2 in cerebral endothelium via tumor necrosis factor alpha*. Glia 1997;21:370-379.

Shavit Y, Wolf G, Goshen I, Livshits D, *Yirmiya R. Interleukin-1 antagonizes morphine analgesia and underlies morphine tolerance*. Pain 2005;115(1-2):50-59.

Sokal, DM. and Chapman, V. *$GABA_B$-receptor antagonism increases nociceptive transmission in vivo.* Neuroreport 2001;12:3247-3250.

Song P, Zhao ZQ. *The involvement of glial cells in the development of morphine tolerance*. Neurosci. Res. 2001;39(3):281-286.

Sorkins, LS., Puig, S., and Jones, DL. *Spinal bicuculline produces hypersensitivity of dorsal horn neurons: effects of excitatory amino acid antagonists*. Pain 1998;77:181-190.

Struzynska L, Sulkowski G. *Relationships between glutamine, glutamate, and GABA in nerve endings under Pb-toxicity conditions*. J Inorg. Biochem. 2004;98:951-958.

Sturgill TW, Ray LB, Anderson NG, Erickson AK. *Purification of mitogen-activated protein kinase from epidermal growth factor-treated 3T3-L1 fibroblasts*. Methods Enzymol. 1991;200:342-351.

Sweitzer SM, Schubert P, DeLeo JA. *Propentofylline, a glial modulating agent, exhibits antiallodynic properties in a rat model of neuropathic pain*. J Pharmacol. Exp. Ther. 2001;297(3):1210-1217.

Tanaka M, Sotomatsu A, Yoshida T, Hirai S and Nishida A *Detection of superoxide production by activated microglia using a sensitive and specific chemiluminescence assay and microglia-mediated PC12h cell death*. J Neurochem. 1994;63:266-270.

Tawfik VL, Nutile-McMenemy N, LaCroix-Fralish ML, DeLeo JA. *efficacy of propentofylline, a glial modulating agent, on existing mechanical allodynia following peripheral nerve injury* Brain Behav. Immun. 2007;2:238-246.

Todd, AJ., and McKenzie, J. *GABA-immunoreactive neurons in the dorsal horn of the rat spinal cord.* Neuroscience 1989;31:799-806.

Tsuda M, Shigemoto-Mogami Y, Koizumi S, Mizokoshi A, Kohsaka S, Salter MW, *Inoue K. P2X4 receptors induced in spinal microglia gate tactile allodynia after nerve injury*. Nature 2003;424(6950):778-783.

Vera-Portocarrero LP, Mills CD, Ye Z, Fullwood SD, McAdoo DJ, Hulsebosch CE. *Rapid changes in expression of glutamate transporters after spinal cord injury*. Brain Res. 2002;927:104-110.

Vikman KS, Duggan AW, Siddall PJ.*Interferon-gamma induced disruption of GABAergic inhibition in the spinal dorsal horn in vivo*. Pain 2007;133(1-3):18-28.

Wang Z, Haydon PG, Yeung ES. *Direct observation of calcium-independent intercellular ATP signaling in astrocytes.* Anal. Chem. 2000;72(9):2001-2007.

Watkins LR, Maier SF. *Glia: a novel drug discovery target for clinical pain*. Nat. Rev. Drug Discov. 2003;2(12):973-985.

Watkins LR, Martin D, Ulrich P, Tracey KJ, Maier SF. *Evidence for the involvement of spinal cord glia in subcutaneous formalin induced hyperalgesia in the rat*. Pain 1997;71(3):225-235.

Watkins LR, Hutchinson MR, Johnston IN, Maier SF. *Glia: novel counter-regulators of opioid analgesia.* Trends Neurosci. 2005;28(12):661-669.

Waxman SG. *Channel, neuronal and clinical function in sodium channelopathies: from genotype to phenotype*. Nat. Neurosci. 2007;10(4):405-409.

Whitmarsh AJ, Davis RJ. *Transcription factor AP-1 regulation by mitogen-activated protein kinase signal transduction pathways*. J Mol. Med. 1996;74(10):589-607.

Widmann C, Gibson S, Jarpe MB, Johnson GL. *Mitogen-activated protein kinase: conservation of a three-kinase module from yeast to human*. Physiol. Rev. 1999;79(1):143-180.

Willis WD, Westlund KN. *Neuroanatomy of the pain system and of the pathways that modulate pain.* J Clin. Neurophysiol. 1997;14(1):2-31.

Woolf CJ, Shortland P, Reynolds M, Ridings J, Doubell T, Coggeshall RE. *Reorganization of central terminals of myelinated primary afferents in the rat dorsal horn following peripheral axotomy.* Comp. Neurol. 1995;360(1):121-134.

Xu XJ, Hao JX, Wiesenfeld-Hallin Z (2002) *Physiological and pharmacological characterization of a rat model of spinal cod injury pain after spinal ischemia*. In "Spinal Cord Injury Pain" pp.175-187. IASP press, Seattle.

Yang L, Blumbergs PC, Jones NR, Manavis J, Sarvestani GT, Ghabriel MN. *Early expression and cellular localization of proinflammatory cytokines interleukin-1beta, interleukin-6, and tumor necrosis factor-alpha in human traumatic spinal cord injury*. Spine 2004;29(9):966-971.

Yu W, Hao JX, Xu XJ, Wiesenfeld-Hallin Z. *Comparison of the anti-allodynic and antinociceptive effects of systemic; intrathecal and intracerebroventricular morphine in a rat model of central neuropathic pain*. Eur. J Pain 1997;1(1):17-29.

Yu CG, Yezierski RP. *Activation of the ERK1/2 signaling cascade by excitotoxic spinal cord injury. Brain Res.* Mol. Brain Res. 2005;138(2):244-255.

Zhang AL, Hao JX, Seiger A, Xu XJ, Wiesenfeld-Hallin Z, Grant G, Aldskogius H. *Decreased GABA immunoreactivity in spinal cord dorsal horn neurons after transient spinal cord ischemic in the rat.* Brain Res. 1994;656:187-190.

Zhang H, Xie W, Xie Y. *Spinal cord injury triggers sensitization of wide dynamic range dorsal horn neurons in segments rostral to the injury*. Brain Res. 2005;1055(1-2):103-110.

Zhao P, Waxman SG, Hains BC. *Extracellular signal-regulated kinase-regulated microglia-neuron signaling by prostaglandin E_2 contributes to pain after spinal cord injury*. J Neurosci. 2007;27:2357-2368.

Zhuang ZY, Gerner P, Woolf CJ, *Ji RR. ERK is sequentially activated in neurons, microglia, and astrocytes by spinal nerve ligation and contributes to mechanical allodynia in this neuropathic pain model.* Pain 2005;114(1-2):149-159.

Zhuang ZY, Wen YR, Zhang DR, Borsello T, Bonny C, Strichartz GR, Decosterd I, *Ji RR. A peptide c-Jun N-terminal kinase (JNK) inhibitor blocks mechanical allodynia after spinal nerve ligation: respective roles of JNK activation in primary sensory neurons and spinal astrocytes for neuropathic pain development and maintenance.* J Neurosci. 2006;26(13):3551-3560.

Zou X, Lin Q, Willis WD. *Role of protein kinase A in phosphorylation of NMDA receptor 1 subunits in dorsal horn and spinothalamic tract neurons after intradermal injection of capsaicin in rats.* Neuroscience 2002;115(3):775-786

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Chapter 6

MULTIPLE NEURONAL AND GLIAL MECHANISMS CAN BE INVOLVED IN THE RESPONSE TO THE ACTIVATION OF NICOTINIC RECEPTORS

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ABSTRACT

Selective activation of brain nicotinic acetylcholine receptors (nAChRs) seems to be a promising treatment strategy for Alzheimer's Disease and related disorders. Nicotine and its related non-toxic compounds improve cognitive ability (including attention, learning, and memory) by acting directly or indirectly on the cortical neurons. The nicotine effects depend on the anatomical distribution and the cellular localization of the different subtypes of nicotinic acetylcholine receptors and are attributable to modifications induced in a wide variety of cellular mechanisms. Nicotinic receptors in the brain are more commonly associated with the modulation of neuronal function than mediation of synaptic transmission. Enhancement of neurotransmitter release (glutamate, dopamine, noradrenaline) via presynaptic nAChRs and increase of intracellular Ca^{2+} by postsynaptic nAChRs, facilitate the conexion with many intracellular signaling pathways. Moreover, glial cells have nAChRs that regulate their functions. Many of the neuronal changes nicotine induces are poorly understood by this complexity of the colinergic systems. Some of the effects of this drug are positive, such as neuroprotection against neurotoxic agents, ageing and pathological situations, but some neuronal changes can be negative, such as the induction of apoptosis, directly or indirectly through the production of free radicals, cytokines and pro-inflammatory derivatives of arachidonic acid. In a model of rat with subchronic nicotine treatment without neuronal apoptosis, our research group has demonstrated that nicotine increases the turnover of the glycolytic pathway and Krebs cycle in the cortex in a layer dependent manner, and the NGF immunoreactivity in neurons and glial cells in the frontoparietal cortex. Moreover, the increase of COX-2 has been observed in an area-, layer- and neuron type-dependent manner in different regions

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of the frontoparietal cortex, hippocampus and cerebellar cortex. The up-regulation of these enzymes and the NGF could have beneficial effects on neuronal function by helping neuronal adaptations involved in the performance of cognitive functions. Such results could help in the development of new treatments for cognitive disorders as well as help us understand the mechanism of action of certain drugs of abuse.

Keywords: Nicotine cholinergic system, nicotine receptors, nicotinergic treatments, nicotine effects, dehydrogenase hyperactivity, cyclooxygenase-2, NGF, oxidative stress, brain, rat.

INTRODUCTION

Nicotinic receptors (nicotinic acetylcholine receptors [nAChRs]) represent a first order therapeutic target in neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and other neuropsychiatric disorders such as schizophrenia, attention deficit/hyperactivity, and certain types of epilepsy (Lloyd et al., 1998; Maelicke et al., 2000; Newhouse et el., 2001; Hogg and Bertrand, 2003; Cincotta et al., 2008; Quik et al., 2008; Boatman et al., 2008; Sharma, 2008; Terry, 2008; Scarr and Dean, 2009). However, the effects of activating these receptors are not well known. In the brain, nicotine, nicotinic acid and nicotine analogues improve cognitive ability (including attention, learning and memory) via their direct or indirect activation of these receptors on cortical neurons (Changeux et al., 1998; Iversen, 1998; Levin and Simon, 1998; Radcliffe and Dani, 1998; Alburquerque et al., 2000; Alkondon et al., 2000; Bartus, 2000; Levin et al., 2006; Hasselmo, 2006; Dani and Bertrand, 2007; Bodor and Offermanns, 2008; Placzek et al., 2009). Nicotine, understood as a prototype activator of nAChRs, has a neuroprotective effect against certain neurotoxic agents and helps prevent the neuronal damage caused by aging and neurodegenerative processes (Shimohama et al., 1996; Li et al., 1999; Belluardo et al., 2000; Jonnala and Buccafusco, 2001; Shimohama and Kihara, 2001; Sofroniew et al., 2001; Pauly et al., 2004; Mudo et al., 2007; Buckingham et al., 2009; Shimohama, 2009). However, some of the effects elicited by the activation of nAChRs are directly or indirectly negative, such as the induction of apoptosis, through the production of free radicals, cytokines and pro-inflammatory derivates of arachidonic acid (Carlson et al., 2001; Jang et al., 2002; Newman et al., 2002; Ziedler et al., 2007; Toledano et al., 2008). Thus, it is vital that we understand the mechanisms involved in the response of all CNS cells – neuronal, glial and even those of the blood vessels – to the activation of nAChR activation. Only in this way will it be possible to develop and use nAChR-specific medications.

The present chapter analyses the possible reasons why such different – sometime contrasting – effects may be seen after the pharmacological activation of nAChRs. Special attention is paid to the activation of certain cellular mechanisms under study at our laboratory, including the activation of glycolysis and the Krebs cycle, the production of neurotrophic factors, the regulation of isoforms of COX, and the relationship wih the oxidative stress.

1. DIVERSITY AND COMPLEXITY OF THE NICOTINIC COMMUNICATION SYSTEM

Our current state of knowledge requires the cholinergic system (i.e., that mediated by acetylcholine) be understood as a general cellular communication system (for cellular communication or cell signalling) since it is used to communicate between neurons and their effector cells (neurons or otherwise) and between non-neuronal cells (Kawashima and Fujii, 2008). Further, since the early days of the study of acetyl choline as a neurotransmitter, the cholinergic system has been known to have two subsystems – the muscarinic and nicotinic subsystems (Langley, 1901; 1905). The key elements of the former subsystem are muscarinic receptors (metabotropic receptors that also couple with secondary messenger), while nicotinic receptors (ionotropic cationic channel receptors) - the nAChRs discussed in the present study - are associated with the latter.

The existence of neuronal and non-neuronal communication systems highlights the complexity of the cholinergic system in the CNS. This fact of this complexity is at its clearest when one analyses the special characteristics and variations of the CNS nicotinergic neurotransmitter system. These are very different to those appreciated in the neuromuscular nicotinergic system. Further, the special characteristics of the nAChRs render them quite different in their behaviour to better known receptors (Dani and Bertrand, 2007; Gotti et al., 2007; Alburquerque et al., 2009). We are gradually discovering the great diversity of subtypes of nAChRs in the brain (Alburquerque et al., 2009; Collins et al., 2009; Millar and Gotti, 2009). The activation of these receptors can induce different effects, and their physiological regulation entails mechanisms not seen in other systems (Dani and Bertrand, 2007; Gaimarri et al., 2007; Lendvai and Vizi, 2008). This complexity and diversity of the nicotinic cholinergic system is of great importance when trying to develop nicotinergic treatments. It might be supposed that the effects of such treatments would be different in different regions of the CNS, depending on the subtypes, densities and exact locations of nAChRs on the neurons and glial cells present. The effects of any nicotinergic medication must therefore be understood in each region and its collateral effects known before it could be used in the clinic.

1. 1. Diversity of Nicotinic Receptors in the Cns

The nAChRs, the structures of which involve a pentameric assembly of identical (homomeric receptors) or homologous subunits (heteromeric receptors), form a heterogeneous and complex family of ionotropic cation receptors (ligand-gated ion channels) (Sugaya et al., 1990; Lindstrom et al., 1995; Changeux et al., 1998; Sihver et al., 1998; Zoli et al., 1998; Cordero-Erausquin et al., 2000; Paterson and Nordberg, 2000; Hogg et al., 2003; Gotti and Clementi, 2004; Dani and Bertrand, 2007; Grady et al, 2007; Steinlein and Bertrand, 2008; Collins et al., 2009). The central ion pore of all nAChR subtypes is permeable to monovalent Na^+ ions once the receptors have been activated by the endogenous neurotransmitter acetylcholine. However, differences in the structure (i.e., the composition and assembly of the subunits) condition the functional and pharmacological properties of each receptor type (Le Novère et al., 2002). In addition to being permeable to Na^+, the monovalent ion K^+ and the divalent ion Ca^{2+} can enter through the pores of some nAChRs (Fucile, 2004;

Dajas-Bailador and Wonnacott, 2004). The entry of Ca^{2+} via these receptors, together with that which enters as a consequence of the activation of the voltage-dependent Ca^{2+} channels by nAChR-induced depolarisation, plays an important role in cell signalling in neurons of different circuits (Dajas-Bailador and Wonnacott, 2004). Two main classes of subunits have been identified in CNS: the alpha (α: α2-10) and the beta (β: β2-4) subunits. The ACh binding site has a primary component in a subunit and a complementary component in other adjacent subunit (Gotti et al., 2006; Jensen et al., 2006). The α2, α3, α4, α6, α7, α9 and α10 subunits of the nAChRs provide the primary binding sites for ACh (and the complementary component in α7-10 homomeric receptors) and the β2 and β4 subunits provide the complementary component of binding sites for ACh in heteromeric receptors. The β3 and α5 subunits in heteromeric receptors do not participate directly in the ACh binding. A pharmacological classification of nAChRs has been made using α-bungarotoxin (αBgtx) binding: the αBgtx-sensitive receptors (homomeric or heteromeric receptors with α7, α8, α9 or α10 subunits), and the αBgtx-insensitive receptors (heteromeric receptors with α2, α3, α4, α6, β2, β3 or β4 subunits). In addition to the ACh binding site, the nAChRs also have different numbers of allosteric binding sites at which a variety of molecules can bind more or less selectively, modifying the function of these receptors (Jensen et al., 2005). These allosteric sites and the selective drugs that act upon them are of special therapeutic interest since they represent an important means of avoiding the side effects that nicotine derivatives provoke. In the brain, the majority of nAChRs contain one type of α subunit and one subtype of β subunit; α4β2 receptors are the most widely distributed. The differences in the α4:β2 ratio defines two subsets of receptors of different stoichiometry, ACh affinity and pharmacological properties. The receptors of the high affinity subset (EC50 approximately 1 μM) are composed of two α4 subunits and three β2 subunits, while those of the low affinity subset contain three α4 and two β2 subunits (Moroni et al., 2006).

nAChRs are present on all neurons of the CNS. The subunit composition is neuron- and CNS region-specific, but more than one subtype of receptor is present on every neuron. The different subtypes of nAChRs are also cell-region specific: the normal physiological functioning of the nicotinergic-cholinergic system is dependent on the exact dendritic, somatic and axonic location of every subtype of nAChR (see the section *Complexity of the nicotinergic system as a neurotransmitter system*). This depends on the correct synthesis trafficking and functional dinamics of their subunits (see the section *Complex regulation of the nAChRs*). At present, we know the nAChRs characteristically present in some CNS regions, in some neurons, and at some synaptic and non-synaptic neuronal sites, but more research will be needed if we are to understand the exact functions of the cholinergic system in the pathophysiology (and the possible effects of nicotinergic treatments) of each CNS region. The most important subset of brain nAChRs is formed by the above-mentioned α4β2 subtypes. These receptors exist in high densities in the brain cortex, hippocampus, amygdala, striatum, hypothalamus, thalamus, brain stem, cerebellum and spinal cord (Champtiaux et al., 2003). The nAChR subtypes that contain α7 subunits form a very complex set of homomeric and heteromeric receptors with very different functions related to their neuronal localization and the characteristics of the subunit(s) they contain. The expression of α7 subunits is very high in the brain cortex, hippocampus and limbic areas, but low in the thalamus and the basal ganglia (Gaimarri et al., 2007). The homomeric α7 receptors are αBgtx-sensitive and the non-α7 receptors αBgtx-insensitive. Kynurenine, an astrocyte-derived factor, is a potent α7

nAChR antagonist of important theoretical and practical interest since this physiological molecule is the intercellular messenger of a glia-to-neuron communication system, highlighting the role of glial cells in the control of neurons (Wu et al., 2007; Zmarowsky et al., 2009). α2 subtypes are species-specific and expressed in small numbers in mammals, being mainly localized to the cortex, retina and interpeduncular nucleus (Drago et al., 2003; Gotti et al., 2006). To date, α8 subunits have not been found in the mammalian brain. α3 subunits, mainly the α3β2 or α3β4 subtypes, have been observed in the spinal cord and cerebellum, and in the hippocampus, locus coeruleus and ventral tegmental area respectively (Gaimarri et al., 2007). α6β4 subtypes are present in the striatum (Gaimarri et al., 2007). In summary, the diversity of subunits, nAChR subtypes and neuronal localization suggests a wide variety of effects may be expected depending on the activation of different intracellular pathways associated with both post-synaptic neuronal receptors and other neurotransmitter systems.

nAChRs are present on different types and subtypes of glial cells of the CNS. The subunit composition is glia- and CNS region-specific, but more than one subtype of receptor is present on certain astroglial and microglial cells (Carnevalle et al., 2007). In consequence, the normal physiological functioning of the nicotinergic-cholinergic system is dependent not only on the exact location and correct function of every subtype of nAChR in the neuronal networks, but the exact location and correct function of the nAChRs in the glial cells sourronding them.

1. 2. Complex Regulation of the Nachrs

The wide variety of nAChRs, and the nAChR subtype specificity of each neuron in the neuronal circuits of the different areas of the CNS are the basis for local differences in the response to ACh, nicotine and nicotine agonists. Moreover, this response to ACh is regulated by different mechanisms, the most important operating at nAChR level. Receptor localization and density are vital variables in normal physiological function, but their regulation by adaptive mechanisms is necessary in many cognitive functions. Many of these mechanisms are elicited by ACh itself but can be brought into action through chronic nicotine administration (e.g., in smokers). The special functional structure of the nicotinic neurotransmission system (see the section *Complexity of the nicotinergic system as a neurotransmitter system*) favours a delay in the breakdown of the neurotransmitter and the consequent continued interaction between the neurotransmitter and its receptors. Over-stimulation induced by a high concentration of ACh can occur in some instances, leading to a reduction in the number of certain types of operative nAChRs. Nicotine in smokers and external nicotine agonists in therapeutic doses can produce a wide variety of effects depending on the local concentration, the type(s) of nAChRs activated and the types of neurons and circuits under cholinergic regulation in different CNS regions. Antagonist would have the effect of increasing the density of certain nAChRs (Gentry and Lukas, 2002). This classical response of nAChRs to the presence of nicotine agonists/antagonists is observed in several biological models (cell cultures or specific CNS regions of experimental animals), but chronic exposure to nicotine or a nicotine agonist can often trigger an increase in the membrane densities of different types of nAChR, a phenomenon known as up-regulation

(Perry et al., 1999; Gentry and Lukas, 2002; Benson et al., 2007; Millar et al., 2008; Picciotto et al., 2008; Takada-Takatori et al., 2008; Walsh et al., 2008). Nicotine acts in this situation as an "antagonist". Up-regulation of the receptors is dependent on many factors, including the type and dose of the agonist, the exposure time, the receptor type, the type of neuron, the CNS region, and the experimental animal (Parker et al., 2004; Mugnaini et al., 2006; Benson et al., 2007). In the brains of animals chronically treated with nicotine or nicotine agonists, the most up-regulated receptor is the $\alpha4\beta2$ subtype (Gentry and Lukas, 2002; Besson et al., 2007); this is seen in smokers (Perry, 1999). The up-regulation of receptors does not seem to be due to an increase in gene expression ($\alpha2$, $\alpha3$, $\alpha4$, $\alpha5$ and $\beta2$ expression does not increase in the mouse brain chronically treated with nicotine - Marks et al., 1992) but rather lies in the modification of post-transcriptional processes. Each subunit is synthesised by specific polyribosomes and assembled into precursors of the functional pentameric receptors in the membranes of the endoplasmic reticulum. Different processes direct the traffic of these internal macromolecules to the cell membrane (the masking of endoplasmic reticulum retention signals, the liberation of export motifs for Golgi and cell membrane transportation, and Golgi processing of the carbohydrates included in the molecules, etc. - Ren et al., 2005; Millar and Harkness, 2008). Gaimarri et al. (2007) indicated four main mechanisms could explain $\alpha4\beta2$ subtype up-regulation: a) a nicotine-induced increase in receptor transport though the secretory pathway (Harkness and Millar, 2002); b) a reduction in surface receptor internalisation that prevents the removal of nAChRs from the cell surface (Peng et al., 1997); c) the isomerisation of surface receptors into a more easily activated high affinity conformation (Vallejo et al., 2005), and d) an increase in subunit assembly and/or a reduction in receptor turnover (Darsow et al., 2005). All these mechanisms are regulated by nicotine agonists acting at different points in different cellular processes. The direct interaction of the nicotine with the extracellular domains of different subunits (for example the $\beta2$ subunits) (Sallette et al., 2004) changes the interaction of the subunits among themselves inside the receptors. The indirect modification of the protein composition and/or configuration of the subunits seems to be another pathway for up-regulation; these characteristics are of prime importance in nAChR assembly and trafficking. Modifications induced in cellular proteins related to assembly and trafficking represent another important pathway for up-regulation since these proteins are responsible for the control of membrane receptors and their intracellular pools (the type, density and neuronal location of the different nAChRs). An important group of these membrane and sub-membrane proteins, the "scaffold" proteins, present molecular motifs by which they interact, participating in the externalisation and internalisation of nAChRs, and the induction of conformational changes in these receptors (Neff et al., 2009). The scaffold proteins are the organizers of a supramolecular assembly of synaptic and extrasynaptic dynamic domains of different nAChRs that can change receptor functionality according to location (extra- or intracellular, accessible or inaccessible to ACh, nicotine or nicotine agonists) via their influence on the conformational structure of the subunits, and through their assembly.

The up-regulation of some nAChRs should not be understood as a movement towards nicotine hyperactivity. Indeed, the constant presence of ACh, nicotine or a nicotine agonist produces a progressive reduction in the response of some nAChRs, a phenomenon known as desensitisation (Giniatullin et al., 2005; Picciotto et al., 2008; Buccafusco et al., 2009). This

desensitisation is dependent on the type of agonist, dose and time of exposure, the type of neuron and CNS region and the animal under chronic agonist exposure.

These special characteristics of the regulation of nAChRs make the study of the effects of nicotine agonists very difficult. Different experimental models and different CNS regions in the brains of experimental animals must therefore be studied (using different doses and times of treatment) for any conclusions to be reached on the effects of nicotinergic drugs in the treatment of brain disease. The activation, up-regulation and desensitization of nAChRs seem to be present in all regions of the CNS, and contribute to normal and pathological regulatory functions of the nicotinic cholinergic communication system in the CNS. Picciotto et al. (2008) indicate that "agents like nicotine itself and partial agonists of nAChRs have the unique ability to regulate network properties of assembles of neurons through the differential activation and desensitisation of nAChRs on excitatory and inhibitory neuronal cell bodies and terminals". Under a nicotinergic treatment, all the different nicotine/nicotine agonist-induced functional states of the receptors need to be considered to evaluate the benefits of the drug (Buccafusco et al., 2009).

1. 3. Complexity of the Nicotinergic System as a Neurotransmitter System

Our long acquaintance with the nicotinergic cholinergic neurotransmitter system of the CNS renders it something of a 'classic' system (Langley, 1901, 1905), and it has been the subject of many papers reporting great advances in the neurosciences. Nonetheless, it might also be considered an atypical neurotransmitter system since it has characteristics unlike those of any other. Only in certain regions of the CNS and in certain subtypes of neuron, especially the cholinergic interneurons of the striatum and some of the hippocampus and cortex (Monzón-Mayor et al., 2000; Zhou et al., 2002; Dani and Bertrand, 2007), can one speak of there being typical synaptic neurotransmission in that the cholinergic buttons release acetylcholine into the synaptic cleft to activate nAChRs on the somatic or dendritic post-synaptic membrane. Indeed, such a synaptic configuration and sequence of events seems to be the minority case in the CNS. In most areas of the brain where the axons of the two main regulatory cerebral cholinergic subsystems are found (the cholinergic basal forebrain and pontine subsystems; Fig. 1), the nAChRs are at some distance from the presynaptic buttons (non-synaptic receptors) (Fig. 2). It has been observed that the majority (>80%) of cortical and hippocampal cholinergic axon varicosities and terminals do not make synaptic contacts on other neurons (Umbriaco et al., 1994, 1995; Descarries et al., 1997; Mechawar et al., 2002; Aznavour et al., 2005; Lendvai and Vizi, 2008). It has also been observed that the main elements of the nicotinergic machinery do not overlap. Choline acetyltransferase (ChAT) and vesicular ACh transport are located at cholinergic axons, but acetylcholinesterase (AChE) and nAChRs are found mainly at sites distant from those of ACh release (Descarries et al., 1997; Lendvai and Vizi, 2008). The above suggests that, functionally, nicotinergic neurotransmission has special but variable characteristics, i.e., special in comparison with classical (or ideal) neurotransmission systems in which all the main elements of this type of cell communication are coincident at the synapse level, and variable with respect to the distances between and the densities of these elements (which suggest the existence of functional subtypes) (Fig. 2). ACh released from the varicosities and terminals of the cholinergic axons diffuses into the extracellular space to reach the nAChRs located in

functionally different areas (dendrites, somata, axons) of more distant neurons as well as glial cells.

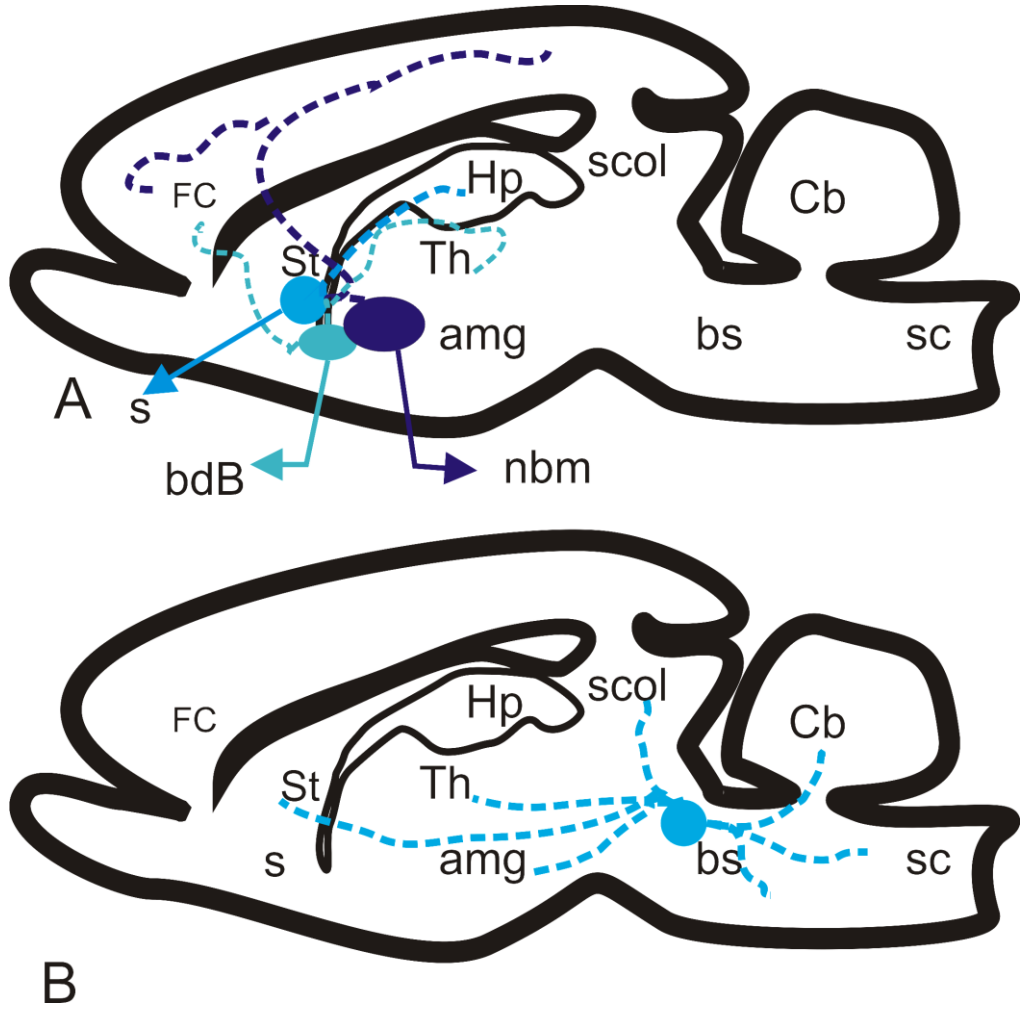

Figure 1. Regulatory cerebral cholinergic subsystems. Diagrams showing the cholinergic "centers" of the basal forebrain (A) and the pontine tegmentun (B). Cholinergic neurons are located in a loosely contiguous axis from the septum to the pontine tegmentum through the telencephalon, the diencephalon and the mesencephalon. These two main regulatory subsystems provide a broad, diffuse and sparse innervation to wide areas of the brain. The basal forebrain cholinergic sybsystem (A) is formed by a continuum of cholinergic cells, classically grouped in three "nuclei" which innervate cortical areas: a) the "septum" (s) (different groups of neurons located in the septal region of the brain which mainly innervate the hippocampus -Hp); b) the "diagonal band of Broca" (dbB) (groups of neurons located in the basal forebrain, from the septum to the preoptic area, which innervate limbic structures such as the cingulate cortex); and c) the "nucleus basalis magnucellularis" (nbm) (nucleus of Meynert in man) (cholinergic neurons located near the internal capsule, close to the globus pallidus, from the anterior commisure to the optic tract, which mainly innervate the brain cortex- FC- and amigdala –amg). The pontine subsystems (B) arise from neurons located in the pedunculopontine tegmentum and the laterodorsal pontine tegmentum and provide widespread innervation to subcortical areas (striatum –St- and septal regions s-, thalamus –Th-, superior colliculus and midbrain regions –scol-, pons, brain stem -bs-, cerebellum –Cb- and spinal cord -sc-). (Adapted from Shute and Lewis, 1967; Mesulam et al., 1983; Butcher and Woolf, 1986; Toledano and Alvarez, 2004).

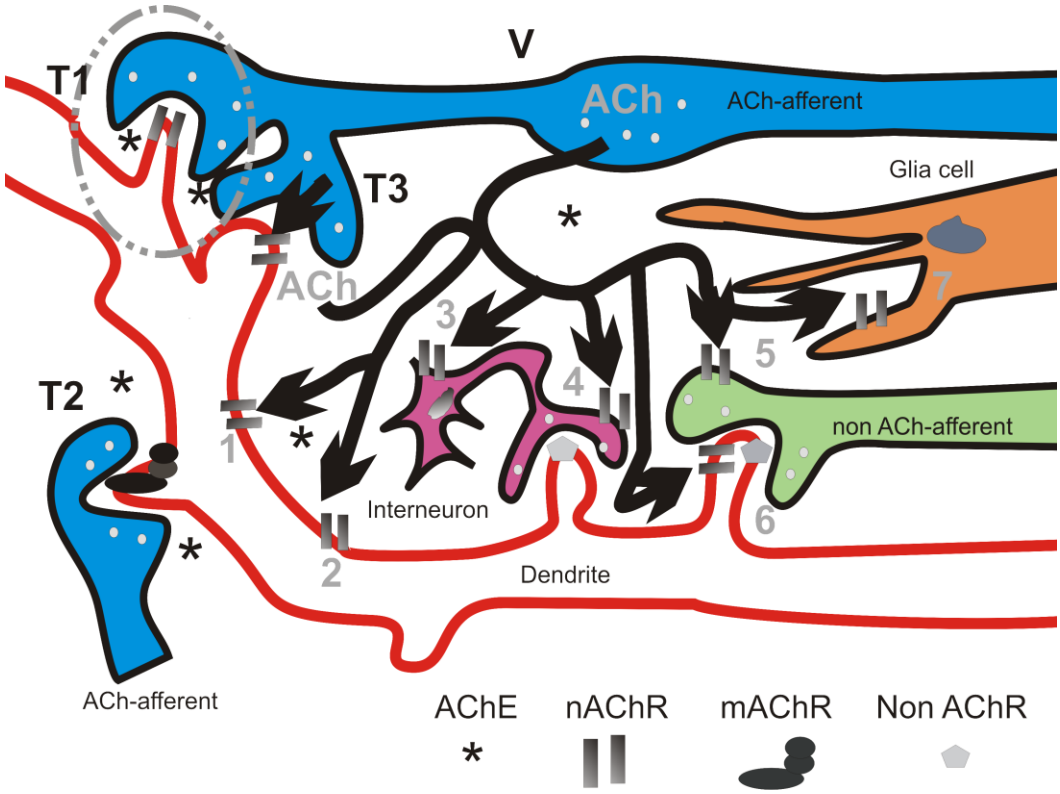

Figure 2. Diagram showing the main features of the cholinergic communication systems in CNS. Cholinergic axons (ACh afferent) release acetylcholine (ACh) at terminals (T 1-3) and preterminal varicosities (V). A few of these terminals mediate rapid nicotinic synaptic transmission similar to that observed at neuromuscular functions or in peripheral nervous system (T-1). In these terminals, the main elements of this type of cell communication (cholin acetyltranspherase –ChAT-; acetylcholinesterase AChE-; nicotinic receptors –nAChRs-) are coincidente at the synapse level, in a similar manner to that observed at muscarinic synapses (T-2) in which nAChRs are substituted by muscarinic receptors (mAChRs). The ACh in these terminals is quickly degraded. However, ACh released from varicosities and terminals with low level of AChE (T-3) diffuse into the extracellular space to reach the nAChRs located in functionally different areas of more distant neurons as well as glial cells (glia). The extent of this ACh diffusion is regulated by the location and concentrentration of AChE (*). This special type of neurotransmission is termed non-sypnatic or volume cholinergic transmission. The nAChRs can be located at dendrites from the efferent neurons, near (1) or far (2) cholinergic synaptic places of their dendrites (dendrites); at dendritic or somatic cholinergic synaptic places of local excitatory or inhibitory neurons (interneurons) (3); at non-cholinergic synapses (nAChRs termed "postsynaptic heteroreceptors" - 6), mixed with GABA or glutamate receptors; at non-cholinergic axons terminals of interneurons (4) or of non-cholinergic afferents (5) (nAChRs termed "presynaptic receptors"); and at glial cells (7).

The extent of this ACh diffusion is regulated by the location and concentration of the extracellular ACh degrading enzyme AChE, while the overall response to the released ACh depends on the number and type of nAChRs activated on different neuronal and non-neuronal cells.

This special type of neurotransmission is termed non-synaptic or volume cholinergic transmission (Umbriaco et al., 1994, 1995; Descarries et al., 1997; Lendvai and Vizi, 2008). It has been experimentally difficult to analyse and characterize this type of neurotransmission in

the brain, but an important body of direct and indirect evidence supports the idea that non-synaptic neurotransmission is the most important ACh regulatory transmission system in broad areas sparsely innervated by the cholinergic neurons of the basalocortical and pontine systems. Moreover, different subtypes of nicotinic transmission have been described on the basis of the location and functionality of the different types of nAChRs. In Figure 1, the possible locations of the nAChRs that might be activated by ACh release are indicated according to the different functional areas of neurons and glial cells: nAChRs in dendritic or somatic areas in post-synaptic positions, or very close to them (classic postsynaptic receptors); nAChRs in neuronal preterminal or terminal areas of axons (presynaptic receptors); nAChRs on dendrites or in somatic areas in extra-synaptic positions (true non-synaptic receptors); and nAChRs on glial cells and endothelial cells.

The postsynaptic receptors may have very different functions. Only a few of these receptors mediate rapid nicotinic synaptic transmission similar to that observed at neuromuscular junctions or in the peripheral nervous system (Horch and Sergent, 1995). Some GABAergic interneurons in the cortex and hippocampus, and a few pyramidal neurons in the cortex, receive rapid and direct excitatory input through ACh release (Lendvai and Vizi, 2008). The operative nAChRs in this transmission are of the α3/ α 5 or α 7 subtype. α4β2 nAChRs most likely correspond to non-synaptic receptors (Buisson and Bertrand, 2001). However, it is important to note that a significant set of postsynaptic receptors is located at non-cholinergic synapses, especially at GABAergic and glutamatergic neurotransmission sites (Fabian-Fine et al., 2001) (Fig. 1). These receptors, termed postsynaptic heteroreceptors, which are mixed with GABA and glutamate receptors (elements of true synaptic neurotransmission at these synapses) are, in a functional sense, non-synaptic receptors from the cholinergic system. The ACh which diffuses into the extracellular space needs to enter the synaptic cleft to reach these nAChRs, in general of the α7 and β2 subtype. The activation of these receptors might modify GABAergic or glutamatergic neurotransmission in specific neurons (Frazier et al., 1998; Adams et al., 2001; Fabian-Fine et al., 2001; Kawai et al., 2002; Wanavaebecq et al., 2007).

Presynaptic neurotransmission is the most prevalent in many regions of the CNS (Fig.1). Via this mechanism the cholinergic system can modulate neurotransmitter release in a wide variety of neuronal circuits in which ACh is not operative as the extracellular interneuronal messenger (Lena et al., 1993; Lena and Changeux 1997; Wonnacot, 1997; Exley and Cragg, 2008; Lendvai and Vizi, 2008). In general, the presynaptic receptors are activated by ACh released at remote sites; no cholinergic varicosities or buttons are observed near the synapses in most neuronal circuits regulated by ACh. Presynaptic neurotransmission is therefore a special case of non-synaptic neurotransmission. The activation of the different types of presynaptic nAChRs in terminals from non cholinergic afferents or local interneurons (Fig. 1) produces the release of neurotransmitters by different mechanisms including depolarisation, calcium entry direct through some nAChRs (mainly of the highly Ca^{2+}-permeable $\alpha 7$ subtype) and calcium entry via the depolarization-induced activation of voltage-gated calcium channels (Castro and Albuquerque, 1995; Soliakov and Wonnacott, 1996; Fucile, 2004; Fayuk and Yakel, 2007). The release of dopamine (especially in the dopamine striatal system – Grady et al., 1992; Soliakov and Wonnacott, 1996; Turner, 2004; Pauly et al., 2004), norepinephrine (Sershen et al., 1997), serotonin (Szasz et al., 2005), GABA and glutamate (Girod et al., 2000; Maggi et al., 2001) can be provoked, stimulated or regulated by direct or indirect intracellular calcium signals elicited by the activation of these type of nAChRs.

Nicotinic stimulation enhances glutamate release and contributes to the induction of synaptic plasticity and long-term potentiation (Mansvelder and McGehee, 2000; Ge and Dani, 2005; Hasselmo, 2006), which are or of great importance in cognitive function. GABA-ergic terminals in different areas of the CNS also have presynaptic receptors (Alkondon et al., 2000). In summary, the activation of nicotinic receptors located in axon preterminal/terminal areas of both afferent fibres and cortical interneurons, together with the activation of other synaptic and non-synaptic dendritic and somatic nAChRs of both efferent and local interneurons, provokes the inhibition and disinhibition of efferent neurons (i.e., the pyramidal cortical neurons), thus regulating the function of each specific area of the CNS. Simultaneously, the nAChRs located on glial and endothelial cell regulate the response of these cells with a view to optimal neuronal function in each area of the CNS.

2. EFFECTS OF ACTIVATING NACHRS

As mentioned above, a variety of effects might be expected in the CNS when nAChRs are activated, a consequence of the variety of these receptors, the diversity of functions they have according to their synaptic or non-synaptic locations at key points in neuronal circuits, the change in response of some nAChRs due to up-regulation and desensitisation, and the intervention of the glia in overall responses in different regions of the CNS. The present section examines some of the effects that have been studied in different CNS regions by our laboratory. These are of great interest with respect to explaining the possible duality of the positive (neuroprotection and neuroplasticity) and negative effects (oxidative stress, apoptosis) of exposure to nicotine/nicotine agonists, and for explaining the neuronal and glial effects that occur in different regions of the CNS.

When the effects of nicotine or nicotinergic agonists in the CNS are studied, special attention must be paid to the animal model to be used; failure to do so could lead to confusing results. The same attention must also be given to any nicotinergic agonist used, the dose employed, the duration of treatment, and the area of the CNS where it its effects are to be studied. The models and protocols that have been used have often been so different that no comparison of the results between studies has been possible. In other studies, the effects elicited have only been studied in one region of the CNS, and this certainly does not permit extrapolations to, say, the rest of the brain. The studies that have been undertaken in attempts to explain the neurotoxicity induced by nicotine in smokers, and to analyse cellular death in different types of neuron, include chronic treatment with medium doses of nicotine/nicotine agonists administered over a long period (>1 month) alongside others that have employed high doses over very short periods (1-3 days). In our work we have preferentially used subchronic treatment models of 15 days, involving doses similar to that of nicotine seen in the brains of smokers. These doses are not associated with apoptosis nor the activation of pro-apoptotic systems (activation of caspases 3, 6 and 9 or an increase in the proteins Bcl-2 and Bclx). This allows more precise assessments to be made of the effects induced by nicotine/nicotine agonists that involve changes in neuronal and glial systems (especially those that might be interpreted as both positive and negative, such as the activation of the isoforms of COX-2 - Toledano et al., 2008). In most cases, the precise intracellular systems set in motion that lead to such different responses to nicotine/nicotine agonist are only imprecisely

known. Many different intracellular signalling pathways are likely involved. In many of the effects studied, especially the development of neuroprotection and neuroplasticity (O'Neil et al., 2002, Dajas-Bailador et al., 2000; Mudo et al., 2007), the changes seen in the Ca^{2+} concentration in certain areas of the neuronal cytoplasm appear to set different Ca^{2+} signalling pathways in motion. The activation of different nAChRs provokes the above-mentioned Ca^{2+} entry through the nicotinergic channels (mainly $\alpha 7$ subtypes) or the voltage-dependent Ca^{2+} channels (activated by nAChR depolarisation) as well as the mobilization of this cation from intracellular stores (Castro and Albuquerque, 1995; Soliakov and Wonnacott, 1996; Fucile, 2004; Fayuk and Yakel, 2007). The modification of the local concentration of Ca^{2+} plays a pivotal role in cellular events such as cell excitability, neurotransmitter release, the production and control of oxidative stress, gene expression and transcriptional changes. All these processes are involved in neuroprotection and neuroplasticity (Messi et al., 1997; Dajas-Bailador et al., 2000). Long-term potentiation (LTP), which is of prime importance in learning and memory, is an effect of nAChR activation. In these intracellular events regulated by nAChR activation, the CREB and ERK/MAPK signalling cascades are operative (Hardingham et al., 2001; Mudo et al., 2007). Nicotine can alter gene expression and transcriptional activation (Gueorguiev et al., 2000; Hardingham et al., 2001). This process seems to be dependent on CaM and MAP kinases (Hu et al., 2002), and provokes the activation of transcription factors such as CREB and an increase in immediate early gene products such as c-Fos (Belluardo et al., 2005). The nicotine-regulated genes produce proteins involved in metabolism, cell signalling and transcription (enzymes, receptors, ion channels, transcription factors, neurotrophic factors – Maggio et al., 1998; Belluardo et al., 1999, 2005; Gueorguiev et al., 2000; Jonnala et al., 2002; Mudo et al., 2007), in both neurons and glial cells.

2.1. Nicotine-Induced Dehydrogenase Activation

We have studied the effects of nicotine on the activity of different dehydrogenases in the frontoparietal region and subcortical nuclei of the rat brain using enzymohistochemical methods (Turégano et al., 2001). Nicotine (sulphate) was intraperitoneally administered as either an acute treatment (1.25 mg of (-) nicotine free base [2.5mg/kg] every day for 3 days), or subchronic treatment (involving an Alzet osmotic pump providing the same daily dose over 15 days). The results showed that forms of administration induced strong increases (60-400%) in glyceraldehyde-3-phosphate (gly3PDH), lactate (LDH), malate (MDH) and succinate (SDH) dehydrogenase activities in the upper layers (all areas of layers I, II and III, and some areas [barrels] of layer IV) of the frontoparietal cortex (cingulate, retrosplenial, motor [motor-1, motor-2 and somatosensory], and the "hindlimb", "forelimb", "barrel field" and somatosensory 2 regions) (fig. 3). However, no significant increases were seen (4-17%) in the deeper layers of the cortex or in the subcortical nuclei (substantia nigra, caudate-putamen, nucleus accumbens or nucleus basalis magnocellularis). Thus, these hyperactivities were produced in brain regions with normally low enzymatic activity (cortex), but not in those with normally greater activity (subcortical nuclei). Cortical layer VI was almost unchanged with regard to the controls. Layer IV, outside the barrels in the somatosensory cortex, showed low reactivity, similar to that of the upper sublayer of layer V. A small but significant difference between motor layer V and somatosensory layer V (+ 10-20%) was

appreciable In cortical layer V of control rats, intense histochemical activity of SDH and LDH was seen in scattered pyramidal-type neurons, but the number of these hyperactive neurons increased (13-37%) in the treated rats. The glial cells also showed an increase in histochemical reactivity in cortical layers I to V.

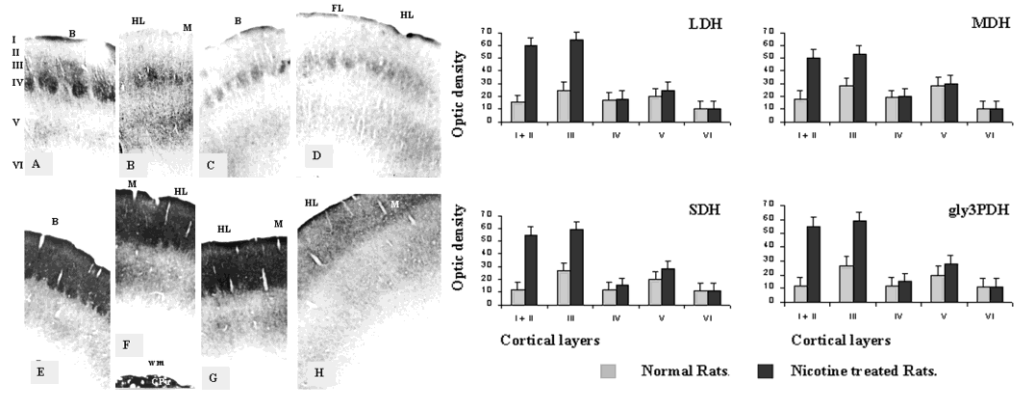

Figure 3. Histochemical increase of lactic- (LDH), malic– (MDH), succinate– (SDH) and glyceraldehyde 3 phosphate– (gly3PDH) dehydrogenases in the frontoparietal cortex of rats under nicotine treatment. In the left side, histochemical demostration in control (A-D) and in acute nicotine-treated (2,5 mg/Kg nicotine free base for 3 days) (E-H) rats of LDH (A, E); MDH (B, F); SDH (C, G) and gly3PDH (D,H). Highg increase of reactivity in layers I-III. In the right ride, graphic representations of the optic density values of the histochemical LDH, MDH, SDH and gly3PDH activities in the cerebral cortex (somatosensory areas) of normal control and subchonic nicotine (2,5 mg/Kg nicotine free base for 15 days) treated rats (statistical significance between groups: $p<0,0001$ in layers I + II and III). (Turégano et al., 2001). (Magnification: 55x).

Both acute (3 days) and chronic (15 days) intraperitoneal treatments with nicotine produced high and specific hyperactivities of these four dehydrogenases: MDH and SDH, mitochondrial enzymes involved in the Krebs cycle; gly3PDH, a key enzyme of glycolysis; and LDH, an important enzyme controlling final glucose utilization in the brain (McIlwain, 1966; Shantha et al., 1968; Laughton et al., 2000). This suggests a rapid and sustained effect of this drug. The observed hyperactivities agreed reasonably well with the strong increase (300-400%) in brain glucose utilization and lactate accumulation elicited by nicotine (McIlwain, 1966). Nicotine acts on blood vessels and cortical neurons (Gitelman and Prohovnik, 1992; Tong and Hamel, 1999; Domino et al., 2000), explaining the increase in glucose utilization. nAChRs associated with cerebral vessels (Kalaria et al., 1994) can regulate the blood flow and increase glucose availability to neurons. Several cellular mechanisms seem to be involved in dehydrogenase hyperactivity since nicotine modulates the expression of a variety of genes and activates transcription (Maggio et al., 1998; Belluardo et al., 1999, 2005; Gueorguiev et al., 2000; Jonnala et al., 2002; Mudo et al., 2007), and favours a liberation of monoaminergic neurotransmitters from aminergic nerve-endings, provoking an increase in glucose utilization. A nicotine-induced overexpression (86%) of mRNA encoding gly3PDH has been reported (Prendergast and Buccafusco, 1998). Whichever neurotransmission system is most involved in activating the cortical neurons during increases in cerebral activity such as stress or seizures, etc., biochemical elevations in the activity of the

glycolytic pathway and in lactic acid production have been observed (McIlwain, 1966; Sokoloff, 1981; Blin et al., 1994). Similarly, histochemical increases in glycolytic and Krebs cycle dehydrogenase have been reported after stimulant consumption (caffeine, amphetamines, etc.) (Martínez-Rodríguez et al., 1984; Martínez-Murillo et al., 1985). In layers II and III, where the dehydrogenase hyperactivities were at their highest, the greatest cortical cell loss has been described after nbM lesion, while the greatest neuroprotective effects have been reported for the nicotinic agonist GTS-21 (Nanri et al., 1997).

Several mismatches exist between the cortical laminar patterns of dehydrogenase maps, cortical cytoarchitecture, local glucose utilization and nicotine receptor localization (Turégano et al., 2001) (Fig. 4). The greatest increases in mitochondrial dehydrogenase activities would be expected in layers III, IV and V by different coincident reasons: the higher mitochondrial density in post-synaptic places, the higher number of synapses in these layers (mainly produced on the pyramidal neurons of the layers III and V - Valverde, 1986) and the reduction in Krebs cycle enzymatic activity that occurs during proximo-distal transport (stronger in axons and less strong in the main dendrites - Toledano et al., 1979. However, the increase in dehydrogenase activity is low in layers IV and V and high in layers I, II and III. The information available suggests a regional effect rather than a general stimulation of the cortical pyramidal cells, suggesting a special sensitivity to nicotine of layer III pyramidal cells and of their regulatory non-pyramidal layer II neurons. All these structures may be involved in nicotinic-regulated cognitive processes.

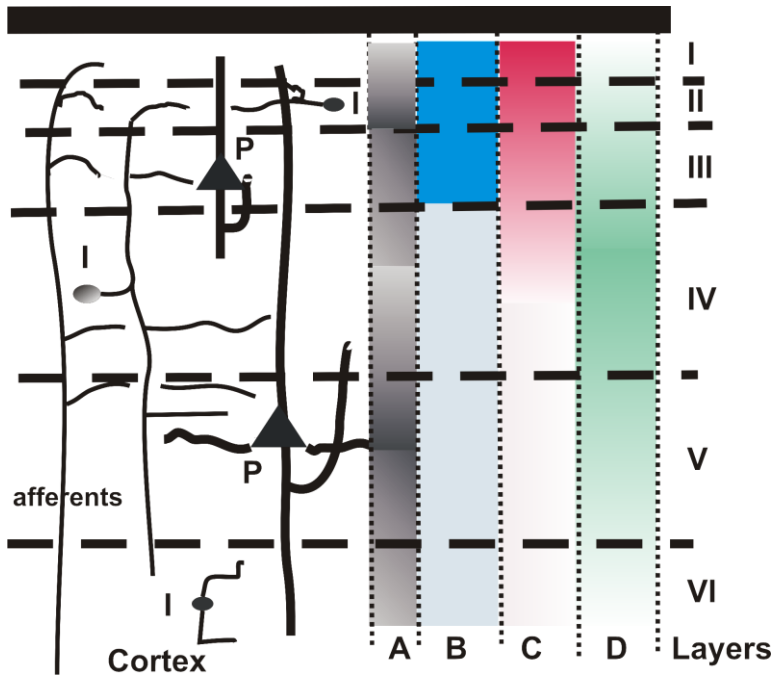

Figure 4. Diagram showing the mismatches existing among the cortical laminar patterns of mitocondrial and synaptic densities (A), dehidrogenase maps of LDH, MDH, SMD and gly3PDH nicotine-induced hyperactivity (B), ^{3}H-methylcaconitine (ligand of high specitificty for α7-nAChRs) autoradiography (C) and ^{3}H-cytisine (ligand of high specificity for $\alpha 4\beta 2$ nAChRs) autoradiography (D). (Adapted from Valverde, 1986; Whiteaker et al., 1999; Happe et al., 1994; Turégano et al., 2001). I-VI = cortical layers ; P= pyramidal neurons. I= cortical interneurons).

Autoradiographic analyses of cortical nAChRs have revealed differences in ligand-binding receptor subtypes among the layers (Clarke et al., 1985; Schultz, 1991; Happe et al., 1994; Perry and Kellar, 1995; Whiteaker et al., 1999). The greatest ^{3}H-nicotine and ^{125}I-α-bungarotoxin (Clarke et al., 1985; Schultz et al., 1991; Whiteaker et al., 1999), ^{3}H-epibatidine (Perry and Kellar, 1995), and ^{3}H-methylcaconitine (Whiteaker et al., 1999) binding is seen in layers I and III, and the highest ^{3}H-cytisine binding (Happe et al., 1994) in layers IV and V. The high specificity of ^{3}H-methylcaconitine for α7 nicotinic receptors, and of ^{3}H-cytisine for α4β2 nicotinic receptors, suggests that the cortical neurons or fibres with the α7 receptor subtype are the primary structures directly affected by nicotine treatment. However, the high ^{3}H-epibatidine binding in layers I-III, more specific for α4β2 N-receptors (Perry and Kellar, 1995; Sihver et al., 1998; Domino et al., 2000), suggests the existence of a complex regional control of metabolism, probably related to specific functions. Different subtypes of receptors, located in different cortical neurons (pyramidal and non-pyramidal) elicit different regional, local or cellular responses, as demonstrated in the hippocampus (Frazier et al., 1998) where interneurons can be nicotinically activated without pyramidal cell stimulation. nAChR up-regulation has been observed in the brain cortex (Sanderson et al., 1993) but it is not cortical-layer specific (Pauly et al., 1991). This non-layered receptor response to nicotine does not correspond to increases in dehydrogenase. It is therefore difficult to explain this layer-specific up-regulation of glucose metabolism. Other discrepancies are seen when comparing the results of this study on nicotinic induction of dehydrogenase hyperactivity with those obtained in studies on local glucose utilization and stimulus-evoked metabolic hyperactivity (Turégano et al., 2001). In general, the metabolic response has been described as more or less homogeneous from layers II to V (Ma et al., 1989). A reduction in glucose metabolism has been documented in senility and AD (Sokoloff, 1981; Mattson et al., 1999). It might be supposed that cortical-specific nicotine agonists could improve brain function by normalizing the nicotinic deficit of the cholinergic system and enhancing the glycolytic and respiratory pathways.

In summary, the neuroprotective, plastic and adaptive effects assigned to nicotine, which are mediated by different mechanisms, might also be related to an ability to activate glycolysis and the Krebs cycle. However, side-effects might originate from this hypermetabolism, including an increase in oxidative stress, which is strongly associated with geriatric brain dysfunctions (Lezza et al., 1994; Mattson et al., 1999; Meier-Ruge and Bertoni-Freddari, 1999; Prasad et al., 1999).

2.2. Nicotine -Induced Increases in Nerve Growth Factor

We have used immunohistochemical and biochemical techniques to examine the effect of nicotine on nerve growth factor (NGF) in the frontoparietal, motor and somatosensory areas of the rat brain cortex. Nicotine was subchronically administered to rats using osmotic pumps (0.35 mg of nicotine base/kg body weight/day for 14 days). An increase in the number and the immunoreactive intensity of NGF immunopositive pyramidal and non-pyramidal neurons was observed in these cortical areas after treatment. In the motor and somatosensory cortical areas of the control animals, only a small number of large pyramidal neurons in layer V, and an even more reduced number of small pyramidal (layer III) and non-pyramidal (layers II to VI)

neurons, all randomly located, showed NGF immunopositivity (and then at low to very low levels) (Fig. 5). The vast majority of the cells were negative in both cortical areas. Positive glial cells were not clearly observed. In the nicotine-treated animals, large numbers of high and low level immunopositive pyramidal cells were observed in layers V and III, as well as many strongly and weakly immunopositive non-pyramidal cells randomly located in layers II to VI (Fig. 5). The intensity of the immunoreaction in the pyramidal cells increased by 35-50% under nicotine treatment, and the number of immunopositive pyramidal neurons increased by 127±4.5% and 82±2.3% (motor and somatosensory areas respectively). The increase in the number of positive non-pyramidal cells was generally very high although variable in all layers (II to VI) of both cortical areas; certain selected sites showed almost all positive cells. Different numbers of NGF-immunopositive glial cells were also observed in the nicotine treated rats (Fig. 5). At the electron microscope level, NGF immunoreactivity was clearly observed in the pyramidal neurons, non-pyramidal neurons and astroglia, with electron-dense deposits in the cytoplasm and prolongations (Fig. 5).

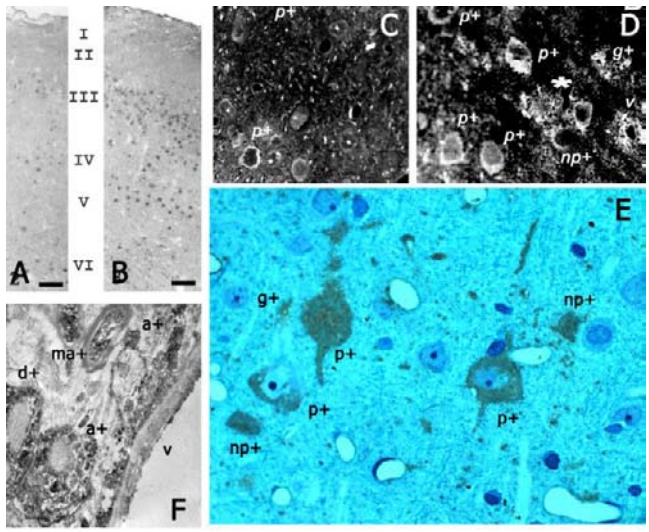

Figure 5. Cortical location of NGF-like immunohistochemical reactivity in somatosensory cortex of control (A) and subchronic nicotine-treated (0.35 mg/Kg nicotine free base/day for 15 days) (B - E) rats. A and B: paraffin embedded sections showing the increase in the number and intensity of immunopositive neurons in layers III to VI. C and D: free floating immunoincubated vibratome secctions. In control rats (C), randomly located immunopositive pyramidal neurons were observed (p+) as well as some immunopositive prolongations. However, most pyramidal neurons showed very low immunopositivity. In nicotine-treated rats (D), a large number of intensely positive pyramidal (p+) and non-pyramidal (np+) neurons were seen. Dense clusters of immunopositive prolongations were observed in the neuropil (*), many of them near blood vessels (v). E, one micron section of plastic-embedded areas from free-floating immunoincubated vibratome section of the motor cortex, layer V, of a nicotine treated rat showing intense immunopositive pyramidal neuron cell bodies (p+), non-pyramidal cell bodies (np+), glial cells (g) and prolongations in the neuropil, with great condensation of prolongations in some areas as well as near some blood vessels (v). F, electron microscope image of a 40nm section of plastic-embedded somatosensory area, layer V, from free floating immunoincubated vibratome sections of a treated rat. Large quantity of strongly immunopositive myelinated (ma+) and non-myelinated (n-ma+) axons, thick and thin dendrites (d+) and glial prolongations (g+) surrounding a blood vessel (v). The long axon (a+) make a synapse (s+) with a large immunopositive dendrite (d+) from a pyramidal cell. Magnification: A, B = 60x; C, D = 160x; E = 300x; F = 20,000x).

Near some capillaries, a network-like structure of immunopositive neuronal prolongations (dendrites and axons, including nerve endings) and astroglial prolongations was seen. These complex structures were numerous and heavily immunostained in treated animals, but very rare in the controls. The increase in NGF was also assessed by Western blotting. The increase (28±6%) after nicotine treatment was statistically significant.

The nicotine-induced increase in NGF seems to be a consequence of enhanced synthesis in the cells that normally produce it. A nicotine-induced NGF increase can be considered a reinforcement of direct nicotinic effects, and the initiation of adaptive mechanisms. The neurotrophin might exert local plastic and neurotransmiter/neuromodulator-like effects (Rattray, 2001; Sofroniew et al., 2001). It can also enhance cholinergic neurotransmission (Knipper et al., 1994) and regulate ionic channels and calcium currents (Nonner et al., 2000). The existence of large, immunopositive myelinated axons suggests the possibility that, under nicotine treatment, NGF might be exported to other brain areas or, on the contrary, that the cortex receives a supplementary quantity of neurotrophin. These effects could be of great therapeutic interest in senility and in neurodegenerative diseases. In old age NGF levels diminish in some cortical regions (Katoh-Semba et al., 1998). In AD, a reduction in neurotrophic factors is reported by some authors (Toledano, 1994; Hefti, 1994), but an increase in NGF, together with a reduction in or a dysfunction of the trkA receptors, has been described by others (Scott and Crutcher, 1994; Siegel and Chauhan, 2000). In addition, increased NGF production in some brain regions might be insufficient for cholinergic maintenance (Rattray, 2001). NGF cooperative effects might include the adjustment of blood flow to the metabolic needs of cortical neurons receiving nicotinic drugs. It has been shown that NGF persistently increases blood flow in patients with AD (Olson et al., 1992). In this respect, the findings suggesting a close relationship between NGF immunopositive neuronal and glial structures and blood vessels are noteworthy. Cholinergic mechanisms and neurotrophin receptor signalling might, together, positively regulate the metabolism of precursor amyloid protein (Rossner et al., 1998). The response of neurotrophic factors to nicotine/nicotine agonists, which can be considered of central importance in nicotinergic treatments, seems to be dependent on the brain-region and nAChR-type. Different factors regulating the functionality of the receptors (up-regulation and desensitisation) could change the neurotrophic factor response as demonstrated with BDNF mRNA in the hippocampus (Kenny et al., 2000).

2.3. Nicotine -Induced Increase in Cyclooxygenase-2

Cyclooxygenase-2 (COX-2) upregulation has been related to both neurodegeneration and physiological processes. In the brains of patients with AD, COX-2 expression has been reported greatly increased (Pasinetti and Aisen, 1998; Deininger et al., 2003). Hoozemans (2001), for example, describes an increase of 95% in the number of COX-2 immunopositive neurons. These results render the anti-neuroinflammatory treatment of sporadic AD of great interest (Giovannini et al., 2003; Eikelenboom and Van Gool, 2004). However, a number of studies (Chang et al., 2000; Dash et al., 2000; Consilvio et al., 2004; Gahring et al., 2005) considering COX-2 as a part of a neuroprotective or restorative system that combats CNS degeneration, suggest that nicotinergic substances and non-steroidal anti-inflammatory drugs (NSAIDs) may be antagonistic. Immunohistochemical and Western blotting studies

performed by us on the frontoparietal cortex, hippocampus and cerebellar cortex of rats treated for 14 days with nicotine (0.35 mg free base/kg/day) showed an up-regulation of COX-2 that can be considered a normal component of the positive effects of nicotine agonists (Fi.6; Tables I and II) (Toledano et al., 2008). The baseline COX-2 immunoreactivity in the brain was very variable, depending on the anatomical area and the type of neuron or glial cell. COX-2 seemed to be selectively expressed by certain neurons in certain regions of the cerebral cortex, hippocampus, amygdala and hypothalamus (Yamagata et al., 1993; Breder et al., 1995; Toledano 2008). In the frontoparietal cortex, variable immunoreactivity against COX-2 was observed, with area- and layer-specific patterns of immunopositive neurons. The number of pyramidal and non-pyramidal COX-2 immunopositive neurons increased progressively from the medial area of the cortex to the perirhinal lateral region (from the primary and secondary motor areas to the insular and ectorhinal areas, passing through the hindlimb, forelimb and trunk primary somatosensory areas, the somatosensory barrell field area, and the secondary somatosensory area) (Table I).

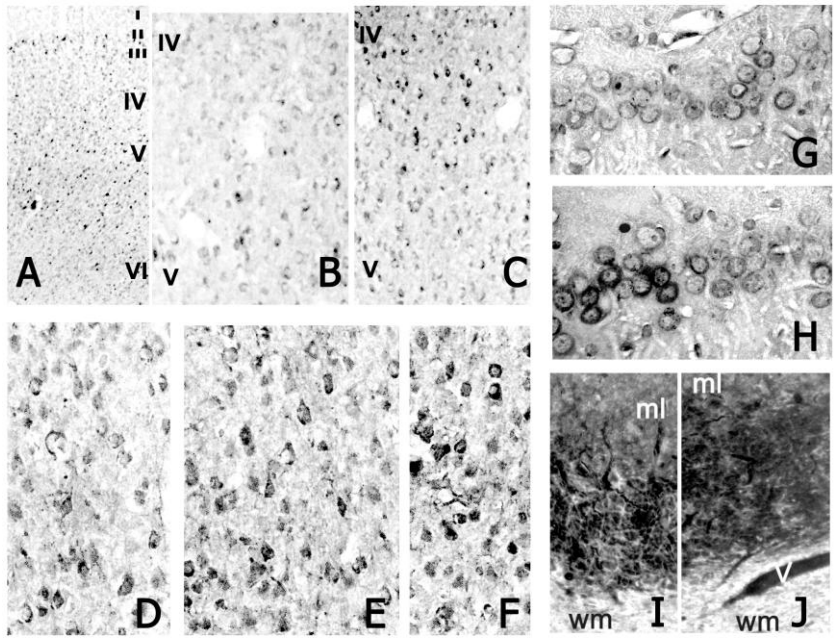

Figure 6: COX-2 immunoreactivity in the frontoparietal cortex from control (A, B, G, I) and treated (C, E, H, J) rats (nicotine: 0.35mg/Kg/day for 14 days). A, B) Normal cortical pattern of hindlimb primary somatosensory cortex in a control rat. Layers I and II were devoid of immunopositive neurons; the largest numbers of immunopositive reactions were seen for the pyramidal neurons of layers V and III. Layers IV and VI showed a variable number of immunopositive neurons. However, in general, the immunopositive reaction was weak. C) Immunopositive neurons in a nicotine-treated animal in layers IV and V. D, E, F) High power magnification of pyramidal neurons in layer V of a control rat (D), a nicotine-treated animal (E), and, for comparison, a 1.46 mg/Kg/day amphetamine-treated animal (F). G, H) Immunopositive pyramidal neurons in the medial area of the CA-1 region of the hippocampus of a control rat (G) and a nicotine-treated (H) rat. No glial cells are COX-2 immunopositive in frontoparietal cortex and hippocampus. I, J) In the cerebellar cortex, only some fibres and glial cells are COX-2 immunopositive (I: control rat; J: nicotine-treated rat). (ml = molecular layer; v = blood vessel; wm = white matter). Magnification: A = 60x; B-F = 100x; G, H = 150x; I, J = 120x).

Table I: Percentages (mean +/- SD) of pyramidal (P) and non-pyramidal (NP) COX-2 immunohistochemically reactive neurons in layers I-VI of the different areas of the frontoparietal cortex (FP CORTEX; gyrus cingularis, primary and secondary motor area [M_1/M_2], hindlimb primary somatosensory area [SHL], and ectorhinal area [Erh]) and pyramidal (P) and granule cells (gc) in regions of the hippocampus (HIPP; CA-1, CA-2; CA-3, dentate gyrus) in control saline and nicotine-treated rats. n = 8 animals/group; ns = non significant; *=p<0.05; **=p<0.005; ***p<0.001).

Anatomical region		Layer (Neuronal type)	Control saline	Nicotine-treated
	Gyrus cingularis	[All layers] Pyramidal (P)	18.2±2.1	28.3±3.2 ***
		Non-pyramidal (NP)	23.2±3.8	27.2±2.2 *
FP CORTEX	M1/M2	Layer II-(NP)	17.8±2.2	18.2±3.4 ns
		Layer III-(NP)	8.1±3.0	14.2±2.6 ***
		Layer III-(P)	14.2±4.0	18.1±2.3 *
		Layer IV-(NP)	6.8±3.2	8.8±4.3 ns
		Layer V-(NP)	6.2±3.1	8.6±3.2 ns
		Layer V-(P)	11.2±4.2	16.3±4.6 *
		Layer VI-(NP)	4.2±2.6	8.2±3.4 *
	SHL	Layer II-(NP)	28.3±2.7	41.1±3.1 ***
		Layer III-(NP)	27.2±3.9	32.1±4.4 *
		Layer III-(P)	34.1±4.4	42.3±3.1 ***
		Layer IV-(NP)	22.3±1.8	28.2±2.2 ***
		Layer V-(NP)	34.5±3.6	38.4±4.3 ns
		Layer V-(P)	28.1±3.8	38.5±4.2 ***
		Layer VI-(NP)	17.0±2.1	27.1±4. ***
	Erh	Layer II/III-(NP)	44.4±3.8	84.2±6.6 ***
		Layer III-(P)	47.23.2	68.8±2.2 ***
		Layer IV-(NP)	39.9±4.4	43.3±7.8 ns
		Layer V-(NP)	47.1±5.5	52.8±6.6 ns
		Layer V-(P)	52.1±6.5	68.2±8.8 ***
		Layer VI-(NP)	23.7±3.2	35.5±5.5 ***
HIPP	CA-1	Pyramidal layer (P)	18.2±2.2	24.9±4.0 ***
	CA-2	Pyramidal layer (P)	4.1±2.1	4.9±2.6 ns
	CA-3	Pyramidal layer (P)	26.1±5.2	38.6±6.1 ***
	Dentate gyrus	Granular layer (gc)	38.2±6.6	51.3±4.8 ***

In the motor areas, COX-2 immunoreactivity was very low, and only a few immunopositive pyramidal neurons in layers III and V and a few positive non-pyramidal elements (mainly in layers II and III) were seen. The highest percentages of COX-2 immunopositive pyramidal and non-pyramidal cells were seen in the insular and ectorhinal/perirhinal regions (Table I). The most intense immunoreactivity (20% greater than

in other regions) was seen in the pyramidal neurons of the secondary somatosensory and perirhinal areas. No glial cells and no neuronal or glial prolongations were immunopositive. In the hippocampus, variable numbers of immunoreactive neurons were seen in the stratum pyramidale of CA-3 and CA-1 (Fig. 6). CA-2, in contrast, was almost negative for this immunostaining. In the cerebellum, no neurons with COX-2 immunopositive cytoplasm were found in the cortex, although immunodeposits were seen in the glial cells, mainly in the Purkinje cell layer, in the ascending mossy and climbing fibres in the granule cell layer, in basket nets surrounding the Purkinje cells, in fibres in the white matter, and in endothelial cells (Fig. 6).

Table II. Percentages (mean +/- SD) of increase in COX-2 content (Western blot immunobiochemical analysis) in the frontoparietal cortex, hippocampus and cerebellum from nicotine-treated rats. (100%= COX-2 content of control saline rats; n= 8 animals/group; ***p<0.001).

CNS Region	Δ COX-2
Frontoparietal cortex	83.3 ± 10.6
Hippocampus	147.2 ± 6.6
Cerebellum	178.6 ± 19.8

The subchronic nicotine-treated animals showed an area- and cell type-dependent up-regulation of COX-2 immunoreactivity (Tables I and II). In the frontoparietal cortex, an increase in the number of COX-2 immunopositive cells was induced, but this enhanced immunoreactivity varied depending on the cortical area and layer. The principal and secondary motor areas showed no important variations, whereas the somatosensory and auditory areas showed significantly more immunoreactive pyramidal and non-pyramidal neurons in some layers than in others (Table I). COX-2 staining in the pyramidal neurons of layer III increased progressively from 23% in the hind- and fore-limb areas to 46% in the secondary somatosensory area, but the pyramidal neurons in layer V showed a similar increase in all these areas (30-37%) (Table I). The intensities of the immunodeposits in the pyramidal neurons were only 10-17% greater than in the controls. The number of COX-2 immunoreactive non-pyramidal neurons varied depending upon the layer: the greatest increase was seen in layers II and III of the ectorhinal area (90%) and in layers II and VI of the hind-limb and fore-limb somatosensory area, as well as in the secondary somatosensory areas (44-60%). In the neuropil, some neuronal fibres (more intense in somatosensory area 2 and the perirhinal area) and randomly located glial cells (bodies and prolongations) showed COX-2 immunopositivity. The endothelial cells showed immunoreactivity similar to that seen in the controls. In the gyrus cingularis, more neurons showed COX-2 immunoreactivity than in the controls: a 55% increase was seen in immunopositive pyramidal cells and a 17% increase in non-pyramidal cells (Table I). In the hippocampus, the dentate gyrus, CA-1 and CA-3 showed a significant increase (Table I) in the number of COX-2 immunopositive pyramidal cells under nicotine treatment (37-50%), but the intensity of the immunodeposits was low except in selected neurons at random locations. In the cerebellar cortex, the nicotine treatment did not change the immunoreactive pattern. Western blot analysis of the brain homogenates of the nicotine-treated rats showed a significant increase in the COX-2 content in the frontal cortex and hippocampus and a reduction in the cerebellum. The constant

presence of specific subsets of COX-2 immunopositive neurons in normal control brains suggests a physiological role for this enzyme in certain neurons. This involves the existence of different functional circuits in areas of similar cytoarchitecture and different possibilities of pathophysiological responses via COX-2 upregulation. The little immunoreactivity in the glial and endothelial cells (except in the cerebellar cortex) suggests a secondary role for COX-2 in these cells in the normal adult brain. Immunopositive fibres coming from the callosal body and entering certain regions of the brain cortex, as well as fine immunodeposits in close association with the cortical neurons, plus the immunopositive mossy and climbing fibres and basket nets surrounding the Purkinje cells in the cerebellar cortex, all suggest a synaptic role for COX-2 in local and extracortical neuronal circuits (Kaufmann, 1997). Nicotine-induced COX-2 up-regulation is only clearly observed in cortical areas where large numbers of neurons showing COX-2 immunopositivity exist. Regions of very small numbers of immunopositive neurons (such as the motor cortex areas, the cerebellar cortex or CA-2), showed no evidence of neuronal COX-2 induction. The most important feature of the COX-2 response to treatment was the strikingly different behaviour of the COX-2 gene and/or its mechanisms of up-regulation in apparently similar neurons and areas in the brain cortex. Similarly intense cholinergic innervation was supposed, and similar nicotinergic receptors seemed to exist in many of the COX-2-responsive and COX-2 non-responsive neurons/areas. The intrinsic characteristics of the neurons can be considered the main factors involved in the response to nicotine. The most general immunoreactive response (an increasing number of immunopositive cells) was shown by non-pyramidal neurons of layers II and III of the frontoparietal cortex, while the most selective response (the most intense neuronal immunodeposits) was shown by pyramidal neurons of layers III and V, as well as selected hippocampal neurons. All this suggests the existence of different subsets of neurons defined by their receptors but also by their metabolic organization. Several papers report COX-2 induction in astroglial, microglial and endothelial cells (Schiltz and Sawchenko, 2002; De Simoni et al, 2005; Choi et al, 2006b) which have nicotine receptors (Choi et al, 2006b), but no nicotine-induced COX-2 up-regulation has been observed. This suggests that the proposed involvement of COX-2 induction in neurodegenerative diseases (mainly related to glial responses) may be associated with other cellular mechanisms of an involutive nature.

This nicotine-induced up-regulation of COX-2 seems to be an important neuroprotective/adaptive effect of nicotine agonist treatments, and it might be important in the treatment of AD. As suggested by Gahring et al. (2005), the administration of such medication and NSAIDs could be antagonistic. Certainly, intrahippocampal infusion of nicotine prevents certain memory deficits induced by NSAIDs (Sharifzadeh et al., 2005).

3. CONCLUSION

The activation of the nAChRs in the CNS produces a great variety of neuronal and glial effects specific to each CNS region. This is the consequence of each region having its own, specific neuronal circuits that are precisely regulated by nicotinergic cholinergic terminals, and the fact that there are many types of nAChR. These receptors are found at strategic places on neurons (at the synapse and elsewhere) and on glial cells. The presence of agonists or antagonists at some nAChRs leads to a series of modifications in their macromolecular

configuration and/or their presence (up-regulation, desensitisation) on the cell surface or intracellular membranes, affecting the responses made. Nicotinergic treatments may therefore have a range of very varied effects, depending on the region of the CNS under study, the type of product used, the dose administered, and the duration of treatment.

The intracellular signalling systems used by the different nAChR subtypes are not well known, but the increase in intracellular Ca^{2+}, which may enter via different subtypes of nAChR, appears to have a crucial role in the activation of many Ca^{2+}-dependent pathways. These pathways are associated with different neuroprotection and neuroplasticity phenomena ranging from the short-term regulation of local neurotransmission to the long-term synaptic regulation of neurotransmission in complex neuronal circuits (vital for learning and memory), and the expression of different genes. The nicotine-regulated genes produce proteins involved in metabolism, cell signalling and transcription (enzymes, receptors, ion channels, transcription factors, neurotrophic factors, etc) in both neurons and glial cells. The activation of intracellular signalling pathways and the regulation of gene expession induced by nicotine agonists modify the behaviour of neurons and glial cells in a region, area and neuronal circuit dependent manner. Our research group has demonstrated that nicotine increases the turnover of the glycolytic pathway and Krebs cycle in the cortex in a layer dependent manner, and the NGF immunoreactivity in neurons and glial cells in the frontoparietal cortex. Moreover, the increase of COX-2 has been observed in an area-, layer- and neuron type-dependent manner in different regions of the frontoparietal cortex, hippocampus and cerebellar cortex. The up-regulation of these enzymes and the NGF could have beneficial effects on neuronal function by helping neuronal adaptations involved in the performance of cognitive functions. Such results could help in the development of new treatments for cognitive disorders as well as help us understand the mechanism of action of certain drugs of abuse.

REFERENCES

Adams CE, Stitzel JA, Collins AC, Freedman R. (2001). *Alpha7-nicotinic receptor expression and the anatomical organization of hippocampal interneurons.* Brain Res. 922:180-190

Albuquerque EX, Pereira EF, Alkondon M, Rogers SW. (2009). *Mammalian nicotinic acetylcholine receptors: from structure to function*. Physiol Rev. 89:73-120

Albuquerque EX, Pereira EF, Mike A, Eisenberg HM, Maelicke A, Alkondon M. (2000). *Neuronal nicotinic receptors in synaptic functions in humans and rats: physilogical and clinical relevance*. Behav Brain Res. 113:131-141

Alkondon M, Pereira EF, Eisenberg HM, Albuquerque EX. (2000) *Nicotinic receptor activation in human cerebral cortical interneurons: a mechanism for inhibition and disinhibition of neuronal networks.* J Neurosci. 20:66-75

Aznavour N, Watkins KC, Descarries L. (2005). *Postnatal development of the cholinergic innervation in the dorsal hippocampus of rat: Quantitative light and electron microscopic immunocytochemical study.* J Comp Neurol. 486:61-75

Bartus RT. (2000). *On neurodegenerative disease models and treatment strategies: lessons learned and lessons forgotten A generation following the cholinergic hypothesis*. Exp Neurol. 163:495-529

Belluardo N, Mudò G, Blum M, Cheng Q, Caniglia G, Dell'Albani P, Fuxe K. (1999). *The nicotinic acetylcholine receptor agonist (+/-)-epibatidine increases FGF-2 Mrna and protein levels in the rat brain.* Brain Res Mol Brain Res. 74:98-110.

Belluardo N, Mudò G, Blum M, Fuxe K. (2000) *Central nicotinic receptors, neurotrophic factors and neuroprotection.* Behav Brain Res. 113:21-34.

Belluardo N, Olsson PA, Mudo' G, Sommer WH, Amato G, Fuxe K. (2005). *Transcription factor gene expression profiling after acute intermittent nicotine treatment in the rat cerebral cortex.* Neuroscience. 133:787-796.

Besson M, Granon S, Mameli-Engvall M, Cloëz-Tayarani I, Maubourguet N, Cormier A, Cazala P, David V, Changeux JP, Faure P. (2007*). Long-term effects of chronic nicotine exposure on brain nicotinic receptors.* Proc Natl Acad Sci U S A. 104:8155-8160

Besson M, Granon S, Mameli-Engvall M, Cloëz-Tayarani I, Maubourguet N, Cormier A, Cazala P, David V, Changeux JP, Faure P. (2007). *Long-term effects of chronic nicotine exposure on brain nicotinic receptors.* Proc Natl Acad Sci U S A. 104:8155-8160

Blin J, Ray CA, Piercey MF, Bartko JJ, Mouradian MM, Chase TN. (1994). *Comparison of cholinergic drug effects on regional brain glucose consumption in rats and humans by means of autoradiography and positron emission tomography.* Brain Res 635:196 –202.

Boatman PD, Richman JG, Semple G. (2008). *Nicotinic acid receptor agonists.* J Med Chem. 51:7653-7662

Bodor ET, Offermanns S. (2008). *Nicotinic acid: an old drug with a promising future*. Br J Pharmacol. 153:S68-S75.

Breder CD, Dewitt D, Kraig RP. (1995). *Characterization of inducible cyclooxygenase in rat brain.* J Comp Neurol 355:296-315.

Buccafusco JJ, Beach JW, Terry AV Jr. (2009). *Desensitization of nicotinic acetylcholine receptors as a strategy for drug development*. J Pharmacol Exp Ther. 328:364-370

Buckingham SD, Jones AK, Brown LA, Sattelle DB. (2009). *Nicotinic acetylcholine receptor signalling: roles in Alzheimer's disease and amyloid neuroprotection.* Pharmacol Rev. 61:39-61

Buisson B, Bertrand D. (2001). *Chronic exposure to nicotine upregulates the human (alpha)4((beta)2 nicotinic acetylcholine receptor function*. J Neurosci. 21:1819-1829

Butcher LL, Woolf NJ. (1986). *Central cholinergic systems: synopsis of anatomy and overview of physiology and pathology*. In: Scheibel AB, Weschler AF, Brazier MAB, eds. The biological substrates of Alzheimer's disease. Academic Pres, Orlando San Diego pp:73-86

Carlson J, Noguchi K, Ellison G. (2001). *Nicotine produces selective degeneration in the medial habenula and fasciculus retroflexus.* Brain Res 906:127-134

Carnevale D, De Simone R, Minghetti L. (2007). *Microglia-neuron interaction in inflammatory and degenerative diseases: role of cholinergic and noradrenergic systems. CNS* Neurol Disord Drug Targets. 6:3883-97

Castro NG, Albuquerque EX. (1995). *alpha-Bungarotoxin-sensitive hippocampal nicotinic receptor channel has a high calcium permeability*. Biophys J. 68:516-524

Chang YW, Jakobi R, McGinty A, Foschi M, Dunn MJ, Sorokin A. (2000). *Cyclooxygenase 2 promotes cell survival by stimulation of dynein light chain expression and inhibition of neuronal nitric oxide synthase activity.* Mol Cell Biol 20:8571-8579.

Changeux JP, Bertrand D, Carriger PJ, Dehoene S, Edelstein S, Lena C, Le Navère N, Marubio L, Picciotto M, Zali M. (1998). *Brain nicotinic receptors: structure and regulation, role in learning and reinforcement*. Brain Res Rev 26:198-216

Choi SH, Langenbach R, Bosetti F. (2006). *Cyclooxygenase-1 and -2 enzymes differentially regulate the brain upstream NF-kappa B pathway and downstream enzymes involved in prostaglandin biosynthesis.* J Neurochem 98:801-811

Cincotta SL, Yorek MS, Moschak TM, Lewis SR, Rodefer JS. (2008). *Selective nicotinic acetylcholine receptor agonists: potential therapies for neuropsychiatric disorders with cognitive dysfunction.* Curr Opin Investig Drugs. 9:47-56.

Clarke PBS, Schwartz RD, Paul SM, Pert CB, Pert A. (1985). *Nicotinic binding in rat brain: autoradiographic comparison of [^{3}H]acetylcholine, [^{3}H]nicotine, and [^{125}I]-α-bungarotoxin.* J Neurosci. 5:1307–1315.

Consilvio C, Vicent AM, Feldman EL. (2004). *Neuroinflammation, COX-2, and ALS. A dual role?* Exp Neurol. 187:1-10.

Cordero-Erausquin M, Marubio LM, Klink R, Changeux JP. (2000). *Nicotinic receptor function: new perspectives from knockout mice*. Trend Pharmacol Sci. 21: 211-217

Dajas-Bailador FA, Lima PA, Wonnacott S. (2000) *The alpha7 nicotinic acetylcholine receptor subtype mediates nicotine protection against NMDA excitotoxicity in primary hippocampal cultures through a Ca(2+) dependent mechanism*. Neuropharmacology. 39:2799-2807

Dash PK, Mach SA, Moore AN. (2000). *Regional expression and role cyclooxygenase-2 following experimental traumatic brain injury*. J Neurotrauma. 17:69-81.

De Simone R, Ajmone-Cat MA, Carnevale D, Minghetti L. (2005). *Activation of alpha7 nicotinic acetylcholine receptor by nicotine selectively up-regulates cyclooxygenase-2 and prostaglandin E2 in rat microglial cultures*. J Neuroinflammation 2:4 (DOI: 10.1186/1742-2094-2-4)

Deininger MH, Bekure-Nemariam K, Trautmann K, Morgalla M, Meyermann R, Schlucsener HJ. (2003). *Cyclooxygenase-1 and -2 in brains of patients who died with sporadic Creutzfeldt-Jakob disease*. J Mol Neurosci 20:25-30.

Descarries L, Gisiger V, Steriade M. (1997). Diffuse *transmission by acetylcholine in the CNS.* Prog Neurobiol. 53:603-625

Domino EF, Minoshima S, Guthrie S, Ohl L, Ni L, Koeppe RA, Zubieta J-K. (2000). *Nicotine effects on regional cerebral blood flow in awake, resting tobacco smokers*. Synapse. 38:313–321.

Drago J, McColl CD, Horne MK, Finkelstein DI, Ross SA. (2003). Neuronal nicotinic receptors: insights gained from gene knockout and knockin mutant mice. Cell Mol Life Sci. 60:1267-80

Eikelenboom P, Van Gool WA. (2004). *Neuroinflammatory perspectives on the two faces of Alzheimer's disease.* J Neural Transm. 111:281-294.

Exley R, Cragg SJ. (2008). *Presynaptic nicotinic receptors: a dynamic and diverse cholinergic filter of striatal dopamine neurotransmission*. Br J Pharmacol. 153 (supp 1):S283-S297

Fabian-Fine R, Skehel P, Errington ML, Davies HA, Sher E, Stewart MG, Fine A. (2001). *Ultrastructural distribution of the alpha7 nicotinic acetylcholine receptor subunit in rat hippocampus*. J Neurosci. 21:7993-8003

Fayuk D, Yakel JL. (2007). *Dendritic Ca2+ signalling due to activation of alpha 7-containing nicotinic acetylcholine receptors in rat hippocampal neurons.* J Physiol. 582:597-611.

Frazier CJ, Rollins YD, Breese CR, Leonard S, Freedman R, Dunwiddie TV. (1998). *Acetylcholine activates an alpha-bungarotoxin-sensitive nicotinic current in rat hippocampal interneurons, but not pyramidal cells*. J Neurosci. 18:1187–1195.

Frazier CJ, Rollins YD, Breese CR, Leonard S, Freedman R, Dunwiddie TV. (1998). *Acetylcholine activates an alpha-bungarotoxin-sensitive nicotinic current in rat hippocampal interneurons, but not pyramidal cells*. J Neurosci. 18:1187-1195

Gentry CL, Lukas RJ. (2002). *Regulation of nicotinic acetylcholine receptor numbers and function by chronic nicotine exposure*. Curr Drug Targets CNS Neurol Disord. 1:359-385

Gharing LC, Meyer EL, Rogers SW. (2003). *Nicotine-induced neuroprotection against N-methyl-D-aspartic acid of beta-amyloid peptide occur through independent mechanisms distinguished by pro-inflammatory cytokines.* J Neurochem. 87:1125-1136.

Gharing LC, Persiyanov K, Days EL, Rogers SW. (2005). *Age-related loss of neuronal nicotinic receptor expression in the aging mouse hippocampus corresponds with cyclooxygenase-2 and PPAR gamma expression and is altered by long-term NS398 administration*. J Neurobiol. 62:453-468

Giniatullin R, Nistri A, Yakel JL. (2005). *Desensitization of nicotinic ACh receptors: shaping cholinergic signaling.* Trends Neurosci. 28:371-378

Giovannini MG, Scali C, Prosperi C, Bellucci A, Pepeu G, Casamenti F. (2003). *Experimental brain inflammation and neurodegenerations as model of Alzheimer's disease: protective effects of selective COX-2 inhibitors.* Int J Immunopathol Pharmacol. 16:S31-S40.

Girod R, Barazangi N, McGehee D, Role LW. (2000) *Facilitation of glutamatergic neurotransmission by presynaptic nicotinic acetylcholine receptors*. Neuropharmacology. 39:2715-2725

Gitelman DR, Prohovnik I. (1992). *Muscarinic and nicotinic contributions to cognitive function and cortical blood flow*. Neurol Biol. 13:313–318.

Gotti C, Zoli M, Clementi F. (2006). *Brain nicotinic acetylcholine receptors: native subtypes and their relevance.* Trends Pharmacol Sci. 27:482-491.

Grady S, Marks MJ, Wonnacott S, Collins AC. (1992). *Characterization of nicotinic receptor-mediated [3H]dopamine release from synaptosomes prepared from mouse striatum*. J Neurochem. 59:848-856.

Grady SR, Salminen O, Laverty DC, Whiteaker P, McIntosh JM, Collins AC, Marks MJ. (2007). *The subtypes of nicotinic acetylcholine receptors on dopaminergic terminals of mouse striatum.* Biochem Pharmacol. 74:1235-1246

Gueorguiev VD, Zeman RJ, Meyer EM, Sabban EL. (2000) *Involvement of alpha7 nicotinic acetylcholine receptors in activation of tyrosine hydroxylase and dopamine beta-hydroxylase gene expression in PC12 cells*. J Neurochem. 75:1997-2005

Happe HK, Peters JL, Bergman DA, Murrin LC. (1994). *Localization of nicotinic cholinergic receptors in rat brain: autoradiographic studies with [3H]cytisine*. Neuroscience. 62:929 –944.

Hardingham GE, Arnold FJ, Bading H. (2001). *Nuclear calcium signaling controls CREB-mediated gene expression triggered by synaptic activity*. Nat Neurosci. 4:261-267

Hasselmo ME. (2006). *The role of acetylcholine in learning and memory*. Curr Opin Neurobiol. 16:710-715

Hefti F. (1994). *Development of effective therapy for Alzheimer's disease based on neurotrophic factors*. Neurobiol Aging. Suppl 2:S193-S194

Hoozemans JJ, Rozemuller AJ, Veerhuis R, Eikelenboom P. (2001). *Immunological aspects of Alzheimer´s disease: therapeutic implication*. Bio Drug 15:325-337.

Horch HL, Sargent PB. (1995). *Perisynaptic surface distribution of multiple classes of nicotinic acetylcholine receptors on neurons in the chicken ciliary ganglion*. J Neurosci. 15:7778-7795

Hu M, Liu QS, Chang KT, Berg DK. (2002). *Nicotinic regulation of CREB activation in hippocampal neurons by glutamatergic and nonglutamatergic pathways*. Mol Cell Neurosci. 21:616-625.

Iversen SD. (1998). *The pharmacology of memory*. CR Acad Sci III. 321:209-215

Jang MH, Shin MC, Jung SB, Lee TH, Bahn GH, Kwon YK, Kim EH, Kim CJ. (2002). *Alcohol and nicotine reduce cell proliferation and enhance apoptosis in dentate gyrus.* Neuroreport. 13:1509-1513.

Jonnala RR, Buccafusco JJ. (2001). *Relationship between the increased cell surface alpha7 nicotinic receptor expression and neuroprotection induced by several nicotinic receptor agonists.* J Neurosci Res. 66:565-572

Jonnala RR, Terry AV Jr, Buccafusco JJ. (2002). *Nicotine increases the expression of high affinity nerve growth factor receptors in both in vitro and in vivo*. Life Sci. 70:1543-1554.

Kalaria RN, Homayoun P, Whitehouse PJ. (1994). *Nicotinic cholinergic receptors associated with mammalian cerebral vessels.* J Auton Nerv Syst 49(Suppl):S3–S7.

Katoh-Semba R, Takeuchi IK, Kato K. (1998). *Age-related changes in levels of brain-derived neurotrophic factor in selected brain regions of rats, normal mice and senescence-accelerated mice: a comparison to those of nerve growth factor and neurotrophin-3*. Neurosci Res. 31:227-234

Kaufman WE, Andreasson KI, Isakson PC, Worley PF. (1997). *Cyclooxygenases and the Central Nervous System.* Prostaglandins. 54:601-624.

Kawai H, Zago W, Berg DK. (2002). *Nicotinic alpha 7 receptor clusters on hippocampal GABAergic neurons: regulation by synaptic activity and neurotrophins*. J Neurosci. 22:7903-7912

Kawashima K, Fujii T. (2008). *Basic and clinical aspects of non-neuronal acetylcholine: overview of non-neuronal cholinergic systems and their biological significance*. J Pharmacol Sci. 106:167-173

Kenny PJ, File SE, Razttray M. (2000). *Acute nicotine decreases and chronic nictotine increases the expression of brain-derived neurotrophic factor mRNA in rat hippocampus.* Mol Brain Res. 85:234-238

Knipper M, da Penha Berzaghi M, Blochl A, Breer H, Thoenen H, Lindholm D. (1994). *Positive feedback between acetylcholine and neurotrophins nerve growth factor and brain derived neurotrophic factor in the hippocampus*. Eur J Neurosci. 4:668-671

Langley JN. (1901). *On the stimulation and paralysis of nerve-cells and of nerve-endings.* Part I. J Physiol. 27:224-236

Langley JN. (1905). *On the reactions of cells and of nerve-endings to certain poisons, chiefly as regard the reaction of striated muscle to nicotine and curari*. J Physiol. 33:374-413

Le Novère N, Corringer PJ, Changeux JP. (2002). *The diversity of subunit composition in nAChRs: evolutionary origins, physiologic and pharmacologic consequences*. J Neurobiol. 53:447-456

Léna C, Changeux JP, Mulle C. (1993). *Evidence for "preterminal" nicotinic receptors on GABAergic axons in the rat interpeduncular nucleus*. J Neurosci. 13:2680-2688

Léna C, Changeux JP. (1997). *Role of Ca2+ ions in nicotinic facilitation of GABA release in mouse thalamus.* J Neurosci. 17:576-585

Lendvai B, Vizi ES. (2008). *Nonsynaptic chemical transmission through nicotinic acetylcholine receptors.* Physiol Rev. 88:333-349

Levin ED, McClernon FJ, Rezvani AH. (2006). *Nicotinic effects on cognitive function: behavioral characterization, pharmacological specification, and anatomic localization*. Psychopharmacology. 184:523-539

Levin ED, Simon BB. (1998). *Nicotinic acetylcholine involvement in cognitive function in animals.* Psychopharmacoly.138:217-230

Lezza AM, Boffoli D, Scacco S, Cantatore P, Gadaleta MN. (1994*). Correlation between mitochondrial DNA 4977-bp deletion and respiratory chain enzyme activities in aging human skeletal muscles*. Biochem Biophys Res Commun. 205:772–779.

Li Y, Papke RL, He YL, Millard WJ, Meyer EM. (1999). *Characterization of the neuroprotective and toxic effects of alpha7 nicotinic receptor activation in PC12 cells.* Brain Res. 830:218-225

Lindstrom J, Anand R, Peng X, Gerzanich V, Wang F, Li Y. (1995). *Neuronal nicotinic receptor subtypes*. Ann N Y Acad Sci. 757: 100-116

Lloyd GK, Menzaghi F, Bontempi B, Suto C, Siegel R, Akong M, Stauderman K, Velicelebi G, Johnson E, Harpold MM, Rao TS, Sacaan AI, Chávez-Noriega LE, Washburn MS, Vernier JM, Cosford ND, McDonald LA. (1998). *The potential of subtype-selective neuronal nicotinic acetylcholine receptor agonists as therapeutic agents.* Life Sci. 62:1601-1606

Ma W, Hohmann CF, Coyle JT, Juliano SL. (1989). *Lesions of the basal forebrain alter stimulus-evoked metabolic activity in mouse somatosensory cortex*. J Comp Neurol. 288:414–427.

Maelicke A, Schrattenholz A, Samochocki M, Radina M, Alburquerque EX. (2000). *Allosterically potentiating ligands of nicotinic receptors as a treatment strategy for Alzheimer's disease*. Behav Brain Res. 113:199-206

Maggi L, Sher E, Cherubini E. (2001). *Regulation of GABA release by nicotinic acetylcholine receptors in the neonatal rat hippocampus*. J Physiol. 536:89-100

Maggi L, Sher E, Cherubini E. (2001). *Regulation of GABA release by nicotinic acetylcholine receptors in the neonatal rat hippocampus.* J Physiol. 536:89-100

Maggio R, Riva M, Vaglini F, Fornai F, Molteni R, Armogida M, Racagni G, Corsini GU. (1998). *Nicotine prevents experimental parkinsonism in rodents and induces striatal increase of neurotrophic factors*. J Neurochem. 71:2439-2446.

Mansvelder HD, McGehee DS. (2000) *Long-term potentiation of excitatory inputs to brain reward areas by nicotine.* Neuron. 27:349-357

Marks MJ, Pauly JR, Gross SD, Deneris ES, Hermans-Borgmeyer I, Heinemann SF, Collins AC. (1992). *Nicotine binding and nicotinic receptor subunit RNA after chronic nicotine treatment.* J Neurosci. 12:2765-8274

Martínez-Murillo R, Martínez-Rodríguez R, Arenas-Díaz G. (1985). *Succinic and malic dehydrogenase activities in cerebral, cerebellar and neostriatum sections incubated in presence of D-amphetamine.* Acta Neuropathol. 67:81– 85.

Martínez-Rodríguez R, Martínez-Murillo R. (1984). *In vitro D-amphetamine action on oxido-reductase activity in several rat nervous centers.* Acta Histochem. 74:85–90.

Mattson MP, Pederson WA, Duan W, Culmsee C, Camandola S. (1999). *Cellular and molecular mechanisms underlying perturbed energy metabolism and neuronal degeneration in Alzheimer disease*. Ann NY Acad Sci. 893:154 –175.

McIlwain H. (1966). *Biochemistry and the central nervous system*. London: Churchill Ltd.

Mechawar N, Watkins KC, Descarries L. (2002). *Ultrastructural features of the acetylcholine innervation in the developing parietal cortex of rat.* J Comp Neurol. 443:250-258

Meier-Ruge WA, Bertoni-Freddari C. (1999). *Mitochondrial genome lesions in the pathogenesis of sporadic Alzheimer disease*. Gerontology. 45:289 –297.

Messi ML, Renganathan M, Grigorenko E, Delbono O. (1997). *Activation of alpha7 nicotinic acetylcholine receptor promotes survival of spinal cord motoneurons*. FEBS Lett. 411:32-38

Mesulam MM, Mufson EJ, Weiner EJ, Levey AI. (1983). *Central cholinergic pathway in the rat: an overview based on an alternative nomenclature (Ch1-Ch6).* Neuroscience. 10:1185-1201

Millar NS, Gotti C. (2009). *Diversity of vertebrate nicotinic acetylcholine receptors.* Neuropharmacology. 56:237-246

Millar NS, Harkness PC. (2008). *Assembly and trafficking of nicotinic acetylcholine receptors.* Mol Membr Biol. 25:279-292

Monzón-Mayor M, Alvarez M, Arbelo-Galván J, Romero-Alemán M, Yanes C, Plaza ML, Rodríguez JR, Rodríguez JJ, Toledano A. (2000) *Long-term evolution of local, proximal and remote astrocyte responses after diverse nucleus basalis lesioning (an experimental Alzheimer model): GFAP immunocytochemical study*. Brain Res. 865:245-258

Mudo G, Belluardo N, Fuxe K. (2007). *Nicotinic receptor agonists as neuroprotective/neurotrophic drugs. Progress in molecular mechanisms*. J Neural Transm. 114:135-47.

Mugnaini M, Garzotti M, Sartori I, Pilla M, Repeto P, Heidbreder CA, Tessari M. (2006). *Selective down-regulation of [^{125}I]Y0-alpha-conotoxin MII binding in rat mesostriatal dopamine pathway following continuous infusion of nicotine*. Neuroscience. 137:565-572

Nanri M, Kasahara N, Yamamoto J, Miyake H, Watanabe H. (1997). *GTS-21, a nicotinic agonist, protects against neocortical neuronal cell loss induced by the nucleus basalis magnocellularis lesion in rats.* Jpn J Pharmacol. 74:285–289.

Neff RA 3rd, Gomez-Varela D, Fernandes CC, Berg DK. (2009). *Postsynaptic scaffolds for nicotinic receptors on neurons*. Acta Pharmacol Sin. 30:694-701

Newhouse PA, Potter A, Kelton M, Corwin J. (2001). *Nicotinic treatment of Alzheimer's disease*. Biol Psychiatry. 49:268-78.

Newman MB, Arendash GW, Shytle RD, Bickford PC, Tighe T, Sanberg PR. (2002). *Nicotine's oxidative and antioxidant properties in CNS*. Life Sci. 71:2807-2820.

Nonner D, Barrett EF, Barrett JN. (2000). *Brief exposure to neurotrophins produces a calcium-dependent increase in choline acetyltransferase activity in cultured rat septal neurons.* J Neurochem 74:268-278

Olson L, Nordberg A, van Holst H, Backman L, Ebental T, Alafuzodf I, Amberla K, Hartvig P, Herlitz A, Lilja A. (1992). Nerve growth factor affects 11C-nicotine binding, blood flow, EEG and verbal episodic memory in an Alzheimer patient. J Neural Transm (Park Dement Section) 4:79-95

O'Neill MJ, Murray TK, Lakics V, Visanji NP, Duty S. (2002). *The role of neuronal nicotinic acetylcholine receptors in acute and chronic neurodegeneration Curr Drug Targets CNS* Neurol Disord. 1:399-411

Parker SL, Fu Y, McAllen K, Luo J, McIntosh JM, Lindstrom JM, Sharp BM. (2004). *Up-regulation of brain nicotinic acetylcholine receptors in the rat during long-term self-administration of nicotine: disproportionate increase of the alpha6 subunit.* Mol Pharmacol. 65:611-622

Parker SL, Fu Y, McAllen K, Luo J, McIntosh JM, Lindstrom JM, Sharp BM. (2004). *Up-regulation of brain nicotinic acetylcholine receptors in the rat during long-term self-administration of nicotine: disproportionate increase of the alpha6 subunit.* Mol Pharmacol. 65:611-622

Pasinetti GM, Aisen PS. (1998). *Cyclooxygenase-2 expression is increased in frontal cortex of Alzheimer's disease brain*. Neurosci. 87:319-324.

Paterson D, Nordberg A. (2000). *Neuronal nicotinic receptors in the human brain. Progress* Neurobiol. 61:75-11

Pauly JR, Charriez CM, Guseva MV, Scheff SW. (2004).*Nicotinic receptor modulation for neuroprotection and enhancement of functional recovery following brain injury or disease.* Ann N Y Acad Sci. 1035:316-34.

Perry DC, Dávila-García MI, Stockmeier CA, Kellar KJ. (1999). *Increased nicotinic receptors in brains from smokers: membrane binding and autoradiography studies*. J Pharmacol Exp Ther. 289:1545-1552

Perry DC, Kellar KJ. (1995). *[^{3}H]epibatidine labels nicotinic receptors in rat brain: an autoradiographic study*. J Pharmacol Exp Neurol. 275:1030–1034.

Picciotto MR, Addy NA, Mineur YS, Brunzell DH. (2008). *It is not "either/or": activation and desensitization of nicotinic acetylcholine receptors both contribute to behaviors related to nicotine addiction and mood.* Prog Neurobiol. 84:329-342

Placzek AN, Zhang TA, Dani JA. (2009). *Nicotinic mechanisms influencing synaptic plasticity in the hippocampus.* Acta Pharmacol Sin. 30:752-760

Prasad KN, Cole WC, Hovland AR, Prasad KC, Nahreini P, Kumar B, Edwars-Prasad J, Andreatta CP. (1999). *Multiple antioxidants in the prevention and treatment of neurodegenerative disease: analysis of biologic rationale*. Curr Opin Neurol. 12:761–770.

Prendergast MA, Buccafusco JJ. (1998). (2)-*Nicotine increases mRNA encoding G3PDH and the vesicular acetylcholine transporter in vivo*. Neuroreport. 9:1385–1389.

Quik M, O'Leary K, Tanner CM. (2008). *Nicotine and Parkinson's disease: implications for therapy.* Mov Disord. 23:1641-1652

Radcliffe KA, Dani JA. (1998). *Nicotinic stimulation produces multiple forms of increased glutamatergic synaptic transmission*. J Neurosci. 18:7075-7083

Rattray M. (2001). *Is there nicotinic modulation of nerve growth factor? Implications for cholinergic therapies in Alzheimer's disease*. Biol Psychiatry. 49:185-193

Ren XQ, Cheng SB, Treuil MW, Mukherjee J, Rao J, Braunewell KH, Lindstrom JM, Anand R. (2005). *Structural determinants of α4β2 nicotinic acetylcholine receptor trafficking*. J Neurosci. 25:6676-6686

Rossner S, Ueberham U, Schiliebs R, Perez-Polo JR. Bigl V. (1998). *The regulation of amyloid precursor protein metebolism by cholinergic mechanisms and neurotrophin receptor signaling.* Prog Neurobiol. 56:541-569

Sanderson EM, Drasdo AL, McCrea K, Wonnacott S. (1993). *Upregulation of nicotinic receptors following continuous infusion of nicotine is brainregion- specific*. Brain Res. 617:349 –352.

Scarr E, Dean B. (2009). *Role of the cholinergic system in the pathology and treatment of schizophrenia*. Expert Rev Neurother. 9:73-86

Schiltz JC, Sawchenko PE. (2002). *Distinct brain vascular cell types manifest inducible cyclooxygenase expression as a function of the strength and nature of immune insults.* J Neurosci. 22:5606-5618.

Schultz DW, Loring RH, Aizenman E, Zigmond RE. (1991). *Autoradiographic localization of putative nicotinic receptors in the rat brain using ^{125}I-neuronal bungarotoxin*. J Neurosci. 11:287–297.

Scott SA, Crutcher KA. (1994). *Nerve growth factor and Alzheimer's disease.* Rev Neurosci. 5:179-211

Sershen H, Balla A, Lajtha A, Vizi ES. (1997).*Characterization of nicotinic receptors involved in the release of noradrenaline from the hippocampus.* Neuroscience. 77:121-130

Sershen H, Balla A, Lajtha A, Vizi ES. (1997).*Characterization of nicotinic receptors involved in the release of noradrenaline from the hippocampus*. Neuroscience. 77:121-130

Sharifzadeh M, Tavasoli M, Naghdi N, Ghanbari A, Amini M, Roghani A. (2005). *Post-training intrahippocampal infusion of nicotine prevents spatial memory retention deficits induced by the cyclo-oxygenase-2-specific inhibitor celecoxib in rats*. J Neurochem. 95:1078-1090.

Sharma G, Vijayaraghavan S. (2008). *Nicotinic receptors containing the alpha7 subunit: a model for rational drug design.* Curr Med Chem. 15:2921-2932.

Shimohama S, Akaike A, Kimura J. (1996). *Nicotine-induced protection against glutamate cytotoxocity. Nicotinic cholinergic receptor-mediated inhibition of nitric oxide formation.* Ann N Y Acad Sci 777: 356-361

Shimohama S, Kihara T. (2001). *Nicotinic receptor-mediated protection against beta-amyloid neurotoxicity.* Biol Psychiat. 49:233-239.

Shimohama S. (2009). *Nicotinic receptor-mediated neuroprotection in neurodegenerative disease models.* Biol Pharm Bull. 32:332-6.

Shute CD, Lewis PR. (1967). *The ascending cholinergic reticular system: neocortical, olfactory and subcortical projections*. Brain. 90:497-520

Siegel GJ, Chauhan NB. (2000). *Neurotrophic factors in Alzhiemer's and Parkinson's disease brain.* Brain Res Rev. 33:199-227

Sihver W, Gillberg PG, Nordberg A. (1998). *Laminar distribution of nicotinic receptor subtypes in human cerebral cortex as determined by [^{3}H](-)nicotine, [^{3}H]cystisine and [^{3}H] epibatidine in vitro autoradiography*. Neuroscience. 85 (4): 1121-1133

Sofroniew MV, Howe CL, Mobley WC. (2001). *Nerve growth factor signaling neuroprotection and neuronal repair*. Annu Rev Neurosci. 24:1217-1281

Sokoloff L. (1981). *The deoxyglucose method for the measurement of local glucose utilization and the mapping of local functional activity in the central nervous system.* Int Rev Neurobiol. 25:287–233.

Soliakov L, Wonnacott S. (1996). *Voltage-sensitive Ca2+ channels involved in nicotinic receptor-mediated [3H]dopamine release from rat striatal synaptosomes*. J Neurochem. 67:163-170

Steinlein OK, Bertrand D. (2008). *Neuronal nicotinic acetylcholine receptors: from the genetic analysis to neurological diseases.* Biochem Pharmacol. 76:1175-1183

Sugaya K, Giacobini E, Chiappinelli VA. (1990). *Nicotinic acetylcholine receptor subtypes in human frontal cortex. Changes in Alzheimer disease*. J Neurosc Res. 27: 349-359

Szász BK, Mayer A, Zsilla G, Lendvai B, Vizi ES, Kiss JP. (2005). *Carrier-mediated release of monoamines induced by the nicotinic acetylcholine receptor agonist* DMPP. Neuropharmacology. 49:400-409.

Takada-Takatori Y, Kume T, Ohgi Y, Fujii T, Niidome T, Sugimoto H, Akaike A. (2008). *Mechanisms of α7-nicotinic receptor up-regulation and sensitization to donepezil induced by chronic donepezil treatment*. Eur J Pharmacol. 590:150-156

Terry AV Jr. (2008). *Role of the central cholinergic system in the therapeutics of schizophrenia.* Curr Neuropharmacol. 6:286-292

Toledano A, Alvarez MI, Caballero I, Carmona P, De Miguel E. (2008). *Immunohistochemical increase in cyclooxygenase-2 without apoptosis in different brain areas of subchronic nicotine- and D-amphetamine-treated rats*. J Neural Transm. 115:1093-1108.

Toledano A, Alvarez MI, Rivas L, Lacruz C, Martinez-Rodriguez R. (1999). *Amyloid precursor proteins in the cerebellar cortex of Alzheimer's disease patients devoid of cerebellar beta-amyloid deposits: immunocytochemical study of five cases.* J Neural Transm. 106:1151-1169.

Toledano A, Alvarez MI. (2004). *Lesions and dysfunctions of the nucleus basalis as Alzheimer's disease models: general and critical overview and analysis of the long-term changes in several excitotoxic models.* Curr Alzheimer Res. 1:189-214.

Toledano A, Barca MA, Pérez C, Martínez-Rodríguez R. (1979). *Histochemical electron microscope study of the enzyme glutamate dehydrogenase in cerebellum* Cell Mol Biol. 24:113–125.

Toledano A. (1994). *Growth factors and aging factors in Alzheimer's disease* In: Selmes S, Selmes J, Portera A, Toledano A, eds. Updating of Alzheimer's disease. Madrid: Fundación Alzheimer España/Alzheimer Europa/Meditor. pp: 59-71

Tong XK, Hamel E. (1999). *Regional cholinergic denervation of cortical microvessels and nitric oxide synthase-containing neurons in Alzheimer disease*. Neuroscience. 92:163–175.

Turégano L, Martínez-Rodríguez R, Alvarez MI, Gragera RR, Gómez de Segura A, De Miguel E, Toledano A. (2001). *Histochemical study of acute and chronic intraperitoneal nicotine effects on several glycolytic and Krebs cycle dehydrogenase activities in the frontoparietal cortex and subcortical nuclei of the rat brain*. J Neurosci Res. 64:626-635

Turner TJ. (2004). *Nicotine enhancement of dopamine release by a calcium-dependent increase in the size of the readily releasable pool of synaptic vesicles*. J Neurosci. 24:11328-11336

Umbriaco D, Garcia S, Beaulieu C, Descarries L. (1995). *Relational features of acetylcholine, noradrenaline, serotonin and GABA axon terminals in the stratum radiatum of adult rat hippocampus (CA1)*. Hippocampus. 5:605-620

Umbriaco D, Watkins KC, Descarries L, Cozzari C, Hartman BK. (1994). *Ultrastructural and morphometric features of the acetylcholine innervation in adult rat parietal cortex: an electron microscopic study in serial sections.* J Comp Neurol. 348:351-373

Valverde F. (1986). *Intrinsic neocortical organization: some comparative aspects*. Neuroscience 18:1–23.

Walsh H, Govind AP, Mastro R, Hoda JC, Bertrand D, Vallejo Y, Green WN. (2008). *Up-regulation of nicotinic receptors by nicotine varies with receptor subtype*. J Biol Chem. 283:6022-6032

Wanaverbecq N, Semyanov A, Pavlov I, Walker MC, Kullmann DM. (2007). *Cholinergic axons modulate GABAergic signaling among hippocampal interneurons via postsynaptic alpha 7 nicotinic receptors.* J Neurosci. 27:5683-5693

Whiteaker P, Davies ARL, Marks MJ, Blagbrough IS, Potter BVL, Wolstenholme AJ, Collins AC, Wonnacott S. (1999). *An autoradiographic study of the distribution of binding sites for the novel a7-selective nicotinic radioligand [^{3}H]-methyllycaconitine in the mouse brain.* Eur J Neurosci. 11:2689 –2696.

Wu HQ, Rassoulpour A, Schwarcz R. (2007).*Kynurenic acid leads, dopamine follows: a new case of volume transmission in the brain?* J Neural Transm. 114:33-41

Yamagata K, Andreasson KI, Kaufmann WE, Barnes CA, Worley PF. (1993). *Expression of a mitogen-inducible cyclooxygenase in brain neurons: regulation by synaptic activity and glucocorticoids.* Neuron. 11:371-386.

Zeidler R, Albermann K, Lang S. (2007). *Nicotine and apoptosis.* Apoptosis. 12:1927-1943.

Zhou FM, Wilson CJ, Dani JA. (2002). *Cholinergic interneuron characteristics and nicotinic properties in the striatum*. J Neurobiol. 53:590-605

Zmarowski A, Wu HQ, Brooks JM, Potter MC, Pellicciari R, Schwarcz R, Bruno JP. (2009). *Astrocyte-derived kynurenic acid modulates basal and evoked cortical acetylcholine release*. Eur J Neurosci. 29:529-538

Zoli M, Léna C, Picciotto MR, Changeux JP. (1998). *Identification of four classes of brain nicotine receptors using β2 mutant mice.* J Neurosci. 18:4461-4472

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Chapter 7

CALCIUM SIGNALING IN GLIAL CELLS OF THE ENTERIC NERVOUS SYSTEM

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ABSTRACT

The enteric nervous system comprises two major cell types: enteric neurons and enteric glia. Enteric glia, which outnumber enteric neurons 4:1, display morphologic and molecular similarities to astrocytes in the central nervous system (CNS); they have irregular shapes, do not synthesize a basal lamina, and have an abundance of glial fibrillary acidic protein. Traditionally, enteric glia have been described as a homogeneous population of cells whose primary function is supporting enteric neurons. Evidence is accumulating challenging the concept that enteric glia are merely passive supports for enteric neurons. This review summarizes the recent advance in calcium signaling events of enteric glia. Enteric glia respond to a variety of neuroligands with intracellular and intercellular calcium signaling. Among these neuroligands are adenosine triphosphate (ATP), endothelins, glutamate and sphingolipids. Mobilization of inositol triphosphate ($InsP_3$) sensitive intracellular calcium stores accounts for the initiation of calcium signaling evoked by activation of these G-protein coupled receptors, while subsequent calcium entry via calcium channels or capacitative calcium entry alters the temporal and spatial patterns of intracellular calcium signaling in enteric glia. Like astrocytes in the central nervous system, enteric glia communicate with each other and with enteric neurons by two mechanisms: (1) propagation of calcium through the gap junction; and (2) release of transmitters triggered by the rise in intracellular calcium. The transmitters released then activate membrane receptors on neighboring cells. The functional consequences following the calcium signaling in enteric glia are also discussed with emphasis toward some of the potential intracellular targets of these calcium transients. We, therefore, propose that enteric glia be considered as an active cellular element participating in the information processing in the enteric nervous system.

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The enteric nervous system (ENS) is a separate class of the autonomic nervous system with the unique ability to function independently from the central nervous system. Like all other peripheral nervous system components, the ENS is derived embryologically from the neural crest and is located throughout the wall of the gastrointestinal tract (Natarajan and Pachnis, 1999; Gershon et al, 1993). This unique component of the autonomic nervous system is organized in two major ganglionated plexuses, namely the myenteric (Auerbach's) plexus and submucosal (Meissner's) plexus (Furness 2006). A variety of functionally distinct neurons are found in the ENS, including primary afferent neurons, interneurons and motor neurons which are synaptically linked to each other in microcircuits. The ENS controls virtually all aspects of gastrointestinal functions ranging from blood flow, absorption, secretion, motility, mucosal growth and local immune response in the absence of extrinsic neural connections, although extrinsic influences often initiate or modulate gastrointestinal reflexes. More specifically, the innermost submucosal plexus is situated beneath the gastrointestinal mucosal layer where it regulates epithelial absorption and secretion. The myenteric plexus, which is embedded between the circular and longitudinal smooth muscle layers, modulates intestinal motility.

Two major cell types comprise the ENS: enteric neurons and enteric glia. Neurons within the ENS have historically been the focus of investigation. Enteric neurons are as numerous as their counterparts in the spinal cord and neural networks have been extensively mapped throughout the gastrointestinal tract. Enteric glia, which outnumber enteric neurons 4:1, display morphologic and molecular similarities to CNS astrocytes; they have irregular shapes, do not synthesize a basal lamina, and have an abundance of glial fibrillary acidic protein (Gabella 1981; Gershon and Rothman 1991). Traditionally, enteric glia have been described as a homogeneous population of cells whose primary function is supporting enteric neurons. Evidence is accumulating challenging the concept that enteric glial cells are merely passive supports for enteric neurons. The aim of this review is to summarize the current knowledge of the calcium signaling in enteric glia. The possible roles of calcium signaling in the regulation of enteric glial functionality under physiological and pathological conditions will also be discussed.

1. BASIC OF CALCIUM SIGNALING

The magic ion –calcium (Ca^{2+}), is defined as a universal signaling molecule because it is essential for a wide variety of cellular functions ranging from muscle contraction, cellular secretion, and cytosolic enzyme activity to regulation of gene expression. Under physiological conditions, intracellular calcium levels are subject to intricate homeostatic mechanisms which achieve a very low intracellular calcium concentration ($[Ca^{2+}]_i$). The cytosolic free calcium concentration is typically below 100 nM in most of cells under resting conditions (Berridge 1994; Verkhratsky et al 1998). This is roughly 40,000 folds lower than the extracellular calcium concentration. This large concentration gradient across the cytoplasmic membrane creates the ideal situation for the use of calcium as a signaling molecule because of its great intrinsic signal-to-noise ratio. In enteric glia, resting $[Ca^{2+}]_i$ levels are approximately 50 nM relative to 2.5 mM for extracellular calcium concentration (Kimball and Mulholland 1996). Because of this steep electrochemical gradient directed towards the cytosol, even a very short time change in the calcium permeability of the cytoplasmic membrane will generate rapidly large signals upon cellular activation. Dependent on the stimulus, dramatic changes in overall $[Ca^{2+}]_i$ can be observed upon cellular activation.

In enteric glia, this can transform to a rise from 50 nM to 1-5 μM (Kimball and Mulholland 1996; Zhang et al 1997). The effective amplitude of the calcium signaling may therefore exceed the basal level by 20-100 orders of magnitude, providing a signal-to-noise ratio at least 20-100 folds greater than the intrinsic noise. Similar electrochemical gradient in calcium concentration exists between the sacroplasmic/endoplamic reticulum and the cytosol. Both sacroplasmic and endoplasmic reticulum appear to have a similar total calcium concentration of 1-10 mM and free calcium concentration around 500 μM. This calcium gradient is large enough to provide a significant calcium flux from the sacroplasmic/endoplasmic reticulum to cytosol. Following sustained elevation in cytosolic $[Ca^{2+}]_{i,}$ calcium sequestration and extrusion occurs by ATP energy-dependent active transportation of calcium. This is mainly mediated by high-affinity ATP dependent calcium pumps present either on the membrane of the intracellular organelles such as sacroplasmic/endoplasmic reticulum and mitochondria, or in the plasma membrane extruding calcium into extracellular milieu. This effective clearance of cytosolic calcium allows for the return of $[Ca^{2+}]_i$ to the pre-stimulation levels and thus renders the cell ready for next stimulation. A variety of mechanisms are involved in this precise control of calcium signaling which comprises the calcium influx from extracellular source, calcium release from the intracellular calcium stores and clearance of cytosolic calcium. The fine tuning of these three aspects of calcium mobilization ultimately determines the temporal and spatial pattern of calcium signaling. Details in mechanism of calcium mobilization are discussed in the following sections.

1.1 Calcium Release from Intracellular Calcium Stores

Calcium signaling is defined as the transient rise in $[Ca^{2+}]_i$. This rise in $[Ca^{2+}]_i$ is achieved by calcium release from intracellular calcium stores, calcium entry from extracellular sources or both. Difference in the mechanism of calcium signaling exists between excitable cells such as neurons and muscle cells, and non-excitable cells such as endothelial cells or lymphocytes. In excitable cells, calcium signaling results from both calcium entry via voltage or ligand-gated calcium channels and calcium release from internal calcium stores. In non-excitable cells, it is however the release of calcium from intracellular stores that dominates. In glial cells which are not capable of generating action potentials and are therefore defined as non-excitable element in the nervous system, the release of calcium from the intracellular calcium stores is the prominent signaling pathway responsible for the rise in $[Ca^{2+}]_i$ (Verkhratsky et al 1998). The general signal transduction cascade responsible for this calcium mobilization involves two principal mechanisms: inositol (1, 4, 5)-triphosphate ($InsP_3$) sensitive receptors or ryanodine receptors mediated activation of intracellular calcium channels present on the endoplasmic reticulum. Although the functional Ca^{2+} -induced release (CICR) via ryanodine receptors has been described on Schwann cells and in cultured astrocytes, this alternative route for calcium release from intracellular stores remains controversial in astrocytes (Verkhratsky et al 1998). Ryanodine receptors have been demonstrated in enteric neurons and are found to involve in the calcium oscillation (Kimball et al 1996). The presence of ryanodine receptors in enteric glia has not been documented. The primary pathway responsible for calcium release from intracellular calcium stores in glial cells is mediated by $InsP_3$ (Kimball and Mulholland 1996; Zhang et al 1997). This pathway is

typically initiated by activation of a metabotropic receptor which is typically G protein-coupled receptors but not infrequently a tyrosine kinase-linked receptor. Coupling of metabotropic receptor to G protein (usually Gαq subunit) activates phospholipase C β to hydrolyse lipid precursor phosphatidyl inositol 4,5-bisphasphate to form $InsP_3$ and diacylglycerol (DAG). To date, three isoforms of $InsP_3$ receptors including type 1, 2 and 3 have been cloned. Type 3 $InsP_3$ receptor has been detected in glial cells by immunocytochemical staining (Verkhratsky et al 1998). The presence of $InsP_3$ receptor in the enteric glia has been demonstrated by double immunofluorescent staining for immunoreactivity to $InsP_3$ receptor and to the glial antigen S-100. Staining for S-100, which is highly expressed in glial cells but not neurons, allows distinguishing the glial cells from neurons. $InsP_3$ receptor type 1 immunoreactivity was detected in the cytoplasm of enteric glia which also stained positive for S-100 (Zhang et al 1997). The alternative pathway to stimulate the release of $InsP_3$ is mediated by tyrosin kinase receptors, which are typically activated by growth factors such as epidermal growth factor, platelet-derived growth factor and nerve growth factor. Unlike G protein-coupled receptors, tyrosin kinase receptors generate $InsP_3$ and DAG by interacting directly with PLC γ instead of PLC β.

1.2 Calcium Influx from Extracellular Source

Glial cells demonstrate a remarkable variability with respect to the calcium signaling. While calcium release from intracellular calcium stores is recognized as the primary mechanism for calcium signaling in glial cell, growing evidences support the concept that extracellular calcium influx contributes to the rise in $[Ca^{2+}]_i$ in many types of glial cells including astrocytes in the central nervous system. Voltage-gated calcium channels have been demonstrated in some glial subtypes, in particular astrocytes, Schwann cells and glial precursor cells (Sontheimer 1994). Other types of glial cells such as Bergmann glial cells or microglial cells lack voltage-gated calcium channels, but appear to possess ligand-gated calcium channels. Two types of ligand-gated channels which are permeable to calcium, namely glutamate receptor and purinoceptors, have been best studied in glial cells. AMPA/kainite glutamate receptos are highly calcium permeable and activation of these receptors leads to an increase in $[Ca^{2+}]_i$ in some type of glial cells such as astrocytes and Bergman glial cells (Verkhratsky et al 1998). In contrast, glutamate receptors in glial precursor cells are low calcium permeable and glutamate-induced calcium signaling in these cells requires the activation of voltage-gated calcium channels. For a long time, NMDA receptors have been considered to be expressed exclusively in neurons. Recent studies have found the functional presence of these high calcium permeable receptors in glial cells (Verkhratsky and Kirchhoff 2007). Purinoceptor subtype P_{2x} and P_{2z} represent another type of calcium permeable ligand-gated channels in glial cells. Both P_{2x} and P_{2z} have been found to couple with non-selective cationic channels. In addition, a unique group of calcium channels which are sensitive to mechanical stimulation have been receiving growing attention in glial cells. These non-selective calcium channels are controlled by mechanosensor and by calcium itself. Since astrocytes can significantly increase their volume, the stretch-activated calcium channels have been proposed to link the regulation of cell volume to intracellular calcium signaling. To date, functional voltage-gated, ligand-gated or mechanosensitive calcium

channels have not been firmly established in the enteric nervous system. Since enteric glial cells have many signaling mechanisms common to other glial cells, it will be interesting to explore their potential involvement in the regulation of calcium signaling in enteric glial cells.

1.3 Capacitative Calcium Entry

Capacitative calcium entry is defined as the process of extracellular calcium influx as a direct consequence of the depletion of intracellular calcium stores (Putney 1986). Capacitative calcium entry can be observed in non-excitable cells after experimental measures that deplete the intracellular calcium stores. Since the capacitative calcium entry is regulated by the state of filling of calcium stores, it is also known as the store-operated calcium entry. The calcium channels responsible for the calcium entry in the capacitative calcium entry have been shown to be the mammalian homologue of drosophila transient receptor potential (TRP) channel protein (Boulay et al 1999). Two major competing theories have been proposed for the activation of these TRP channels: they are based on either the involvement of a diffusible messenger which is generically referred to as the calcium influx factor (CIF) or the mechanical linkage of $InsP_3$ receptors to store-operated calcium entry through endoplasmic reticulum-cytoplasmic membrane interactions (Putney 1990; Irvine 1990). The second mechanism is mostly favored by current evidences, although the mechanism has not been completely revealed and both theories remain viable possibilities. In their elegant study, Boulay et al (1999) provided convincing data to support the concept that TRP channels are activated by a physical coupling with $InsP_3$ receptors. This finding is in line with the discovery by Patterson et al (1999) that capacitative calcium entry is selectively affected by F-actin redistribution.

In enteric glial cells, capacitative calcium entry is observed in the scenario when intracellular calcium stores are depleted by thapsigargin: an irreversible endoplasmic membrane Ca^{2+} ATPase inhibitor or by release of intracellular calcium stores induced by G protein-coupled receptors such as purinergic receptor and endothelin receptors (Zhang et al 1998; Sarosi et al 1998). Data from our studies clearly show that capacitative calcium entry is the predominant mechanism responsible for the sustained elevation in $[Ca^{2+}]_i$ following the initial spike triggered by neuromodulators such as ATP and endothelin in cultured enteric glial cells. The calcium channels accounting for the capacitative calcium entry are sensitive to Ni^{2+} and Ln^{3+}, but insensitive to L-, N-, or P-type calcium channel blockers. The mechanism regulating the capacitative calcium entry in enteric glia is currently unknown. Studies from our laboratory suggest that it may involve the protein kinase C and nitric oxide signaling pathways. Depletion of PKC activity by either prolong exposure of enteric glial cells to phorbol 12-myristate 13-acetate (PMA) or by treatment of these cells with specific PKC inhibitors partially attenuates the capacitative calcium entry. Depletion of PKC activity produces a 67±7% inhibition relative to the control, while treatment of enteric glial cells with PKC inhibitors: staurosporine and chelerythrine inhibits the capacitative calcium entry up to 55±2%. Similar results are observed using a series of NO synthase (NOS) inhibitors including N^G-Nitro-L-Arginine (L-NA), N^G-Monomethyl-L-Arginine Monoacetate (L-NMMA), L-N^5-(1-iminoethyl) Ornithine (L-NIO) or S-methyl-L-thiocitrulline (Me-TC). Dose-dependent inhibition is observed with 1 to 1000μM L-NA with maximal inhibition of 35±3%. L-NA attenuated capacitative calcium entry is restored by simultaneous treatment of enteric glial

cells with 10mM sodium nitroprusside (SNP): an NO donor, demonstrating the specific effect of NOS inhibition. It is noteworthy that although PKC or NOS inhibition alone causes only partial inhibition of capacitative calcium entry in enteric glial cells, the combination of inhibitors for these two signaling pathways causes near-total suppression of the capacitative calcium entry. The synergistic effect of PKC and NOS inhibitors indicates that PKC and NO may act on different pathways to regulate the capacitative calcium entry in enteric glial cells.

The potential relationship of the cellular cytoskeletal architecture to the mechanisms of capacitative calcium entry in enteric glia has also been explored in our laboratory. Disruption of the conformational coupling between the plasma membrane receptor and $InsP_3$ receptor by altering the cellular cytoskeletal structure significantly inhibits the capacitative calcium entry in enteric glial cells. Cytochalasin D is an alkaloid produced by Helminthosporium and other moulds which acts to destabilize F-actin by binding to G-actin, the monomeric form of actin, thus creating a state of G-actin depletion. As turnover and re-polymerization are constantly occurring within cells, existing F-actin depolymerizes as the effective concentration of free G-actin becomes limiting. In enteric glial cells, disruption of actin microfilaments using cytochalasin D diminishes the capacitative calcium entry compared to the vehicle-treated control. The microtubule cytoskeletal network is similarly investigated using nocodazole, which binds to β-tubulin and induces microtubule depolymerization. Enteric glial cells treated with nocodazole demonstrate a diminished capacitative calcium entry compared with the control response. The proportion of cells exhibiting capacitative calcium entry inhibition is significantly greater in the nocodazole-treated group (89 ± 2%) compared with control (40 ± 9%, $P < 0.05$). These results demonstrate that the cytoskeleton plays an integral role in communicating the state of intracellular calcium stores to facilitate their replenishment from the external environment in enteric glial cells.

1.4 Recovery Following Calcium Signaling

It is now clear that life is impossible when calcium is absent. However, when calcium is too much, cells die. The temporal and spatial nature of calcium signals appears to be pertinent in the coding of messages and their ultimate termination seems essential for preventing the initiation of apoptosis signals which occur following sustained increase in $[Ca^{2+}]_i$. Such process to maintain the homeostasis of $[Ca^{2+}]_i$ is facilitated by calcium sequestration and extrusion via plasma membrane bound calcium channels. These high-affinity ATP-dependent calcium pumps are present either in the plasma membrane, extruding calcium into the extracellular space or on the membrane of the intracellular organelles involved in calcium storing. Another calcium pool involved in the recovery of the low resting $[Ca^{2+}]_i$ following a calcium response is the mitochondrial store. Using mitrotracker green FM to identify the mirochondrial in enteric glial cells, we are able to monitor the change of calcium concentration in mitochondria by didydro-rhod-FF/AM (unpublished data). Significant rise in mitochondrial calcium concentration is observed following the increase of cytosolic free calcium concentration induced by agonists such as ATP or endothelin in cultured enteric glial cells. Inhibition of mitochondrial calcium uptake by depolarizing the mitochondrial membrane potential using p-trifluoromethoxy carbonyl cyanide phenyl hydrazone (FCCP) increases the cytosolic free calcium concentration and inhibits the recovery of low basal

$[Ca^{2+}]_i$ following the initial response to ATP or endothelin in enteric glial cells. All these data suggest that mitochondria are involved in the regulation of $[Ca^{2+}]_i$ in enteric glial cells.

2. INTRACELLULAR CALCIUM SIGNALING

As described above, the calcium ion is a critical second messenger that serves to tranduce extracellular signals into numerous intracellular events in a variety of cell types. These intracellular events include both short-term responses to extracellular signals such as exocytosis and neurotransmitter release, contraction, intercellular communication, movement of cells, modulation of ion channels and long-term regulation of gene expression, protein trafficking, development and growth, and apoptosis. Indeed, Ca^{2+} is involved in almost all the cellular functions, with very few exceptions (e.g., the sodium-dependent action potentials). How this universal signaling used for activating many pathways triggers one or another specific functional response relies on the intricate mechanisms which control precisely the temporal and spatial patterns of the calcium signaling. The spreading of the calcium signaling takes two forms: the intracellular calcium signaling which defines the temporal and spatial change of calcium within the cell, and the intercellular calcium which is the mobilization of calcium between cells. In the last decade, our studies have revealed that enteric glia, much like other cells, are capable of responding to a wide variety of neurotransmitters and neuroligands with a rise in intracellular calcium signaling (Kimball and Mulholland 1996; Zhang et al 1997; Segura et al 2004a; Segura et al 2004b). This initiation of intracellular calcium signaling spreads both within the cytosol and between the enteric glia and neurons, demonstrating the presence of intracellular and intercellular calcium signaling in the enteric glia. The intracellular calcium $[Ca^{2+}]_i$ signaling in enteric glia is well defined relative to the intercellular calcium signaling. To date, a variety of agonists have been revealed to cause intracellular calcium signaling in enteric glial cells. Members of these agonists include neurotransmitters, inflammatory mediators and bio-lipid molecules (Table 1).

Table 1. List of agonists capable of inducing $[Ca^{2+}]_i$ signaling in enteric glial cells.

Agonist	Dose	Maximal % Responding	Reference
Neurotransmitters			
ATP	0.5 -1000μM	100% (100μM)	Kimball et al (1996)
UTP	100μM	100%	Kimball et al (1996)
ADP	100 μM	15%	Kimball et al (1996)
βγ-MeATP	100 μM	7%	Kimball et al (1996)
Glutamate	1 mM	72% (1 mM)	Segura et al (2001)
Serotonin	100 μM	4%	Kimball et al (1996)
Inflammatory Mol			
Endothelin 1,2,3	0.01nM-0.1 μM	100% (1 nM)	Zhang et al (1997)
Histamine	100μM	31%	Kimball et al (1996)
Bradykinin	10μM	11%	Kimball et al (1996)
PAR 1	10 nM-2 μM	70% (1 μM)	Garrido et al (2002)
PAR 2	10 nM-1μM	89% (100 nM)	Garrido et al (2002)

Bio-lipids			
LPA	0.1 nM-10 μM	85% (1μM)	Segura et al (2004)
S-1-P	1 nM -10 μM	80% (1μM)	Segura et al (2004)
SPC	1μM	66% (1μM)	Segura et al (2004)
SMase	1μM	48% (1μM)	Segura et al (2004)
D-SPH	1μM	28% (10μM)	Segura et al (2004)

PAR: protease-activated receptor; LPA: lysophosphatidic acid; S-1-P: sphingosine-1-phosphate; SPC: sphingosylphosphorylcholine; D-SPH: D-sphingosine; SMase: sphingomyelinase

2.1 Neurotransmitter-Mediated $[Ca^{2+}]_I$ Response

In the central nervous system, functional receptors for a wide variety of neurotransmitters have been found in the glia, demonstrating the active involvement of glial cells in synaptic transmission. Activation of these neurotransmitter receptors causes a remarkable variability in the calcium signaling in glial cells both in culture or in situ. Activation of ionotropic GABA or glutamate receptors typically induces an intracellular calcium signaling characterized by a brief monophasic rise in $[Ca^{2+}]_i$ which quickly returns to baseline within seconds. In contrast, a biphasic response characterized by an initial rapid increase followed by a prolong elevation in $[Ca^{2+}]_i$ is often observed in glial cells upon activation of metabotropic receptors such as metabotropic glutamate receptors, purinergic receptors, and α-adrenergic receptors (Verkhratsky et al 1998). This diversity of calcium signaling in response to neurotransmitters occurs as a consequence of activation of different systems which determine the time course and amplitude of calcium mobilization. Ionotropic receptors such as $GABA_A$, AMPA (a-amino-3-hydroxy-5-methyl-isoxazolepropionic acid), Kainate and NMDA (N-methyl-D-aspartate) receptors are typically coupled to the ligand-gated channels. Activation of these receptors in glial cells triggers a single $[Ca^{2+}]_i$ spike as a result of calcium entry through these calcium permeable ligand-gated channels. In contrast, more-complex calcium signaling is observed when metabotropic receptors in glial cells are activated.

Enteric glia, like astrocytes in the central nervous system, possess receptors coupled to multiple signal transduction pathways including phosphoinositide turnover. Among these receptors are a variety of neurotransmitter receptors including purinergic receptor, glutamatergic receptor. Previous work in our laboratory has revealed that activation of these neurotransmitter receptors causes $[Ca^{2+}]_i$ signaling in cultured enteric glia. Purines are the first group of neuroligand identified to cause $[Ca^{2+}]_i$ signaling in enteric glial cells (Kimball and Mulholland 1996). Based on the percentage of enteric glial cells responding in the rise of $[Ca^{2+}]_i$ upon stimulation with ATP and related nucleotides, the rank order of potency is ATP=UTP>ADP>β,γ-MeATP>>2-MeS-ATP=α,β Me-ATP=AMP=adenosine. In responding enteric glial cells, peak $[Ca^{2+}]_i$ levels achieved by these neuroligands at 100μM are as follows: UTP>ATP>ADP>β,γ- MeATP. Both the percentage of enteric glial cells responding to purinergic agonists and the peak $[Ca^{2+}]_i$ levels induced by these neuroligands are consistent with action at a purinergic receptor subtype P_{2U}. This conclusion is further supported by the observation that neither suramin: a trypanocidal agent that has been shown to antagonize both P_{2x} and P_{2Y}-mediated response, nor RB2, an anthraquinone sulfonic acid derivative and specific P_{2Y} antagonist, shows any effect to reduce ATP-induced $[Ca^{2+}]_i$ transients in cultured enteric glial cells. The $[Ca^{2+}]_i$ signaling induced by ATP is dose-dependent within a range of

0.5 to 1000μM. Repetitive exposure of enteric glial cells to ATP attenuate the $[Ca^{2+}]_i$ transients by 41%, suggesting a receptor-mediated event. The $[Ca^{2+}]_i$ signaling triggered by ATP comprises two components: the initial spike occurred rapidly within milliseconds and the subsequent sustained elevation maintained during the presence of the agonist. The initial response in ATP-induced $[Ca^{2+}]_i$ signaling is mediated by the release of calcium from the $InsP_3$ sensitive calcium stores because (1) that U73122, a specific PLC inhibitor, nearly abolishes the calcium response; (2) depletion of $InsP_3$ sensitive calcium stores by thapsigargin: an endoplasmic reticulum Ca^{2+}-ATPase pump inhibitor, completely blocks the $[Ca^{2+}]_i$ transients in response to ATP; (3) the initial peak response in $[Ca^{2+}]_i$ is not affected by elimination of extracellular calcium from the perfusion buffer. The plateau response following the initial peak in ATP-mediated $[Ca^{2+}]_i$ signaling in enteric glia occurs as the result of influx of extracellular calcium because the sustained elevation in $[Ca^{2+}]_i$ observed in the calcium-containing buffer is abolished in calcium-free buffer.

Another neurotransmitter shown to induce $[Ca^{2+}]_i$ signaling is glutamate (Segura 2001). Up to 72% of cultured enteric glial cells respond to glutamate with a rapid, robust increase in $[Ca^{2+}]_i$ ($\Delta[Ca^{2+}]_i$ 185±9 nM). The glutamatergic receptor on enteric glial cells appears to be the NMDA receptor subtype because NMDA similarly activates $[Ca^{2+}]_i$ signaling in 51% of enteric glial cells, whereas AMPA demonstrates no effect. However, the possibility that enteric glial cells also bear functional metabotropic glutamate receptors coupled to the mobilization of intracellular calcium stores cannot be ruled out because their responsiveness to glutamate in the absence of extracellular calcium is maintained even though such responses are diminished in their amplitudes.

2.2 Inflammatory Molecule-Evoked $[Ca^{2+}]_I$ Response

The gastrointestinal tract contains a resident population of inflammatory cells which outnumber the total lymphocytes from all other parts of the immune system combined. In this respect, the ENS is located in a unique environment and therefore may differ substantially from other parts of the autonomic nervous system. Recent studies (Ruhl 2005; Lin et al 2007) have provided evidences to support the ideal that enteric glial cells participate actively in the course of inflammation by (1) production of cytokines such as interleukin 1β (IL1β), IL6 and tumor necrosis factor α (TNFα); (2) antigen presentation via phagocytosis and cytokine-inducible expression of major histocompatibility complex class II (MHC II); (3) secretion of matrix metalloproteinases (MMPs) into the extracellular environment in response to cytokine stimulation; (4) proliferation in response to the inflammation in vivo and in vitro. Our studies focusing on $[Ca^{2+}]_i$ signaling have revealed that a number of inflammatory mediators activate enteric glial cells by causing $[Ca^{2+}]_i$ signaling, thus processing the intestinal inflammatory signals to the enteric nervous system. These inflammatory molecules include histamine, bradykinin, endothelin and PAR receptors (Kimball and Mulholland 1996; Zhang et al 1997; Garrido et al 2002). Of these compounds, endothelin and PAR receptors have been extensively studied and are discussed in depth as follows.

Endothelins (ET) are a family of 21 amino acid isopeptides (ET1, ET2, ET3), initially linked to the regulation of vasoreactivity. Molecular cloning has revealed two endothelin receptor subtypes (ET_A and ET_B). These receptors are coupled to either pertussis toxin (PTX)

sensitive or PTX insensitive G proteins. In the gastrointestinal system, endothelins have been found to regulate the intestinal contraction, colonic secretion and the development of the enteric nervous system. Targeted disruption of ET_B receptor gene or deletion of its ligand ET_3 gene in mice results in aganglionic megacolon resembling human Hirschprung's disease. Corresponding genetic mutations are also identified in patients with Hirschprung's disease. These findings suggest that endothelins actively involve in the regulation of gastrointestinal function. We have reported that cultured enteric glial cells respond to each isopeptide of endothelin (ET1, ET2 and ET3) with biphasic increase in $[Ca^{2+}]_i$ in a dose-dependent manner over a range of 10^{-11} to 10^{-7} M. This action is a receptor-mediated event. The receptor responsible for the action of endothelin in enteric glial cells appears to be the ET_B subtype. 4ala-ET1: a specific agonist for ET_B receptor, causes similar response in a dose-dependent manner. The responses to endothelin are attenuated by BQ-788: a specific antagonist for ET_B, whereas specific ET_A receptor antagonist BQ610 demonstrates no effect. The endothelin-evoked $[Ca^{2+}]_i$ change can be divided into two components: the initial spike that appears first at low concentrations of endothelin and a prolong elevation in $[Ca^{2+}]_i$ observed at higher concentrations. The initial response occurs as a result of calcium release from the $InsP_3$ sensitive calcium stores. Several evidences support the involvement of $InsP_3$ calcium stores in endothelin-induced $[Ca^{2+}]_i$ signaling in enteric glial cells. The aminosteroid U73122, an inhibitor for PLC-coupled response, completely abolishes the response to endothelin, while U73343, an inactive analog of U73122, has no effect in endothelin-caused increase in $[Ca^{2+}]_i$. Depletion of $InsP_3$ sensitive intracellular calcium stores by thapsigargin blocks the $[Ca^{2+}]_i$ response in the subsequent perfusion to endothelin. Introduction of $InsP_3$ receptor antagonist heparin into enteric glial cells by radio frequency electroporation significantly inhibits the $[Ca^{2+}]_i$ responses to endothelin while the negative control chondroitin sulfate demonstrates no effect. The presence of InsP3 receptor in enteric glial cells is further confirmed by the co-localization of $InsP_3$ receptor immunoreactivity with S-100, a glial marker. Following the initial rapid increase in $[Ca^{2+}]_i$ is the distinct plateau response. This sustained response persists even after termination of endothelin treatment and may last over 10 minutes. This plateau response is due to the influx of extracellular calcium because exposure of enteric glial cells to endothelin under calcium free condition yields a $[Ca^{2+}]_i$ response without a subsequent plateau response. In addition, Ni^{2+} (1 mM): a divalent cation which blocks a variety of calcium channels, reversibly inhibits the plateau response by 85±3% in all enteric glial cells. Neither nifedepine nor conotoxin demonstrates any effect on the plateau $[Ca^{2+}]_i$ response, suggesting that voltage dependent calcium channels do not contribute to the subsequent calcium influx in enteric glial cells.

Protease-activated receptors (PARs) belong to the family of G-protein-coupled receptors. Activation of PAR receptors involves a unique mechanism that requires proteolytic cleavage of the extracellular N-terminal domain, exposing a new N-terminus that acts as a tethered ligand for binding and activating the receptor itself. To date, four distinct PAR receptors have been identified. PAR1 and PAR3 are activated by thrombin; PAR2 is activated by trypsin and tryptase; and PAR4 is activated by both trypsin and thrombin. In the ENS, PAR receptors have been found in submucosal and myenteric neurons. Study from our laboratory reveals that PAR1 and PAR2 are present and functionally active in enteric glial cells (Garrido et al 2002). Thrombin within the range from 7.3 nM to 7.3 μM causes dose-dependent increments in $[Ca^{2+}]_i$ in enteric glial cells. Maximum increase in $[Ca^{2+}]_i$ of 183±18nM is elicited in

61±17% of enteric glial cells at the dose of 7.3 μM. Similar responses to synthetic PAR1 peptide, PAR2 peptide and trypsin are observed, demonstrating the functional presence of PAR1 and PAR2 receptors. The $[Ca^{2+}]_i$ response caused by activation of PAR receptor includes the initial rapid $[Ca^{2+}]_i$ increase due to the calcium release from the intracellular calcium stores and subsequent elevation in $[Ca^{2+}]_i$ resulting from extracellular calcium influx, which gradually returns to baseline upon withdrawal of agonist. It is noteworthy that different mechanisms exist for calcium mobilization following activation of PAR1 and PAR2 in enteric glial cells. Activation of PAR1 leads to calcium release from intracellular calcium stores via PLC-dependent mechanism, while PAR2-mediated increase in $[Ca^{2+}]_i$ requires the synergistic actions of PLC and sphingosine kinase activity.

2.3 Bioactive Lipid-Evoked $[Ca^{2+}]_I$ Response

Bioactive lipids are a family of signaling molecules and growth factors, which include sphingosine-1-phosphate (S1P) and lysophosphatidic acid (LPA). S1P is enzymatically produced from membrane lipid sphingomyelin, whereas LPA is derived from phosphatidylcholine. Despite of being lipids, S1P and LPA act as extracellular signaling molecules in a receptor-mediated manner. A family of G protein-coupled receptors, known as endothelial differentiation gene (EDG) receptors, has been described having high affinity for S1P (EDG1, EDG3, EDG5, EDG6 and EDG8) and LPA (EDG2, EDG4 and EDG7). We have recently reported that enteric glial cells are responsive to bioactive lipids while enteric neurons do not respond with $[Ca^{2+}]_i$ signaling to any of the lipids investigated (Segura et al 2004a; Segura et al 2004b). S1P, LPA and sphingosylphosphorylcholine (SPC) are the most potent in the induction of $[Ca^{2+}]_i$ signaling. The maximum percentage responding triggered by S1P, LPA or SPC is 80±14%, 85±6% and 66±20% of enteric glial cells, respectively. Sphingomyelinase (Smase) and D-sphingosine (D-SPH) cause a moderate $[Ca^{2+}]_i$ change in 48±31% and 28±9% of enteric glial cells respectively. Sphingomyelin (SM) and C2-ceramide produce negligible effect. The $[Ca^{2+}]_i$ signaling evoked by S1P and LPA involves both intracellular calcium release and extracellular calcium influx. Although distinct receptors are activated following treatment of enteric glial cells with S1P or LPA, no major difference in $[Ca^{2+}]_i$ signaling is observed between these two agonists. Both agonists mobilize the calcium release from the $InsP_3$ sensitive calcium stores based on the finding that the $[Ca^{2+}]_i$ signaling caused by S1P or LPA is attenuated by U73122-mediated inhibition of PLC activity or by 2-aminoethoxydiphenyl borate (2-APB): a cell permeable antagonist for $InsP_3$ receptor.

From the discussion presented here, it is clear that enteric glial cells respond to a wide range of neuroligands with $[Ca^{2+}]_i$ signaling. How this universal signaling molecule can activate specifically distinct functions in enteric glial cells remains unknown. One attractive explanation focuses on the dynamic change of calcium signal. In this theory, calcium signals can be coded either in a continuous analogue manner: amplitude modulation of the size of calcium signals, or in a digital discrete manner: frequency modulation of the number of individual calcium molecules occurring per unit of time. Each of these modes of coding takes place in a different domain: the amplitude modulation in the spatial domain and the frequency domain in the temporal domain. It is in this coding of the signal molecule that ultimately determines the functional specificity of calcium signaling. Emerging evidences have been reported to support this proposal. Although it is too immature to assume that this mechanism

holds true in the determination of functional specificity for the enteric glial cells, distinct temporal patterns in $[Ca^{2+}]_i$ response to agonists have been noted. These differences lie in the time required for reaching the peak response, duration and amplitude of the subsequent sustained elevation in $[Ca^{2+}]_i$. In particular, sustained plateau response occurs following exposure of enteric glial cells to high dose of endothelin (Zhang et al 1997). This sustained plateau response is distinct in two aspects. First, it lasts long (up to 10 minutes) after the withdrawal of endothelin. Second, the subsequent elevation of $[Ca^{2+}]_i$ induced by endotheloin reaches a plateau indicating a dynamic balance between the calcium influx and clearance from the cytosol, a unique response not observed in those induced by other agonists such as ATP and LPA. It is noteworthy that the link between the coding of calcium signal with the newly discovered functions of enteric glial cells remains speculative. Further investigation is required to fully reveal this relationship.

3. INTERCELLULAR CALCIUM SIGNALING

In the nervous system, neuronal signaling is not confined within a single cell. Communication between neurons, glial cells, and neuron-glial cells enables the synchronization of actions of neighboring cells and cells at remote sites necessary to produce complex behavior. It is now apparent that glial cells are able to communicate with each other and exchange information with neurons via a slow form of communication and signaling: intercellular calcium waves (Scemes and Giaume 2006). These calcium waves enable glial cells to integrate spatially and temporally neurotransmitter-mediated signals and respond in a stimulus-specific manner to regulate neuronal activity. Studies from our laboratory have also demonstrated that intercellular calcium waves are a communication feature of glial cells in the enteric nervous system (Zhang et al 2003).

Enteric glial cells are connected with each other, forming a synchronized network. Intracellular injection of fluorescent dye: Lucifer Yellow, into a single enteric glial cell reveals the dye coupling between neighboring cells in cultured enteric glial cells (Zhang et al 2003). Similar dye coupling in enteric glial cells in situ has been reported by Hanani et at (1989). This dye coupling appears to be mediated via gap junctions as revealed by freeze-fracture techniques (Gabella 1981). All these studies suggest the existence of low-resistance pathways between enteric glial cells.

The possibility of an enteric glial network with potential functional synchronization is raised by our studies showing that enteric glial cells are capable of transmitting increase in $[Ca^{2+}]_i$ from a single cells to surrounding cells in the form of intercellular calcium waves (Zhang et al 2003). Both mechanical or chemical stimulations of a single enteric glial cells are able to evoke propagation of $[Ca^{2+}]_i$ in a wave form. Mechanical stimulation induces increases in $[Ca^{2+}]_i$ in the stimulated cell with a mean change of 432±14 nM. This increment in $[Ca^{2+}]_i$ propagates outward from the site of stimulation to 36±3% of surrounding enteric glial cells present in the microscopic field. Focal application of ATP (100 μM) or endothelin (1 μM) elicits a widespread propagation of intercellular calcium waves in enteric glial cells, which extends beyond the microscopic field being examined. These intercellular calcium waves in enteric glial cells are independent of extracellular calcium and involve a sequence of intracellular steps in which PLC and $InsP_3$ play critical roles. Depletion of intracellular

calcium stores with 1μM of thapsigargin is able to abolish the intercellular calcium waves. Inhibition of PLC activity by U73122 significantly attenuates the propagation of calcium waves between cultured enteric glial cells. Similar inhibition in the propagation of intercellular calcium waves is achieved when $InsP_3$ receptors are competitively inhibited by 2APB. All these data demonstrate that $InsP_3$ sensitive intracellular calcium pool is the predominant source for initiation and propagation of intercellular calcium waves in enteric glial cells. Uncoupling gap junction communication by octanol and heptanol significantly inhibits propagation of intercellular calcium waves in enteric glial cells, suggesting a requirement for functional gap junction. In addition to this direct communication via gap junction, release of extracellular ATP appears to contribute to the propagation of intercellular calcium waves in cultured enteric glial cells. Two observations support the involvement of ATP release as the alternative mechanism responsible for the initiation and propagation of intercellular calcium waves: (1) the extent of intercellular calcium waves initiated by mechanical stimulation is significantly reduced by pretreatment of enteric glial cells with apyrase: an ATPase. It is noteworthy that such inhibition is incomplete even at a high dose of apyrase (80 U/ml); (2) pretreatment of enteric glial cells with 100 μM ATP to densensitize purinergic receptors significantly attenuates, but does not abolish, the extent of mechanically induced intercellular calcium waves from 47±10% to 22±4%.

4. ROLE OF CALCIUM SIGNALING

Enteric glial cells are now considered critical in the development of enteric neurons and in the maintenance of the functional and structural integrity of enteric neurons. Reported functions of enteric glial cells include information processing, maintenance of intestinal integrity (Bush et al 1998; Savidge et al 2007), synthesis and secretion of cytokines and matrix metalloproteinase 2 and 9 (Lin et al 2007), antigen presentation (Ruhl 2005), proliferation and apoptosis (Ruhl 2005). It is likely that calcium signaling is involved in the regulation of these functions in enteric glial cells.

4.1 Calcium Signaling and Information Processing in the Enteric Nervous System

It is increasingly recognized that enteric neurons do not act in isolation, instead coordinating their activity with that of surrounding non-neuronal cells. In the last decade, emerging data has demonstrated that enteric glial cells function as an integral part of information processing units of the enteric nervous system because they are able to respond to neuronal signals and to evoke response in enteric neurons. This duo communication between enteric neurons and glial cells centers on the calcium signaling. Neurotransmitters released during activation of enteric neurons could mediate signaling from enteric neurons to perisynaptic enteric glial cells. A number of neurotransmitters have been shown to activate enteric glial cells by causing $[Ca^{2+}]_i$ transients (Kimball and Mulholland 1996). Increase in $[Ca^{2+}]_i$ has been found to propagate between enteric glial cells themselves (Zhang et al 2003) and between enteric neurons and glial cells (unpublished data), making it as a form of enteric

glial excitability. The importance of information processing in the development of enteric neurons and in the maintenance of the functional and structural integrity of enteric neurons is supported by a study demonstrating that targeted disruption of either ET_B receptor or its ligand ET3 in enteric glial cells results in aganglionic megacolon in mice (Hosoda et al 1994).

4.2 Calcium Signaling and Cellular Functions in Enteric Glial Cells

In the central nervous system, calcium signaling has been shown to facilitate the coordination of several independent glial activities involving in synaptic regulation of ions and neurotransmitters such as buffering of extracellular potassium, uptake and conversion of glutamate into glutamine. It is likely that enteric glial cells, which are similar to astrocytes in many aspects, also involve in maintenance of the functional homeostasis of synapses in the enteric nervous system although direct evidence is still lacking.

Accumulating data demonstrate that enteric glial cells can produce and secret a variety of peptides and proteins such as cytokines and matrix metalloproteinase (Ruhl 2005; Lin et al 2007). Secretion of these molecules typically occurs through exocytosis. The process of secretary granule precursors by budding off from the trans-Golgi network as well as their maturation towards releasable vesicles is closely regulated by intracellular calcium. Calcium signaling in enteric glial cells might therefore exercise a crucial influence on the secretion of cytokines and matrix metalloproteinase.

Enteric glial cells are able to respond to physiological and pathophysiological stimuli with long term functional changes including gene expression, cell proliferation.

Stimulation of central vagal efferent neurons has been found to induce c-fos gene expression in enteric glial cells in the rat stomach. Increase in synthesis of glial fibrillary acidic protein (GFAP) has been demonstrated in enteric glial cells relative to astrocytes. In animal model of intestinal inflammation, enteric glial cells have been shown to proliferate in response to inflammation. Although the mechanism responsible for enteric glial cell proliferation is still unclear, many signaling pathways regulating cell proliferation involve calcium sensitive cytosolic enzymes. It is therefore possible that physiological and pathophysiological stimuli affect the long term functions of enteric glial cells by the mediation of calcium signaling.

Although proliferation of enteric glial cells occurs during intestinal inflammation, apoptosis of these cells cannot be excluded. Unlike other agonists, a high dose of endothelin causes a sustained plateau response in $[Ca^{2+}]_i$ which may last up to 10 minutes. Since excessive calcium loading, exceeding the capacity of calcium regulatory mechanism, may inappropriately activates calcium dependent processes to cause cell death either directly or by the formation of toxic reaction products, such a sustained plateau response induced by enodthelin may cause apoptosis in enteric glial cells.

Despite the discussion of many potential functions of calcium signaling in enteric glial cells, it is noteworthy that direct evidences linking calcium signaling and functions of enteric glial cells are still lacking and many of these discussion remain highly imaginative. That is not to say that calcium signaling is not important; rather it is to say that dissecting out the functions that calcium signaling in enteric glial cells may play within the complexity of the enteric nervous system is not a trivial task.

REFERENCES

Berridge MJ. *The biology and medicine of calcium signalling*. Mol Cell Endocrinol. 1994;98:119-24.

Boulay G, Brown DM, Qin N, Jiang M, Dietrich A, Zhu MX, Chen Z, Birnbaumer M, Mikoshiba K, Birnbaumer L. *Modulation of Ca(2+) entry by polypeptides of the inositol 1,4, 5-trisphosphate receptor (IP3R) that bind transient receptor potential (TRP): evidence for roles of TRP and IP3R in store depletion-activated Ca(2+) entry*. Proc Natl Acad Sci U S A. 1999;96:14955-60.

Bush TG, Savidge TC, Freeman TC, Cox HJ, Campbell EA, Mucke L, Johnson MH, Sofroniew MV. *Fulminant jejuno-ileitis following ablation of enteric glia in adult transgenic mice*. Cell. 1998;93:189-201.

Furness JB 2006. *The Enteric Nervous System*. Blackwell, Oxford, pp 274

Gabella G. *Ultrastructure of the nerve plexuses of the mammalian intestine: the enteric glial cells.* Neuroscience 1981; 6:425:36.

Garrido R, Segura B, Zhang W, Mulholland M. *Presence of functionally active protease-activated receptors 1 and 2 in myenteric glia.* J Neurochem. 2002;83:556-64

Gershon MD, Chalazonitis A, Rothman TP. *From neural crest to bowel: development of the enteric nervous system*.J Neurobiol. 1993; 24:199-214.

Gershon MD, Rothman TP. *Enteric glia*. Glia. 1991; 4:195-204.

Hanani M, Zamir O, Baluk P. *Glial cells in the guinea pig myenteric plexus are dye coupled.* Brain Res. 1989;497:245-9.

Hosoda K, Hammer RE, Richardson JA, Baynash AG, Cheung JC, Giaid A, Yanagisawa M. *Targeted and natural (piebald-lethal) mutations of endothelin-B receptor gene produce megacolon associated with spotted coat color in mice*. Cell. 1994;79:1267-76.

Irvine RF. *'Quantal' Ca2+ release and the control of Ca2+ entry by inositol phosphates--a possible mechanism*. FEBS Lett. 1990;263:5-9

Kimball BC, Mulholland MW. *Enteric glia exhibit P2U receptors that increase cytosolic calcium by a phospholipase C-dependent mechanism*.J Neurochem. 1996;66:604-12.

Kimball BC, Yule DI, Mulholland MW. *Extracellular ATP mediates Ca2+ signaling in cultured myenteric neurons via a PLC-dependent mechanism*. Am J Physiol. 1996;270:G587-93.

Lin T, Zhang W, Fan Y, Mulholland M. *Interleukin-1beta and interleukin-6 stimulate matrix metalloproteinase-9 secretion in cultured myenteric glia*. J Surg Res. 2007;137:38-45.

Natarajan D and Pachnis V 1999. In Sanderson IR and Walker WA (Editors): *Development of the gastrointestinal tract, Page 197*. BC Decker Inc., Hamilton, Ontario.

Patterson RL, van Rossum DB, Gill DL. *Store-operated Ca2+ entry: evidence for a secretion-like coupling model*. Cell. 1999;98:487-99.

Putney JW Jr. *Capacitative calcium entry revisited*.Cell Calcium. 1990;11:611-24.

Putney JW Jr. *A model for receptor-regulated calcium entry*. Cell Calcium. 1986;7:1-12.

Rühl A. *Glial cells in the gut. Neurogastroenterol* Motil. 2005;17:777-90.

Sarosi GA, Barnhart DC, Turner DJ, Mulholland MW. *Capacitative Ca2+ entry in enteric glia induced by thapsigargin and extracellular ATP*. Am J Physiol. 1998;275:G550-5.

Savidge TC, Newman P, Pothoulakis C, Ruhl A, Neunlist M, Bourreille A, Hurst R, Sofroniew MV. *Enteric glia regulate intestinal barrier function and inflammation via release of S-nitrosoglutathione*. Gastroenterology. 2007;132:1344-58

Scemes E, Giaume C. *Astrocyte calcium waves: what they are and what they do*. Glia. 2006;54:716-25.

Segura BJ, Zhang W, Cowles RA, Xiao L, Lin TR, Logsdon C, Mulholland MW. *Lysophosphatidic acid stimulates calcium transients in enteric glia*. Neuroscience. 2004a;123:687-93.

Segura BJ, Zhang W, Xiao L, Turner D, Cowles RA, Logsdon C, Mulholland MW. Sphingosine-1-phosphate mediates calcium signaling in guinea pig enteroglial cells .J Surg Res. 2004b;116:42-54.

Segura BJ, Zhang W, Cowles RA, Mulholland MW. *Evidence of glutamatergic signaling in enteric glia.* FASEB J 2001; 15:A826.

Sontheimer H. *Voltage-dependent ion channels in glial cells*. Glia 1994; 11: 156-172.

Verkhratsky A, Kirchhoff F. NMDA *Receptors in glia*.Neuroscientist. 2007;13:28-37

Verkhratsky A, Orkand RK, Kettenmann H. *Glial calcium: homeostasis and signaling function.* Physiol Rev. 1998;78:99-141

Zhang W, Sarosi GA Jr, Barnhart DC, Mulholland MW. *Endothelin-stimulated capacitative calcium entry in enteric glial cells: synergistic effects of protein kinase C activity and nitric oxide.* J Neurochem. 1998;71:205-12.

Zhang W, Sarosi G Jr, Barnhart D, Yule DI, Mulholland MW. *Endothelin-activated calcium signaling in enteric glia derived from neonatal guinea pig*.Am J Physiol. 1997;272:G1175-85.

Zhang W, Segura BJ, Lin TR, Hu Y, Mulholland MW. *Intercellular calcium waves in cultured enteric glia from neonatal guinea pig*. Glia. 2003;42: 252-62.

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Chapter 8

COCAINE AND CRAVING: FROM NORMAL TRANSMISSION TO PATHOLOGICAL FUNCTIONING

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ABSTRACT

Cocaine belongs to the psychomotor stimulant drug class, members of which are united by their common action of increasing the synaptic availability of monoamine neurotransmitters. These drugs are also united by the fact that they produce craving in users. However, the means by which psychostimulant effects on normal transmission evolve into a 'hunger for drugs' remains uncertain. Our recent work has begun to elucidate one aspect of this complex process – how drug actions on sensory systems may aid the association of environmental stimuli ('cues'), via classical conditioning, with the effects of the drug, triggering craving and relapse. We have shown that administration of cocaine can enhance evoked responses in the primary sensory cortex of experimental animals. Given that the speed of learning in classical conditioning is affected by the intensity of the conditioned stimulus (CS), and that cocaine enhances the neural representation of sensory stimuli in the primary sensory cortex in a manner similar to an increase in intensity, we propose that cue-induced craving in human addicts is facilitated by the drug. In short, cocaine speeds the process that leads to craving. This proposal is supported by the fact that cocaine enhances sensory responses in humans and leads to an improvement in attention (the putative intermediary between enhanced sensory responses and facilitated learning). Furthermore, cocaine affects neural loci which are known to play a role in learning and facilitates classical conditioning when present during acquisition. In addition, related drugs like d-amphetamine and ecstasy (which themselves produce craving) affect sensory processing and attention, and in the case of d-amphetamine facilitate human learning. It is therefore possible that cocaine itself plays a – previously under-appreciated – role in the formation of associations between drug and drug-related environmental cues by enhancing primary sensory responses. A corollary of this is that, as with other intense CSs, the established association may be particularly resistant to extinction, potentially explaining why cues continue to elicit craving months or even years after the last cocaine use.

INTRODUCTION

Cocaine belongs to the psychomotor stimulant drug class, members of which are united by their common action of increasing the synaptic availability of monoamine neurotransmitters [1]. These drugs are also united by the fact that they produce craving in users. Craving, a 'hunger for drugs' [2], is one of the cardinal features of drug dependence in humans, and its importance in perpetuating the consumption of a range of drugs of abuse has been widely acknowledged (e.g. [3]). In relation to cocaine, Gawin [4] identified two types of craving following abstinence in human addicts: 'anhedonic craving', arising when memories of cocaine-induced euphoria contrast with the dysphoric state present in the first few weeks of abstinence, and 'cue-induced craving', arising when objects or situations ('cues') encountered at the time of drug taking (e.g. abuse objects like syringes) are re-encountered during abstinence. In the case of cocaine, the strength of cue-induced craving is positively correlated with the severity of cocaine dependence [5], and craving elicited by drug-related cues plays an important role in relapse [6]. The impact of drug-related cues on relapse also extends to animal models of cocaine addiction [7].

The pivotal role of craving, in particular cue-induced craving, in relapse to cocaine use suggests that conquering craving is an important therapeutic goal. However, the search for pharmacotherapies to treat cocaine craving has been largely unsuccessful [8]. This is primarily because so little is known about the processes involved in the establishment and maintenance of this cardinal feature of addiction, and the corollary neuroadaptations. Recent theories of addiction emphasise learning [9], and work in animals has begun to reveal some of the structures and circuits within which drugs of abuse act to affect learning [10]. However, the major focus here has been the conversion of goal-directed behaviour into habits, with the spotlight inevitably on operant conditioning.

Cue-induced craving on the other hand is assumed to involve classical conditioning, the assumption being that through repeated pairing of the drug (the unconditioned stimulus, UCS) and environmental stimuli (cues) present at the time of drug taking (conditioned stimuli, CSs), the latter eventually come to elicit an incentive motivational state (the conditioned response; CR, possibly involving memories of cocaine euphoria [4]) similar to that elicited by the UCS itself (the unconditioned response, UCR [11]), subjectively experienced as craving. Although strictly speaking only an assumption, the involvement of classical conditioning in cue-induced craving has high face validity and is treated almost axiomatically in the addiction literature. Furthermore, work using conditioned place preference (where subjects learn to associate drug effects and an environmental context [12]), and work with more discrete cues [13], demonstrate that general environmental features and specific stimuli can acquire incentive properties via classical conditioning following repeated pairings with cocaine, firmly supporting the idea that a similar process is involved in craving.

Theories concerning the neurobiology of craving have been largely dominated over the past 10-15 years by the phenomenon of sensitisation. Repeated, intermittent administration of psychostimulants like cocaine to laboratory animals leads to a long-lasting augmentation of certain behavioural responses (e.g. locomotor activation) elicited by the drug (see [14] for a review). Robinson and Berridge [11] have argued that following administration of drugs of abuse in humans, a sensitisation-like process causes excessive incentive salience to be attached to drug-related stimuli, which leads to craving in addicts when these stimuli are re-

encountered later during withdrawal. The central idea is that drug-related cues (CSs) elicit an incentive motivational state in the addict that is supra-normal - craving rather than wanting - because the process of sensitisation has made the system which attributes 'salience' to incentives (like drug-paired CSs) hyper-responsive. Salience will be an important concept in this chapter, and hence it is useful to define it here. In accordance with Rumbaugh et al. [15], we consider salience to be 'a property of a stimulus that causes an organism to focus attention toward that stimulus'. As a consequence of sensitisation, the salience attributer (which is argued to be the mesoaccumbens dopamine system in the brain) attributes too much salience to drug-related stimuli (which have gained incentive value by pairing with the drug). The conditioning process itself, which links previously neutral stimuli to the drug, is not considered to be affected by sensitisation [11]. Indeed, there is probably a significant temporal gap between exposure to the drug taking context and the neurobiological processes which induce sensitisation [16], making it unlikely that sensitisation affects the formation of associations between drug-related stimuli and drug effects. However, whilst sensitisation does not affect the associative process, other effects of the drug could well do.

CLASSICAL CONDITIONING

Here we argue that cocaine (and related drugs of abuse – see later) affects the process of craving by interacting with the conditioned association between environmental CSs and the drug UCS, via an effect on stimulus salience. To avoid terminological confusion, we need to point out that 'salience' here has nothing to do with the salience which stimuli accrue when they are imbued with incentive properties following the establishment of CS-UCS associations. This attribution of incentive salience is the point at which the work on sensitisation becomes relevant. The salience we are concerned with here is the 'attention grabbing' properties of environmental CSs when they are *neutral with respect to the UCR*, i.e. before conditioning. In particular, we will focus on one specific determinant of stimulus salience, namely intensity. Intensity has a strong impact on the direction of attention in humans [17], and therefore can be considered a parameter which has an important influence on salience. Indeed, some researchers in learning theory use intensity and salience interchangeably (e.g. [18]).

Theoretical accounts of associative learning indicate a strong relationship between the intensity of a stimulus and its capacity to enter into a conditioned association. Thus, Pearce and Hall [18], following on from the seminal work of Rescorla and Wagner (e.g. [19]), theorise that the change in associative strength accruing to CS A on conditioning trial N is given by:

$$\triangle V_A^N = S_A \mid \lambda^{N-1} - V_A^{N-1} \mid \lambda^N$$

Where $\triangle V_A$ is the change in associative strength gained by CS (A) on trial (N); S_A is a parameter which depends on the intensity of A, and λ (the asymptote of learning) is a parameter which depends on the intensity of the US. Hence, the change in associative strength, or learning, on any one trial (the *speed of acquisition* of the association) is directly related to the intensity of the CS. In other words, the number of trials required to achieve a

given level of association between the CS and UCS will be inversely related to the intensity of the CS.

Empirical data support the idea of a strong relationship between the intensity of a CS and its capacity to enter into a conditioned association. Kamin and Schaub [20] investigated the acquisition of a conditional emotional response (press suppression) by rats, using an auditory CS at three intensities and a shock UCS. They found that although the groups all reached the same learning asymptote during acquisition, acquisition speed varied positively and linearly with sound intensity. Similar effects were found using a visual CS [20]. An additional interesting feature of the work by Kamin and Schaub [20] was that subjects trained with an intense CS were more resistant to extinction than those trained with a weaker CS (when all groups had reached asymptotic performance during acquisition).

How might stimulus intensity affect learning? Stimulus intensity is linearly related to neural activity, such that increases in intensity produce increases in activity in the primary sensory cortices (e.g. somatosensory [22]; auditory [23]; visual [24]). Increasing the neural representation of sensory stimuli in the primary sensory cortices effectively mimics the impact that attention has on this stage of sensory processing. The literature on visual attention suggests that 'attended to' stimuli are associated with an increase in the activity of neurons in the primary sensory cortices. For example, attention-related activation has been described in the human primary visual cortex [25], and responses of neurons in monkey primary visual cortex increase when stimuli are made reward contingent [26]. Similarly with audition: tones that are attended to produce larger event-related potentials in the human primary auditory cortex than tones that are not attended to [27]. As a result, attention may well act as an intermediary, linking stimulus intensity to speed of learning. Attention is a process that is external to and independent of memory and learning, but one that is known to strongly influence the memory induction process [28].

We have recently found that these changes in neural activity in the primary sensory cortex that accompany increases in stimulus intensity and/or the direction of attention are mimicked by cocaine. Local field potential, extracellular multiunit and haemodynamic responses (obtained using optical imaging spectroscopy and laser-Doppler flowmetry) in the barrel cortex of anaesthetised rats to mechanical stimulation of the whiskers are all significantly, and relatively rapidly (within 11-19 min), enhanced by cocaine (0.5 mg/kg i.v. [29,30]; see Figure 1 for an example of the effects of cocaine on field potential responses). These findings are consonant with reports from other laboratories that intravenous cocaine produces a rapid enhancement of single unit responses in the barrel cortex of anaesthetised [31] and unanaesthetised rats [32], as well as barrel cortex multiunit responses in the latter [32]. Initial indications are that cocaine's enhancement of neural activity in the primary somatosensory cortex may be effected by an interaction with 5-HT mediated transmission [33].

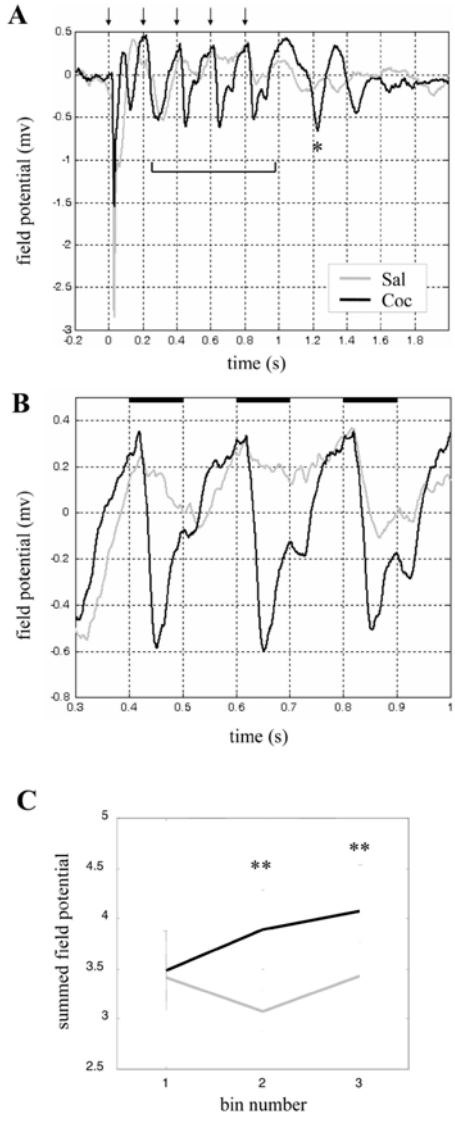

Figure 1. Local field potentials in the rat barrel cortex in response to whisker stimulation are enhanced following cocaine. Field potentials were recorded from the cortex of anaesthetised rats using 16 channel linear probes during repetitive (air puff) stimulation of the whiskers using 1 s, 5 Hz trains of stimuli. (A) Example of field potential responses elicited by whisker stimulation after saline and cocaine infusions (0.5 mg/kg). The featured data (averages of all trials from an individual rat) comprise the dominant response (1st principal component) of the field potentials collapsed across the 16 channels. Arrows denote stimulus onset; (B) Close-up (bracketed region in A) of the responses to stimuli 3 to 5 in the stimulus train. Periods of forced whisker movement are shown as black bands at the top of the figure. The responses to these later stimuli are markedly enhanced, and in the presence of cocaine further field potential components emerge after the train (asterisk in 'A'). (C) Summing the stimulation-induced field potentials over a two second period following the first stimulus in the train, and averaging over 3 time periods ('bins') post-drug (bins starting 2, 11 and 19 min post-drug), it can be seen that cocaine induced changes are relatively rapid (11-19 min). Responses after saline infusion in grey, cocaine infusion in black; **P < 0.01 (saline vs. cocaine).

COCAINE, SENSORY PROCESSING AND LEARNING

Given that the speed of learning in classical conditioning is affected by the intensity of the CS, and that cocaine enhances the neural representation of sensory stimuli in the primary sensory cortex in a manner similar to an increase in intensity, we propose that cue-induced craving in human addicts, which involves the association via classical conditioning of drug-related stimuli and the effects of the drug, is facilitated by cocaine. In short, cocaine speeds the process that leads to craving. With that in mind, we now turn to consider evidence for the proposal. Based on the hypothesised chain of events which link drug administration to cue-induced craving, a number of key predictions can be made that must be true if the envisioned facilitation of learning is taking place. These predictions are as follows: cocaine should enhance sensory responses in humans and lead to an improvement in attention (the putative intermediary between enhanced sensory responses and facilitated learning); cocaine should affect neural loci which are known to play a role in learning, and cocaine should actually facilitate learning in classical conditioning when present during acquisition. Finally, these previous predictions should also be true for related drugs which themselves produce craving. Importantly, there is support for each of these predictions and we now turn to consider that evidence.

Cocaine and Sensory Processing in Humans

Cocaine has been known for some time to produce hallucinations in a range of sensory modalities in addicts: visual, tactile, olfactory, auditory and gustatory [34]. For example ‘snow lights’, a flimmering of bright slivers of light in the periphery of the visual field, are common [35]. Snow lights can be perceived even with the eyes closed [34], which suggests that the drug raises baseline (spontaneous) activity in the sensory systems. In addition, perceptual distortions can occur, such that environmental stimuli are over interpreted as threatening or ominous [35], suggesting perhaps that stimulus-related activity is also enhanced by cocaine, leading to an increase in stimulus salience.

These inferences from the self reports of cocaine users are largely supported by findings from human neuroimaging work. Hence, cocaine increases the baseline blood oxygen level dependent (BOLD) functional magnetic resonance (fMRI) signal in the primary visual cortex in humans [36], and enhances the BOLD fMRI response in the occipital cortex (including the primary visual cortex) to diffuse flashing photic stimulation [37]. As a consequence, given the linear relationship between intensity and neural activity in the primary visual cortex [24], following cocaine the visual cortex in humans responds to a given visual stimulus as if it was more intense.

Cocaine and Attention in Humans

Given this increase in apparent stimulus intensity, it is perhaps not surprising that cocaine appears to enhance attention to stimuli under experimental conditions in humans. For example, cocaine enhances several long latency (‘slow’ and ‘late’) components of the

auditory event-related potential (ERP), the so called 'endogenous' components which are related to aspects of cognitive processing [38]. Specifically, cocaine increased the amplitude of N100 to a warning stimulus (the letter X) in a continuous performance task, where subjects had to respond to a letter B following X in a list of spoken letters, and the contingent negative variation (CNV) following correctly detected 'Bs' [39]. In addition, it increased the amplitude of the P3B and slow wave (SW) components of the auditory event-related potential to 'oddball' stimuli (rare tones in a sequence of frequent tones [40]). Although these long latency components of the auditory ERP reflect particular aspects of information processing, they are all positively modulated by arousal and attention [38], and in the case of the CNV may actually reflect the deployment of the attentional resources themselves [41]. As a consequence, given the broad range of the ERP components that appear to be affected by cocaine, the most parsimonious interpretation of these ERP data is that cocaine enhances attention in humans.

In addition to this electrophysiological evidence that cocaine enhances attention in humans, there is also confirmatory behavioural evidence. Cocaine increases the number of correct responses on the Rapid Visual Information Processing Task (a test of attention in which subjects have to respond to the presentation of successive odd or even digits in a visually presented series [42]), and speeds reaction times in Posner et al.'s [43] test of covert shifts in visual attention (in which changes in reaction times to a target are measured when covert attention is shifted towards or away from the target [44]). It therefore appears that, perhaps because of cocaine-enhanced stimulus salience, stimuli engage attentional processes more readily in the presence of cocaine, presumably producing a corresponding facilitation of learning with respect to those stimuli.

Locus of Action for Cocaine and its Implications for Learning

In the context of learning, and the impact of cocaine on learning, the results of further work on cocaine's enhancement of sensory processing in the rat barrel cortex is pertinent. As we mentioned above, intravenous cocaine enhances sensory responses in the rat barrel cortex [29,30]. However, the extent of this enhancement is not uniform across the cortical layers. Enhancement is more marked in the upper cortical layers (layers II/III; Figure 2). Layers II/III have been implicated in experience-dependent plasticity [45] and cortical-map remodelling [46]. Indeed, these layers retain the ability to adapt into adulthood, whilst the subjacent layer IV loses that ability by adolescence [47]. Plasticity at the functional level is probably supported by long-term potentiation (LTP) and/or long term depression (LTD) at the synaptic level, phenomena which many believe to underlie learning and memory (e.g. [48]). Therefore, it is perhaps not surprising that LTP following thalamic stimulation is strongest in layers II and III [49], and these layers (along with layer 1) have the highest density of NMDA receptors [50], which play a crucial role in 'classical' LTP and LTD [51,52]. Establishing a functional connection between the capacity for plasticity in these outer layers and actual learning, Siucinska and Kossut [53] have demonstrated that a classically conditioned association between mechanical deflection of the whiskers (CS) and tail shock (UCS) in mice results in an increase in the size of the representation of the trained whiskers in the lower part of layer III. Together, the above findings suggest that cocaine preferentially affects cortical

layers that have a particular role to play in learning, including the learning that underlies classical conditioning.

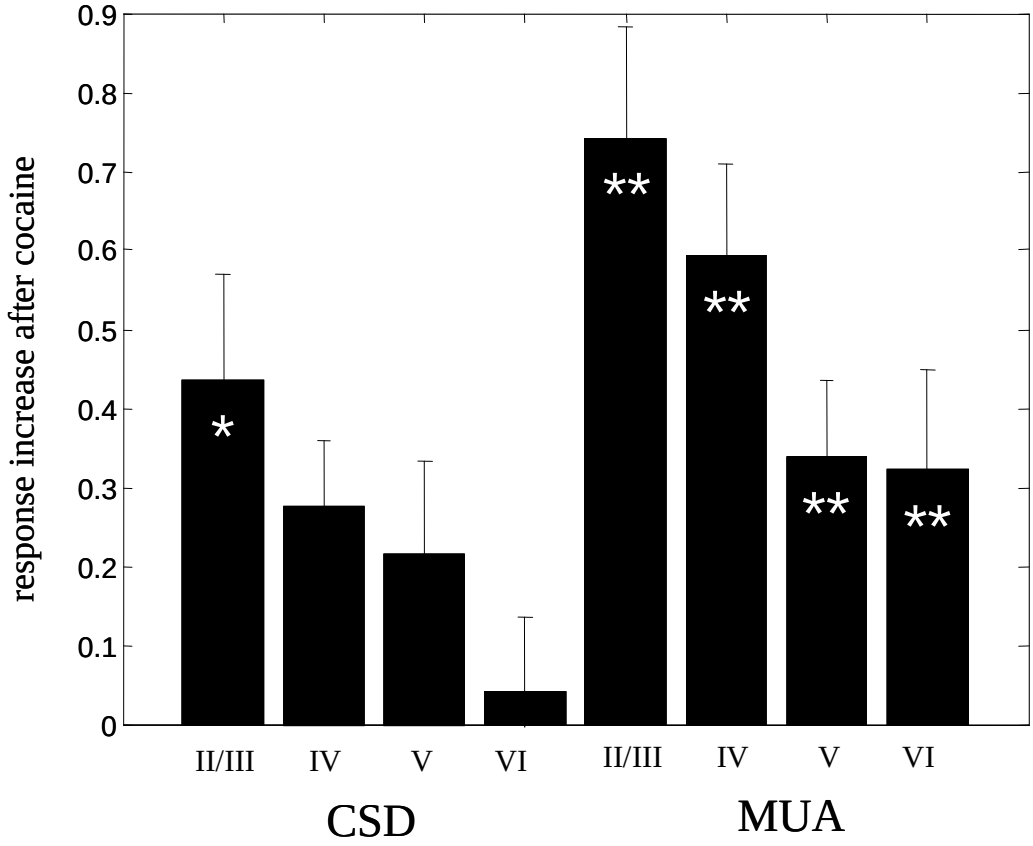

Figure 2. Enhanced neural responses to whisker stimulation following cocaine are more marked in the upper cortical layers. Field potentials and multiunit activity (MUA) were recorded from the cortex of anaesthetised rats using 16 channel linear probes during repetitive (air puff) stimulation of the whiskers using 5 s, 1 Hz trains of stimuli. Field potentials have been converted to current source density (CSD) measurements by calculating the second spatial derivative of the field potential responses across the cortex. The responses to the final three stimuli in the train following saline have been averaged and subtracted from the responses after cocaine in order to summarise the overall effects of cocaine in the different layers (II/III, IV, V and VI). Significant differences (saline vs. cocaine) in the layers indicated are indicated by asterisks; * = $p < 0.05$; ** = $p < 0.01$).

Cocaine and Classical Conditioning

Given that cocaine enhances sensory responses in the primary sensory cortex, and that enhancement is greatest in the layers of the cortex which appear to play a particular role in learning (including classical conditioning), if our contention is correct that these cocaine-induced sensory changes speed acquisition in classical conditioning, then cocaine should facilitate classical conditioning. Although this issue has been poorly researched, evidence from conditioned fear does indeed suggest that this is the case. Wood et al. [54] examined the

effects of cocaine on the learning of an association between a tone (CS) and shock (UCS) in mice, measured in terms of freezing in the presence of the tone 24 and 48 hours later. Low dose cocaine (0.1 mg/kg) was found to significantly enhance learning at both time points, as measured by an increase in freezing relative to saline-treated controls. Higher doses (1 and 5 mg/kg) were still effective but less so, although a much higher dose (15 mg/kg) retarded learning. In this regard it is interesting that if the dose of cocaine is sufficiently high it can actually depress sensory responses in the cortex [31].

Evidence from other Drugs Related to Cocaine

As mentioned earlier, cocaine belongs to the psychomotor stimulant drug class, members of which are united by their common action of increasing the synaptic availability of monoamine neurotransmitters [1]. They are also united by the fact that, like cocaine, other psychostimulants produce craving in users. Given the commonality in the mode of action of these compounds, work concerning these substances can be informative with respect to the proposed link between sensory enhancement and cue-induced craving. For example, amphetamines have been reported to produce cue-induced craving [55]. As a consequence, if our proposal concerning sensory enhancement and cue-induced craving is correct, there should be evidence of sensory enhancements with amphetamines.

Preliminary work suggests that this is indeed the case. D-amphetamine augments the amplitudes of visual evoked potentials in hyperkinetic children [56], produces a dose-dependent increase in the startle response to auditory stimuli in mice [57], and enhances the middle latency auditory ERP N50 response to a test stimulus in the rat, in a paradigm where that response is attenuated due to the presentation of a prior auditory cue [58]. The N50 ERP response appears to have an auditory cortex origin [59], thus suggesting that auditory responses are enhanced at the cortical level by d-amphetamine.

Although work with d-amphetamine is limited, work with a related drug, ecstasy (3,4-methylenedioxymethamphetamine; MDMA), is also generally supportive of a sensory enhancement following administration. Although cue-induced craving with ecstasy has not been examined (indeed there is very little literature on craving and ecstasy, although the phenomenon does exist [60]) heightened visual sensations and (at high doses) visual hallucinations occur with human ecstasy use [61]. Ecstasy increases baseline activity in the human occipital cortex [62], the monkey primary visual cortex [63,64], and sensorimotor cortex [64], and it also produces enhanced visual cortical responses to photic stimulation in monkeys [63,64].

In the case of d-amphetamine, in addition to the studies above which indicate that the drug enhances sensory responses, there is also evidence that there is a corollary enhancement in the salience of CSs in classical conditioning paradigms. In particular, work with latent inhibition (where prior exposure to a stimulus without consequence retards subsequent learning involving that stimulus) has demonstrated that d-amphetamine blocks latent inhibition [65]. It also increases conditioning to relatively uninformative (less salient) predictors in other classical conditioning paradigms [66,67]. Indeed, the fact that d-amphetamine disrupts 'blocking' ([68]: where prior conditioning to stimulus A disrupts learning to stimulus B, when both are later presented together as a compound) can also be interpreted within a stimulus salience framework. Salience of a stimulus presumably affects

the amount of attention paid to it during conditioning, and hence it may not be surprising that there is an extensive literature which shows that d-amphetamine enhances sustained attention in normal humans (see [69] for a review) and rats [70].

Studies with d-amphetamine have gone even further to show that the drug improves learning in humans. D-amphetamine enhances the speed of explicit learning (the learning of non-word names for novel objects via explicit teaching [71]), and also implicit learning (learning of new words for familiar objects without explicit teaching, based on the relative occurrences of correct to incorrect picture-to-new word pairs [72]). Recent evidence suggests that d-amphetamine facilitates experience-dependent plasticity and reorganisation in the human cortex [73], which may provide the neural substrate for some of the learning enhancements induced by the drug.

CONCLUSION

Given that the speed of learning in classical conditioning is affected by the intensity of the CS, and that cocaine enhances the neural representation of sensory stimuli in the primary sensory cortex in a manner similar to an increase in intensity, we propose that cue-induced craving in human addicts, which involves the association via classical conditioning of drug-related stimuli and the effects of the drug, is facilitated by cocaine. In short, cocaine speeds the process that leads to craving. Although inevitably the evidence is to an extent indirect and incomplete, there is support for several key features of the hypothesised chain of events which link drug administration to cue-induced craving. In particular, cocaine enhances sensory responses in humans and leads to an improvement in attention. Furthermore, cocaine affects neural loci which are known to play a role in learning, and facilitates classical conditioning when present during acquisition. In addition, related drugs like d-amphetamine and ecstasy (which themselves produce craving) affect sensory processing and attention, and in the case of d-amphetamine facilitate human learning. It is therefore possible that cocaine plays a – previously under-appreciated – role in the formation of associations between drug and drug-related environmental cues by enhancing primary sensory responses. A corollary of this is that, as with other intense CSs [20], the established association may be particularly resistant to extinction, potentially explaining why cues continue to elicit craving months or even years after the last cocaine use [4]. These actions of the drug are independent of the sensitising neuroadaptations which have dominated much of the literature on craving for many years. Indeed, preliminary work in our laboratory suggests that the cocaine induced sensory enhancement itself does not sensitise (Overton and Devonshire, unpublished observations). From a theoretical perspective this might be expected on the grounds that sensitisation across administrations would produce a continuously changing CS, militating against the establishment of a CS-UCS association.

The hypothesis that cocaine facilitates craving by speeding the acquisition of the cue-drug association concerns the establishment or induction of craving, and therefore it has little explicitly to say about craving once it is established. However, given that craving in cocaine addicts seems to be so readily elicited by drug associated cues, and the fact that, from a classical conditioning point of view, the best CS to elicit the CR is the one used in training (variants, for example less intense stimuli, are effective due to generalisation, but less so), it

might also be hypothesised that stimulus representations remain enhanced during withdrawal. Recent evidence suggests that this is likely to be the case. Thus, visual stimuli elicit larger responses in the occipital cortex of cocaine users [74], and the area of the occipital cortex activated by visual stimuli is more diffuse in cocaine users ([74], and MDMA users [75]), extending beyond the primary visual cortex. Interestingly, the magnitude of activity in cortical sensory areas of cocaine-dependent individuals upon exposure to drug-related cues can be used to predict future relapse [76]. If sensory responses do continue to be enhanced during withdrawal, then this may represent a potential therapeutic target. Pharmacotherapeutic 'dimming' of the cues (by altering the sensory responsiveness of the relevant cortical areas) should weaken their ability to elicit the CR.

A direct test of the proposal that cocaine-induced sensory enhancements speed acquisition of cue-induced craving in humans will be difficult for many reasons, not the least of which are certain ethical impediments. Given that, a potentially fruitful way forward would be to try to fill in some of the evidential gaps in the hypothesised chain of events connecting sensory enhancements to learning. Perhaps the most straightforward of these would be to determine whether cocaine enhances stimulus salience (like d-amphetamine does), which it should do if our contention is correct. Many methods have been developed to assess salience: e.g. classical conditioning – blocking, latent inhibition; operant conditioning - discrimination [77]. Presumably, cocaine could be screened for salience effects via a combination of these methods in animals and also possibly humans. Additional studies might be usefully directed at a more complicated issue: establishing a clear link between drug-induced sensory enhancements and speed of acquisition in classical conditioning. To establish this, a more complex set of investigations would be required, almost certainly limited to animal subjects. One possible approach would be to use a press-suppression task where whisker stimuli are associated with mild footshock. Following initial training to press a lever to receive a reward, electrophysiological responses in the cortex to whisker stimulation could be assessed before and after cocaine administration. Once sensory enhancement was maximal, conditioning trials could be instituted in which paired whisker-shock stimuli (trains of whisker stimuli, terminating for example in footshock) are administered at random intervals as the animal presses. Speed of acquisition would be apparent by how long it takes for whisker stimulation to produce response suppression. Correlating acquisition speed and degree of drug-induced enhancement should suggest whether sensory enhancements have functional consequences.

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REFERENCES

[1] Feldman RS; Meyer JS; Quenzer LF. *Fundamentals of neuropsychopharma-cology*. 3rd Edition. Massachusetts: Sinauer Associates; 1997.

[2] Shiffman S. *Comments on craving*. Addiction, 2000 95, S171-S175.

[3] Wise RA. *The neurobiology of craving: implications for the understanding and treatment of addiction*. J Abnorm Psychol, 1988 97, 118-32.

[4] Gawin FH. *Cocaine addiction: psychology and neurophysiology*. Science, 1991 251, 1580-1586.

[5] Modesto-Lowe V; Burleson JA; Hersh D, Bauer LO; Kranzler HR. (1997*) Effects of naltrexone on cue-elicited craving for alcohol and cocaine*. Drug Alcohol Depend, 1997, 499-16.

[6] Wallace BC. *Psychological and environmental determinants of relapse in crack cocaine smokers.* J Subst Abuse Treat, 1989 6, 95-106.

[7] Shalev U; Grimm JW; Shaham Y. *Neurobiology of relapse to heroin and cocaine seeking: a review*. Pharmacol Rev, 2002 54, 1-42.

[8] Kosten TR; McCance E. *A review of pharmacotherapies for substance abuse*. Am J Addict, 1996 5, 58-65.

[9] Everitt BJ; Dickenson A; Robbins TW. *The neuropsychological basis of addictive behaviour*. Brain Res Rev, 2001 36, 129-138.

[10] Everitt BJ; Wolf ME. *Psychomotor stimulant addiction: A neural systems perspective*. J Neurosci, 2002 22, 3312-3320.

[11] Robinson TE; Berridge KC. *The neural basis of drug craving: an incentive-sensitization theory of addiction*. Brain Res Rev, 1993 18, 247-91.

[12] Spyraki C; Fibiger HC; Phillips AG. *Cocaine-induced place preference conditioning: lack of effects of neuroleptics and 6-hydroxydopamine lesions*. Brain Res, 1982 253, 195-203.

[13] Uslaner JM; Acerbo MJ; Jones SA; Robinson TE. *The attribution of incentive salience to a stimulus that signals an intravenous injection of cocaine*. Behav Brain Res, 2006 169, 320-4.

[14] Robinson TE; Becker JB. *Enduring changes in brain and behavior produced by chronic amphetamine administration: a review and evaluation of animal models of amphetamine psychosis.* Brain Res, 1986 396, 157-98.

[15] Rumbaugh DM; King JE; Beran MJ; Washburn DA; Gould KL. *A salience theory of learning and behavior: With perspectives on neurobiology and cognition*. Int J Primatol, 2007 28, 973-996.

[16] Dommett EJ; Simpson J; Clark D; Overton PG. *Identification of a excitatory amino acid mediated component of the ventral tegmental area local field potential response induced by medial prefrontal cortex stimulation: effect of acute d-amphetamine*. J Neural Transm, 2007 114, 161-72.

[17] Parkhurst D; Law K; Niebur E. *Modeling the role of salience in the allocation of overt visual attention.*Vision Res, 2002 42, 107-23

[18] Pearce JM; Hall G. *A model for Pavlovian learning: variations in the effectiveness of conditioned but not of unconditioned stimuli*. Psychol Rev, 1980 87, 532-52.

[19] Rescorla RA; Wagner AR. *A theory of Pavlovian conditioning: Variations in the effectiveness of reinforcement and nonreinforcement.* In: Black AH, Protasy WF, editors. Classical Conditioning II. New York: Appleton-Century-Crofts; 1972; 64-99.

[20] Kamin LJ; Schaub RE. *Effects of conditioned stimulus intensity on the conditioned emotional response.* J Comp Physiol Psychol, 1963 56, 502-7.

[21] Kamin LJ. *Temporal and intensity characteristics of the conditioned stimulus*. In: Prokasy WF, editor. Classical conditioning: a symposium. New York: Appleton-Century-Crofts; 1965; 118-47.

[22] Hewson-Stoate N; Jones M; Martindale J; Berwick J; Mayhew J. *Further nonlinearities in neurovascular coupling in rodent barrel cortex*. Neuroimage, 2005 24, 565-74.

[23] Phillips DP; Semple MN; Calford MB; Kitzes LM. *Level-dependent representation of stimulus frequency in cat primary auditory cortex*. Exp Brain Res, 1994 102, 210-26.

[24] Kinoshita M; Komatsu H. *Neural representation of the luminance and brightness of a uniform surface in the macaque primary visual cortex*. J Neurophysiol, 2001 86, 2559-70.

[25] Martinez A; DiRusso F; Anllo-Vento L; Sereno MI; Buxton RB; Hillyard SA. *Putting spatial attention on the map: timing and localization of stimulus selection processes in striate and extrastriate visual areas*. Vision Res, 2001 41, 1437-57.

[26] Haenny PE; Schiller PH. *State dependent activity in monkey visual cortex. I. Single cell activity in V1 and V4 on visual tasks*. Exp Brain Res, 1988 69, 225-244.

[27] Woldorff MG; Gallen CC; Hampson SA; Hillyard SA; Pantev C; Sobel D, Bloom FE *Modulation of early sensory processing in human auditory cortex during auditory selective attention*. Proc Natl Acad Sci USA, 1993 90, 8722-9726.

[28] Posner MI; Petersen SE. *The attention system of the human brain*. Annu Rev Neurosci, 1990 13, 25-42.

[29] Devonshire IM; Berwick J; Jones M; Martindale J; Johnston D; Overton PG; Mayhew JE. *Haemodynamic responses to sensory stimulation are enhanced following acute cocaine administration*. Neuroimage, 2004 22, 1744-53.

[30] Devonshire IM; Mayhew JEW; Overton PG. *Cocaine preferentially enhances sensory processing in the upper layers of the primary sensory cortex*. Neuro-science, 2007 146, 841-51.

[31] Bekavac I; Waterhouse BD. *Systemically administered cocaine selectively enhances long-latency responses of rat barrel field cortical neurons to vibrissae stimulation*. J Pharmacol Exp Ther, 1995 272, 333-42.

[32] Drouin C; Waterhouse BD. *Cocaine-induced vs. behaviour-related alterations of spontaneous and evoked discharge of somatosensory cortical neurons*. Eur J Neurosci, 2004 19, 1016-26.

[33] Waterhouse BD; Gould EM; Bekavac I. *Monoaminergic substrates underlying cocaine-induced enhancement of somatosensory-evoked discharges in rat barrel field cortical neurons*. J Pharmacol Exp Ther, 1996 279, 582-92.

[34] Siegel RK. *Cocaine hallucinations*. Am J Psychiatry, 1978 135, 309-14.

[35] Cohen S. Cocaine: *Acute medical and psychiatric complications*. Psychiatr Ann, 1984 14, 747-749.

[36] Brieter HC; Gollub RL; Weisskoff RM; Kennedy DN; Makris N; Berke JD; Goodman JM; Kantor HL; Gastfriend DR; Riorden JP; Mathew RT; Rosen BR; Hyman SE. *Acute effects of cocaine on human brain activity and emotion*. Neuron, 1997 19, 591-611.

[37] Levin JM; Kaufman MJ; Ross MH; Roc AC; Maas LC; Mendelson JH; Cohen BM; Renshaw PF. *Cocaine increases BOLD fMRI response to photic stimulation*. Proc Intl Soc Magn Reson Med, 1999 7, 838

[38] Oken BS. (1997) *Endogenous event-related potentials*. In: Chiappa KH, editor. Evoked potentials in clinical medicine. 3rd Edition. Philadelphia: Lippincott-Raven; 1997; 529-63.

[39] Herning RI; Hooker WD; Jones RT. *Cocaine effects on electroencephalographic cognitive event-related potentials and performance*. Electroencephalogr Clin Neurophysiol, 1987 66, 34-42.

[40] Herning RI; Glover BJ; Guo X. Effects of cocaine on P3B in cocaine abusers. Neuropsychobiology, 1994 30, 132-42.

[41] Kropp P; Linstedt U; Niederberger U; Gerber WD. *Contingent negative variation and attentional performance in humans.* Neurol Res, 2001 23, 647-50.

[42] Johnson B; Overton D; Wells L; Kenny P; Abramson D; Dhother S; Chen YR; Bordnick P. *Effects of acute intravenous cocaine on cardiovascular function, human learning, and performance in cocaine addicts*. Psychiatry Res, 1998 77, 35-42.

[43] Posner MI; Snyder CR; Davidson BJ. *Attention and the detection of signals*. J Exp Psychol, 1980 109, 160–174.

[44] Stillman R; Jones RT; Moore D; Walker J; Welm S. *Improved performance 4 hours after cocaine.* Psychopharmacology, 1993 110, 415-20.

[45] Huang W; Armstrong-James M; Rema V; Diamond ME; Ebner FF. *Contribution of supragranular layers to sensory processing and plasticity in adult rat barrel cortex*. J Neurophysiol, 1998 80, 3261-71.

[46] Hickmott PW; *Merzenich MM. Local circuit properties underlying cortical reorganization.* J Neurophysiol, 2002 88, 1288-301.

[47] Fox K. *Anatomical pathways and molecular mechanisms for plasticity in the barrel cortex.* Neuroscience, 2002 111, 799-814.

[48] Malenka RC; Bear MF. *LTP and LTD: an embarrassment of riches*. Neuron, 2004 44, 5-21.

[49] Heynen AJ; Bear MF. *Long-term potentiation of thalamocortical transmission in the adult visual cortex in vivo*. J Neurosci, 2001 21, 9801-13.

[50] Rema V; Ebner FF. *Postnatal changes in NMDAR1 subunit expression in the rat trigeminal pathway to barrel field cortex*. J Comp Neurol, 1996 368, 165-84.

[51] Bliss TV; Collingridge GL. *A synaptic model of memory: long-term potentiation in the hippocampus.* Nature, 1993 361, 31-9.

[52] Artola A; Singer W. *Long-term depression of excitatory synaptic transmission and its relationship to long-term potentiation*. Trends Neurosci, 1993 16, 480-7.

[53] Siucinska E; Kossut M. *Short-lasting classical conditioning induces reversible changes of representational maps of vibrissae in mouse SI cortex--a 2DG study*. Cereb Cortex, 1996 6, 506-13.

[54] Wood SC; Fay J; Sage JR; *Anagnostaras SG. Cocaine and Pavlovian fear conditioning: dose-effect analysis.* Behav Brain Res, 2007 176, 244-50.

[55] Topp L; Lovibond PF; Mattick RP. *Cue reactivity in dependent amphetamine users: can monistic conditioning theories advance our understanding of reactivity?* Drug Alcohol Rev, 1998 17, 277-88.

[56] Saletu B; Saletu M; Simeon J; Viamontes G; Itil TM. *Comparative symptomato-logical and evoked potential studies with d-amphetamine, thioridazine, and placebo in hyperkinetic children.* Biol Psychiatry, 1975 10, 253-75.

[57] Kokkinidis L; MacNeill EP. *Potentiation of d-amphetamine and L-dopa-induced acoustic startle activity after long-term exposure to amphetamine*. Psychopharmacology (Berl), 1982 78, 331-5.

[58] Adler LE; Rose G; Freedman R. *Neurophysiological studies of sensory gating in rats: effects of amphetamine, phencyclidine, and haloperidol*. Biol Psychiatry, 1986 21, 787-98.

[59] Haenschel C; Vernon DJ; Dwivedi P; Gruzelier JH; Baldeweg T. *Event-related brain potential correlates of human auditory sensory memory-trace formation*. J Neurosci, 2005 25, 10494-501.

[60] Hopper JW; Su Z; Looby AR; Ryan ET; Penetar DM; Palmer CM; Lukas SE. *Incidence and patterns of polydrug use and craving for ecstasy in regular ecstasy users: an ecological momentary assessment study*. Drug Alcohol Depend, 2006 85, 221-35.

[61] Peroutka SJ; Newman H; Harris H. *Subjective effects of 3,4-methylenedioxymethamphetamine in recreational users*. Neuropsychopharmacology, 1988 1, 273-7.

[62] Gamma A; Buck A; Berthold T; Liechti ME; Vollenweider FX. *3,4-Methylenedioxymethamphetamine (MDMA) modulates cortical and limbic brain activity as measured by [H(2)(15)O]-PET in healthy humans*. Neuropsychopharmacology, 2000 23, 388-95

[63] Meyer JS; Brevard ME; Piper BJ; Ali SF; Ferris CF. *Neural effects of MDMA as determined by functional magnetic resonance imaging and magnetic resonance spectroscopy in awake marmoset monkeys.* Ann N Y Acad Sci, 2006 1074, 365-76.

[64] Brevard ME; Meyer JS; Harder JA; Ferris CF. *Imaging brain activity in conscious monkeys following oral MDMA ("ecstasy").* Magn Reson Imaging, 2006 24, 707-14.

[65] Warburton EC; Joseph MH; Feldon J; Weiner I; Gray JA. *Antagonism of amphetamine-induced disruption of latent inhibition in rats by haloperidol and ondansetron: implications for a possible antipsychotic action of ondansetron*. Psychopharmacology, 1994 114, 657-64.

[66] O'Tuathaigh CM; Moran PM. *Evidence for dopamine D(1) receptor involvement in the stimulus selection task: overshadowing in the rat*. Psychopharmacology, 2002 162, 225-31.

[67] Norman C; Cassaday HJ. *Amphetamine increases aversive conditioning to diffuse contextual stimuli and to a discrete trace stimulus when conditioned at a higher footshock intensity*. J Psychopharmacol, 2003 17, 67-76

[68] Crider *A; Solomon PR; McMahon MA. Disruption of selective attention in the rat following chronic d-amphetamine administration: relationship to schizophrenic attention disorder*. Biol Psychiatry, 1982 17, 351-61.

[69] Koelega HS. *Stimulant drugs and vigilance performance: a review*. Psychopharmacology, 1993 111, 1-16.

[70] Grilly DM. *A verification of psychostimulant-induced improvement in sustained attention in rats: effects of d-amphetamine, nicotine, and pemoline*. Exp Clin Psychopharm, 2000 1, 14-21.

[71] Whiting E; Chenery H; Chalk J; Darnell R; Copland D. *Dexamphetamine enhances explicit new word learning for novel objects.* Int J Neuropsycho-pharmacol, 2007 10, 805-16.

[72] Breitenstein C; Wailke S; Bushuven S; Kamping S; Zwitserlood P; Ringelstein EB; Knecht S. *D-amphetamine boosts language learning independent of its cardiovascular and motor arousing effects.* Neuropsychopharmacology, 2004 29, 1704-14.

[73] Dinse HR; Ragert P; Pleger B; Schwenkreis P; Tegenthoff M. *Pharmacological modulation of perceptual learning and associated cortical reorganization*. Science, 2003 301, 91-4.

[74] Lee J-H; Telang FW; Springer CS; Volkow ND. *Abnormal brain activation to visual stimulation in cocaine abusers*. Life Sci, 2003 73, 1953-1961.

[75] Cowan RL; Haga E; deB Frederick B; Dietrich MS; Vimal RL; Lukas SE; *Renshaw PF. MDMA use is associated with increased spatial BOLD fMRI visual cortex activation in human MDMA users.* Pharmacol Biochem Behav, 2006 84, 219-28.

[76] Kosten TR; Scanley BE,; Tucker KA; Oliveto A; Prince C; Sinha R; Potenza MN; Skudlarski P; Wexler BE. *Cue-induced brain activity changes and relapse in cocaine-dependent patients.* Neuropsychopharmacology, 2006 31, 644-50.

[77] Bushnell PJ. *Behavioral approaches to the assessment of attention in animals*. Psychopharmacology, 1998 138, 231-259.

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Chapter 9

EPILEPSY AND COGNITIVE FUNCTIONS: AN INVERSE RELATIONSHIP OF THE UNDERLYING MECHANISMS

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ABSTRACT

Impairment of cognitive functions in patients with epilepsy is a major problem and a very complicated area with many possible causes and implications. The challenge is complex as both the underlying pathology (the disease process) and the therapeutic measures (drugs and surgery) can adversely affect cognitive functions. A close look at the scientific literature reveals an inverse relationship between the mechanisms involved in epilepsy and cognitive functions. Both epilepsy and cognition has been linked to abnormalities in the excitatory amino acid transmission, long-term potentiation and GABAergic inhibition in an opposite manner. Further, epilepsy and memory are reported to share the same anatomical loci in the brain in such a way that the regions of brain, considered important for memory, may provoke a seizure. Improving one condition may thus deteriorate another. Such biological/ pharmacological antagonism has been responsible for compromises in the therapeutic approach towards drug therapy and the management of epilepsy. In this chapter, an attempt has been made to review the underlying mechanisms and the anatomical loci /shared circuits between epilepsy and memory along with the relationship of various neurotransmitters involved.

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Memory impairment, mental slowing and attention deficits are the most frequently reported cognitive disorders in patients with epilepsy and represents a significant burden under conditions of an already debilitating disease. It has been estimated that 44% of patients with epilepsy report difficulties with learning, 45% report that they are slow thinkers and 63% report that the adverse effects of antiepileptic drugs (AEDs) prevent them from achieving their activities or goals (Meador, 2006). It's a complicated area with many possible causes and implications. While the phenomenon of deficient learning, memory, and cognition in epilepsy has been well established and recognized way back in 1942, its place in the pathophysiology of epilepsy continues to be a subject of debate (Lennox, 1942). Difficulty in learning and memory may critically influence the daily life of epileptic patients and greatly compromise with their quality of life (Hermann & Seidenberg, 2007; Harden et al., 2007; Thompson, 1992). The problem is complex because both the underlying pathology (the disease process) (Halgren et al., 1991) and the therapeutic measures (drugs and surgery) (Vermeulen and Aldenkamp, 1995) can adversely affect cognitive function. A variety of factors such as the underlying disease process, psychosocial environment, assessment procedures used, mono- or multiple drug therapy, duration of the disease and treatment etc. are responsible for the observed cognitive deficits (Hendriks et al., 2004; Thompson, 1991). An attempt to look at one factor in isolation is not justified.

THE DISEASE PROCESS

The association of memory disturbances with epilepsy was known long before the widespread availability of anticonvulsant medication (Thompson, 1991). Thus, the etiology, age of onset, seizure type and severity, duration, antiepileptic medication and other factors all contribute to the cognitive dysfunction observed in epileptic patients (Carreno et al., 2008). Given the complexity of memory and cognitive mechanisms, as well as the diversity of underlying neuronal processes, it is unlikely that impaired memory and cognition in epilepsy can be explained by a single mechanism. Factors that contribute to the observed memory deficits include:

a) **Etiology:** Etiology of epilepsy is a factor in determining cognitive function and intellectual changes over time. The main distinction is between symptomatic epilepsy (which has an identified cause such as stroke or cortical dysplasia) and idiopathic epilepsy (which has no identified cause other than genetic factors). The various etiological factors attributed to this disease include - head trauma, CNS infections and poisoning, brain tumors, cerebrovascular disorders, chronic alcoholism etc (Nashef, 1996). All these conditions can lead to neurological dysfunction including loss of memory even in the absence of epilepsy. It has been reported that patients with known etiology exhibit greater memory problems vs those in whom the cause of epilepsy has not been ascertained (Thompson, 1991). Thus, the underlying brain pathology appears to be crucial in associated cognitive deficits.

b) **Seizures:** Cognitive dysfunction has long been known to accompany seizure activity. The type, frequency, severity and duration of seizures as well as the age of the affected person at the first seizure episode can all influence such dysfunction in epileptic patients.

(i) **Type:** People with seizures originating in the temporal lobe are at increased risk for memory problems. The human medial temporal lobe (MTL) plays a critical role both in experimental memory and clinical situations involving complex partial seizures (CPS) (Halgren et al., 1991). Comparison of partial and generalized seizures reveals more specific deficits associated with partial epilepsy (Rausch et al., 1978). Memory deficits are more frequent and severe with partial seizures than with primary generalized epilepsies. Although early onset of generalized seizures is associated with poorer outcome than is early onset of partial seizures, in one study, no significant difference between the neuropsychological functioning of these two groups was observed overall (O'Leary et al., 1983). The cognitive effects of partial epilepsy differ based on lateralization and localization of the seizure focus, with left temporal, right temporal, and frontal lobe epilepsy each producing different phenomena (Powell et al., 1987). Classically, left temporal lobe epilepsy (left TLE) causes verbal memory deficits, whereas right TLE causes visuospatial impairment (Rodin et al., 1986; Strauss et al., 1995; Hermann et al., 1987). Hermann et al (1997) however, found that the overall effects of the laterality of TLE on hemisphere-dependent cognitive functions were the exception rather than the rule. Medial TLE is associated with generalized cognitive impairment in several areas (Hermann et al., 1997; Glowinski, 1973). The potential of status epilepticus (SE) to cause permanent neurologic sequelae also has long been recognized (Oxbury & Whitty, 1971). Among the risk factors for the development of cognitive and behavioral decline after an episode of SE are early age of onset (Aicardi & Chevrie, 1970), longer duration of SE (Rowan & Scott, 1970; Aminoff & Simon, 1980), failure to treat with antiepileptic drugs (Aicardi & Chevrie, 1970; Lawson et al., 1990; Maytal et al., 1989) and symptomatic etiology (Rowan & Scott, 1970; Maytal et al., 1989). The association of absence seizures with cognitive decline is not clear.

(ii) **Age of onset/duration:** Data from experimental models provide evidence that both prolonged and brief seizures can cause irreversible impairment in spatial and emotional learning and memory. Onset of seizures at an early age and a longer duration are implicated with greater memory impairments (Thompson, 1991). Jokeit & Ebner (1999) separated patients with epilepsy into groups on the basis of seizure duration. Their statistics showed that patients with epilepsy of more than 30 years' duration had significantly lower Full-Scale IQ scores than patients with duration of epilepsy of 15 to 30 years or fewer than 15 years. Most studies, however, indicate that early age of onset of seizures is a more important predictor of poor cognitive outcome in patients with epilepsy (Dodrill, 1991; Dodrill & Matthews, 1992; Gomez et al., 1982; O'Leary et al., 1983; Saykin et al., 1989; Strauss et al., 1995).

(iii) **Frequency:** A higher frequency of seizure has a greater disruptive influence on memory (Mohan et al., 1976). In monozygotic twins with the same form of epilepsy but different seizure frequency, the twin with the more frequent seizures showed greater cognitive impairment (Dodrill & Troupin, 1976). The findings, however, have not always been consistent. Dodrill (1986) suggested the total life time number of seizures rather than the frequency to be more important. Several

studies have reported that seizure frequency was negatively correlated with cognitive outcome (Farwell et al., 1985; Trimble, 1988). Other researchers found that this was not the case, however (Bourgeois et al., 1983; Giordani et al., 1983).

(iv) **Severity:** Seizures become severe when they are of longer duration or when they result in secondary injuries e.g. head injury. This can cause severe memory deficits (Thompson, 1991).

c) **EEG Abnormalities:** The relationship between epilepsy and epileptiform EEG abnormalities continues to be debated. Such cognitive impairment occurs exclusively in direct relation to episodes of epileptiform EEG discharges and must be distinguished from (post) ictal seizure effects and from the non-periodic long-term "stable" interictal effects caused by the clinical syndrome or the underlying etiology (Aldenkamp & Arends, 2004). Some investigators consider the epileptiform abnormalities an epiphenomenon reflecting underlying brain pathology rather than the direct cause of cognitive disorder (Holmes et al., 1981). They cite the lack of clear correlation between the status of the epilepsy and EEG abnormalities and the status of cognitive or behavioral disorder, as well as the lack of consistent response to medication. Other researchers believe that the cognitive impairment is a direct consequence of the epileptiform activity (Harden et al., 2007). According to Binnie & Marston (1992), generalized 3 Hz spike wave bursts lasting at least 3 sec are most likely to produce transitory impairments. Evidence also exists that subclinical epileptiform discharges in the EEG of epileptics are accompanied by impairments of the short term working memory (Thompson, 1991).

d) **Psychological/ Social factors:** It has been suggested that the non-cognitive determinants of cognitive function may be important. Conditions that affect patient's mood and other factors such as alertness, motivation, attention and ability to sustain concentration; cognitive skills etc can all influence the performance of epileptic patients. In addition to these, a person's emotional state is also important to consider. There is evidence that depression may impair memory that performance by epileptics (Thompson, 1991).

TREATMENT

a) **Surgery:** Human MTL resection is the most common surgery for intractable epilepsy because of its proven efficacy in seizure control. However, patients who may benefit from the procedure might be deterred from surgical evaluation due to concerns of postoperative cognitive decline (Langfitt et al., 2007). Decline in verbal memory occurs frequently after resections of the left temporal lobe (Marla et al., 2008; Tellez-Zenteno & Weibe, 2008). Although memory decline observed on testing is not typically accompanied by functional decline, a small proportion of patients do experience reductions in occupational or academic status. However, Shin et al (2009) has recently reported no impairment of neuropsychological functioning after surgery, with the exception of auditory immediate memory in patients with left TLE. In addition, the patients with right TLE also showed improvement in post-operative

neuropsychological functioning. Hence it has been suggested that temporal lobectomy does not harm the neuropsychological functioning of patients with intractable TLE and that it improves cognitive functions of the contralateral hemisphere (Shin et al., 2009). Frontal lobe surgery may also impair memory. Children who have undergone surgical resection of the dominant frontal lobe frequently show declines in verbal fluency, and sometimes verbal IQ, visual confrontation naming, and conceptual reasoning. Adult surgical cases have shown the most specific frontal lobe findings, including reduced word fluency with relatively small lesions of the dominant dorsolateral frontal cortex, the analogous finding of impaired nonverbal fluency with nondominant frontal lesions, and other executive deficits following large resections of prefrontal cortex bilaterally (Marla et al., 2008). Recent advances hold promise for better prediction and protection of cognitive functioning due to functional imaging and refinements in preoperative mapping (Hamberger & Drake, 2006). Additionally, results from studies comparing cognitive outcome among different surgical techniques suggest that more restricted resections benefit some patients, whereas more extended resections might be appropriate in a select group of well-defined patients (Halgren et al., 1991). Damage of functional tissue, low mental reserve capacity, and poor seizure outcome increase the risk for postsurgical memory impairment whereas functional release due to seizure freedom counteracts negative impact (Risse, 2006). Preliminary findings indicate that postsurgical training improves memory deficits and encourage further research (Hoppe et al., 2007).

b) **Antiepileptic drugs:** One of the most important obstacles in defining the progressive cognitive and behavioral decline of epilepsy is the need to separate toxicity of AEDs from the effects of seizures. The AED that provides better seizure control for an individual may be more likely to provide a cognitive or behavioral benefit than another AED that has a statistically better cognitive-behavioral profile in controlled clinical studies (Aldenkamp et al., 2003). Although a variety of factors may affect cognition and behavior in patients with epilepsy, the effects of AEDs are of particular concern to the clinician as they constitute the major therapeutic modality to treat seizures (Mula & Trimble, 2009; Meador, 1998; Meador et al.,1991) and a considerable number of patients require AED therapy for many years, or perhaps even a lifetime which emphasizes the need to focus on the long-term adverse effects of these drugs on cognition (Trimble & Dodson, 1994). Studies that demonstrate stable or improving cognitive function over time may actually reflect benefits gained from seizure control. The interest in formally evaluating the cognitive adverse effects of AEDs started in the early 1970s. It was stimulated by the awareness of patients' subjective complaints, the monitoring of AED blood concentrations and the widening range of possibilities of drug treatment (Mula & Trimble, 2009; Aldenkamp, 2002). The results of several clinical studies in epileptic and healthy volunteers reveal that all major AEDs can cause some cognitive side effects (Perucca, 2007; Aldenkamp, 2001; Meador et al., 1990; Smith, 1991; Aldencamp et al., 1994; Vermeulen & Aldencamp, 1995). The risks for such effects are shown to be increased with polypharmacy and with increasing doses/ antiepileptic blood levels (ABLs) (Brunbech & Sabers, 2002; Meador et al., 1990, 1991). However, there are reports where such deficits have occurred even

when the ABLs were well within the established therapeutic range (Ortinski & Meader, 2004; Becker et al., 1995).

(i) **Experimental evidence:** The data available from animal studies is meager and is not consistent so far. Investigations reporting no ill effects of AEDs on memory, an improvement of memory and detrimental effects are available (Majak & Pitkanen, 2004; Meador, 2003; Thompson, 1991). However, the latter evidence is accumulating more as a dose-dependent worsening of the electroshock-induced amnesia following phenytoin, valproic acid, phenobarbitone and ethosuximide has been reported (Mondadori & Classen, 1984). In contrast, carbamazepine was found to be associated with an improved memory functioning. Consistent with these observations, an investigation in rats showed an improved retention performance with carbamazepine but not with sodium valproate and phenytoin in the active avoidance tasks (Rostock & Siegemund, 1993). Sudha and coworkers (1995) studied the adverse cognitive effects following chronic treatment with phenytoin on the T-maze and passive avoidance paradigm in rats. Such effects were found even when the plasma levels were maintained within the required therapeutic range of the drug (Sudha et al., 1995).

(ii) **Clinical evidence:** Mula and Trimble (2009)) reviewed over 100 clinical investigations on the evaluation of the cognitive side-effects of chronic AEDs and found differential effects in different investigations. Older AEDs (phenobarbital, phenytoin, carbamazepine and valproate) have been found to possess detrimental effects on cognition. Several studies report an adverse effect of phenytoin on cognition (Thompson, 1992; Meador et al., 1990, Sudha et al., 1995; Vermeulen and Aldencamp, 1995). Phenytoin shows an overall trend towards poorer performance on several cognitive tasks when compared to carbamazepine. Such effects of phenytoin are reported even with the therapeutic plasma concentration range (Aldencamp et al., 1994; Thompson et al., 1981). Carbamazepine generally produces fewer adverse effects when compared with phenobarbitone or phenytoin (Meador et al., 1990). A brief review of the past studies by Meador (1998), however, revealed greater adverse cognitive effects for phenobarbitone and no clinically significant differences between carbamazepine, phenytoin and sodium valproate. All four AEDs exhibited adverse effects when compared with the non drug conditions. Clonazepam and clobazam have also been reported to show impairments on the tests of memory (Thompson, 1991). Among the newer AEDs, more studies on the cognitive effects have been conducted with vigabatrin than with any of the other agents. Fewer adverse effects on cognition or quality of life measures are reported with vigabatrin (Tartara et al., 1989). A number of studies reported on the long-term safety of vigabatrin shows mild and transient CNS-related adverse effect (Remy & Beaumont, 1989; Ylinen et al, 1995) and the majority of authors agree that vigabatrin does not have a negative influence on cognitive test scores. Lamotrigine has been reported to have some positive effects on the quality of life but cognitive function has not been adequately assessed (Meador, 1998) Preliminary evidence suggesting a small impact of lamotrigine on cognitive functions was reported in a limited number of studies (Placidi et al., 2000;

Sabers et al., 1995; Banks & Beran,1991). A double-blind, crossover, placebo controlled, add-on, dose-ranging study of 21 patients with refractory focal epilepsy showed no significant differences in cognition at the end of 4-week treatment period with Lamotrigine (Blum et al., 2006).Gabapentin and tiagabine were reported to exhibit some positive effects on cognitive function (Meador, 1998; Martin et al., 1999). In a retrospective study that evaluated neuropsychological tests in 18 patients treated with topiramate, a possible decline in verbal functions has been suggested (Burton & Harden, 1997). However, a preliminary study has suggested that zonisamide adversely affects memory and verbal learning was impaired in a dose- or concentration dependent manner during the acute stage (Berent et al., 1987). With levetiracetam, no changes in cognitive performance were reported in an observational study (Gomer et al., 2007). Still, the effect of new compounds vs old drugs on cognitive function remains uncertain. Though a large number of data is generated from experimental and clinical studies on the effects of AEDs on cognitive function, the effects observed have been differential. There is little reason to recommend any of the first-line AEDs as the drugs of choice on the basis of cognitive side effects.

NEUROCHEMICAL RELATIONSHIP

a) Synaptic plasticity/Long term potentiation/ Excitatory amino acids:

Several forms of plasticity, either increasing or decreasing synaptic transmission, exist in the mammalian CNS. The best characterized example is a long-lasting enhancement of the excitatory synaptic transmission termed long-term potentiation (LTP). LTP can be observed at hippocampal synapses and is induced by high frequency tetanus (Foster, 1999).

- **Cognitive functions:** LTP is considered as a candidate for neural mechanisms underlying learning and memory (L&M). It has been suggested to underlie memory function and storage. It has been shown that pharmacological or genetic manipulations that impair LTP induction also impair acquisition of hippocampal-dependent behavior (Foster, 1999). Thus, blocking the induction of LTP in the hippocampus via NMDA receptor antagonists also impairs acquisition of a spatial learning task in rats (Morris, 1989). Further, mutant mice (lacking a gene controlling LTP) also perform poorly in the spatial learning tasks. In addition, it has been shown that repeated induction of LTP can occlude formation of new spatial memories (Reid & Stewart, 1997). Thus, several studies have demonstrated experimentally a link between LTP and L&M.
- **Epilepsy:** It is well known that excitatory pathways that utilize NMDA receptors or abnormalities in the EAA neurotransmission are involved in the initiation, spread and maintenance of the abnormal electrical events that underlie seizure activity (Reid & Stewart, 1997). Thus, NMDA, when injected i.p, induces convulsions in mice while NMDA-receptor antagonists have been

demonstrated to act as anticonvulsants in various experimental models of epilepsy (Meldrum, 1992).

- **Evidence linking LTP, Epilepsy and Cognition:** Electrophysiological studies of the rodent hippocampus have shown that repeated seizure activity has a profound deleterious effect on LTP. It has been suggested that seizure activity causes an indiscriminate and wide-spread induction of LTP and consumes the synaptic plasticity available in otherwise healthy brain tissue such that the formation of new neurons is disrupted. This may represent a mechanism by which cognitive function is impaired in epileptics (Reid & Stewart, 1997). In addition to seizures, AEDs also modulate LTP. A study by Lee et al. (1996) demonstrated that while phenobarbitone potently blocked NMDA LTP and inhibited VDCC LTP, phenytoin reduced only VDCC LTP and valproic acid abolished the expression of NMDA LTP. On the basis of such differences observed in this study, it was proposed that AEDs have different cellular effects on synaptic plasticity and thus may possess different amnesic properties. Thus, both seizures and AEDs have been linked to LTP, known to be involved in L&M.

b) Cholinergic system

- **Cognitive functions:** Cholinergic modulation of L&M processes is now well accepted based on extensive experimental and clinical data (Sharma et al., 2008; Christiane, 2003). The degree of cholinergic dysfunction in Alzheimer's disease patients is correlated with the degree of cognitive deterioration (Bartus et al., 1985; Decker & McGaugh, 1991). Tacrine and donepezil has beneficial effects in Alzheimer's disease due to anticholinesterase actions (Sharma et al., 2008; Freeman & Dawson, 1991). L&M deficits can be produced in experimental animals by cholinergic blockade. There is now extensive evidence that systemic treatment with muscarinic cholinergic antagonists such as scopolamine and atropine disrupts the performance of experimental animals in a wide variety of tasks (Sellin et al., 2008; Howland et al., 2008; Decker & McGaugh, 1991; Dilts & Berry, 1967; Calhoun & Smith, 1968).
- **Epilepsy:** Cholinergic system is known to be involved in the expression of convulsions and in the anticonvulsant effect of drugs. Applied to the cortex, acetylcholine (ACh) acts as a convulsant and physostigmine, an anticholinesterase agent, exhibits proconvulsant properties. Massive release of ACh from the cortex is known to occur with a concomitant decrease in total ACh levels in the brain during PTZ-induced seizures (Subramaniam et al., 1986). It has been recently reported that altered cholinergic modulation may initiate seizure events in the epileptic temporal cortex (Zimmerman et al., 2008).

c) GABA

- **Cognitive functions:** The negative effects of GABA enhancement are well known clinically as a result of studies on benzodiazepines in healthy volunteers. Sedation, drowsiness, psychomotor slowing, anterograde amnesia and difficulties learning new materials are reported adverse effects with short term administration (Stewart, 2005). On the other hand, the reported dysfunctions

during long-term treatment includes impairment of visuospatial and visuomotor abilities, decreased IQ, motor incoordination, slowing of psychomotor speed, decreased speed of information processing, verbal learning and concentration, and delayed response times (Stewart, 2005). The experimental evidence indicating a role for GABA in learning and memory also exists extensively. For instance, retention of recently acquired information is shown to be impaired by post-training systemic injections of either GABA agonist muscimol or GABA transaminase inhibitor aminoxyacetic acid. GABA antagonists and chloride channel blockers, on the other hand, have been shown, in low doses, to facilitate L&M in various experimental tasks. For example, picrotoxin (PTX) enhanced maze learning and performance in the active avoidance tasks, Metrazol (PTZ) and bemegride facilitated learning and bicuculline enhanced retention of the learned response etc. (Singh & Dhawan, 1992; Decker & McGaugh, 1991; Gold, 1995).

- **Epilepsy:** Activation of GABAergic system causes neuronal inhibition and prevents epileptiform activity. GABA agonists and drugs augmenting GABAergic transmission have proved to be protective in many seizure models (Mares & Kubova, 2008; Treiman, 2001; MacDonald & Kelly, 1993). The long duration of $GABA_B$ -mediated potential is responsible for some pro-epileptic effects. In models of absence seizures, $GABA_B$ agonists such as beclofen exacerbate the spike-wave discharge antagonists reduce their occurrence (Clark & Wilson, 1997; Dichter, 1997, 1998). Hence, it is possible to speculate that AEDs with prominent GABAergic properties may show a similar pattern of cognitive adverse effects.

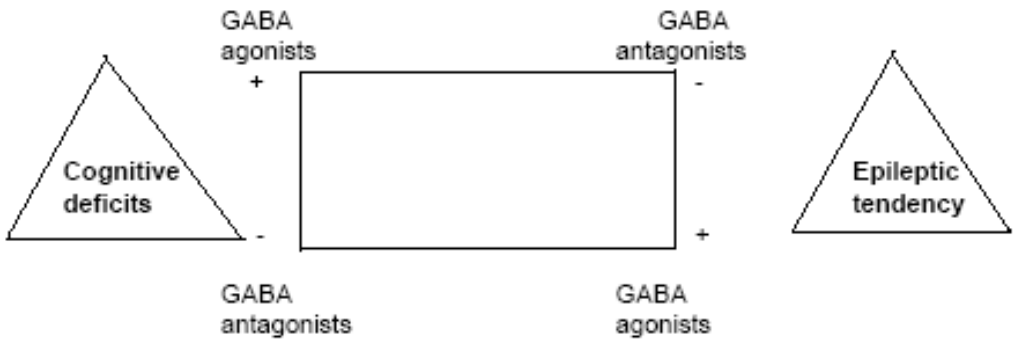

Figure 1 Relationship between epilepsy, cognitive function and GABA

d) **Shared circuits by epilepsy and memory:** The information presented above indicates that epilepsy and memory share the same anatomical locus in the medial temporal lobe (MTL) and use the same synaptic mechanisms. There appears to be an inverse relationship between epilepsy and cognitive functions (as evidenced from Table 1). Improving one condition may thus deteriorate another. Thus, a careful approach is needed in treating cognitive deficits in an epileptic patient.

Table 1 Evidence of biological antagonism between epilepsy and cognitive functions

	Epilepsy	Cognitive function
NEURO-PHARMACOLOGY		
AEDs	Therapeutic	Impairment
GABA GABA agonists GABA antagonists	Antiepileptic Proconvulsants	Impairment Facilitation
Cholinergic system Cholinergic agonists Cholinergic antagonists	Proconvulsants -	Facilitation Impairment
NMDA NMDA receptor agonists NMDA receptor antagonists	Proconvulsants Anticonvulsants	Facilitation Impairment
NEUROCHEMISTRY **Brain Regions** Medial temporal lobe resection (Hippocampus, Amygdala & Parahippocampal gyrus)	Therapeutic (Complex partial seizures)	Impairment
Frontal cortex resection	Therapeutic	Impairment
Intracellular calcium	Proconvulsant	Facilitation / Impairment??

CONCLUSION

Given the complexity of memory and cognition mechanisms, as well as the diversity of underlying neuronal processes, it is unlikely that impaired memory and cognition in epilepsy can be explained by a single mechanism. Indeed, a variety of factors (e.g., neuronal cell loss, recurrent seizures, and sustained tonic dysfunction of limbic circuits) likely contribute to the impairments of learning and memory.

Pharmacological approaches to combine one or more drug with the standard AED may provide beneficial for some. Recent research indicates a variety of novel targets that could be explored for better seizure control (in case of intractable seizures or otherwise) as well as for a positive effect on memory & cognitive functions. Though most of the neurotransmitters important for seizures share an inverse relationship between seizure control and cognitive functions (as is shown in table 1), there are a few transmitters that have a beneficial role in both the processes. An example of such neurotransmitter is histamine. Drugs enhancing the histaminergic transmission as also the recently developed selective H_3 receptor antagonists proved to be beneficial in epilepsy and learning/ memory processes (Vohora &Pillai, 2009). Another possible therapeutic approach could be antioxidants supplementation. There is evidence of a positive correlation between certain antioxidants and seizure control as well as

cognitive improvement (Devi et. al, 2006; 2008). However, we have to keep in mind that all these reports are the result of only preliminary studies conducted in rodents though clinical trials have been conducted for some of the agents discussed above. Readers are requested to refer to other articles for details about the same. Whether such an approach would be beneficial to epileptic patients in their pharmacotherapy remains to be seen.

In spite of accumulated data on various factors/ mechanisms contributing to memory impairments in epileptics, the question still remains to be answered: do learning and memory impairments in epileptics require special dedicated treatment or would merely getting rid of the epileptic foci or seizures be sufficient?

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REFERENCES

Aicardi J, Chevrie JJ. Convulsive status epilepticus in infants and children. Epilepsia 1970; 11: 187–197.

Aldenkamp AP, Alpherts WCJ, Diepman L, Van't Slot B, Overweg J, Vermeulen J. Cognitive side effects of phenytoin compared with carbamazepine in patients with localization related epilepsy. Epilepsy Research 1994; 19: 37-43.

Aldenkamp AP, Arends J. Effects of epileptiform EEG discharges on cognitive function: is the concept of "transient cognitive impairment" still valid? Epilepsy Behav.. 2004; 5(1); S25-34.

Aldenkamp AP, De Krom M, Reijs R. Newer antiepileptic drugs and cognitive issues. Epilepsia. 2003;44(suppl 4):21-29.

Aldenkamp AP. Antiepileptic drug treatment and epileptic seizures: effects on cognitive function. In: Trimble MR, Schmitz B, editors. The neuropsychiatry of epilepsy Cambridge: Cambridge University Press, 2002: 256-65

Aldenkamp AP. Effects of antiepileptic drugs on cognition. Epilepsia 2001; 42 Suppl. 1: 46-9.

Aminoff MJ, Simon RP. Status epilepticus: causes, clinical features, and consequences in 98 patients. Am J Med 1980; 69:657–666.

Banks GK, Beran RG. Neuropsychological assessment in lamotrigine treated epileptic patients. Clin Exp Neurol 1991; 28: 230-7

Bartus RT, Dean RL, Pontecorvo MJ, Flicker C. The cholinergic hypothesis: A historical review, current perspective and future directions. Ann NY Acad Sci. 1985; 444: 332-358.

Becker A, Grecksch G, Brosz M. Antiepileptic drugs – their effects on kindled seizures and kindling – induced learning impairments. Pharmacol Biochem Behav. 1995; 52: 453-459.

Berent S, Sackellares JC, Giordani B, et al. Zonisamide (CI-912) and cognition: results from preliminary study. Epilepsia 1987; 28: 61-67.

Binnie CD, Marston D. *Cognitive correlates of interictal discharges*. Epilepsia 1992; 33(6): S11-S17.

Blum D, Meador K, Biton V, et al. *Cognitive effects of lamotrigine compared with topiramate in patients with epilepsy*. Neurol. 2006; 67: 400-406.

Bourgeois BFD, Prensky AL, Palkes HS, et al. *Intelligence in epilepsy: a prospective study in children*. Ann Neurol. 1983;14:438–444.

Bridgman PA, Malamut BL, Sperling MR, et al. *Memory during subclinical hippocampal seizures*. Neurol. 1989;39:853–856.

Brunbech L, Sabers A. *Effect of antiepileptic drugs on cognitive function in individuals with epilepsy: a comparative review of newer versus older agents*. Drugs 2002; 62: 593-604.

Burton LA, Harden C. *Effect of topiramate on attention*. Epilepsy Res. 1997; 27: 29-32.

Calhoun WH, Smith AA. *Effect of scopolamine on acquisition of passive-aviodance*. Psychopharmacol. 1968; 13: 201-209.

Carreno M, Donaire A, Sanchez-Carpintero R. *Cognitive disorders associated with epilepsy: diagnosis and treatment*. Neurologist. 2008; 14(6 Suppl 1): S26-34.

Christiane M. Thiel. *Cholinergic modulation of learning and memory in the human brain as detected with functional neuroimaging*. Neurobiol Learn Memory 2003; 80(3): 234-244.

Clark S, Wilson W. *Mechanisms of epileptogenesis and the expression of epileptiform activity*. In: Willie E, ed. The treatment of epilepsy: Principles and Practice.1997 2nd ed, 53-81. Baltimore: Willaims and Wilkins.

Decker MW, McGaugh JL. *The role of interactions between the cholinergic system and other neuromodulatory systems in learning and memory*. Synapse 1991; 7: 151-168.

Devi PU, Pillai KK, Vohora D. *Modulation of PTZ-induced seizures and oxidative stress parameters by sodium valproate in the absence and presence of N-acetylcysteine*. Fundamental & Clinical Pharmacol. 20: 247-253.

Devi PU, Manocha A and Vohora D. Seizures, Antiepileptics, *Antioxidants and Oxidative stress : An insight for researchers*. Expert Opin Pharmacother. 2008: 9: 3169-3177.

Dichter MA. *Basic mechanisms of epilepsy: targets for therapeutic intervention*. Epilepsia 1997: 38(9): S2-S6.

Dichter MA. *Mechanism of action of new antiepileptic drugs*. Adv Neurol. 1998; 76: 1-9.

Dilts SL, Berry CA. *Effect of cholinergic drugs on passive avoidance in the mouse*. J Pharmacol Exp Ther. 1967; 158: 279-285.

Dodrill CB, Matthews CG. *The role of neuropsychology in the assessment and treatment of persons with epilepsy*. Am Psychol. 1992;47:1139–1142.

Dodrill CB, Troupin AS. *Seizures and adaptive abilities*. Arch Neurol. 1976; 3: 04–607.

Dodrill CB. *Correlates of generalized tonic-clonic seizures with intellectual, neuropsychological, emotional and social functions in patients with epilepsy*. Epilepsia 1986, 27: 399-411.

Dodrill CB. *Neuropsychological aspects of epilepsy*. Psychiatr Clin North Am. 1991;15:383–394.

Farwell JR, Dodrill CB, Batzel LW. *Neuropsychological abilities of children with epilepsy*. Epilepsia 1985;26:395–400.

Foster FC. *Involvement of hippocampal synaptic plasticity in age-related memory decline*. Brain Res Rev. 1999; 30: 236-249.

Freeman SE, Dawson RM. *Tacrine: a pharmacological review*. Prog Neurobiol. 1991; 36: 257-258.

Giordani B, Sakellares JC, Miller S, et al. *Improvement in neuropsychological performance in patients with refractory seizures after intensive diagnostic and therapeutic intervention*. Neurology 1983;33:489–493.

Glowinski H. *Cognitive deficits in temporal lobe epilepsy. An investigation of memory function.* J Ment Dis. 1973;137:129–137.

Gold PE. *Modulation of emotional and nonemotional memories: Same pharmacological systems, different neuroanatomical systems*. In: McGaugh JL, Weinberger NM and Lynch G, eds. Brain and Memory: Modulation and Mediation of neuroplasticity, pp 41-74, New York: Oxford Univ. Press. 1995

Gomer B, Wagner K, Frings L, et al. *The influence of antiepileptic drugs on cognition: a comparison of levetiracetam with topiramate*. Epilepsy Behav. 2007; 10: 486-94

Gomez MR, Kuntz NL, Westmoreland BF. *Tuberous sclerosis, early onset of seizures, and mental subnormality: study of discordant homozygous twins*. Neurology 1982; 32:604–611.

Halgren E, Stapleton J, Domalski p et al. *Memory dysfunction in epilepsy patients as a derangement of normal physiology*. Adv Neurol. 1991, 55: 385-410.

Hamberger MJ, Drake EB.*Cognitive functioning following epilepsy surgery*. Curr Neurol Neurosci Reports. 2006; 6; 319-326.

Harden CL, Maroof DA, Nikolov B, Fowler K, Sperling M, Liporace J, Pennell P, Labar D, Herzog A. *The effect of seizure severity on quality of life in epilepsy*. Epilepsy Behav.. 2007;11(2):208-11.

Hendriks MPH, Aldenkamp AP, Alpherts WCJ, Ellis J, Vermeulen J, Van DerVlugt H. *Relationships between epilepsy-related factors and memory impairment.* Acta Neurol Scand. 2004; 110: 291-300.

Hermann B, Seidenberg M. *Epilepsy and cognition*. Epilepsy Curr. 2007, 7: 1-6.

Hermann BP, Seidenberg M, Schoenfeld J, Davies K. *Neuropsychological characteristics of the syndrome of mesial temporal lobe epilepsy*. Arch Neurol. 1997;54:369–376.

Hermann BP, Wyler AR, Richey ET, Rea JM. *Memory function and verbal learning ability in patients with complex partial seizures of temporal lobe origin*. Epilepsia 1987;28:547–554.

Holmes H, McKeever M, Saunders Z. *Epileptiform activity in aphasia of childhood: an epiphenomenon?* Epilepsia 1981;22:631–639.

Hoppe C, Elger CE, Helmstaedter C. *Long term memory impairment in patients with focal epilepsy*. Epilepsia. 2007; 48 Suppl 9; 26-29.

Howland J, Rohsenow DJ, Minsky S, Snoberg J, Tagerud S, Hunt SK, Almeida A, Greece J, Allensworth-Davies D. *The effects of transdermal scopolamine on simulated ship navigation and attention/reaction time.* Int J Occup Environ Health. 2008;14(4):250-6.

Jokeit H, Ebner E. *Long-term effects of refractory temporal lobe epilepsy on cognitive abilities: a cross sectional study*. J Neurol Neurosurg Psychiatr. 1999; 67: 44–50.

Langfitt JT, Westerveld M, Hamberger Mj, Walczak TS, Ciccetti DV, Berg AT, Vickrey BG, Barr WB, Sperling MR, Masur D, Spencer SS. *Worsening the quality of life after epilepsy surgery: effect of seizures and memory decline.* Neurol. 2007; 68(23): 1988-1994.

Lawson JS, Inglis J, Delva NJ, et al. *Electrode placement in ECT: cognitive effects*. Psychol Med. 1990; 20: 335–344.

Lee GYP, Brown LM, Teyler TJ. *The effects of anticonvulsant drugs on long term potentiation (LTP) in the rat hippocampus*. Brain Res Bull. 1996; 39: 39-42.

Lennox WG. *Brain injury, drugs and environment as a cause of mental decay in epilepsy*. Am J Psychiatr. 1942, 99: 174-180.

MacDonald RL, Kelly KM. *Antiepileptic drug mechanisms of action*. Epilepsia. 1993; 34(5): S1-S8.

Majak K, Pitkanen A. *Do seizures cause irresistible cognitive damage?* Evidence from animal studies. Epilepsy Behav.. 2004; 5(1); S35-44.

Mares P, Kubová H. *What is the role of neurotransmitter systems in cortical seizures?* Physiol Res. 2008;57 Suppl 3:S111-20.

Marla J. Hamberger , Evan B. Drake. *Long-term seizure and psychosocial outcomes of epilepsy surgery*. Curr treat options Neurol. 2008; 10(4); 253-259.

Martin R, Kuzniecky R, Ho S, Hetherington H, Pan J, Sinclair K, Gilliam F, Faught E. *Cognitive effects of topiramate, Gabapentin and Lamotrigine in healthy young adults.* Neurol. 1999; 52: 321-327.

Maytal J, Shinnar S, Moshé SL, Alvarez LA. *Low morbidity and mortality of status epilepticus in children.* Pediatrics 1989; 83: 323–331.

Meador KJ, Loring DW, Allen ME, et al. *Comparative cognitive effects of carbamazepine and phenytoin in healthy adults.* Neurol.1991; 41: 1537-1540

Meador KJ, Loring DW, Huh K, Gallagher BB, King DW. *Comparative cognitive effects of anticonvulsants.* Neurology 1990, 40: 391-394.

Meador KJ. *Cognitive and behavioral assessment in AED trial*. Adv Neurol. 1998; 76: 231-238.

Meador KJ. *Cognitive and memory effects of the new antiepileptic drugs*. Epilepsy Res. 2006; 68: 63-67.

Meador KJ. *Newer anticonvulsants: dosing strategies and cognition in treating patients with mood disorders and epilepsy*. J Clin Psychiatry. 2003; 64(8); 30-34.

Meldrum BS. *Excitatory aminoacids in epilepsy and potential novel therapies.* Epilepsy Res. 1992; 12: 189-196.

Mohan V, Varma VK, Sahrey BB. *Intellectual and memory functions in epileptics*. India Neurol. 1976, 24: 110-111.

Mondadori C, Classen WC. *The effect of various antiepileptic drugs on E-shock induced amnesia in mice: Dissociability of effects on convulsions and effects on memory*. Acta Neurol Scand. 1984; 69(99): 125-129.

Morris RGM. *Synaptic plasticity and learning: Selective impairment of learning in rats and blockade of long-term potentiation in vivo by the N-methyl-D-aspartate receptor antagonist AP5.* J Neurosci. 1989; 9: 3040-3057.

Mula M, Trimble MR. *Antiepileptic drug induced cognitive adverse effects: Potential Mechanisms and Contributing Factors*. CNS Drugs 2009; 23(2): 121-137

Nashef L. *The definitions, aetiologies and diagnosis of epilepsy*. In: Shorvon S, Driefuss F, Fish D, Thomas D, Eds. The Treatment of Epilepsy, pp-66-96, 1996 London: Blackwell Science.

Neimann H, Boenick HE, Schmidt RC, et al. *Cognitive development in epilepsy: the relative influence of epileptic activity and of brain damage*. Eur Arch Psychiatry Neurol Sci. 1985;234:399–403.

O'Leary DS, Lovell MR, Sackellares JC, et al. *Effects of age of onset of partial and generalized seizures on neuropsychological performance in children*. J Nerv Ment Dis. 1983;171:624–629.

Ortinski P, Meador KJ. *Cognitive side effects of antiepileptic drugs*. Epilepsy Behav. 2004; 5 Suppl. 1: S60-5

Oxbury JM, Whitty CWM. *Causes and consequences of status epilepticus in adults: a study of 86 cases.* Brain 1971; 94:733–734.

Perucca E. *Withdrawing antiepileptic drugs in seizure-free patients: what are the cognitive benefits?* Nat Clin Pract Neurol. 2007; 3: 194-5

Placidi F, Marciani MG, Diomedi M, et al. *Effects of lamotrigine on nocturnal sleep, daytime somnolence and cognitive functions in focal epilepsy.* Acta Neurol Scand 2000; 102: 81-86.

Powell GE, Polkey CE, McMillan T. *The new Maudsley series of temporal lobectomy: short-term cognitive effects*. Br J Clin Psychol. 1987;24: 09–124.

Rausch R, Leib JB, Crandall PA. *Neuropsychological correlates of depth spike activity in epileptic patients*. Arch Neurol. 1978;35: 99–705.

Reid IC, Stewart CA. *Seizures, memory and synaptic plasticity*. Seizure1997; 6: 357-359.

Remy C, Beaumont D. *Efficacy and safety of vigabatrin in the long-term treatment of refractory epilepsy*. Br J Clin Pharmacol 1989; 27 Suppl. 1: 125-9S

Risse Gl. *Cognitive outcomes in patients with frontal lobe epilepsy*. Epilepsia. 2006; 47(2); 87-89.

Rodin EA, Schmaltz S, Twitty G. *Intellectual functions of patients with childhood-onset epilepsy.* Dev Med Child Neurol. 1986;28:25–33.

Rostock A, Siegemund C. *Antiepileptic drugs and cognitive functions: Investigations in rats.* Epilepsia, 1993; 34(2): 51-52.

Rowan AJ, Scott DF. *Major status epilepticus: a series of 42 patients*. Acta Neurol Scand. 1970; 46: 573–584.

Sabers A, Moller A, Dam M, et al. *Cognitive function and anticonvulsant therapy: effect of monotherapy in epilepsy*. Acta Neurol Scand. 1995; 92: 19-27

Saykin AJ, Gur RC, Sussman NM, et al. *Memory deficits before and after temporal lobectomy: effect of lateralization and age of onset*. Brain Cogn. 1989; 9:191–200.

Sellin AK, Shad M, Tamminga C. *Muscarinic agonists for the treatment of cognition in schizophrenia.* CNS Spectr. 2008 ;13(11):985-996.

Sharma B, Singh N, Singh M. *Modulation of celecoxib- and streptozotocin-induced experimental dementia of Alzheimer's disease by pitavastatin and donepezil*. J Psychopharmacol. 2008 ;22(2):162-171.

Shin MS, Lee S, Seol SH, Lim YJ, Park EH, Sergeant JA, Chung C. *Changes in neuropsychological functioning following temporal lobectomy in patients with temporal lobe epilepsy.* Neurol Res. 2009 (in press)

Singh HK, Dhawan BN. *Drugs affecting learning and memory*. In: Tandon Pn, Bijlani V and Wadhwa S, eds. Lectures in Neurobiology, 1992, pp 189-207, New Delhi: Wiley Eastern.

Smith DB. *Cognitive effects of antiepileptic drugs*. Adv Neurol. 1991; 55: 197-212.

Stewart SA. *The effects of benzodiazepines on cognition*. J Clin Psychiatry 2005; 66 Suppl. 2: 9-13

Strauss E, Loring D, Chelune G, et al. *Predicting cognitive impairment in epilepsy: findings from the Bozeman epilepsy consortium.* J Clin Exp Neuropsychol. 1995;17:909–917.

Subramaniam S, Ghosh SS, Sankaranarayanan A. *Effect of scopolamine on physostigmine potentiated convulsions produced by pentylene tetrazole in rats*. Indian j Exp Biol. 1986; 24: 541-542.

Sudha S, Lakshmana MK, Pradhan N. *Chronic phenytoin induced impairment of learning and memory with associated changes in brain acetylcholine esterase activity and monoamine levels.* Pharmacol Biochem Behav. 1995;52: 119-124.

Tartara A, Manni R, Galimberti CA, et al. *Vigabatrin in the treatment of epilepsy: a long-term follow-up study.* J Neurol Neurosurg Psychiatry 1989; 52: 467-71

Tellez-Zenteno JF, Weibe S. *Long term seizure and psychosocial outcomes of epilepsy surgery.* Curr Treat Options Neurol. 2008; 10(4): 253-259.

Thompson P, Huppert FA, *Trimble M. Phenytoin and cognitive function: effects on normal volunteers and implications for epilepsy*. Br J Clin Psychol. 1981; 20: 155-62

Thompson PJ. *Antiepileptic drugs and memory*. Epilepsy 1992, 33(6): S37-S40.

Thompson PJ. *Memory functions in patients with epilepsy*. Adv Neurol. 1991, 55: 369-384.

Tomson T, Lindbom U, Nilsson BYI. *Nonconvulsive status epilepticus in adults: thirty-two consecutive patients from a general hospital population*. Epilepsia 1992;33:829–835.

Treiman DM. *GABAergic mechanisms in epilepsy*. Epilepsia. 2001;42 Suppl 3:8-12.

Trimble MR, Dodson WE. *Epilepsy and quality of life*. New York: Raven Press, 1994

Trimble MR. *Cognitive hazards of seizure disorders*. Epilepsia 1988;29[Suppl]:S19–S24.

Vermeulen J, Aldenkamp AP. *Cognitive side effects of chronic antiepelptic drug treatment: A review of 25 yers of research.* Epilepsy Res. 1995, 22: 65-95.

Vohora D and Pillai KK. Epilepsy. In: Vohora D (Ed) *The Third Histamine Receptor:* Selective ligands as novel therapeutic agents in CNS disorders. 2009: pp 305-328.

Ylinen A, Kalviainen R, Riekkinen Sr SJ. *Long-term efficacy and cognitive effects of vigabatrin.* Acta Neurol Scand. 1995; 162: 47-50.

Zimmerman G, Njunting M, Ivens S, Tolner EA, Behrens CJ, Gross M, Soreq H, Heinemann U, Friedman A. *Acetylcholine-induced seizure-like activity and modified cholinergic gene expression in chronically epileptic rats*. Eur J Neurosci. 2008 Feb;27(4):965-75.

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Chaper 10

PATHOGENESIS OF SCIATICA: INTRARADICULAR BLOOD FLOW ANALYSIS UNDER THE TENSION OF NERVE ROOT INDUCED BY LUMBAR DISC HERNIATION

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ABSTRACT

Objective. Leg pain is a common and disabling symptom in lumbar disc herniation. The femoral nerve stretch test (FNST) and straight leg raising (SLR) test have been one of the simplest and surest methods of making a clinical diagnosis of lumbar disc herniation. It is generally believed that nerve root compression is caused by the hernia when the legs are extended and raised, thus resulting in the onset of lumbar pain or leg pain. However, it is unknown whether intraradicular blood flow (IRBF) changes during the FNST and SLR test in patients with lumbar disc herniation. A nerve root stretch test was conducted in patients with lumbar disc herniation to observe the changes of IRBF, which were then compared with the clinical features. Methods. The subjects were 37 patients with disc herniation who underwent microdiscectomy. Patients were asked to adopt the prone position immediately before surgery, so that FNST test (N=12) was performed to confirm at which anterolateral thigh pain developed or SLR test (N=25) was performed to confirm the angle at which sciatica developed. The needle sensor of a laser Doppler flow meter was inserted into each nerve root immediately above the hernia and the change of IRBF was measured during intraoperative nerve root stretch test. After removal of the hernia, a similar procedure was repeated and IRBF was measured again.

Result. The intraoperative nerve root stretch test showed that the hernia compressed the nerve roots and there was marked disturbance of gliding, which was reduced to only a few millimeters. During the test, intraradicular blood flow showed a sharp decrease at

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the angle that produced root pain, which lasted for one minute. Intraradicular flow decreased by 92.8~100% in the L4 nerve root and by 40~98% in the L5 or S1 nerve roots relative to the blood flow before the test. After removal of the hernia, all the patients showed smooth gliding of the nerve roots during the test and there was no marked decrease of intraradicular blood flow.

Conclusion. This study demonstrated that the blood flow in the nerve root is reduced when the nerve root is compressed *in vivo*.

KeyWords: lumbar disc hernia, sciatica, nerve root, blood flow, straight leg raising test, femoral nerve stretch test

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INTRODUCTION

Mixter and Barr [1] were the first to describe prolapse of the intervertebral disc as a causative agent in the production of low back pain and leg pain. It now is commonly acknowledged that derangements of the intervertebral disc lead to many cases of low back pain and sciatica. It has been considered that symptoms are induced by mainly mechanical factors; deforming nerve fibers and changes in the intraneural circulation may be the secondary factor in the nerve root. However, acute compression of a normal nerve root does not always cause pain; instead, it can cause numbness, paresthesia, and motor weakness. Compressed nerve roots can exist without causing any pain. These facts suggest that some secondary changes in and around the nerve root may be a critical factor in radicular pain. Recent developments in diagnostic imaging procedures such as MR imaging have made it easy to visualize nerve root compression by hernias, assisting in the diagnosis of lumbar disc herniation. A compressed nerve root does not always cause pain. Approximately 20% to 30% of individuals without any history of sciatica have abnormal findings on imaging examinations [2-5]. However, it is difficult to obtain information relevant to pain from such diagnostic imaging, so taking a history and neurological examination are still important.

BLOOD SUPPLY TO THE LUMBAR NERVE ROOT

There have been a number of morphologic studies on the radicular vessels [6-10]. Corbin described anatomic details of radicular arteries and classified them into three groups: artères radiculo-grèles, artères radiculo-piemerriennes, and artères radiculo-medullares [6]. First two arteries were named as distal and proximal radicular arteries by Parke and colleagues [8] and were through to be nutrient arteries of the nerve roots. They described that each lumbosacral spinal nerve root receives its intrinsic blood supply from both distal and proximal radicular arteries, through which the blood flows toward a mutual anastomosis in the proximal one

third of the root. They postulate that the region of relative hypovascularity formed below the conus by the combined areas of anastomoses in the cauda equina may provide an anatomic rationale for the suspected neuroishchemic manifestations concurrent with degenerative changes in the lumbar spine. Crock and associates [9], based on their studies, hold a different view: that there is no area of hypovascularity in the region of the middle third of the cauda equina. Kobayashi and colleagues [10] also examined the vasculature of the nerve roots in dogs with the aid of high-speed serial photography after injecting India ink into the aorta. Consequently, the blood flow direction of the radicular artery was ascending in the extradural nerve root and descending in the cauda equina nerve root., and there existed a so-called watershed of the blood stream in the radicular artery itself near the root of the dural sleeve. When the stream of the ascending radicular artery was intercepted by compression, however, the blood flow direction changed quickly and the blood supply was compensated by the descending radicular artery. These observations indicate that there is no relatively hypovascular region in the nerve root, which is vulnerable in the course of degenerative changes of the lumbosacral spine, although there exists a so-called watershed of the bloodstream in the radicular artery itself, the site of which is, however, changeable due to circumstances.

FEMORAL NERVE STRETCH TEST (FNST) AND STRAIGHT LEG RAISING (SLR) TEST

Nerve stretch tests, such as femoral nerve stretch test (FNST) and straight leg raising (SLR) test, are carried out to determine whether there is evidence of nerve root irritation, usually as a consequence of prolapse of a lumbar disc. The FNST is one of the most important signs with anterior thigh pain as it indicates mechanical fixation of the root, a state amenable to surgery [11-15]. Its absence is a discouragement to operation. It is generally believed that nerve root compression is caused by the hernia when the knee joint is flexed with the hip joint kept in hypertension, thus resulting in the onset of lumbar pain or anterior thigh pain. However, it remains unclear exactly how the nerve roots of the upper lumber region are affected by the FNST in vivo. And also, we have to turn our attention to that a false-positive FNST has been reported with osteoarthritis of the hip, diabetic neuropathy [16], ruptured aortic aneurysm [17], retroperitoneal hemmorrhage [18], epidural hematoma [19], anticoagulation therapy [20], and retroperitoneal tumors [21]. The FNST may compromise the nerve roots contributing to the femoral nerve.

The SLR test has been one of the most significant of clinical signs when making a clinical diagnosis of lumbar disc herniation and checking the progress of this condition since Lasègue is credited with the introduction of the SLR test in 1864 [22]. Principally described in 1881 by Forst [23], a student of Lasègue, the SLR test today is regarded as probably the most important clinical test for evaluation lumbar nerve root tension caused by disc herniation. Charnley [24] in 1951, stated that the SLR test was more important than that of all the other clinical or radiological signs put together. Currently, it is generally believed that the hernia causes nerve root compression when the legs are extended and raised, thus resulting in the onset of lumbar pain or leg pain [25,26]. However, it remains unclear exactly how the nerve roots of the lumbosacral region are affected by the FNST or SLR test in vivo. Basic

research on nerve root circulation [6-10] and nerve root compression [27-35] has shown that blood flow disturbances resulting from compression may induce nerve dysfunction, however, it is not clear if an acute reduction of blood flow may induce pain. Therefore, it is interesting to study to what extent blood flow is disturbed in the nerve roots when sciatica is experienced in lumbar disc herniation.

SUBJECTS OF INTRAOPERATIVE FNST AND SLR TEST

In this study, an intraoperative FNST or SLR test was performed, with attention given to thigh pain or sciatica experienced during the nerve stretch test. In the FNST or SLR test performed intraoperatively, we evaluated the extent of the intraradicular blood flow disturbed at various angles, which caused leg pain to develop, using a laser Doppler blood flow meter.

Patients

The subjects were 37 patients with lumbar disc herniation who underwent microdiscectomy (25 men and 12 women, aged 32.8 years on average [range, 19-63]) from October 2003 to December 2007. All of the patients initially received adequate conservative treatment such as administration of analgesics and epidural block and were checked by MRI once three months, but selected to have surgery because their sciatica was not alleviated. The details of this study were explained to the patients in obtaining their informed consent in advance. The test was performed only on those who gave consent. Regarding operated disc levels, 12 patients were operated on L3/4 disc, 15 patients were operated on L4/5 disc and 10 patients on L5/S1 disc. The mean duration of sciatic pain (ie, pain in one leg radiating into the foot) before operative treatment was 6 months (range, 3-18).

Intraoperative FNST

Before conducting the intraoperative FNST, in order not to cause excessive distraction of the nerve roots under anaesthesia, we asked the patients to lie on the operating table in the prone position before anaesthesia was induced and performed the FNST test to confirm which anterolateral thigh pain occurred (Fig.1A). All patients revealed the positive of preoperative FNST test, but there were painless restriction of SLR test. The operation was performed under general anaesthesia, and each patient was placed in the prone position with both legs extended. First, we confirmed that the nerve roots were compressed by the hernia by observation under an operating microscope, and then performed an intraoperative FNST by flexing the patient's knee joint on affected side with the hip joint kept in hypertension.

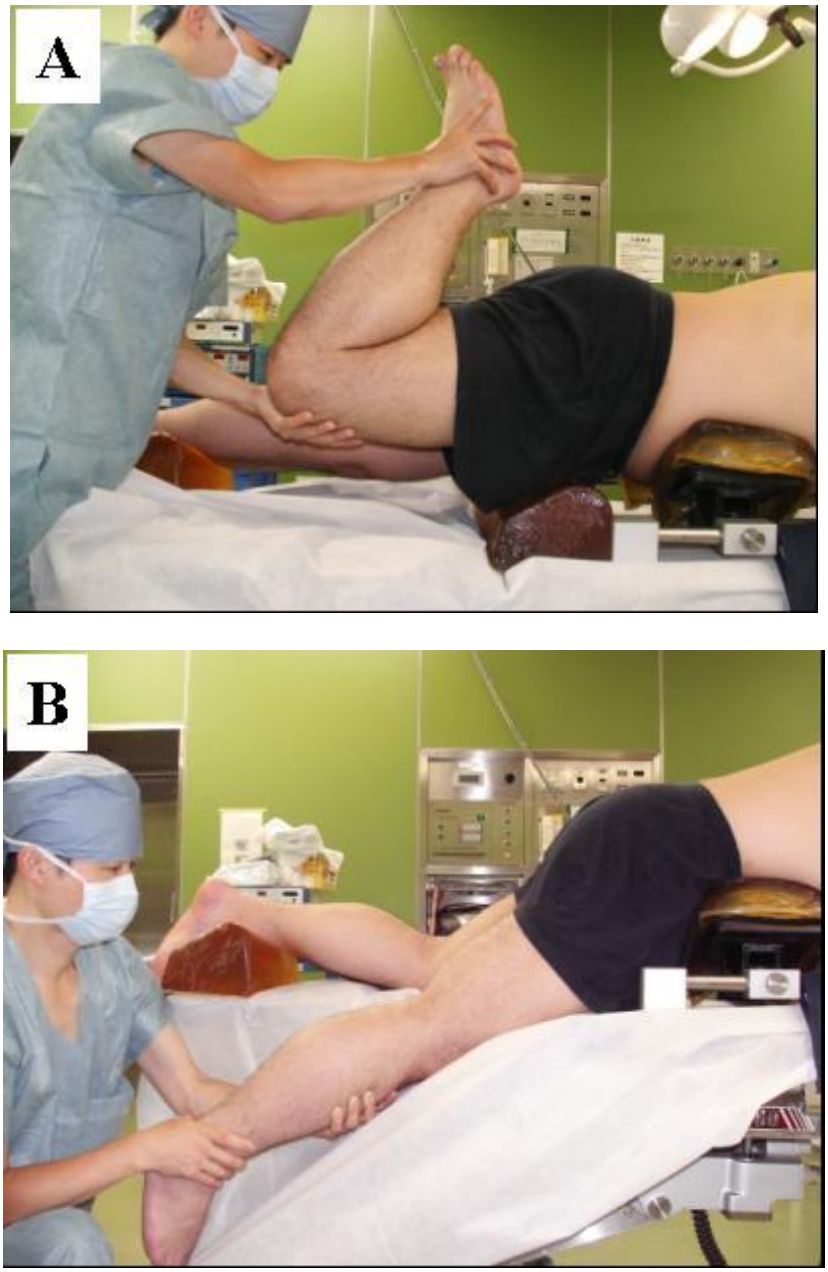

Figure 1. Intraoperative femoral nerve stretch test (FNST) [A] and straight leg raising (SLR) test [B].

Intraoperative SLR Test

Before conducting the intraoperative SLR test, in order not to cause excessive distraction of the nerve roots under anaesthesia, we asked the patients to lie on the operating table in the prone position before anaesthesia was induced and performed the SLR test to confirm the angle at which sciatica occurred (Fig.1B). We took care not to exceed this angle during the operation. The preoperative SLR test revealed that 3 patients experienced sciatica at an angle

of 10 degrees, 5 patients at 30 degrees, 2 patients at 40 degrees and 2 patients at 60 degrees. These angles were the same as those previously measured with the patients lying supine on a bed before surgery. Each patient was placed in the prone position with both legs extended under general anaesthesia. First, we confirmed that the nerve roots were compressed by the hernia by observation under an operating microscope, and then performed an intraoperative reverse SLR test by hanging down the patients' leg on the affected side from the operating table with the knees extended while the nerve roots were observed under the microscope.

Nerve Root Movement During FNST and SLR Test

There have been several reports about measurement of the gliding of nerve roots (Table 1) [24,36-45] or the pressure on the nerve roots during the SLR test [41,46,47]. Smith et al. [40] performed a detailed evaluation of the movement of the lower lumbosacral nerves during the SLR test using fresh cadavers. They confirmed that the dorsal root ganglion at L5 and S1 moved by 0.4~4.5 mm and 1~5 mm, respectively, that L5 and S1 were stretched by 2.8% and 3.4%, respectively, and that the dorsal root ganglion moved towards the pedicle of the vertebra at the SLR angle of 60 degrees. In addition, Matsuzaki et al. [41] performed the SLR test at the time of surgery for disc herniation and measured the movement of the nerve roots. They reported that the distance moved was 3.4±1.9 mm on average as the SLR angle increased from zero to 90 degrees and that the gliding distance increased to 4.6±1.1 mm on average after removal of the hernia. However, they did not mention the influence of adhesions between the nerve root and the hernia.

Table 1. Measurement of the gliding of nerve roots by FNST or SLR test.

AUTHORS	SUBJECTS	NERVE ROOT	NERVE ROOT MOTION
Inman VT, et al.[36] (1942)	Cadavers	L5,S1,2	2-7 mm
Falconer MA, et al.[37] (1948)	Fresh cadavers	L5,S1	2-6 mm
Charnley J, et al.[24] (1951)	Cadavers	L5	4-8 mm
Goddard MD, et al.[38] (1965)	Cadavers	L5 S1	3 mm 4-5 mm
Smith et al. [40] (1993)	Fresh cadavers	L5 S1	0.4-4.5 mm 1-5 mm
Matsuzaki K, et al.[41] (1998)	Intraoperate	L5 and S1	before discectomy: 3.4±1.9 mm after discectomy: 4.6±1.1 mm
Kobayashi S, et al.[42] (2003)	Intraoperate	L5 S1	before discectomy: 0.5±0.8 mm after discectomy: 3.8±0.5 mm before discectomy: 0.3±0.5 mm after discectomy: 4.1±0.4 mm
Kobayashi S, et al.[43] (2003)	Intraoperate	L4	before discectomy: 0.25±0.43 mm after discectomy: 3.75±0.5 mm
Kerry GK, et al.[44,45] (2007)	Cadavers		Parallel distal displacement
		L4	0.53±0.83 mm
		L5	0.48±0.55 mm
		S1	0.51±0.73 mm
			Perpendicular lateral displacement
		L4	0.17±0.71 mm
		L5	-0.04±0.49 mm
		S1	-0.15±0.58 mm

Kobayashi et al. [42,43] observed adhesions between the hernia and nerve root during FNST or SLR test (Fig. 2,3). Whether or not the herniated nucleus pulposus pieced the posterior longitudinal ligament, the nerve root was placed under tension from the site of the hernia to the periphery during the FNST or SLR test, with almost no gliding adjacent to the herniated mass and flattening of the nerve root by compression. However, after removal of the hernia, the nerve roots showed a clear improvement of gliding and smoother movement. Thus, it seems that tension on the nerve root caused by adhesion to the hernia resulting from the effects of chemical inflammation may be of more importance than nerve root compression by the hernia with respect to the onset of sciatica during the SLR test. Olmarker and associates [32] showed that the average minimum pressure in the inflated balloon compressing the nerve roots of the pig cauda equina required to stop the flow in the capillaries was 40 mmHg and in the venules 30 mmHg, although Pedowitz and colleagues[48] demonstrated that 2 or 4 hours of 50 mmHg compression did not produce significant changes in conduction amplitudes. Higher compression pressures induced increasing conduction deficit in the pig cauda equina, which may be related to mechanical deformation of the nerve tissue. So it is thought that impairment of intraneural circulation in the nerve roots caused by chronic compression even at these low pressures may induce intra- and extraneural fibrosis that leads to dysfunction of the nerve fibers.

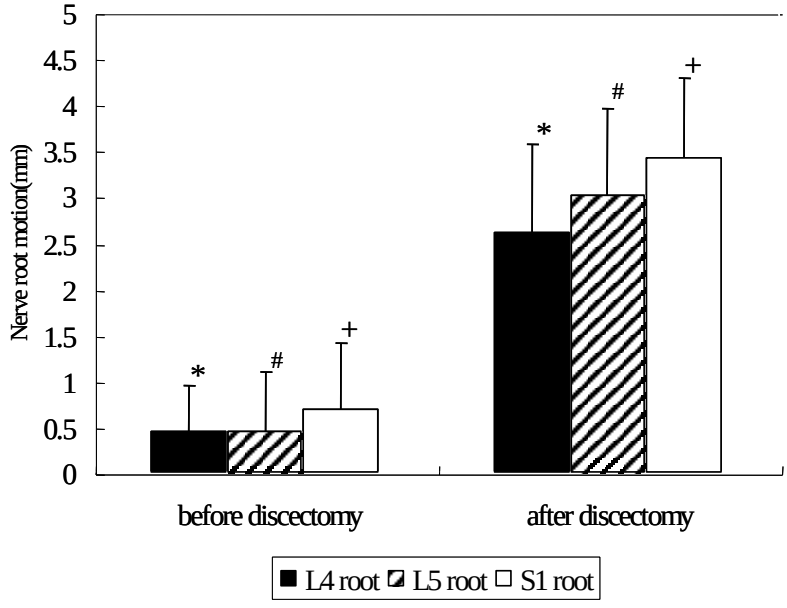

Figure 2. Changes of nerve root movement before and after discectomy.During the operation, microscopic observation revealed that a hernia mass was adhered to dura mater of the nerve roots in all the patients. The intraoperative FNST or SLR test showed that the hernia caused nerve root compression in all patients. In 19 out of 37 patients, the nerve roots showed no gliding at the angle which caused sciatica before the operation, and their movement was clearly disturbed, being only 0~1 mm at L4 (0.46 ± 0.50 mm on average), 0~2 mm at L5 (0.47 ± 0.64 mm on average) and 0~1.5 mm at S1 (0.70 ± 0.71 mm on average). The restriction of gliding caused tension extending from the hernia to the periphery of the nerve root. After removal of the hernia, the nerve roots showed smooth gliding in all patients, with the gliding distance of the L4 nerve root being 1.5~4.0 mm (3.02 ± 0.93mm) that of the L5 nerve root being 2~4.6 mm (3.02 ± 0.93mm) and that of the S1 nerve root being 2~4.6 mm (3.44 ± 0.87 mm) at an angle of 60 degrees. [*, #, +: $p<0.05$]

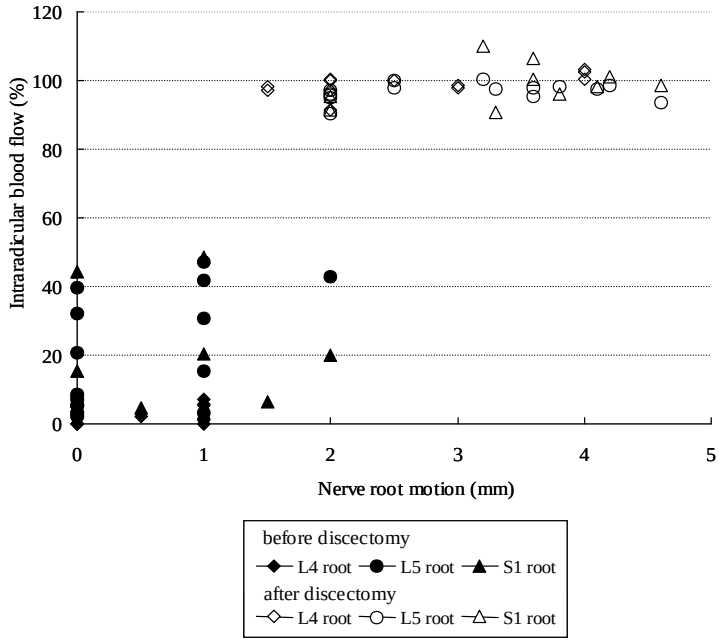

Figure 3. Changes of nerve root movement and intraradicular blood flow (IRBF) before and after discectomy. The adhesions between the henia and the nerve root reduced the mobility of the nerve root during FNST or SLR test. This lesds to severe tension or compression on the nerve root, thus causing disturbabce of intraradicular blood flow. After removal of hernia, the nerve roots showed smooth gliding and no significant decrease of intraradicular blood flow during FNST or SLR test.

That is, even when the nerve root is compressed by the hernia, absence of adhesions to the hernia will allow the nerve root to remain mobile and decrease the development of sciatica. In fact, Suzuki et al. [49] have reported a patient in whom the hernia did not disappear, even though MRI showed that the patient was cured by conservative treatment and the SLR test was negative. In addition, Bankart [50], Holmes et al. [51], Edgar et al. [52] and Thelander et al. [53] have all reported that the result of the SLR test is not related to the size of the hernia. Therefore, it is considered that radiculopathy is attributable to a complicated mechanism based on chemical inflammation, rather than to direct compression by the hernia itself.

CHANGES OF INTRARADICULAR BLOOD FLOW (IRBF) INDUCED BY INTRAOPERATIVE FNST AND SLR TEST

No previous studies have measured nerve root blood flow related to symptomatic lumbar disc herniation in humans. IRBF was measured by using a laser Doppler flow meter (LFB-III, Biomedical Sci. Co., Japan). The needle sensor of a laser Doppler flow meter (diameter: 500μm, LFN-50, Biomedical Sci. Co., Japan) was inserted into each nerve root immediately above the hernia, and the changes of IRBF was measured during an intraoperative FNST or SLR test. After removal of the hernia, a similar procedure was repeated and IRBF was measured again.

Intraoperative FNST

During the FNST, there was a sharp decrease of IRBF in L4 nerve root at which anterolateral thigh pain was experienced in all the patients, and this decrease lasted during the minute of FNST. IRBF was decreased to 8.7% and 3.2% at 20 and 50 seconds after initiating the test, respectively. When the angle of the knee was retuned to zero degrees, IRBF showed an immediate improvement and the value recovered to that obtained before the FNST. The IRBF recovered by 107.0% and 101.6% at 20 and 50 seconds after completion of the test, respectively (Fig 3,4A).

After removal of the hernia, the nerve roots showed smooth gliding in all patients. The intraoperative FNST conducted after removal of the hernia showed no significant decrease of IRBF in L4 nerve roots, and confirmed that discectomy had improved IRBF (Fig 3,4B). When the FNST was performed at 1 week after the operation, all the patients were negative and did not develop anterolateral thigh pain, unlike the results obtained preoperatively.

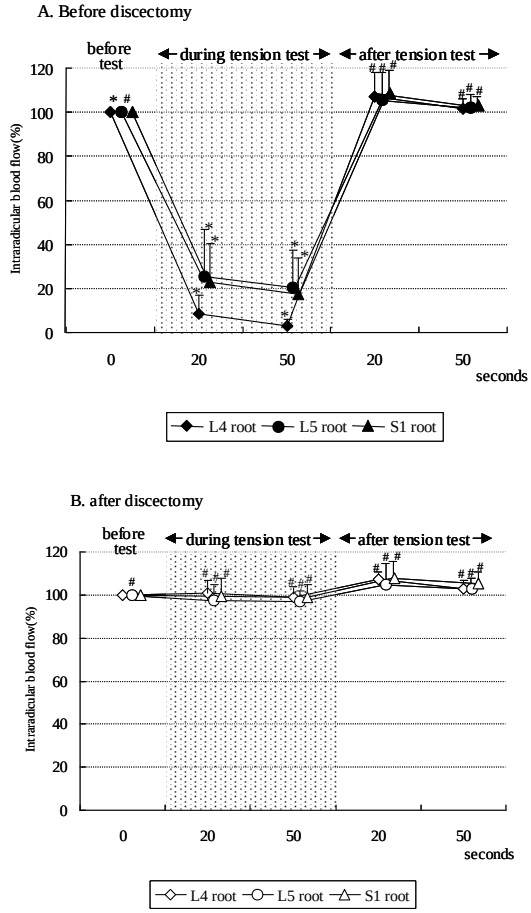

Figure 4. Changes of intraradicular blood flow (IRBF) during FNST or SLR test. A. Before discectomy: Intraradicular flow decreased to 3.16 ± 3.00 % (average ± SEM.) in the L4 nerve root, to 20.3 ± 16.9 % in the L5 nerve root and to 17.5 ± 16.5 % in the S1 nerve roots during test. B. After discectomy: The intraoperative test conducted after removal of the hernia showed no significant decrease of intraradicular blood flow. [*: $p<0.05$, #: p=n.s.]

Intraoperative SLR Test

During the reverse SLR test, there was a sharp decrease of intraradicular blood flow at the angle at which sciatica was experienced in all the patients, and this decrease lasted during the minute of SLR. Intraradicular blood flow at L5 was decreased to 25.5% and 20.0% at 20 and 50 seconds after initiating the test, respectively, while flow at S1 was decreased to 22.9% and 17.0% at 20 and 50 seconds, respectively (Fig 3,4A). When the angle of the legs was retuned to zero degrees, intraradicular blood flow showed an immediate improvement and the value recovered to that obtained before the SLR test. The intraradicular blood flow at L5 recovered by 105.4 3% and 102.0% at 20 and 50 seconds after completion of the test, respectively while S1 flow recovered by 108.4% and 103.0% at 20 and 50 seconds, respectively. These series of changes in intraradicular blood flow was confirmed to be reproducible by repeating the test 3 times consecutively.

After removal of the hernia, the nerve roots showed smooth gliding in all patients. The intraoperative reverse SLR test conducted after removal of the hernia showed no significant decrease of intraradicular blood flow in either the L5 or S1 nerve roots, and confirmed that discectomy had improved intraradicular flow (Fig 3,4B). When the SLR test was performed at 1 week after the operation, all the patients were negative and did not develop sciatica, unlike the results obtained preoperatively.

Report of a Case

A 29-year-old man had a 5-month history of sciatica. MRI shows L5/S1 disc herniation (Fig. 5A,B). During the straight-leg raising test when his leg was passively raised up to about 40 degrees, he experienced not only sudden deep pain in the leg, but also distinct paresthesiae in the S1 dermatome.

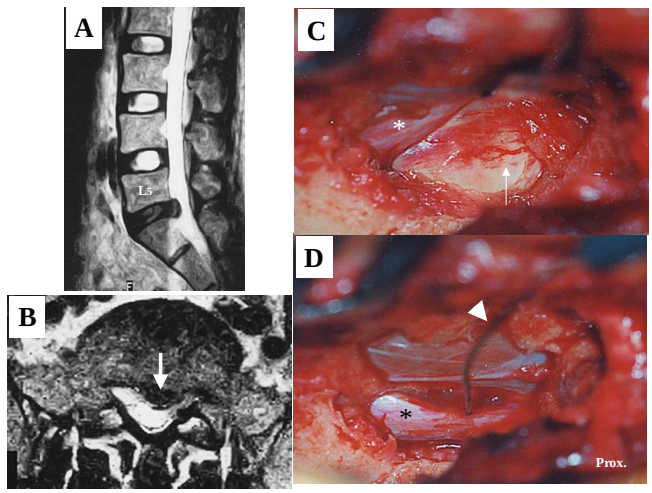

Figure 5. Disc herniation at L5/S1 disc in the reported case. A, MRI. T2-weight (2000/15, Sagittal). B, MRI. T2-weight (2000/15, Axial). ↓: Disc herniation. C, Operative findings. The type of hernia was subligamentous extrusion (Arrow). *: S1-nerve root. D, The blood flow sensor (arrow head) was inserted into S1-nerve root (*) above the hernia.

He was treated conservatively, but owing to increasing symptoms the S1 was explored using a microsurgical technique. The root sleeve had compressed by hernia and was found slightly adherent to the disc hernia. The type of hernia was subligamentous extrusion (Fig. 5C). The blood flow sensor was inserted into S1-nerve root above the hernia (Fig. 5D).

Intraradicular blood flow at S1 was decreased by 14.9% and 7.3% at 20 and 50 seconds after initiating the test, respectively (Fig. 6A). The intraoperative reverse SLR test conducted after removal of the hernia showed no significant decrease of intraradicular blood flow in the S1 nerve roots, and confirmed that discectomy had improved intraradicular flow (Fig. 6B).

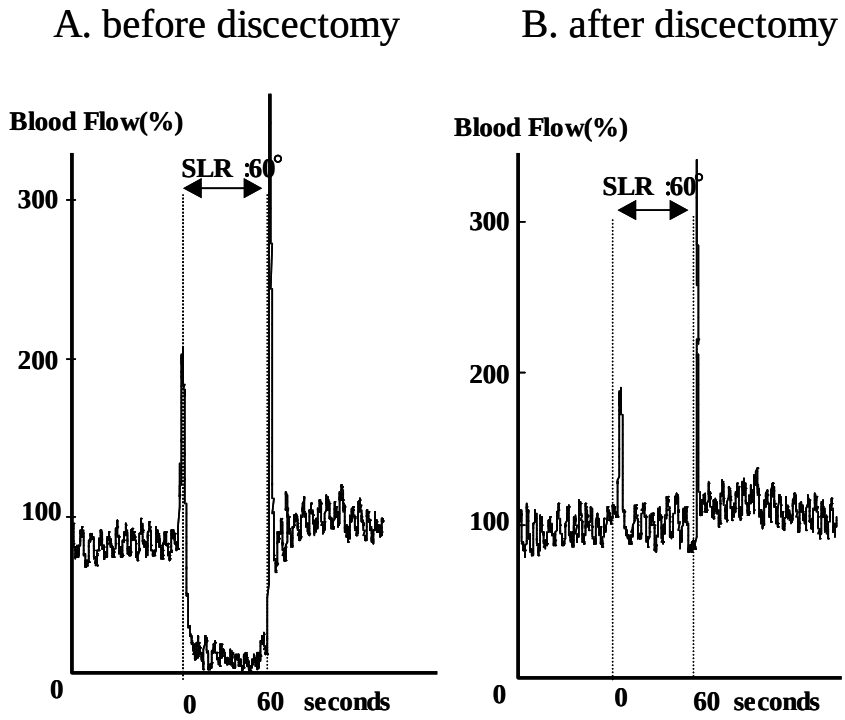

Figure 6. Changes of the intraradicular blood flow during intraoperative- SLR test in the reported case.During the test, intraradicular blood flow showed a sharp decrease at the angle that produced sciatica, which lasted for one minute (A). At one minute after completing the test, intraradicular blood flow returned to the value obtained at baseline (B).

NERVE ROOT PRESSURE DURING NERVE STRETCH TEST

We think that the primary cause of radicular pain after herniation of a lumbar intervertebral disc will be mechanical force of the nerve root induced by adhesion between hernia mass and nerve root. However, it is very difficult to distinguish acute from chronic compression to the nerve root as the cause of radicular pain. The nerve roots of the lumbosacral spine always move with movement of the lower extremities, and the dynamic limit is dependent on the positional relationship between the nerve root and the surrounding tissues. Thus, when narrowing of the spinal canal and the nerve root canal is caused by herniation of an intervertebral disc, movement of the nerve root along with movement of the

lower extremities becomes limited, and consequently compression and traction on the nerve root cause radicular pain. Chronic compression is the result of repeated episodes of mild acute compression. According to our clinical experience, radicular pain is frequently alleviated by rest in patients with lumbar disc herniation and is intensified by application of acute compression to the nerve root during walking or the SLR test. Takahashi et al. [54] intraoperatively measured the pressure applied by the herniated mass to the nerve root in 34 patients with lumbar disc herniation and reported that the pressure was 7~255 mmHg (mean 53 mmHg). Take et al. paid attention to the SLR test for diagnosis of lumbar disc herniation, and measured the pressure applied by the herniated mass to the nerve root [55]. When SLR was 0, 30, 60, and 90 degrees, the pressure was 9~60 mmHg (mean 32 mmHg), 20~235 mmHg (111 mmHg), 65~367 mmHg (186 mmHg), and 130~600 mmHg (294 mmHg), respectively, so the pressure increased with an increasing angle. They concluded that compression by the hernia was responsible for radiculopathy because an increase of the SLR angle caused an increase of pressure on the nerve root. However, they did not evaluate adhesions between the hernia and the nerve root.

PATHOPHYSIOLOGY OF NERVE ROOT COMPRESSION DURING NERVE STRETCH TEST

We previously performed an experiment in which the nerve roots of dogs were compressed with a clip and the action potential was measured at 1 cm proximal to the site of compression [56]. Compression at 60 gram force (about 942mmHg) for 1 hour caused a reduction of the amplitude by about 80% and the amplitude did not show improvement at 1 hour after the release of compression. In contrast, compression at 30-gram force (about 471 mmHg) caused a transient increase of the amplitude immediately after the onset of compression, although the amplitude was decreased by about 30% after 60 minutes. The amplitude then returned to the baseline by 1 hour after the release of compression. We considered that the changes of amplitude at 60-gram force reflected severe damage (neurotomesis and axonotomesis) to the nerve root, while 30 gram force of compression mainly caused changes due to disturbed blood flow or neuropraxia. Matsuzaki et al. measured the nerve root action potential during the intraoperative SLR test in patients with disc herniation [41]. In 2 out of 5 patients, the action potential could be obtained at an SLR angle of zero degrees before removal of the hernia, but disappeared when the SLR angle was set at 90 degrees for 5 minutes. Then the action potential returned to the baseline value after 10 minutes when the angle was decreased to 30 degrees. These changes probably resulted from disturbed blood flow in the nerve roots since the same manoeuvres did not alter the potential after removal of the hernia. In our study, it was difficult to make a clear differential diagnosis of whether ectopic discharge in the nerve roots, which were deeply related with development of pain, was attributable to direct injury of nerve fibers due to mechanical compression or extraction caused by hernia mass, or was responsible for the results of disturbed blood flow. It seems that the nerve roots become hypoxic when pain is experienced during the SLR test (or in the case of neuropraxia) since there was a relatively sharp decrease of intraradicular blood flow observed when we conducted the SLR test and Matsuzaki et al. obtained similar results by their electrophysiological study [41]. Because compression of normal nerves does not

cause pain, it is highly likely that repeated mechanical stress on the nerve roots during walking may lead to development of intraradicular inflammatory reaction, such as edema [29,32,57,58], demyelination and/or invasion of macrophage [59,60], in patients with disc herniation. Accordingly, when inflammation occurs in a nerve root, mechanical stress on the root during the SLR test can lead to ischemia and hypoxia, thus inducing ectopic firing [61,62] and giving rise to sciatica (Fig. 7).

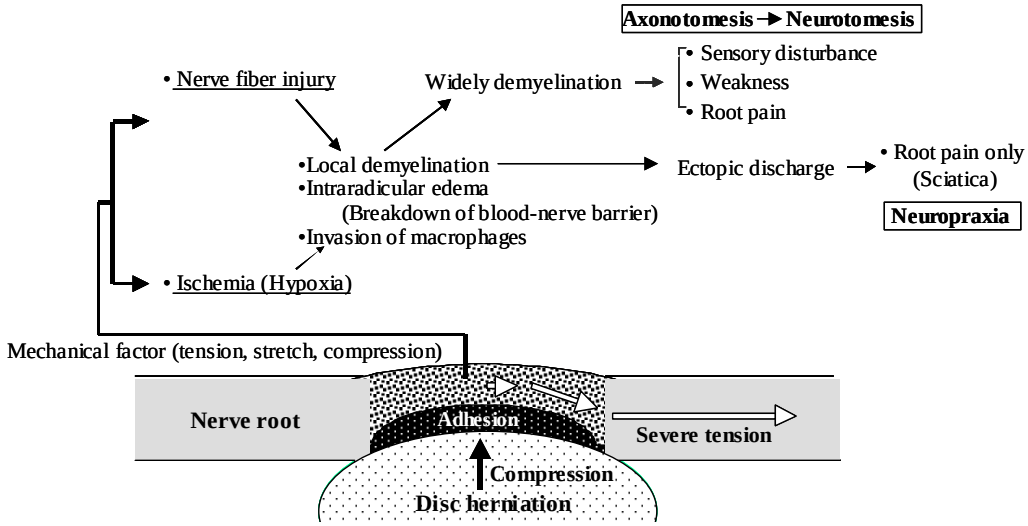

Figure 7. Pathomechanisms of nerve root dysfunction induced by compression of hernia.

Olmarker et al. [63] reported that chemical factors from the nucleus pulposus could cause demyelination of porcine nerve roots. They also reported that cytokines such as TNF in the hernia tissue were radiculopathy-causing factors [64]. However, they did not mention how adhesions between the nucleus pulposus and nerve root could influence the nerve root during walking. The present study suggested that the inflammatory reaction around the hernia after disc herniation may be different in mechanism from the inflammatory reaction associated with demyelination of nerve roots since the SLR test performed after removal of the hernia showed no disturbance of intraradicular blood flow and none of the patients had sciatica after the operation. It is suggested that the epidural inflammatory reaction may cause adhesions between the lesion and the nerve root, which then may reduce the mobility of the nerve root during movement of the legs. This led to severe tension or compression on the nerve root [36,38,43,65], thus causing disturbance of intraradicular blood flow and breakdown of the blood-nerve barrier, resulting in intraradicular inflammatory changes such as edema [29,32,57,58] and demyelination [58,59]. On the other hand, it is apparent from the natural history of lumbar disc herniation that an inflammatory reaction around the hernia is essential for spontaneous involution of the mass [66-72], although it produces adhesions between the hernia and the nerve roots. Therefore, control of the inflammatory reaction is an important challenge when treating patients with disc herniation.

REFERENCES

[1] Mixter WJ, Barr JS. (1934) *Rupture of the intervertebral disk with involvement of the spinal canal*. N Engl J Med 211:210-215.

[2] Boden S, Davis DO, Dina TS, et al. (1990) *Abnormal magnetic resonance scans of the lumbar spine in asymptomatic subjects.* J Bone Joint Surg[Am] 72:403-408.

[3] Edgar MA, Park WM. (1974) *Induced pain patterns on passive straight leg raising in lower lumber disc protrusion: A prospective clinical, myelographic and operative study in fifty patients*. J Bone Joint Surg 56A:658-667.

[4] Hiselberger WE, Witten RM. (1968) *Abnormal myelograms in asymptomatic patients*. J Neurosurg 28:204-206.

[5] Wisel SW, Tsourmas N, Feffer HL, et al. (1984) *A study of computer-assisted tomography: The incidence of positive CAT scans in an asymptomatic group of patients.* Spine 9:549-551.

[6] Corbin JL. (1961) *Anatome et pathologie arterielles de la molle*. Masson et Cie, Paris.

[7] Crock HV, Yoshizawa H. (1977) *The blood supply of the vertebral column and spinal cord in man.* Springer-Verlag, New York.

[8] Parke WW, Gammell K, Rothman RH. (1981) *Arterial vascularization of the cauda equina.* J Bone Joint Surg 63A:53-62.

[9] Crock HV, Yamagishi M, Crock MC.(1986) *The conus medullaris and cauda equina in man.* New York, Wien: Springer Verlag.

[10] Kobayashi S, Yoshizawa H, Nakai S. (2000) *Experimantal study on the dynamics of lumbosacral nerve root circulation*. Spine 25: 298-305.

[11] Asquier C, Troussier B, Chirossel JP, et al. (1996) Femoral neuralgia due to degenerative spinal disease. *A retrospective clinical and radio-anatomical study of one hundred cases.* Rev Rhum Engl Ed. 63:278-284.

[12] Estridge MN, Rouhe SA, Johnson NG. (1982) *The femoral stretching test. A valuable sign in diagnosing upper lumbar disc herniations.* J Neurosurg 57: 813-817.

[13] Kreitz BG, Cote P, Yong-Hing K. (1996) *Crossed femoral stretching test.* A case report. Spine 21: 1584-1586.

[14] McCombe PF, Fairbank JCT, Cockersole BC, et al. (1989) *Reproducibility of physical signs in low-back pain.* Spine 14: 908-918.

[15] Postacchini F, Cinotti G, Gumina S. (1993) *The knee flexion test: a new test for lumbosacral root tension*. J Bone Joint Surg 75B: 834-835.

[16] Calverly JR, Mulder DW. (1960) *Femoral neuropathy*. Neurology 10: 963-967.

[17] Razzuk MA, Linton RR, Darling RC. (1967) *Femoral neuropathy secondary to ruptured abdominal aortic aneurysms with false aneurysms*. JAMA 201: 817-820.

[18] Seddons HJ. (1930) *Haemophilia as a cause of lesions in the nervous system*. Brain 53: 306-310.

[19] Kingery WS, Seibel M, Date ES, et al. (1994) *The natural resolution of a lumbar spontaneous epidural hematoma and associated radiculopathy*. Spine 19: 67-9, 1994.

[20] McCombe PF, Fairbank JCT, Cockersole BC, et al. (1989) *Reproducibility of physical signs in low-back pain*. Spine 14: 908-918.

[21] Woltman HW. (1944) *Neuritis and other disease or injures of peripheral nerves*, in Tice F (ed): Practice of Medicine. Hagerstown, Md: WF Prior, Vol 9, pp 355-356.

[22] Lasègue EC. (1864) *Consideration su la sciatique*. Arch Gen de Med 6: 558-580.
[23] Forst JJ.(1881) *Contribution a L'Etude Clinique de la Sciatique*, Paris. These No.33.
[24] Charnley J. (1951) *Orthopaedic sign in the diagnosis of disc protrusion. With special reference to the straight-leg-raising test.* Lancet 27:186-192.
[25] Jönsson B, Strömqvist B. (1995) *The straight leg raising test and the severity of symptoms in lumbar disc herniation. A preoperative and postoperative evaluation*. Spine 20:27-30.
[26] Shiqing BX, Quanzhi Z, Dehao AF, et al. (1987) *Significance of the straight leg raising test in the diagnosis and clinical evaluation of lower lumbar intervertebral disc protrusion*. J Bone Joint Surg 69A:517-522.
[27] Gelfan S, Tarlov IM. (1956) *Physiology of spinal cord, nerve root, and peripheral nerve compression*. Am J Physiol 185:217-229.
[28] Delamarter RB, Bohlman HH, Dodge LD et al. (1990) *Experimental lumbar spinal stenosis: Analysis of the cortical evoked potential, microvasculature, and histopathology,* J Bone Joint Surg 72A:110-120.
[29] Kobayashi S, Yoshizawa H, Hachiya Y, et al. (1993) *Volvo Award Winner in Basic Science Studies; Vasogenic edema induced by compression injury to the spinal nerve root: Distribution of intravenously injected protein tracers and gadolinium-enhanced magnetic resonance imaging,* Spine 18:1410-1424.
[30] Iwamoto H, Kuwahara H, Matsuda H, et al. (1995) *Production of chronic compression of cauda equina in rats for use in studies of lumbar spinal canal stenosis.* Spine 20:2750-2757.
[31] Kobayashi S, Yoshizawa H. (2002) *Effect of mechanical compression on the vascular permeability of the dorsal root ganglion.* J Orthop Res 20: 730-739.
[32] Olmarker K, Rydevik B, Holm S, et al. (1989) *Effects of experimental graded compression on blood flow in spinal nerve roots: A vital microscopic study on the porcine cauda equina.* J Orthop Res 7:817-823.
[33] Yoshizawa H, Kobayashi S, Kubota K. (1989*) Effect of compression on intraradicular blood flow in dogs.* Spine 14:1220-1225.
[34] Yoshizawa H, Kobayashi S, Hachiya Y. (1991) *Blood supply of nerve roots and dorsal root ganglia*. Orthop Clin North Am 22:195-211.
[35] Yoshizawa H, Kobayashi S, Morita T. (1995) *Chronic nerve root compression: Pathophysiologic mechanism of nerve root dysfunction*. Spine 20:397-407.
[36] Inman VT, Saunders JB. (1942) *The clinico-anatomical aspects of the lumbosacral region.* Radiology 38:669-678.
[37] Falconer MA, Mcgeorge M, Begg AC. (1948) *Observations of the cause and mechanism of symptom-production in sciatica and low-back pain*. J Neurol Neurosurg Psychiatr 11:13-26.
[38] Goddard MD. (1965) *Movements induced by straight leg raising in the lumbo-sacral roots, nerve and plexus, and in the intrapelvic section of the sciatic nerve*. J Neurol Neurosurg Psychiatr 28:12-18.
[39] Graham GE. (1981) *Intraoperative straight-leg raising during laminectomy and disk excision for sciatica.* Clin Orthop 154:343-344.
[40] Smith SA, Massie JB, Chesnut R, et al. (1993) *Straight leg raising. Anatomical effects on the spinal nerve root without and with fusion.* Spine 18:992-999.

[41] Matuzaki K, Tokuhashi T, Wakabayashi K, et al. (1998) *A study on straight leg raising test in lumbar herniated intervertebral disc.* J Jpn Orthop Assoc 72:S327.(Jpn)

[42] Kobayashi S, Shizu N, Suzuki Y, et al. (2003) *Changes of nerve root motion and intraradicular blood flow during an intraoperative straight-leg-raising test*. Spine 28: 1427-1434.

[43] Kobayashi S, Suzuki Y, Asai T, et al. (2003) *Changes of nerve root motion and intraradicular blood flow during an intraoperative femoral nerve stretch test*. Report of four cases. J Neurosurg 99: 298-305.

[44] Kerry GK, Jean-Michel B, Dwayne CL, et al. (2007) *Lumbosacral nerve root displacement and strain: Part 1. A novel measurement technique during straight leg raise in unembalmed cadavers.* Spine 32: 1513-1520.

[45] Kerry GK, Jean-Michel B, Dwayne CL, et al. (2007) *Lumbosacral nerve root displacement and strain: Part 2. A comparison of 2 straight leg raise conditions in unembalmed cadavers.* Spine 32: 1521-1525.

[46] Breig A, Troup JD.(1979) *Biomechanical considerations in the straight leg raising test. Cadaveric and clinical studies of the effects of medial hip rotation*. Spine 4: 242-250.

[47] Spencer DL. (1999) *The anatomical basis of sciatica secondary to herniated lumbar disc:* A review. Neurol Res 21:S33-36.

[48] Pedowitz RA, Garfin SR, Massie JB, et al. (1992) *Effects of magnitude and duration of compression on spinal nerve root conduction.* Spine17:194-199.

[49] Suzuki S, Urushidani H, Kikkaw K, et al. (1986) *Study on negative cases of Lasegue test about lumbar disc herniation.* Seikeigeka 37:313-317. (Jpn)

[50] Bankart AS. (1943) *Spinal arthritis and sciatica*. Proc R Soc Med 36:329-330.

[51] Holmes JM and Sworn BR. *Sciatic neuritis*. Br Med J 1945;2: 3501.

[52] Edgar MA, Park WM. (1974) *Induced pain patterns on passive straight leg raising in lower lumber disc protrusion: A prospective clinical, myelographic and operative study in fifty patients.* J Bone Joint Surg 56A:658-667.

[53] Thelander U, Fagerlund M, Friberg S, et al. (1992) *Straight leg raising test versus radiologic size, shape, and position of lumbar disc hernias.* Spine 17: 395-398.

[54] Takahashi K, Shima I, Porter RW. (1999) *Nerve root pressure in lumbar disc herniation,* Spine 24:2003-2006.

[55] Take O, Matuzaki H, Hoshino M, et al. (1989) *Pathology and pressure of the nerve root at the herniated site during straight leg raising test ; Lumbar disc herniation*. J Jpn Orthop Assoc 63: S1008. (Jpn)

[56] Kobayashi S, Yoshizawa H, Nakai S. (1999) *Neurophysiological changes of the nerve root induced by mechanical compression. In: H. Takahashi H, eds. Mechanical Loading of Bones and Joints.* Tokyo: Springer-Verlag, pp245-258.

[57] Olmarker K, Rydevik B, Holm S. (1989) *Edema formation in spinal nerve roots induced by experimental, graded compression. An experimental study on the pig cauda equina with special reference to differences in effects between rapid and slow onset of compression.* Spine 14:569-573.

[58] Kobayashi S, Uchida K, Takeno K, et al. (2006) *Imaging of the cauda equina edema in lumbar canal stenosis using Gadolinium-enhanced MR imaging*: *Experimental constriction injury*. Am J Neuroradiol 27: 346-353.

[59] Kobayashi S, Yoshizawa H, Yamada S. (2004) *Pathology of lumbar nerve root compression. Part 1: Intraradicular inflammatory changes induced by mechanical compression.* J Orthop Res 22: 170-179.

[60] Kobayashi S, Baba H, Uchida K, et al. (2005) *Effect of mechanical compression on the lumbar nerve root: Localization and changes of intraradicular inflammatory cytokines, nitric oxide, and cyclooxygenase*. Spine 30:1699-1705.

[61] Howe JF, Loeser JD, Calvin WH. (1977) *Mechanosensitivity of dorsal root ganglia and chronically injured axons: A physiological basis for the radicular pain of nerve root compression.* Pain 3:25-41.

[62] Nordin M, Nyström B, Wallin U, et al. (1984) *Ectopic sensory discharges and parasthesiae in patients with disorders of peripheral nerves, dorsal roots and dorsal columns*, Pain 20:231-245.

[63] Olmarker K, Rydevik B, Nordborg C. (1993) *Antologous nucleus pulposus induce neurophysiologic and histologic changes in porcine cauda equina nerve roots.* Spine 18:1425-1432.

[64] Olmarker K, Myers RR. (1998) *Pathogenesis of sciatic pain: role of herniated nucleus pulposus and deformation of spinal nerve root and dorsal root ganglion*. Pain 78: 99-105.

[65] O'Connell J.E. (1943) *Sciatica and the mechanism of the production of the clinical syndrome in protrusions of the lumbar intevertebral discs*. Br Jr Surg 30: 315-327.

[66] Lindblom L, Hultqvist G. (1950) *Absorption of protruded disc tissue*. J Bone Joint Surg 32A: 557-560.

[67] Teplick JG, Haskin ME. (1985) *Spontaneous regression of herniated nucleus pulposus*. AJNR 6:331-335.

[68] McCarron RF, Wimpee MW, Hudkins PG, et al. (1987) *The inflammatory effect of nucleus pulposus; A possible element in the pathogenesis of low-back pain*. Spine 12:760-764.

[69] Hirabayashi S, Kumano K, Tsuiki T, et al. (1990) *A dorsally displaced free fragment of lumbar disc herniation and its interesting histologic findings : a case report*. Spine 15: 1231-1233.

[70] Maigne JY, Rime B, Delignet B. (1992) *Computed tomographic follow-up study of forty-eight cases of nonoperatively treated lumbar intervertebral disc herniation*, Spine 17:1071-1074.

[71] Komori H, Shinomiya K, Nakai O, et al. (1996) *The natural history of herniated nucleus pulposus with radiculopathy.* Spine 21: 225-229.

[72] Kobayashi S. (2000) *Mechanisms of spontaneous regression of lumbar disc herniation: Preliminary report of MRI and pathological findings*, In: Brock M, Schwarz W, Wille C eds. First Interdisciplinary World Congress on Spinal Surgery and Related Disciplines. Monduzzi Editore, Appendix, pp25-30.

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Chapter 11

HEREDITARY CHOROIDAL DYSTROPHIES

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ABSTRACT

This chapter involves a survey on the morphological and functional characteristics of hereditary choroidal dystrophies. These belong among a heterogenous group of diseases in which the common feature is pigment epithelium damage and atrophy (loss) of the choriocapillaries, which results in progressive involvement of the photoreceptor layer. There are three characteristic forms of the disease: gyrate atrophy, choroideremia and geographical choroidal dystrophy. The latter can be divided into three subentitites: the central areolar, the peripapillary and the generalized forms. The chapter puts a special emphasis on generalized choroidal dystrophy that is regarded as a rare disease. We describe our experiences with the follow-up of 15 patients including a family of seven members. The most important conclusion of our study is that electrophysiological examination methods including multifocal ERG greatly facilitate the diagnosis and care of this disease that seems to be much more frequent than is widely believed.

INTRODUCTION

The hereditary choroidal dystrophies belong among a heterogenous group of diseases in which the common feature is pigment epithelium damage and atrophy (loss) of the choriocapillaries, which results in progressive involvement of the photoreceptor layer.

Later, the choroidal vessels become fully sclerotized and the white color of the pure sclera shines through the residual sclerotic choroidal vessels. There are three characteristic forms of the disease: gyrate atrophy, choroideremia and geographical choroidal dystrophy. The latter can be divided into three subentitites: the central areolar, the peripapillary and the generalized forms.

Gyrate atrophy, choroideremia and the central areolar form of geographical choroidal dystrophy are rare.They furnish characteristic ophthalmoscopic pictures when they are in the developed stage, and their recognition therefore poses no difficulty.

There are generally no differential diagnostic problems, though in some patients the visual symptoms may be present before the appearance of the ophthalmoscopic alterations, and in other cases only a careful ophthalmological examination, performed because of a positive family history, can indicate the disease before the visual symptoms are manifested.

In contrast, the generalized form of choroidal dystrophy poses a real differential diagnostic problem throughout, from the early period up to the terminal stage. This chapter will lay particular emphasis on this form of the disease.

Our interest in this topic was initiated by the fact that 4 members of a family of 7 who were referred to our Department were found to be affected by this disease. We were able to follow the manifestation and progression of the disease for more than a decade, during which time we detected another 8 individual cases with the same diagnosis.

To the best of our knowledge this total of 15 patients is the largest group reported to date. In this description of our experience, we survey the symptomatology and diagnostic problems of these dystrophies and related diseases. From this aspect, the introduction of modern electrophysiological techniques meeting the standards of the International Society for Clinical Electrophysiology of Vision provided great help. We have made use of all the standard methods of electroretinography (rod ERG, cone ERG, maximal ERG (maxERG), oscillatory potentials (OPs), flicker ERG, multifocal ERG (mfERG) and pattern ERG (PERG), together with visual evoked potentials (VEPs).

I. Gyrate Atrophy of the Choroid and Retina

Gyrate atrophy, a rare choroidal dystrophy, was identified in 1896 by Fusch [1].

Several publications have subsequently appeared concerning the progression and the biochemical alterations in this disease (see the review paper by Francois) [2]. It may be congenital, although the youngest patient reported by Francois [3] was already 6 years old.

The diagnosis is based predominantly on the family history and on the characteristic ophthalmoscopic picture.

Inheritance and Pathology

It is usually inherited as an autosomal recessive disorder. The biochemical abnormalities in this disease, described by Simell and Takki in 1973 [4], include a 10-to-20- fold elevation in the plasma ornithine level, hypolysinemia, hyperornithinuria and either the absence or a marked reduction in ornithine alfa-aminotransferase activity in the cultured skin fibroblasts and lymphocytes. Some patients display a lowering of their plasma ornithine levels after vitamin B6 treatment, while others respond to a low-protein, low-organic diet [5].

Symptoms

In this disease the symptoms generally appear between 20 and 30 years of age, though visual complaints may arise much earlier. In addition to night blindness, the patients experience a progressive visual impairment in the peripheral field. In the final stage, the central vision is also decreased. Myopia is invariably present. The progression of the disease is generally very slow.

Ophthalmoscopic Alterations

The abnormality begins in the peripheral or mid-peripheral fundus. The affected area exhibits multiple, initially discrete, irregular, yellowish patches (Fig. 1). The lesions of the pigment epithelium and choriocapillaries gradually increase in number, enlarge and become confluent. Later, the progressive atrophy involves the larger choroidal vessels, too. The affected areas are separated from the normal-appearing retina by scalloped borders, which begin as isolated islands and then merge to assume the form of a garland. Pigment clumps are often seen in and around the atrophic patches. The optic disc may retain its normal color or becomes atrophic and waxy-like, as in pigmentary retinopathy. Likewise, in some patients the retinal vessels are at times normal, but more often attenuated. As the disease progresses, total atrophy of the pigment epithelium and choriocapillaries develops. Eventually, of the entire choroid disappears, exposing the white sclera.

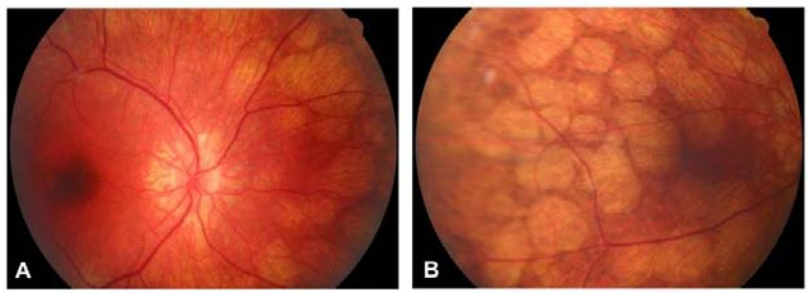

Figure 1. Ophthalmoscopic picture of a patient with gyrate atrophy on the central (left) and peripheral (right) part of the retina of the right eye.

The central vision is usually spared or becomes impaired later, because of the involvement of the macula by the disease progress or by cystoid macular edema.

Fluorescein Angiography (FLA)

FLA reveals a loss of the choriocapillaries in the affected areas, with a sharp border between the normal and the abnormal-appearing retina.

Electrophysiological Alterations

In the earliest stage normal ERGs may be detected, but later the amplitudes of the cone and rod ERGs become subnormal, markedly reduced or nondetectable. We are not aware of any publication on mf ERG in this disease.

In one of our patients, a central peak was detected and the response amplitudes of the mf ERG decreased toward the periphery. This alteration resembled that seen in retinitis pigmentosa.

Differential Diagnosis

It should be distinguished from pavestone degeneration, choroideremia, high myopia and thioridasine retinopathy.

It is noteworthy that gyrate atrophy may be found in families with pigmentary retinopathy [6, 7, 8, 9] or with retinitis pigmentosa albescens [10]. According to Franceschetti and Dieterle [10], the two diseases may even coexist in the same eye.

II. CHOROIDEREMIA

The condition was first described by Mauthner in 1871 [11]. Goedbloed [12] noted "salt and pepper" fundal changes in the mother and one sister of a patient, which suggested the carrier properties.

The youngest case reported was a boy aged 22 months, who displayed definite fundal changes in the form of pigmentation [13, 14]. The youngest carrier detected was a girl of 4.5 months with peripheral pigmentary changes [14].

Inheritance

This X-linked recessive chorioretinal dystrophy manifests itself in the first or second decade. All daughters of the patients are carriers. Half of the male offspring of the female carriers develop the disease, and 50% of the female offspring are carriers.

The carriers of X-linked choroideremia are generally asymptomatic. They often exhibit patchy depigmentation and clumping of the retinal pigment epithelium in the midperiphery, and mild pigmentary changes in the macular area [15].

The genetic background of the disease is a DNA fragment polymorphism (DXYS1), located on the long arm of the X chromosome at the locus Xq 13-21 [16, 17].

Symptoms

The major complaint of the patients is poor night vision, which appears usually in the first decade. The condition slowly progresses both anatomically and functionally. A relatively good central vision is preserved until late in the disease. The peripheral fields are generally moderately depressed even in the early stage, with severe peripheral field restriction occurring in the fifth-sixth decades of life.

Ophthalmoscopic Alterations

The initial fundus appearance is "salt and pepper" pigment mottling in the far periphery and the posterior pole of the retina. Later, the underlying choroid may appear patchy because of the loss of the choriocapillaries and choroidal vessels and the atrophy of the pigment epithelium.The alterations become larger, and more numerous, and progress centrally. The macula is affected last. In the final stage, the entire fundus displays a diffuse yellowish-white reflex of the underlying sclera. At this stage the fundus may resemble gyrate atrophy. In contrast with primary retinal dystrophies, the optic disc and retinal blood vessels remain relatively normal.

The fundus of carrier females may show a "moth-eaten" appearance of the pigment epithelium, or the "salt and pepper" type of pigmentary alterations.

Fluorescein Angiography

Besides a normal retinal vasculature, the FLA may show the patchy loss of choriocapillaries and choridal vessels in the early stage, when the choroid appears ophthalmoscopically normal. There is hypofluorescence in the center of the macula, with surrounding diffuse hyperfluorescence corresponding to the atrophic areas. Some choroidal vessels supplying the macula remain relatively intact, which explains the persistence of central vision until the final stage.

Electrophysiological Alterations

Even in the initial stages of the disease, the ERG and EOG can be markedly subnormal. The rod ERG usually becomes nonrecordable and the cone ERG is severely reduced during progression [18]. In an examination of mf ERG in choroidemia carriers, Vajarand found a mosaic pattern of retinal dysfunction even in those who displayed a normal-appearing macula and good visual acuity [19].

Differential Diagnosis

The possibilities of retinitis pigmentosa (RP), gyrate atrophy, albinism and thioridasine retinopathy must be considered from the aspect of differential diagnostics.

In retinitis pigmentosa, however, there are severely attenuated retinal arterioles which distinguishes it from choroideremia. Gyrate atrophy is inherited in an autosomal recessive and not an X-linked recessive manner. The ophthalmoscopic alterations of the female carriers in choroideremia are rather characteristic, which can facilitate the diagnosis. In albinism, nystagmus is generally present because of the macular hypoplasia. Thioridasine (Mellaril) is administered for the treatment of psychosis. The history of this medication can promote the diagnosis.

Although gyrate atrophy and choroideremia are rare diseases, they are well described in numerous publications. They can be recognized from the family history (mode of inheritance), the early manifestation of the symptoms, and the characteristic fundus picture in the developed stage, not only in the affected males, but in the carrier females, too. The ophthalmoscopic alterations spreading toward the central retina during progression result in a visual field constriction; the central vision is preserved until late in the disease. This is in contrast with generalized choroidal dystrophy, which is generally manifested at the central retina and progresses toward the periphery.

III. GEOGRAPHIC CHOROIDAL DYSTROPHIES

III.1. Central Areolar Choroidal Dystrophy

In 1884, the name central senile areolar choroidal atrophy was applied by Nettleship to describe a bilateral, relatively sharply circumscribed, more or less regular oval area in the

central retina, extending from the disc to well beyond the macula, with varying stages of atrophy of the choroidal vessels, which led to a grave impairment of vision [20].

Since then, the histological and genetical bases, the inheritance, and the progression of this disease have been widely discussed [21, 22, 23, 24, 25, 26, 27].

Inheritance

An autosomal dominant inheritance is widely accepted. The locus of the affected gene for central areolar choroidal dystrophy on chromosome 17p [28], and the cis Arg-142 trp mutation in the peripherin/RDS gene were determined by Hoyng [29]. The variable expressivity of a family with central areolar pigment epithelium dystrophy was discussed by Keithahn [30].

Symptoms

The first symptoms are manifested in the third or fourth decade of life, with progressive visual loss. However, ophthalmoscopically visible lesions may be present for some years without symptoms.

Ophthalmoscopic Alterations

The alterations affect the posterior pole of the retina, with a definite border between the involved and the normal retina (Fig. 2). The early lesion consists of a nonspecific area of granular hyperpigmentation at the macula with an exudative reaction around it. This stage reveals nothing pathognomic, and might well be mistaken for macular dystrophy or a central chorioretinal lesion. After several years, a pathognomic zone of definite circumscript para-foveal pigmentary changes develops. Later, patches of atrophy develop in the macular area, affecting the retina, the retinal pigment epithelium and the choriocapillaries, with enhanced visibility of the choroidal vessels. The involvement of the fovea results in blindness in middle or old age.

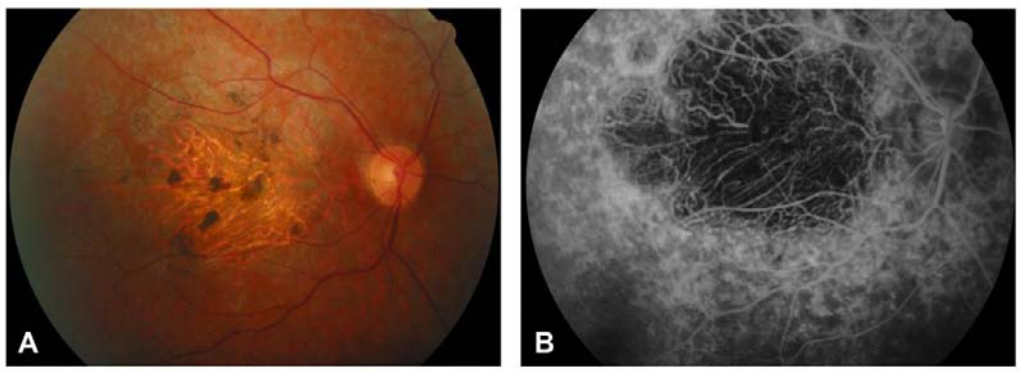

Figure 2. Ophthalmoscopic picture (left) and FLA photo (right) of a patient of ours with central areolar choroidal dystrophy. Granular hyperpigmentation and choroidal vessels of enhanced visibility can be seen in the macular area of the right eye. FLA reveals the loss of pigment epithelium and choriocapillaries and increased visibility of moderately enlarged choroidal vessels in the macular area, accompanied by a hyperfluorescent border outlining the atrophic lesion.

Fluorescein Angiography

The characteristic FLA alterations, loss of the choriocapillaries, increased visibility of the intermediate and large choroidal vessels and a hyperfluorescent border outlining the dystrophic lesion have been repeatedly reported [31, 32].

Electrophysiological Alterations

The single-flash dark-adapted ERGs and rod ERGs are almost normal, while the cone and flicker ERGs can present slightly reduced amplitudes [33] The VEPs to pattern reversal stimulation are generally greatly affected [28, 34] In the clinically normal youngest generation of an affected family, Lotery detected bilaterally abnormal VEPs and PERGs [28] The mf ERG displays reduced amplitudes not only in the visibly atrophic area, but also in areas with no visible alteration. The peak time can be slightly delayed [35, 36, 37].

Differential Diagnosis

In the differential diagnosis, the possibilities that may arise include the cone dystrophies, macular coloboma or North Carolina dystrophy, Stargardt's disease, Best vitelliform dystrophy, and later age-related macular dystrophy. However, macular coloboma and North Carolina dystrophy exhibit an onset at birth, and Stargardt's dystrophy has an inheritance of autosomal recessive instead of autodomal dominant. In cone dystrophy, even if the fundus alterations can be deceiving, the photophobia and color defect are early signs. In age-related macular dystrophy the area of the circumscribed degeneration in the posterior pole generally demonstrates other alterations such as drusen.

The fully developed picture of central areolar choroidal sclerosis is unmistakable, because its three components, the central position, the characteristic changes in the exposed choroidal vessels, and the sharp demarcation of the lesion from the surrounding background, are clearly defined.

III.2. Peripapillary Choroidal Dystrophy

The diagnosis of this type of choroidal dystrophy is rare, because there are generally no visual symptoms. It is discovered by chance during ophthalmoscopic examinations for glaucoma or before the prescription of glasses. In the course of the progression, the atrophy reaches the macula, after which the differentiation from generalized choroidal dystrophy is difficult.

A continuum among central, peripapillary and generalized choroidal dystrophy has been suggested [38].

Inheritance

The inheritance of this disease can be autosomal dominant or autosomal recessive, but in many cases a positive family history is lacking.

Symptoms

The onset is probably similar that of the central areolar variety, although the symptoms may occur later, when the macula will be affected and the vision is reduced. The peripheral field is full and there is no night blindness.

Ophthalmoscopic Alterations

The retinal pigment epithelium and choroidal loss of tissue begin in the region surrounding the optic disc, with a slow enlargement in a finger-like projection nasally along the temporal vessels and into the macula, eventually occupying the entire posterior pole. This area demonstrates a generalized loss of pigment epithelium with irregular borders, separating the abnormal central retina from the normal-appearing periphery. Peripheral to the involved area the retinal function is normal. In some cases, perivascular bone spicule pigmentation and several pigment strands are also seen within the affected regions.

Fluorescein Angiography

No FLA study has been reported previously. Since the pathology is the same as that of central areolar or generalized choroidal dystrophy, the angiographic alteration may be similar in these diseases, with the distribution of the choroidal atrophy starting around the disc and spreading toward the periphery.

Electrophysiological Alterations

We have found no releveant publication. In the early stages, ERG alterations may not be observed. In one of our patients in an advanced stage, we detected a considerable amplitude reduction in VEP, PERG and mf ERG.

Differential Diagnosis

The most frequent misdiagnoses include halo senilis, myopic conus temporalis and peripapillary conus. In these diseases there is no visual loss. If the atrophy reaches the macular area, it causes a visual loss that is milder than that in generalized choroidal dystrophy (Fig. 3).

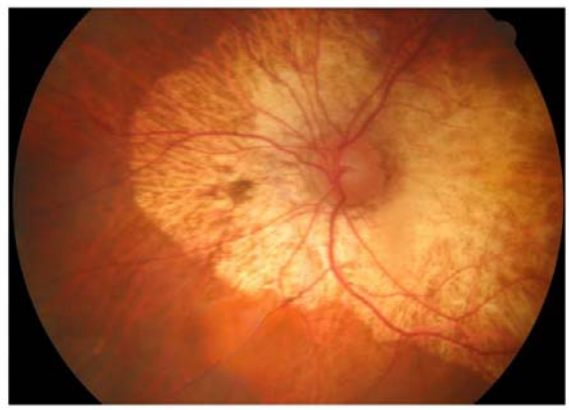

Figure 3. Ophthalmoscopic picture of the left eye of a patient with peripapillary choroidal dystrophy. There is a sharp border separating the abnormal central retina from the normal-appearing periphery.

III.3. Generalized Choroidal Dystrophy

This clinical entity was described as long ago as in the 19th century. Nevertheless, it is rarely detected and does not appear to be well established in the literature or in clinical practice. The disease may be named choroidal sclerosis after the ophthalmoscopic picture, choroidal atrophy after the histological findings, or choroidal dystrophy as an indication of the hereditary origin. Only two major review articles have been published on this disease, the more recent one 30 years ago [2]. The rarity of the diagnosis can be attributed either to its true rarity or to the diagnostic problems stemming from the great variability in the manifestations and in the interpretation of the signs and symptoms.

Our experience suggests that the condition is not particularly uncommon.

Inheritance

Although Sorsby [39] raised the possibility of autosomal dominant inheritance in 1939, no firm genetic basis has yet been found. The abnormal gene locus has not been identified. Additionally, several independent, individual cases have been described.

Histopathology

There is atrophy of the choroid affecting all of its elements, including the blood vessels [40] There is no arteriosclerosis of the choroidal network and no fibrous replacement of the media, but vascular atrophy [21, 41] . At least in the central part of the retina, there is a total absence of choriocapillaries [42]. Important changes are seen in Bruch's membrane too. The pigment epithelium and the other outer layers of the retina are absent.

Symptoms

Night blindness is generally an early symptom, but the symptoms associated with the photopic system (decreased visual acuity, light aversion and photophobia) predominate. A varying degree of field contraction is frequently detected. Interestingly, a central scotoma is rarely mentioned.

Our clinical observations revealed that the first symptoms of the disease may be manifested as early as the age of 24 years or as late as 66 years (mean age 48 years). The first symptoms in some patients were monocular metamorphopsia and a visual impairment. Marked side differences were noted in visual acuity and ophthalmoscopic alterations. Night blindness was mentioned only when we asked about it.

Although this disease can spread and involve the whole of the retina, the level of visual field constriction in our patients was mild. Only in the terminal stage was there a marked peripheral field loss. The most characteristic defect was an enlarged blind spot and/or a central scotoma gradually covering the central retina, which resulted in a centro-cecal scotoma with serious visual loss during progression.

The progression of the disease was found to vary. It was rapid, leading to severe visual loss within 1-2 years in some cases, and it was rather slow in some others. In general, no close relation was found between the duration and the severity of the disease. Similarly, there was no correlation between the age of the patients and the visual impairment. The age at manifestation and the severity of retinal and choroidal alterations in family occurrences were also variable. The oldest family member, with a duration of the disease of 24 years, still had

reading vision, while his youngest brothers, with durations of 4 and 6 years displayed severe visual loss.

Ophthalmoscopic Alterations

Generalized choroidal dystrophy is characterized by an ophthalmoscopic picture of bilateral atrophy of the pigment epithelium and of the retina with the formation of choroidal vessels, which appear as a yellowish or white sharply-defined network of broad lines [43].

There are discrepancies in the literature as concerns the forms and stages of this disease.

McKay [43] differentiated two forms of chorioretinal dystrophy: central areolar and diffuse choroidal dystrophy. In the chapter of Hereditary Fundus Dystrophy of his book of Clinical Ophthalmology Kanski referred to the forms of the choroidal dystrophy as central areolar choroidal dystrophy, diffuse choroidal atrophy, helicoidal parapapillary chorioretinal degeneration and pigmented paravenous retinochoroidal atrophy [44]. Fishmann distinguished four forms: central areolar, central, peripapillary and diffuse forms, the last three of which may represent a continuum [38]. Our experience supports this notion.

The events in this type of dystrophy seem to follow the sequence described by Sorsby [45]:

1. Early stage: mottling of the central areas, reminiscent of macular dystrophy.
2. Intermediate stage: extensive central lesion, edematous, atrophic or pigmentary proliferation, suggestive of generalized fundus atrophy or inflammation.
3. Developed stage: exposure of the choroidal vessels in the central areas, with conversion of the vessels into white streaks. This means the beginning of the pathognomic appearance of generalized choroidal (chorioretinal) dystrophy.
4. Late stage: the central lesion is spreading toward the periphery, so the entire fundus exhibits generalized choroidal sclerosis with its characteristic appearance. The retinal vessels display only age-related sclerosis, and the attenuation is not as severe as in retinitis pigmentosa. The optic discs are pale, and scattered irregular pigment clumping can be found in the peripheral retina.
5. Terminal stage: absorption of the exposed choroidal vessels gives an appearance reminiscent of choroideremia.

Our experience is based on the 15 patients we have detected and followed up during the last 10 years. In one case, an asymptomatic member of a family with generalized choroidal sclerosis, marked pigmentary alterations were observed in the peripheral retina: tigroid-type or cobweb-like patterns bilaterally, without choriocapillary atrophy. Although there was pigment clumping in the left macula, his visual acuity was 1.0. No complaint of night blindness was mentioned (Fig. 4).

In 2 individual cases, one eye revealed pigmentary alterations in the macula without visual loss, while the other eye demonstrated generalized choriocapillary atrophy, which facilitated the diagnosis.

In the other cases, the characteristic ophthalmoscopic picture of macular and peripheral choroidal atrophy, pigment alterations, and a pale optic disc with only mild age-related attenuation of the retinal vessels was observed (Fig. 5).

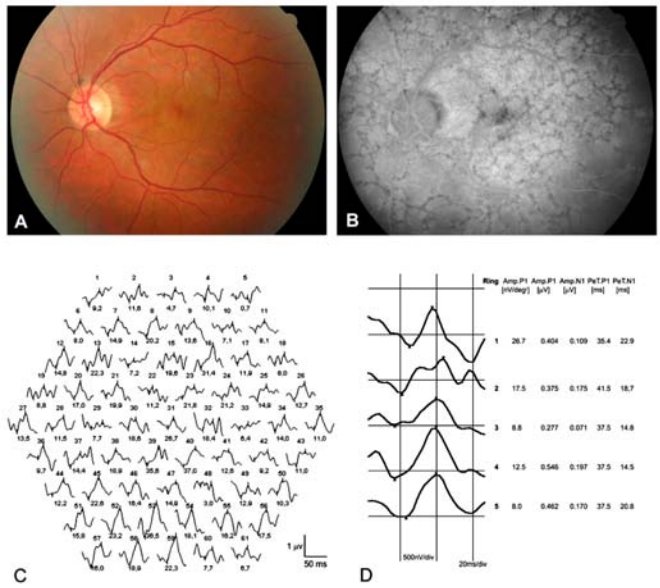

Figure 4. Ophthalmoscopic picture (top left), FLA photograph (top right), trace array of multifocal ERG (bottom left) and ring analysis of the multifocal ERG (bottom right). The mild pigment mottling in the macular area did not result in any visual loss (visual acuity: 1.0). A characteristic cobweb-like pigmentary alteration was visualized by FLA. Multifocal ERG revealed the mosaic pattern of damage in the macular area. There were almost extinguished and almost normal responses scattered in the 30 degree central part of the retina. Ring analysis of the multifocal ERG revealed the most severe alterations in rings 2 and 3.

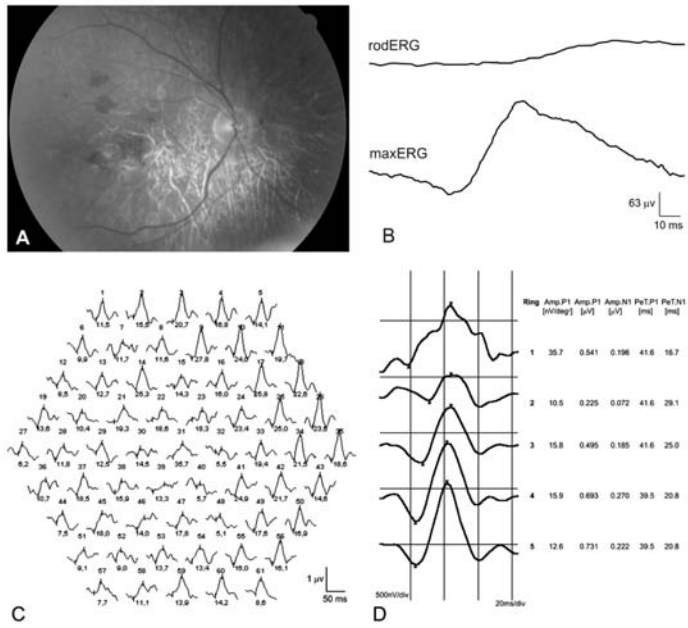

Figure 5. FLA photograph (top left), ERG (rod ERG, upper curve), and mixed rod and cone ERG (max ERG, lower curve), trace array of mf ERG (bottom left) and ring analysis of mf ERG (bottom right). The rough pigment clumps and the attenuation of the retinal arterioles are reminiscent of retinitis pigmentosa. This diagnosis was excluded by the recorded ERGs. Rod ERG was subnormal, but not extinguished and max ERG displayed a characteristic waveform: the depression of the ‘a’ wave was more enhanced than that of the ‘b’ wave.

Fluorescein Angiography

Widespread disturbance of the retinal pigment epithelium is the first sign of this disease. The loss of pigment results in hyperfluorescent spots due to the increased visualization of the dye in the intact choriocapillaries underneath the defective pigment epithelium. The progression of the disease is accompanied by disappearance of the choriocapillaries, though this is never complete. Residual islets with leaked dye are always seen in the affected area. Dye also leaks from intact choriocapillaries at the margin of the diseased area. The delayed filling of some of the isolated islets of choriocapillaris indicates the early involvement of these vessels. The leakage of dye from the choriocapillaries increases with time. Later, some of the choroidal veins fill directly from the precapillary arterioles, which apparently act as arterial-venous shunts. The caliber of the choroidal vessels is frequently considerably larger after dye injection, sometimes even doubling [31, 32]. These data are in line with the histological finding that the choroidal vessels are not sclerotic in degenerative choroidal sclerosis [21, 40, 41].

FLA studies clearly distinguish between generalized and local dystrophies, too. The diagnosis of generalized choroidal dystrophy in our patients was based on the FLA findings. In the early stage, when there was no choriocapillary atrophy, but only pigmentary alterations indicated by FLA, either the family history or the characteristic alterations in the fellow eye helped the diagnosis.

Electrophysiology

There is a controversy in the literature as regards the ERG and EOG alterations in generalized choridal dystrophy. Some early reports considered that they are extinguished, whereas others have claimed that they can be severely altered, but never extinguished [2, 43].

Since then, new electrophysiological methods have been developed. With the aid of these, we performed a search in our patients for characteristic electrophysiological alterations and for new information on this disease, and followed up the progression. In order to examine the function of the outer layers of the retina, rod ERG, cone ERG, mixed cone and rod ERG: max ERG and mf ERG have been tested, and to recognize the inner retinal layer damage, the OPs and PERGs have been recorded. VEPs were employed to assess optic nerve and visual pathway function.

The electrophysiological signs seem to be rather consistent. The rod ERG reveals a mild delay in the implicit time, and the amplitude of the ‘b’ wave is reduced in line with the progression.

The max ERG does not demonstrate any substantial implicit time delay or reduction in ‘b’ wave amplitude. However, because of the explicit reduction of the ‘a ‘wave amplitude, the shape of the curve is rather characteristic. During progression, not only the ‘a’ wave, but also the ‘b’ wave decreases in amplitude; the shape of the curve remains similar. None of our patients had an extinguished max ERG even after an extensive duration of the disease.

The cone ERGs, OPs, and 30-Hz flicker ERGs are subnormal too and reflect the side differences in the functional loss.

MfERG reveals functional alterations in the central 30-degree area of the retina. The most severely decreased responses are the central ones, especially in rings 2 and 3. Toward the periphery (rings 4 and 5), the amplitudes of the responses increase, even in the developed stage, when the atrophy spreads towards the periphery.

PERGs were hardly detectable in many of our patients with a delayed implicit time.

The VEPs depended on the foveal involvement of the retina. When all the central visual field was affected by the atrophy, the visual acuity decreased rapidly and the VEP became undetectable.

Differential Diagnosis

Diagnostic problems can be posed by differentiation from inflammatory macular diseases, Sorsby's pseudoinflammatory disease, macular dystrophies, age-related macular degeneration, retinitis pigmentosa and choroideremia in the terminal stage.

The ophthalmoscopic signs depend on the stage of the disease and this was the probable main cause of the several misdiagnoses in our patients. In the early stage, when there was only pigment mottling in the macular area, without any visual loss, the macular alteration was revealed by chance. In the second stage, when the inflammatory process had developed, the misdiagnosis was generally central serous chorioretinopathy or macular dystrophy. In the third, developed stage, age-related macular degeneration was generally the first diagnosis in our patients. When pigment clumps appear that resemble bone spicule formation, the false diagnosis can be retinitis pigmentosa.

Electrophysiological Differential Diagnosis

In our patients, the rod ERG exhibited progressively subnormal amplitudes. In the advanced stage, they were hardly detectable. The max ERG had a characteristic shape because of the more severe 'a' wave depression as compared that of the 'b' wave.

TABLE 1. Electrophysiological differential diagnostics.

		Macular dystrophy (AMD)	Retinitis Pigmentosa (RP)	Generalized choroidal sclerosis (GCS)
ERG	Scot	normal	subnormal → extinguished	normal → subnormal
	Max	normal	subnormal → extinguished	normal → subnormal
	OP	subnormal	subnormal → extinguished	subnormal
	Phot	normal → subnormal	subnormal → extinguished	subnormal
	Flicker	normal → subnormal	subnormal → extinguished	subnormal
mfERG		crater-like central depression, increasing amplitude towards periphery	central peak decreasing amplitude towards periphery	subnormal central, in 2. and 3. rings severely depressed responses, increasing amplitude towards periphery
PERG		subnormal → extinguished	subnormal → extinguished	subnormal → extinguished
VEP		subnormal	normal → subnormal	severely subnormal

This is in contrast with RP, when the ERG can already be nondetectable in the early stage of the disease, and with macular degeneration, when the full-field ERG is generally normal.

Analysis of the averaged values of mf ERG in 5 rings around the fovea provides further information on the residual function of the macula and may help the differential diagnosis, too. In retitinis pigmentosa, there is a central peak in the mf ERG and the responses are

progressively diminished toward the periphery. In macular degeneration, the characteristic alteration is a crater-like central depression and the responses improve toward the periphery. In generalized choroidal dystrophy, there is neither a central peak nor a central depression.

Histological analysis proved that the atrophy of the choriocapillaries is not homogenous; there are capillary areas that are better preserved [21]. Mf ERG seems to be important in the localization of these small islets of lesser involved areas.

The VEPs are already subnormal in the early stage of the disease. In retinitis pigmentosa, the VEP can be almost normal, even in the late stage of the disease.

The electrophysiological differential diagnostic aspects are summarized in Table 1.

CONCLUSION

The common features in chorioretinal dystrophy are the loss of choriocapillaries, involvement of the retinal pigment epithelium and the photoreceptor layer, and in the advanced stage the entire choroid. Some of these progress from the peripheral retina toward the macula, while others originally involve the macula and progress toward the periphery.

Gyrate atrophy, choroideremia and the central form of geographical choroidal dystrophy are well described in the literature. They afford characteristic ophthalmoscopic pictures, and the inheritance and the pathology of this disease were soon clarified. Further, the ophthalmoscopic status of the carriers can promote the diagnosis. However, the diagnosis of generalized chorioretinal dystrophy can not be made by relying exclusively on the symptoms and the ophthalmoscopic alterations because of the high variability in the manifestation and progression of this disease. The diagnosis and the care of patients are further complicated by the high intrafamilial variability, and by the lack of a close correlation between the duration and the severity, and between the ophthalmoscopic picture and the viual loss. The involvement of the second eye and the progression of the atrophy are also almost unpredictable.

Electrophysiological examinations may be of decisive help in the diagnosis, in the assessment and in the localization of any functional loss. The follow-up of the condition of the patients by means of electrophysiological methods may provide further information on the pathophysiology of this disease, which may not be as rare as is widely believed.

REFERENCES

[1] Fusch, E. *Über zwei der retinitis pigmentosa verwandte Krankheiten (Retinitis punctata albescens und Atrophia gyrata chorioideae et retinae).* Arch Augenheilk, 1896 32, 111-116.

[2] Francois, J. *Heredity of the choroidal dystrophies*. Adv Ophthalmol, 1978 35, 1-25.

[3] Francois, J; Verriest, G; De Rouck, A. *Les functions visuelles dans les degénérescences tapéto-rétinienes*. Bibliotheca Ophthal No 43. Basel: Karger 1956.

[4] Simel, O; Takki, K. *Raised plasma ornithine and gyrate atrophy of the choroid and retina.* Lancet, 1973 1, 1031-1033.

[5] Weleber, RG; Kennaway, NG; Buist, NRR. *Vitamin B6 in management of gyrate atrophy of choroid and retina.* Lancet, 1978 2, 1213.
[6] Jacobson, E. *Ein Fall von Retinitis Pigmentosa Atypical*. Klin Mbl. Augenheilk, 1888 26, 2002-2006.
[7] Böhm, FF. *Über einen eigentümlichen Fall von Retinitis pigmentosa mit Atrophie der Aderhaut (Atrophia gyrata). Klin Mbl Augenheilk,* 1919 63, 381-386.
[8] Davis, WT; Sheppard, E. *Gyrate atrophy of retina and choroid following retinitis pigmentosa. 2 cases.* Arch Ophthal (Chicago), 1940 23, 1252-1256.
[9] Ohrt, V.von. *Gyrate atrophy of choroid and retina and inverse retinitis pigmentosa in the same patients*. Acta Ophthal, 1957 35, 26-31.
[10] Franceschetti, A; Dieterle, P. *Die differentialdiagnostische Bedeutung des ERG bei taptoretinalen Degenerationen.* Bibliotheca Ophthal No 48 pp 161-181 Basel: Karger.
[11] Mauthner, L. *Ein Fall von Chorioideremia. Naturwissenschaften,* 1871 2, 191.
[12] Goedbloed, J. *Mode of inheritance of choroideremia*. Ophthalmologica (Basel), 1942 104, 308.
[13] Kurstjens, IH. *Choroideremia and gyrate atrophy of the choroid and retina*. Doc Ophthalmol, 1965 19, 1.
[14] McCulloch, JC; McCulloch, RJP. *A hereditary and clinical study of choroideremia*. Trans Am. Acad. Ophthal. Oto-Lar, 1948 52, 160.
[15] Wald, KJ; Hirose, T. *Clinical methods for detecting the carrier state of X-chromosome-linked retinal disorders*. Int Ophthalmol Clin, 1993 33, 203-217.
[16] Nussbaum, RL; Lewis, RA; Lesko, JG; Ferrel, L. *Choroideremia is linked to restriction fragment length polymorphism DXYS1 at Xq 13-21. Am J Hum Genet,* 1985 37, 473-481.
[17] Lewis, RA; Nussbaum, Rl; Ferrel, RF. *Mapping X-linked ophthalmic diseases. Provisional assignment of the locus for choroideremia to Xq 13-24.* Ophthalmology, 1985 92, 800-806.
[18] Sieving, PA; Niffenegger, JH; Berson, EL. *Electroretinographic findings in selected pedigrees with choroideremia*. Am J Ophthalmol, 1986 101, 361-367.
[19] Vajaranant, TS; Fishman, GA; Grant-Jordan, P; Szlyk, JP; Lindeman, M; Seiple, W. *Detection of mosaic retinal dysfunction in choroideremia carriers elecroretinographic and psychophysical testing*. Ophthalmology, 2008 115, 723-729.
[20] Nettleship, E. *Central senile areolar choroidal atrophy.* Trans Ophthalmol Soc UK, 1884 4, 165-166.
[21] Ashton, N. *Central areolar choroidal sclerosis; a histo-pathological study*. Br J Ophthalmol, 1953 37, 140-147.
[22] Sorsby, A; Crick, RP. *Central areolar choroidal sclerosis.* Br J Ophthalmol, 1953 37, 129-139.
[23] Hoyng, CB; Deutman, *AF. The development of central areolar choroidal dystrophy*. Graefes Arch Clin Exp Ophthalmol, 1996 234, 87-93.
[24] Sandvig, K. *Familial, central areolar choroidal atrophy of autosomal dominant inheritance*. Acta Ophthalmol (Copenh), 1955 33, 71-78.
[25] Carr, RE. *Central areolar choroidal dystrophy*. Arch Ophthalmol, 1965 73, 32-35.
[26] Ferry, AP; Llovera, I; Shafer DM. *Central areolar choroidal dystrophy*. Arch Ophthalmol, 1972 88, 39-43.
[27] Noble, KG. *Central areolar choroidal dystrophy*. Am J Ophthalmol, 1977 84, 310-318.

[28] Lotery, AJ; Silvestri, G; Collins, AD. *Electrophysiology findings in a large family with central areolar choroidal dystrophy.* Doc Ophthalmol, 1998-99 97, 103-119.
[29] Hoyng, CB; Heutink, P; Testers, L; Pinkers, A; Deutman, AF; Oostra, BA. *Autosomal dominant central areolar choroidal dystrophy caused by a mutation in codon 142 in the* peripherin/RDS gene. Am J Ophthalmol, 1996 121, 623-629.
[30] Keithahn, MA; Huang, M; Keltner, JL; Small, KW; Morse LS. *The variable expressivity of a family with central areolar pigment epithel dystrophy*. Ophthalmology, 1996 103, 406-415.
[31] Archer, D; Krill, AE; Newell, FW. *Fluorescein studies of choroidal sclerosis*. Am J Ophthalmol, 1971 71, 266-285.
[32] Noble, KG; Carr, RE; Siegel, IM. *Fluorescein angiography of the hereditary choroidal dystrophies.* Br J Ophthalmol, 1977 61, 43-53.
[33] Ponjavic, V; Andreasson, S; Echinger, B. *Full-field electroretinograms in patients with central areolar choroidal dystrophy. Acta Ophthalmol*, 1994 72, 537-544.
[34] Adachi-Usami, E; Murayama, K; Yamamoto, Y. *Electroretinograms and pattern visually evoked cortical potentials in central areolar choroidal dystrophy*. Doc Ophthalmol, 1990 75, 33-40.
[35] Nagasaka, K; Horiguchi, M; Shimada, Y; Yuzawa M. *Multifocal electroretinograms in central areolar choroidal dystrophy*. Invest Ophthalmol Vis Sci, 2003 44, 1673-1679.
[36] Hartley, KL; Blodi, BA; VerHoeve, JN. *Use of the multifocal electroretinogram in the evaluation of a patient with central areolar choroidal dystrophy*. Am J Ophthalmol, 2002 133, 852-854.
[37] Feigl, B; Haas, M. *Multifocal ERG in central areolar choroidal dystrophy*. Ophthalmologe, 2001 98, 1074-1078.
[38] Fishman, GA. *The electroretinogram in retinal disease: Chorioretinal dystrophies* In: Fishman, GA, Sokol, S. editors. Electrophysiologic Testing in Disorders of the Retina, Optic Nerve, and Visual Pathway, American Academy of Ophthalmology 1990, 1-13.
[39] Sorsby, A. *Choroidal angio-sclerosis with special reference to its hereditary character*. Br J Ophthalmol, 1939 23, 433-443.
[40] Sarks, SH. *Senile choroidal sclerosis*. Br. J. Ophthalmol, 1973 57, 98-109.
[41] Babel, MJ. *Le role de la choriocapillaire dans les affections degeneratives du pole posterieur.* Bull Soc Fr Oph, 1958 71, 389-398.
[42] Howard, GM; Wolf, E. *Central choroidal sclerosis. A clinical and pathologic study.* Trans Am. Acad. Ophthal. Oto-Lar, 1964 68, 647-660.
[43] McKay, RA; Spivey, BE. *Generalized choroidal angiosclerosis.* Arch Ophthalmol, 1962 67, 727-735.
[44] Kanski, JJ. *Hereditary fundus dystrophy*. In: Kanski, JJ. Editor. Clinical Ophthalmology. A Systematic Approach Vth edition. Butterworth-Heinemann 2003 487-515.
[45] Sorsby, A; Davey, JB. *Generalized choroidal sclerosis; course and mode of inheritance*. Br J Ophthalmol, 1955 39, 257-276.

(Noble)
(see in Francois review paper).
Simell and Takki in 1973.
(Weleber, Simmel).
(Jacobson 1888, Böhm 1919 Davis and Sheppard 1940)
(Franceschetti and Dieterle 1957).
Mauthner in 1871.
Goedbloed (1942)
(Kurstjens 1965- A Carr kék könyvben, Mc Culloch: aFrancois cikkben 1948).
(Mc Culloch 1948 a Francois cikkből).
(Nusbaum, Lewis).
(Amalrich).
(Sieving).
Vajarand
(Nettleship).
(Ashton, Sorsby 1953, Hoyng).
(Lotrery),
Hoyng
Keithahn
(Archer, Noble).
(E-Adachi-Ushami Lottery).
Nagasaka, Hartley Feigel)
(Fishman).
Sorsby raised 1933,
(Sharks).
(Ashton1953, Babel 1958, Sarks 1973)
(Babel, Howard and Wolf1964).
Important changes are seen in the Bruch's membrane too. Ó
(Francoise).
(McKay).
Mc Kay (1962).
In the book of Hereditary Fundus Dystrophy (451 old)
Fishmann distinguished 4
Sorsby (1955)
(Nobble, Archer
(Aston, Babel, Shark).
(MacKay.Francois).
(Ashton).

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Chapter 12

NEUROPLASTICITY IN THE DORSAL VAGAL COMPLEX

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ABSTRACT

The dorsal vagal complex (DVC), located dorsally in the caudal brainstem, comprises three distinct structures: the sensory nucleus of the solitary tract (NST), the area postrema (AP), and the dorsal motor nucleus of the vagus (DMV). The DVC integrates both peripheral and central signals and is the major center providing innervation to the cardiovascular and gastrointestinal systems to modulate autonomic function. New lines of evidence indicate that DVC neuronal networks undergo neuroplastic changes either in physiological states such as development and aging or as a consequence of pathological conditions. This review summarizes our current knowledge on functional and structural plasticity in the DVC. Emphasis will be given to the following three aspects of neuroplasticity in DVC: (1) changes in neurochemical phenotypes and structural reorganization including synaptic plasticity; (2) alterations in neuronal excitability; and (3) survival and neurogenesis. Several lines of evidence support the presence of proliferative neuronal precursor cells and neurogenesis in adult DVC. These neuronal precursor cells are located in the wall of the fourth ventricle and appear to be closely associated with the glial fibrillary acidic protein (GFAP) positive radial-like cells. These neuronal precursor cells in the adult DVC respond to several growth factors including epidermal growth factor (EGF), basic fibroblast growth factor (bFGF) and ghrelin with increase in proliferation. The potential implications of neuroplasticity in DVC in the adaptation of gastrointestinal function to physiological and inflammatory conditions are discussed. A better comprehension of neuroplasticity in DVC could provide insights into new therapeutic strategies for patients with gastrointestinal dysfunction.

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1. INTRODUCTION

The gastrointestinal system receives input from two distinct sources: the intrinsic enteric nervous system and the extrinsic nervous system. The dorsal motor nucleus of the vagus (DMV) contains neurons that provide parasympathetic efferent outflow to the gastrointestinal system; and the DMV modulates virtually all aspects of gastrointestinal functions including blood supply, secretion, absorption and motility. As a result, the DMV is considered the major center that provides direct output to the gastrointestinal system to regulate its functions. Together with the sensory nucleus of the solitary tract (NST) and the area postrema (AP), the DMV comprises the dorsal vagal complex (DVC). This complex integrates peripheral and central signals from three distinct sources (Travagli et al 2006). First, the dorsal vagal complex possesses the characteristics of a circumventricular organ and is essentially devoid of a blood-brain barrier. Thus, the DVC is capable of monitoring blood-borne and cerebrospinal fluid-borne factors and of changing vagally-mediated autonomic functions accordingly. Second, afferent neurons innervating the gastrointestinal organs project to and terminate within the DMV either directly or via interneurons in the NST. Third, the DVC conveys signals from the neuroendocrine control center of the hypothalamus.

The ability of vagal neurons to undergo adaptive changes has long been considered limited due to the so called “logic” assumption that neurons in the medulla are vital and should remain stable and well-protected from the influence of noxious factors such as inflammation and infection. This concept is directly challenged by data from our laboratory, and from others. The purpose of this review is to summarize recent studies in the neuroplasticity of DVC neurons.

2. BRAINSTEM VAGAL CIRCUITS

Vagal neuronal circuits comprise vagal afferent inputs, NST interneurons and vagal efferent projections to the upper gastrointestinal tract. Vagal afferent nerve fibers carry a large volume of information from the gastrointestinal tract to the DVC, informing this parasympathetic nervous center of the physiological and pathological status of the gut. Gastrointestinal information is processed by NST neurons which serve as interneurons to bridge gastrointestinal signals to DMV neurons. The DMV neurons then provide efferent vagal nerves to modulate gastrointestinal functions. There exist two orders of synaptic connections in the DVC complex. The first order of synaptic connections occurs as visceral vagal afferent fibers from bipolar neurons in the nodose ganglia terminate on NST neurons. Electrophysiological studies have revealed several types of neurotransmitter receptors and two type of afferent nerve fibers: unmyelinated C fibers and myelinated A fibers. Despite a diversity of functions and conduction velocities, all vagal afferent fibers use glutamate as the primary neurontransmitter to transfer information to NST neurons (Hornby 2001). Release of presynaptic glutamate activates both NMDA and non-NMDA receptors. The second order of synaptic connections is formed by NST neuronal endings on DMV neurons. Neurophysiological studies demonstrate two neural circuits in the second order synaptic connections in the vagal complex. One circuit, regulated by the gamma-aminobutyric acid (GABA)ergic neurons, controls cholinergic vagal output in the DMV (Travagli et al. 2006).

The other circuit, modulated by glutamatergic neurons, activates an inhibitory nonadrenergic noncholinergic (NANC) pathway to the gut (Travagli et al 2006). Under physiological conditions, the balance between these two neuronal pathways accounts for changes in gastrointestinal function in response to distention, acidity, fat and nutrient composition. In disease states such as inflammation and injury, alterations in neuronal chemical profiles and synaptic activity in the two synaptic connections in the DVC ultimately affect the function of DMV neurons.

3. PLASTICITY IN NEUROCHEMICAL PROFILES

3.1 Change During Development

Recent studies have revealed alteration in neurochemical profiles of the dorsal vagal complex at early postnatal days P3-4 and especially at P12. Similar to other brain stem nuclei (the only exception being the cuneate nucleus), NST and DMV neurons shows a developmental trend for neurochemical profiles. These changes include: (1) although the expression of glutamate increases with age, there exists a major reduction in its expression at P12; (2) the expression of the excitatory neurotransmitter receptor N-methyl-D-aspartate receptor subunit 1 (NMDAR1) declines at P12; expression of another glutamate receptor-glutamate receptor subunit 2 (GluR2) increases at P12; (3) a marked increase in GABA expression occurs at P12, although its expression decreases postnatally; (4) expression of inhibitory neurotransmitter $GABA_B$ receptor declines with age, but there is a distinct peak at P12; (5) glycine receptors show an increase in expression with age. Thus, there is a reduction in the expression of excitatory neurotransmitters and receptors, while expression of inhibitory neurotransmitters and their receptors are increased at P12. Since activation of GluR2 receptor reduces the permeability of DL-a-amino-3-hydroxy-5-methyl-isoxazole-propionic acid (AMPA) receptors to Ca^{2+} and therefore reduces neuronal excitation, expression of inhibitory neurotransmitter receptors suggests an overall reduction in the neuronal excitability in the dorsal vagal complex at P12. These observations strongly suggest that the neuronal structure in NST and DMV undergoes active synaptic re-organization during postnatal development and that there exists a critical period during the maturation of NST and DMV. During this period (around P12 in rats), neuronal circuits are dominated by inhibitory drive due to a transient imbalance between excitatory and inhibitory neurotransmission. Whether such period exits in humans is unknown.

3.2 Physiological Adaptation

Vago-vagal reflexes involve sensory afferent fibers of bipolar neurons from the nodose ganglion which terminate on NST neurons, making first order synaptic connections. NST neurons provide innervation to DMV neuronal soma, forming second order synaptic connections. Preganglionic efferent fibers from DMV neurons project to visceral organs such as stomach, intestine and pancreas, making third order synaptic connections within vago-

vagal circuits. The strategic organization of this neuronal circuitry provides virtually limitless control sites and therefore numerous mechanisms by which vago-vagal reflex selectivity and sensitivity are regulated. In addition, portions of the DVC are located outside the blood-brain barrier, making them accessible to a variety of circulating hormones and cytokines.

At first order synaptic connections, vagal afferent neurons have been shown to respond to a variety of neurotransmitters and/or neuromodulators such as substance P and serotonin (5-hydroxytryptamine, 5-HT). The pharmacological responses of nodose ganglion neurons demonstrate a striking regional difference, providing a structural basis for differential regulation of neuronal activity. Responses of nodose ganglion neurons to 5-HT have been demonstrated to be subject to modulation. In guinea pig airway vagal afferent neurons, naïve neurons are insensitive to neurokinins. However, antigen inhalation can unmask previously silent NK-2 tachykinin receptors for a period from hours to days ((Moore et al 2000; Undem and Weinreich 1993). Rat gastrointestinal vagal afferent neuons have also been demonstrated to uncover in response to application of serotonin. Unlike guinea pig airway vagal afferent neurons in which the sensitizing effect of 5-HT involves activation of 5-HT3 receptors, the unmasking of neurokinin receptors on gastrointestinal vagal afferent neurons appears to involve 5-HT2 receptors (Browning and Mendelowitz 2002). Another example of neuronal plasticity of nodose ganglion neurons is illustrated by the finding that voltage-gated calcium channels at the soma of aortic baroreceptor neurons highly correspond to the release of neuronal transmitters at their synaptic terminals in the NST (Mendelowitz et al 1995). Voltage-gated calcium currents in the soma of baroreceptor neurons have been shown to be suppressed by μ-opioid agonist or activation of metabotropic glutamate receptors (Hamra et al 1999; Hay and Junze 1994). This inhibition at the soma typically depresses glutamate-mediated excitatory postsynaptic potentials in NST neurons evoked by stimulation of the solitary tract ((Rhim et al 1993).

At the second order synaptic connection in vago-vagal reflexes, three types of neurons including glutamatergic, GABAergic and catecholaminergic neurons have been identified. Among these neuronal inputs, GABA is the principal neurontransmitter at the NST-DMV synapse. While glutamatergic transmission is subject to modulation by a variety of neurotransmitters/neuromodulators such as 5-HT、 neuropeptide Y、glucagon-like peptide-1 (GLP-1) and orexin (Browning and Travagli 1999; Browning and Travagli 2003; Wan et al 2007; Davis et al 2003), GABAergic synaptic transmission is resistant to the majority of these neurotransmitters (Browning and Travagli 1999; Browning and Travagli 2003). The level of cAMP-protein kinase A (PKA) appears to be critical for determining the state of activation of GABAergic synaptic transmission. Activity of cAMP-PKA within GABAergic NST nerve terminals is dependent on vagal afferent nerve input. Under normal conditions, the level of cAMP-PKA is low because ongoing vagal afferent activation of group II but not group III metabotropic glutamate receptor acts as a brake on the cAMP-PKA pathway in NST GABAergic circuits (Browning and Travagli 2006). Following activation of the cAMP-PKA pathway either by extrinsic afferent denervation or by neuromodulators, the activity level of cAMP-PKA is elevated, causing trafficking of internalized μ-opioid receptors to the cell membrane (Browning et al 2004).

3.3 Pathological Changes

Neurochemical profiles of vagal afferent neurons are also subject to alteration during pathological insults. The level of sensory neuropeptides in nodose neurons has been shown to be elevated during allergic inflammation of guinea pig airways. The proportion of substance P and calcitonin gene-related peptide (CGRP) positive neurons increases from <1 % to over 30% during allergic stimulation (Myers et al. 2002). Viral infection has also been reported to lead to a qualitative change in the chemical coding of tachykinins in the vagal afferent innervation of guinea pig airways (Carr et al 2002). These alterations in the neurochemical codings imply that simple nonnoxious alterations in the normal state could potentially result in the release of neuropeptides associated with noxious stimuli, therefore inducing a potential hypersensitivity with inflammation.

4. ALTERATIONS IN NEURONAL EXCITABILITY

Plasticity in the excitability of vagal afferent neurons has dramatic consequences for the regulation and modulation of gastrointestinal vago-vagal reflexes. Studies using extracellular recording techniques demonstrate that vagal afferent neurons have a low level of spontaneous activity and that their action potential frequency increases upon stimulation of the afferent terminal field. Tonic vagal inputs act as a brake on excitability of DMV neurons, providing a basis for regulation of the gastrointestinal functions during physiological and pathological states.

4.1 Changes in Physiological States

At the first order synaptic connection, communication between subpolulations of nodose ganglion neurons has been suggested by the observation that stimulation of a subpolulation of nodose neurons can augment the activity of unstimulated neighboring cells. In a study by Oh and Weinreich (2002), cross-depolarization and cross-excitation as manifest by membrane depolarization (approximately 4 mV), the presence of spontaneous action potentials, and a decreased spike threshold are observed between somata of vagal afferents (nodose ganglion neurons), suggesting the possibility that communication between different visceral organs may occur at the level of primary vagal afferent neurons. Part of this cross-excitation appears to be due to the release of a diffusible transmitter because it can be blocked by cadmium, a nonselective calcium channel antagonist. In addition, activation of NST neurons has been demonstrated to be subject to the modulation of nodose ganglion neurons by neurotransmitters such as μ-opioid agonist and glutamate. Both μ-opioid agonist and glutamate have been reported to act to depress excitatory postsynaptic potentials in NST neurons evoked by stimulation of the solitary tract (Hay and Kunze 1994; Rhim et al 1993). These effects appear to be mediated by inhibition of ω-conotoxin-GIVA sensitive N-type voltage-gated calcium currents in the soma of nodose ganglion neurons.

Plasticity at the second order of synaptic connection within the vago-vagal reflexes is best illustrated in an elegant series of studies by Browning and Travagli (2004; 2006). Based

on their findings, cAMP-PKA within the GABAergic NST nerve terminal is proposed as a brake governing the excitability of DMV neurons. Under normal conditions, neuromodulators such as μ-opioid demonstrate no effect on GABAergic transmission at NST-DMV synapses. Low resting activity of the cAMP-PKA pathway of GABAergic transmission contributes to this resistance because activation of this pathway either by agonists of adenylate cyclase such as forskolin, thyrotropin-releasing hormone (TRH), or cholecystokinin (CCK), or by inhibiting phosphodiesterase-mediated degradation of cAMP unlocks the ability of μ-opioid receptor agonists to act at presynaptic receptors to inhibit GABAergic synaptic transmission. Consistent with these observations, inhibition of the cAMP-PKA pathway using inhibitors of adenylate cyclase or PKA prevents the effect of adenylate cyclase activators to uncover the presynaptic inhibitory actions of μ-opioid peptide (Browning and Travagli 2006). Further experiments using chemical C-fiber ablation or surgical rhizotomy to selectively remove vagal afferent inputs have suggested that vagal afferent inputs control the level of cAMP within NST GABAergic nerve terminals via activation of group II metabotropic glutamate receptors (Browning and Travagli 2006). Physiological signals regarding the status of the gastrointestinal tract can influence excitability of DMV neurons by altering cAMP levels at the NST GABAergic nerve terminals.

4.2 Alterations During Pathological Conditions

Alterations in nodose neuronal excitability in response to pathological challenges have also been reported. Exposure to hypoosmotic solution significantly increases conductance in the cell bodies of aortic baroreceptor neurons as well as in some additional neurons from the nodose ganglia (Cunningham et al 1995). The increase in conductance produced by hypoosmolality can be blocked by gadolinium, a putative blocker of mechanosensitive channels (Cunningham et al 1995). In response to inflammatory or antigenic challenge, vagal afferent respiratory neurons have been shown to be more excitable in a study by Undem and Weinreich (1993). Histamine was able to depolarize the membrane potential of the C-type neurons in a majority of nodose ganglion neurons. Bradykinin, prostacyclin, and leukotriene C4 were found to cause membrane depolarizations in a subset (73%, 31%, and 50%, respectively) of nodose ganglion neurons. A slowly developing, long-lasting afterhyperpolarization (AHPslow) is abolished by bradykinin, prostacyclin, and in a subset of neurons, by leukotriene C4. Inhibition of the AHPslow is accompanied by a change in the accommodative properties of the neurons, reflected by the increased frequency at which the neurons elicit repetitive action potentials. These results demonstrate that inflammatory mediators influence the excitability of nodose ganglion neurons by producing subthreshold membrane depolarization and by modulating the rate of neuronal discharge.

Another example of the plasticity of nodose ganglion neurons is illustrated by a recent finding that vagal afferent neurons become more excitable after the induction of experimental gastric ulcers (Gabhart et al. 2002). Significant changes in the characteristics of voltage-sensitive sodium channels have been observed in nodose ganglion neurons projecting to the stomach in rats with gastric ulcers induced by injection of acetic acid into the stomach wall. Significant enhancement in tetrodotoxin (TTX)-resistant sodium current is observed, while the TTX-sensitive sodium current remains inactive for a shorter period of time compared with

saline treated control neurons. Changes in sodium currents render gastric nodose ganglion neurons more excitable, leading to the development of gastric hyperalgesia.

Excitability of nodose ganglion neurons can be altered in response to vagal nerve injury. In contrast to many primary sensory neurons that are thought to become hyperexcitable following section of their axons, nodose ganglion neurons demonstrate a marked decrease in electrical excitability following vagotomy (Lancaster et al 2001). Vagotomy increases action potential threshold by over 200% compared with control values. The number of action potentials evoked by a 3-time threshold 750-ms depolarizing current decreases by >70% from 8.3 to 2.3 action potentials, whereas the number of action potentials evoked by a standardized series of (0.1-0.9 nA, 750 ms) depolarizing current steps decreases by over 80% from 16.9 to 2.6 action potentials in vagotomized nodose ganglion neurons (Lancaster et al 2001). Vagotomy also induces a depolarizing after potential in a subpopulation of nodose ganglion neurons (Lancaster et al 2002). This effect is attributed to activation of a calcium-dependent chloride channel subsequent to increased calcium influx during the action potential. Despite this depolarizing after potential following vagotomy, these neurons are still less excitable compared to control neurons (Lancaster et al 2002).

At the second level of synaptic connection in vago-vagal reflexes, vagal deafferentation has been reported to modulate neuronal excitability of DMV neurons (Browning et al 2006). Following chronic vagal deafferentation, the opioid agonist methionine-enkephalin (ME) inhibits the amplitude of evoked IPSC (eIPSC) in rat DMV neurons, without exogenous enhancement of cAMP levels. The ME-induced inhibition appears to be mediated by group II metabotropic glutamate receptors because this effect is prevented by the group II metabotropic GluR-selective agonist 2R, 4R-4-aminopyrrolidine-2,4-dicarboxylate (APDC). Although ME demonstrates no effect in brainstem slices of control rats, perfusion with the group II metabotropic GluR-selective antagonist 2s-α-ethylglutamic acid (EGLU) renders DMV neurons responsive to ME in brainstem slices of control rats. These data show that by tonically dampening the levels of cAMP within the GABAergic synaptic contacts, activated group II mGluRs prevent modulation of this synapse by endogenous opioids.

5. NEUROGENESIS

Neurogenesis in adult DVC neurons has long been considered limited due to the so called "logic" assumption that neurons in medulla are vital and should remain stable and well-protected from the influence of noxious factors such as inflammation and infection. This notion has been recently challenged by our findings and others' studies.

The potential of adult DVC neurons to regenerate in vivo and in vitro will be covered in detail in the following sections.

5.1 Presence of Neural Stem Cells in Adult DVC

Neural stem cells, precursor cells with self-renewal and multilineage potential, are present in the embryonic and adult mammalian forebrain. These mitotically active cells have been demonstrated within the subependyma of the adult forebrain lateral ventricles (Lois and

Alvarez-Buylla1993), in the olfactory bulb (Lois and Alvarez-Buylla 1994), within the dentate gyrus of the adult rodent hippocampus (Cameron et al.1993) and in the spinal cord (Weiss et al 1996). When explanted into culture, these precursor cells can generate neurons and glia (Lois and Alvarez-Buylla1993; Weiss et al. 1996). Similar self-renewing stem cells have been isolated from the third and fourth ventricles of adult mice (Weiss et al 1996). Unlike neural stem cells in the region of the lateral ventricles, primary multipotent stem cells isolated from the region of third and fourth ventricles require the combination of both epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) for the formation of neurospheres (Weiss et al 1996). In addition, the frequency of neurospheres generated from cells of third and fourth ventricles is markedly lower when compared with the lateral ventricles and spinal cord (Weiss et al 1996).

Using the in vitro neurosphere assay, Charrier et al (2006) have demonstrated that cells directly dissociated from adult DVC tissues by micro-dissection give rise to floating round-shaped clusters of cells, i.e. primary spheres. Relative to the neurospheres from the subventricular zone which can be identified 2-3 days in vitro, DVC primary spheres grow much slower and cannot be observed until 4-5 days in vitro. The yield of primary spheres from the DVC is 76±17 primary spheres per 10,000 viable cells relative to 257±29 per 10,000 viable cells from the subventricular zone. The growth capacity of the sphere-generating cells from adult DVC is estimated to be about 2/3 of those from the subventricular zone. The mean diameter of the DVC primary spheres is 50±3μm versus 73±2μm for those from the subventricular zone. Passage of primary neurospheres from the adult DVC yields secondary and tertiary neurospheres, clearly demonstrating the self-renewing ability of DVC colony-forming cells. When proliferation of secondary spheres is stopped by transferring single spheres onto poly-lysine-coated coverslips and culturing in a medium with no added growth factors, cells are able to differentiate into three individual lineages including β-III tubulin positive neurons, GFAP immunoreactive astrocytes and oligodendroglial progenies containing both immature NG2 positive progenitors as well as mature O4 positive oligodendrocytes. Regarding astrocyte and oligodendrocyte generation, DVC neurospheres are similar to those from subventricular zone. DVC and subventricular zone neurospheres strongly differ in their ability to generate neurons. Indeed, β-III tubulin immunoreactive neurons are only found in a small fraction of DVC neurospheres, while they could be observed in almost all neurospheres from subventricular zones.

The most direct evidence supporting the existence of multipotent neuronal precursor cells in DVC comes from the observation that colonies of neuron-like cells or glial-like cells form from dissociated single cell suspensions when plated in culture at clonal density. This density allows individual founder cells to form spatially distinct colonies so that colony composition can be assessed. In an unpublished study, we applied this technique to examine whether multipotent neural stem cells exist in DMV. DMV cells were cultured at clonal density. The colonies were examined daily. After 14 days in culture, cells were stained immunocytochemically for neuronal markers (Hu) and glial markers (GFAP). DMV cells cultured at the clonal density gave rise to colonies positive for either the neuronal marker (Hu), or the glial lineage marker (GFAP). These results demonstrate cells from both glial and neuronal lineages and suggest that multipotential stem cells exist in the DMV.

Taken together, these results demonstrate the existence of neural stem cells within the DVC. These DVC neural stem cells are likely candidates to participate in the generation of new neurons and astrocytes in the adult rat brainstem.

5.2 Role of Radial Glial Cells

The finding of neural stem cells in the floor of fourth ventricle bordering the DVC leads to another question: what is the nature of neural precursor cells in the adult DVC? Are these neural stem cells in the adult DVC similar to radial glial cells observed in the adult forebrain germinative centers (Alvarez-Buylla and Lim 2004)? In a study by Pecchi et al. (2007), glial fibrillary acidic protein (GFAP)-positive radial-like cells were identified in the vicinity of proliferative progenitors in the nucleus tractus solitarius of adult rat. Two types of GFAP-immunoreactive cells are present in the NST and DMV throughout their whole rostrocaudal extent. Most GFAP-positive cells exhibit typical features of differentiated stellate astrocytes. A small percentage of GFAP-immunoreactive cells with atypical morphology are found within NST. These cells possess cuboidally shaped cell bodies. GFAP-positive cell are located in the rostral part of the NST lining the 4th ventricle and their processes radiate rostrocaudally into the NST parenchyma. Double immunolabeling reveals that most of these GFAP positive cells co-express vimentin and nestin, two intermediary filament proteins known to be expressed by immature astrocytes (Wiese et al. 2004). These characteristics are shared with radial glial cells present in the developing nervous system. However, it is still unclear whether these radial glial cells in the adult DVC are capable of giving rise to neuronal cells. A study by Pecchi et al. (2007) demonstrates that newborn cells labeled with 5-bromo-2-deoxyuridine (BrdU) and Ki-67 are also positive for GFAP immunoreactivity in the adult DVC, suggesting that radial glial cells in adult DVC are capable of proliferating. Moreover, doublecortin, a 40-kDa microtubule-associated protein specifically expressed in neural precursor cells, is observed in close association with the radiating processes of GFAP positive cells after stimulation of neural proliferation in the caudal brainstem either by injection of growth factors or by NST deafferentation.

Radial glial cells in the adult DVC are proposed to serve as a scaffold for the migration of cells arising from the wall of the 4th ventricle. However, the possibility that radial glial cells in adult DVC give rise to newborn neural cells cannot be ruled out because colocalization of doublecortin and GFAP exists in a proportion of radial glial cells on the floor of fourth ventricle.

5.3 Neural Proliferation in Vivo

The existence of neural stem cells in the vicinity of DVC suggests that neurogenesis may occur in adult DVC. To address this question, we used BrdU labeling to examine neurogenesis in the adult rat medulla. BrdU administered intraperitoneally at a dose of 50 mg per kg of body weight twice daily for 6 days is able to cross the brain blood barrier and incorporate into cells during the DNA replication phase of the cell division cycle. Under normal conditions, BrdU labeling is rare in adult DVC. With cervical vagotomy, there is a significant increase in BrdU labeling in the ipsilateral NST and DMV of adult rats (Zhang et al 2004; Zhang et al 2005). Similar observation of BrdU labeled cells within adult DVC following vagal deafferentation has also been reported by Bauer et al. (2005). Using double immunofluorescent staining and confocal microscopy, BrdU labeled cells within adult DVC were further characterized as neural precursor cells. Several neuronal markers are found to co-localize with BrdU in the adult DVC. The neuronal nuclear antigen NeuN, which labels

mature neurons, is detected in 9.2% of total BrdU labeled cells in the DVC of adult rats with vagal deafferentation. The DVC also contains a few BrdU positive nuclei colocalizing with the RNA-associated protein HuC/D which is expressed by mature and immature neurons (Barami et al. 1995), and with the microtubule-associated protein doublecortin (DCX) which labels neuronal progenitors and migrating immature neurons of the adult hippocampus (Brown et al 2003). Nestin, an intermediate filament protein found in neuroepithelial precursor cells (Hockfield and McKay, 1985) and used as a marker of neural stem/progenitor cells, is also found to colocalize with some of BrdU labeling cells within the adult DVC. About 7% of BrdU labeled cells in the DVC are positive for S100, a marker for astrocytes. Taken together, these studies demonstrate that newly generated BrdU labeled cells in the adult rat DVC can differentiate into either neuronal or astrocytic cell types in vivo.

5.4 Neural Proliferationin Vitro

To further study mechanisms of neurogenesis, we have established an in vitro model of culture of DVC neurons (Zhang et al 2004; Zhang et al 2005). NST and DMV neuron cultures at 6–7 days contain mature neurons as demonstrated by expression of the neuronal marker, MAP-2, as well as precursor cells as demonstrated by expression of the immature neuronal marker, Hu. GFAP immunopositive cells in these cultures are rare, indicating that these cultures are free of contaminating astrocytes. When cultured neurons are induced to differentiate by growing in medium without βFGF, the number of fully differentiated neurons increases significantly as demonstrated by the increment of MAP-2 positive cells. DVC precursor cells cultured in vitro are actively proliferating as demonstrated by incorporation of BrdU. These mitotic cells, identified by BrdU labeling, also stain positive for HuC/D, suggesting neuronal characteristics.

5.5 Regulation of Neurogenesis

In a study by Weiss et al (1996), self-renewing and expandable spheres from the fourth ventricle region of adult mice were generated only with the addition of both EGF and basic FGF to the culture medium. Consistent with this observation, Bauer et al (2005) showed that formation of neurospheres derived from DVC tissues of adult rats is dependent on the presence of EGF and basic FGF. Removal of these two growth factors allows cultured neurospheres to differentiate into mature neurons, astrocytes and oligodendrocytes. These results indicate that EGF and basic FGF regulate the survival and self-renewal of neural stem cells within the adult DVC.

Ghrelin, a novel 28 amino acid peptide secreted by gastric oxyntic glands and circulated in blood, has also been demonstrated to stimulate neuronal proliferation in NST and DMV both in vivo and in vitro (Zhang et al 2004; Zhang et al 2005). mRNA corresponding to ghrelin receptor is detected in DVC tissues, while expression of ghrelin receptor is verified by localization of ghrelin receptor immunoreactivity in DVC neurons using a specific antibody against the C-terminal sequence 330–366 of the human ghrelin receptor 1a. Systemic administration of ghrelin increases vagotomy-induced BrdU incorporation in the NST and DMV in adult rats. In vitro, cultured NST and DMV neurons respond to ghrelin with

increased BrdU incorporation in both dose- and time-dependent manners. Pretreatment of cultured

DMV neurons with ghrelin receptor antagonists such as D-Lys3-GHRP-6 (growth hormone release peptide, GHRP) and [D-Arg1, D-Phe5,D-Trp(7,9),Leu11]-substance P (SP) significantly attenuates the mitotic effect of ghrelin (Figure 1). These data suggest that ghrelin is a neurotropic signal for DVC tissues.

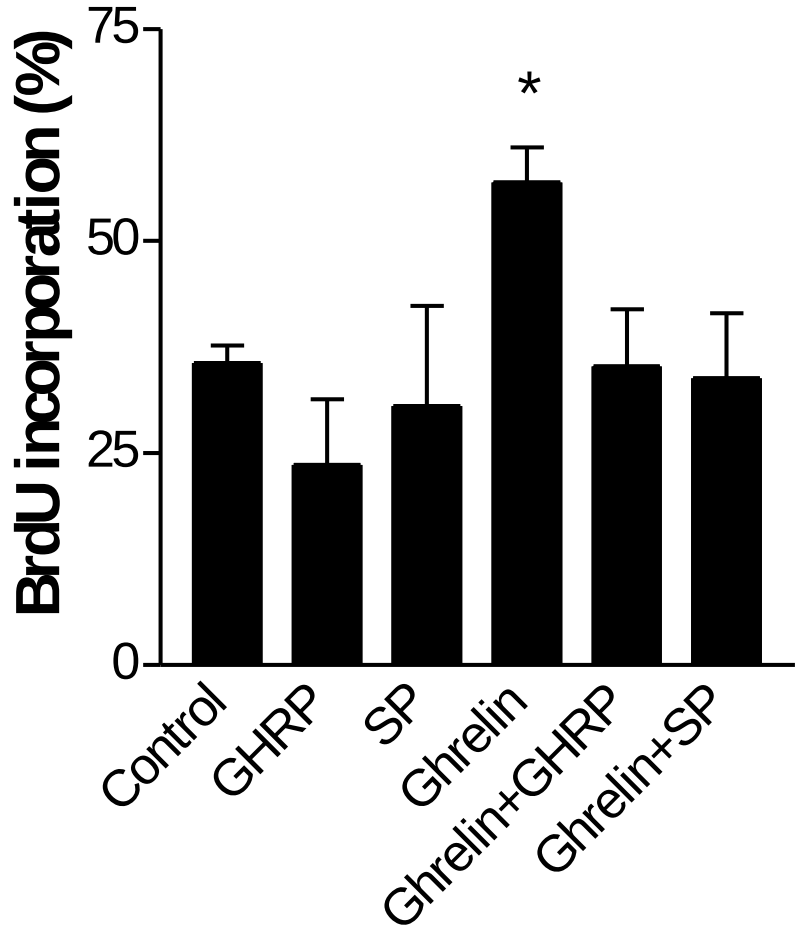

Figure 1. Antagonists of ghrelin receptor block the ghrelin-evoked increase in neuronal proliferation in cultured DMV cells.

Severe derangement of gut motility has been observed in gastrointestinal inflammation such as inflammatory bowel disease. The effects of intestinal inflammation on the DVC are largely unknown. A recent study demonstrates for the first time that systemic cytokines which

have been found to be elevated during intestinal inflammation may adversely affect the survival and proliferation of DMV neurons (Ammori et al. in press). Exposure of cultured DMV neurons to interleukin 1β (IL-1β), interleukin 6 (IL-6), or tumor necrosis factor α (TNF-α) significantly inhibits proliferation in a dose-dependent manner. The addition of ghrelin to cytokine treatment increased cellular proliferation compared to cytokine alone, returning proliferation rates to control levels.

6. CONCLUSION

The DVC has long been postulated to be an unchanging neural network. Emerging evidence now indicates that the DVC has the potential for activity-dependent plasticity. Diverse plasticity has been characterized at the cellular and molecular levels. These findings suggest that autonomic reflex adaptations in the adult involve plasticity mechanisms in the DVC (Berthoud 2003; Feldman et al. 2003). The link between the neuronal plasticity within the DVC and the control of digestive processes remains to be uncovered at the integrative physiological level. Future study should directly address the relation of DVC plasticity to experience-dependent adjustments of autonomic reflexes both in vitro and in vivo.

REFERENCES

Alvarez-Buylla A, Lim DA. *For the long run: maintaining germinal niches in the adult brain.* Neuron 2004; 41: 683-686.

Ammori JB, Zhang W, Li J-Y, Chai B-X, Mulholland MW. *Effects of Ghrelin on Neuronal Survival in Cells Derived from Dorsal Motor Nucleus of the Vagus*. Surgery. 2008;144:159-67.

Bauer S, Hay M, Amilhon B, Jean A, Moyse E. *In vivo neurogenesis in the dorsal vagal complex of the adult rat brainstem*. Neuroscience. 2005;130:75-90

Barami K, Iversen K, Furneaux, and Goldman SA. *Hu protein as an early marker of neuronal phenotypic differentiation by subependymal zone cells of the adult songbird forebrain*. J Neurobiol 1995; 28:82–101.

Berthoud HR. *Neural systems controlling food intake and energy balance in the modern world.* Curr. Opin. Clin. Nutr. Metab. Care 2003;6:615–620.

Brown JP, Couillard-Despres S, Cooper-Kuhn CM, Winkler J, Aigner L and Kuhn HG. *Transient expression of doublecortin during adult neurogenesis*. J Comp Neurol 2003; 467:1–10.

Browning KN, Zheng Z, Gettys TW, Travagli RA. *Vagal afferent control of opioidergic effects in rat brainstem circuits*. J Physiol. 2006;575:761-76.

Browning KN, Travagli RA. *Short-term receptor trafficking in the dorsal vagal complex: an overview*. Auton Neurosci. 2006;126-127:2-8.

Browning KN, Travagli RA. *Neuropeptide Y and peptide YY inhibit excitatory synaptic transmission in the rat dorsal motor nucleus of the vagus* J Physiol. 2003; 549:775-85

Browning KN, Travagli RA. *Characterization of the in vitro effects of 5-hydroxytryptamine (5-HT) on identified neurones of the rat dorsal motor nucleus of the vagus (DMV).* Br J Pharmacol. 1999;128:1307-15.

Browning KN, Kalyuzhny AE, Travagli RA. *Mu-opioid receptor trafficking on inhibitory synapses in the rat brainstem.* J Neurosci. 2004;24:7344-52.

Browning KN and Mendelowitz D. *Musings on the wanderer: what's new in our understanding of vago-vagal reflexes?* II integration of afferent signaling from the viscera by the nodose ganglia. Am J Physiol Gastrointest Liver Physiol 2003; 284:G8-14.

Cameron HA, Woolley CS, McEwen BS, Gould E. *Differentiation of newly born neurons and glia in the dentate gyrus of the adult rat.* Neuroscience 1993; 56:337-344.

Carr MJ, Hunter DD, Jacoby DB, Undem BJ. *Expression of tachykinins in nonnociceptive vagal afferent neurons during respiratory viral infection in guinea pigs*. Am J Respir Crit Care Med. 2002;165:1071-5.

Charrier C, Coronas V, Fombonne J, Roger M, Jean A, Krantic S and Moyse E. *Characterization of neural stem cells in the dorsal vagal complex of adult rat by in vivo proliferation labeling and in vitro neurosphere assay.* Neuroscience 2006;138:5-16.

Cunningham JT, Wachtel RE and Abboud FM. *Mechanosensitive curreNST in putative aortic baroreceptor neurons in vitro.* J Neurophysiol 1995;73:2094-2098.

Davis SF, Williams KW, Xu W, Glatzer NR, Smith BN. *Selective enhancement of synaptic inhibition by hypocretin (orexin) in rat vagal motor neurons: implications for autonomic regulation*. J Neurosci. 2003;23:3844-54.

Feldman JL, Mitchell GS and Nattie EE. *Breathing: rhythmicity, plasticity, chemosensitivity*. Annu Rev Neurosci 2003;26:239–266.

Gebhart GF, Bielefeldt K and Ozaki N. *Gastric hyperalgesia and changes in voltage gated sodium channel function in the rat.* Gut. 2002;51 Suppl 1: i15-8

Hay M and Kunze DL. *Glutamate metabotropic receptor inhibition of voltage-gated calcium curreNST in visceral sensory neurons*. J Neurophysiol 1994; 72:421-430.

Hamra M, McNeil RS, Runciman M and Kunze DL. *Opioid modulation of calcium current in cultured sensory neurons:m-modulation of baroreceptor input*. Am J Physiol Heart Circ Physiol 1999; 277: H705-713.

Hornby PJ. Receptors and transmission in the brain-gut axis. II. *Excitatory amino acid receptors in the brain-gut axis.*Am J Physiol Gastrointest Liver Physiol. 2001;280:G1055-60.

Lancaster E, Oh EJ, Weinreich D. *Vagotomy decreases excitability in primary vagal afferent somata*.J Neurophysiol. 2001;85:247-53

Lancaster E, Oh EJ, Gover T, Weinreich D.*Calcium and calcium-activated curreNST in vagotomized rat primary vagal afferent neurons* .J Physiol. 2002;540:543-56

Lois C, Alvarez-Buylla A. *Proliferating subventricular zone cells in the adult mammalian forebrain can differentiate into neurons and glia*. Proc Natl Acad Sci USA 1993; 90:2074-2077.

Lois C, Alvarez-Buylla A. *Long distance neuronal migration in the adult mammalian brain.* Science 1994; 264:1145-1148.

Mendelowitz D, Reynolds PJ and Anfresen MC. *Heterogeneous functional expression of calcium channels at sensory and synaptic regions in nodose neurons*. J Neurophysil 1995; 73: 872-875.

Moore KA, Undem BJ and Weinreich D. *Antigen inhalation unmasks NK-2 tachykinin receptor-mediated responses in vagal afferNST*. Am J Respir Crit Care Med 2000;161:232-236.

Myers AC, Kajekar R, Undem BJ. *Allergic inflammation-induced neuropeptide production in rapidly adapting afferent nerves in guinea pig airways*. Am J Physiol Lung Cell Mol Physiol 2002;282:L775-81.

Oh EJ, Weinreich D. *Chemical communication between vagal afferent somata in nodose Ganglia of the rat and the Guinea pig in vitro*. J Neurophysiol. 2002;87:2801-7.

Pecchi E, Dallaporta M, Charrier C, Pio J, Jean A, Moyse E, Troadec JD. *Glial fibrillary acidic protein (GFAP)-positive radial-like cells are present in the vicinity of proliferative progenitors in the nucleus tractus solitarius of adult rat.* J Comp Neurol. 2007;501:353-68.

Rhim H, Glaum SR and Miller RJ. *Selective opioid agonists modulate afferent transmission in the rat nucleus tractus solitarius.* J Pharmacol Exp Ther 1993; 264: 795-800.

Travagli RA, Hermann GE, Browning KN, Rogers RC. *Brainstem circuits regulating gastric function.* Annu Rev Physiol. 2006; 68:279-305

Undem BJ and Weinreich D. *Electrophysiological properties and chemosensitivity of guinea pig nodose ganglion neurons in vitro*. J Auton Nerv Syst 1993; 44:17-34.

Wan S, Browning KN, Travagli RA. *Glucagon-like peptide-1 modulates synaptic transmission to identified pancreas-projecting vagal motoneurons*. Peptides. 2007;28:2184-91

Weiss S, Dunne C, Hewson J, Wohl C, Wheatley M, Peterson AC, Reynolds BA. *Multipotent CNS stem cells are present in the adult mammalian spinal cord and ventricular neuroaxis.* J Neurosci. 1996;16:7599-609.

Wiese C, Rolletschek A, Kania G, Blyszczuk P, Tarasov KV, Tarasova Y, Wersto RP, Boheler KR, Wobus AM. *Nestin expression - a property of multi-lineage progenitor cells?* Cell Mol Life Sci 2004; 61:2510-2522.

Zhang W, Hu Y, Lin TR, Fan Y, Mulholland MW. *Stimulation of neurogenesis in rat nucleus of the solitary tract by ghrelin*.Peptides. 2005;26:2280-8

Zhang W, Lin TR, Hu Y, Fan Y, Zhao L, Stuenkel EL, Mulholland MW. *Ghrelin stimulates neurogenesis in the dorsal motor nucleus of the vagus.* J Physiol. 2004;559:729-37.

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Chapter 13

VAGUS NERVE STIMULATION IN FOOD INTAKE CONTROL

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ABSTRACT

The vagus nerve is the principal exponent of the parasympathetic nervous system. It is originated in the central nervous system and is distributed broadly through the thorax and abdominal viscera. It regulates a wide variety of organic functions such as heart rate, gastric emptying, gastric and intestinal secretion and motility, etc. Regarding microscopic anatomy, it should be highlighted that it is mainly composed of afferent fibres.

Due to its macroscopic and microscopic anatomic characteristics vagus nerve stimulation has been applied in different treatments; some of them broadly used and its possible effects widely demonstrated, such as in the refractory epilepsy (Vagus Nerve Stimulation) and depression. Some others have not been much studied, for instance, in pain, memory enhancement and food intake control.

Based on the exceptional situation of the vagus nerve, specifically the abdominal vagal afferent fibres originate from the conjunction of gastrointestinal tract receptors and they synapse within the central nervous system. This occurs predominately within the nucleus tractus solitarius from which second order fibres connecting to the limbic system and subcortical centres involved with satiety are originated. We attempt to act on the perception of satiety by either stimulating/inhibiting afferent and/or efferent vagal fibres in order to reduce food intake and, consequently, body weight.

Up to this point results have been controversial; in rabbits after a 21-day continuous stimulation food intake was reduced. However, in swine despite causing no alteration in food intake pattern, vagal nerve stimulation caused changes in systemic gatrin and insulin concentrations.

During our studies in this research line and based on the results observed in rabbits and swine, many questions have arisen and are still waiting to be answered. For instance, how does the vagus nerve respond to different stimulating voltages? How does the response to the stimulation change in time? Do the microscopical anatomical changes

(fibrosis, etc.) have any influence on the response to the stimulation? Should the stimulation parameters be changed in the course of time?

With this short communication we intend to show our experience in the abdominal vagus nerve stimulation to control food intake, and response the questions that have arisen during the development of this research line.

INTRODUCTION

The vagus nerve is the principle exponent of the parasympathetic nervous system. In order to understand the current interest caused by this nerve in the treatment of conditions as diverse as epilepsy, depression, pain, obesity, etc., it is fundamental to be aware of its ample distribution, through central nerve nuclei, and through thoracic and abdominal viscera.

The vagus nerve leaves the medulla by means of the retro-olivary fossa. It then descends through the neck, accompanying the carotid artery to the thorax, where it is located on both sides of the oesophagus, and is known as the left and right vagus nerve. From here it gives out branches that go towards the heart and the respiratory tract. Before crossing the oesophageal hiatus, the left and right vagus nerves merge, creating the anterior/ventral and posterior/dorsal vagal trunks. Fibres of the right vagus nerve are predominant in the dorsal or posterior vagal trunks, and *vice versa* [1,2]

In turn, the anterior or ventral vagal trunk gives rise to the common hepatic branch, the accessory celiac branches and the ventral branch. The latter supplies innervation to the ventral face of the stomach, to the circular musculature of the pyloric sphincter and to the proximal duodenum.

The dorsal vagal trunk provides the dorsal or posterior gastric branch and the dorsal celiac branch. The former innervates the stomach and the duodenum, while the dorsal celiac branch joins the ventral celiac branch close to the source of the left gastric artery and they innervate the small and large intestine.

As regards the distribution of the afferent fibres through the central nervous nuclei, more specifically, the sensations coming from the gastrointestinal tract synapse in the nodular ganglia, which sends information to the following central nervous system centres (mainly through the nucleus of the solitary tract –NTS-):

- The dorsal motor nucleus of the vagus nerve [3-5], the ambiguous nucleus and the intermediolateral columns [*6*].
- The motor nuclei of the cranial nerves [*4]*.
- The parabrachial nucleus, which, in turn, sends information to most of the centres related to the NTS and the pre-frontal and insular areas of the cerebral cortex [*7]*. The latter seem to form the substrate for conscious visceral sensations.
- The fourth and last category that receives projections from the NTS is the nuclei of the hypothalamus and the areas adjacent to the limbic area [8]. The limbic system is, in turn, linked to the stria terminalis [*9]* and the central nucleus of the amygdale [5,10]. All of the above centres play an important role in the integration of metabolic, neuroendocrinal and behavioural responses involved in homeostasis. The projections of the NTS to the lateral reticular formation, specifically the ambiguous nucleus, like

the dorsal motor nucleus of the vagus nerve, are related to the autonomous cardiorespiratory and alimentary reflexes [3].

Another factor that has contributed to applying the stimulation of the said nerve to treat conditions whose origins are located in the central nervous system is its microscopic characteristics.

The vagus nerve of mammals is composed of 80-90% afferent fibres. According to *Asala et al* [*11]*, only 23% of the efferent fibres of the cervical vagus nerve reach the abdomen and 50% of the afferent vagal fibres originate in the abdomen.

Taking into account the ample distribution of the vagus nerve, it is not surprising that it participates in numerous physiological functions and that its stimulation can have an effect on the activity of the central nervous system.

In 1973, *Cooper et al [12]* developed the process of cerebral stimulation for the treatment of epilepsy. Years later, the lack of favourable results and the inconvenient nature of intracranial surgery limit its use.

The theoretical bases of cerebral stimulation will be dealt with once more at a later stage, but this time they will be applied to the stimulation of the vagus nerve. Contrary to what would happen with cerebral stimulation, the vagus nerve acts as a conductor, thereby facilitating the dispersal of the impulse all over the brain and not only in the areas where the electrodes were placed.

Bailey et al [*13]* were the first to describe how vagal nerve stimulation (VNS) in cats lead to the synchronisation of the orbital cortex. *Zabara [14,15]* subsequently demonstrated the anticonvulsive action of the VNS. Finally, and supported by the positive results obtained in animal models, in 1988, VNS was applied to humans [*16]*.

The application of VNS in the treatment of epilepsy was extended to the treatment of depression. It has also been demonstrated that VNS can facilitate or inhibit nociceptive sensations according to the parameters applied during stimulation. Therefore, high intensity electrical impulses inhibit pain, while low intensity impulses accentuate it [17].

The benefits of VNS in memorisation processes have also been described [*18]*. The last application -the line of research developed in this study- of vagus nerve stimulation, is to control the food intake, for the purpose of weight loss.

The latter application is becoming more and more fashionable, since obesity is considered to be difficult to prevent and challenging to treat.

The world health organization estimates that a billion adults have a body mass index greater than 25kg/m and 300 million people are obese, with a BMI of over 30kg/m2 [19].

The importance of obesity as a public health problem stems from the morbidity and the healthcare costs generated by it. On the one hand, being overweight is a primary risk factor for a large number of illnesses. Top of this list are cardiovascular conditions, stroke, hypertension and diabetes [20,21]. It is also associated with diseases of the gall-bladder (cholethiasis), urinary incontinence, increased uric acid, hormonal problems in women, sleep apnoea [*22]*, degenerative diseases of the joints (arthrosis of the extremities and lumbar region) and certain forms of cancer [23].

As regards the healthcare costs arising from obesity, it has been estimated that the health costs of overweight and obese persons are associated with approximately 10% of national healthcare expenditure [24].

The most appropriate therapeutic option in cases of morbid obesity is surgical treatment (bariatric surgery). Although this can lead to significant weight loss, reducing the cardiovascular risk factors [*25]* and alleviate associated conditions such as diabetes mellitus [*26]*, hyperlipidemia, etc., these techniques are not without complications, such as a deficit of nutrients, failure of the staples [*27]*, vomiting [28], etc. As a result, new, more effective and less traumatic medical and surgical treatments continue to be researched.

The most recent research has developed a subcutaneous pacemaker, whose electrodes are implanted in the pyloric antrum, in order to induce an early sensation of satiety [*29,30]*. Related to this technique, and based on studies of vagal stimulation in the treatment of epilepsy, according to *Reddy et al* [*31]* the stimulation of the vagus nerve in dogs provokes changes in food behaviour, i.e. it favours lower food intake and as a result leads to weight loss.

As described in the section on the topographical anatomy of the vagus nerve, it is not surprising that the stimulation of that nerve leads to changes in food intake, since the vagus nerve is distributed through nuclei in the central nervous system involved in satiety and through the digestive tract, meaning that the stimulation of the nerve, as demonstrated by various authors -*Reddy et al [31] and Roslin et al [32]* in dogs, *Krolczyk et at [33]* in rats *and Sobocki et al [34,35]* in rabbits-, could lead to changes in motor or sensory functions of the gastrointestinal tract, and/or satiety.

The principle objective of the study is to control food intake. When stimulating the vagus nerve, we should provoke an excitation/inhibition of the upper centres related to the sensation of satiety, and/or inhibiting/exciting motor neurons (neurons that synapse with the smooth gastric and intestinal muscle cells), and/or the gastric and intestinal secretor cells. In the event of achieving a reduction in food intake, the electrical vagal stimulation will entail an innovative therapeutic option in the treatment of morbid obesity.

The second objective is to describe the action mechanisms involved in the changes caused by electrical vagal stimulation on daily food intake. Although numerous hormones are involved in the sensation of satiety, in our experimental work we studied the variation in the concentration of three hormones that are strictly linked to the stimulation of satiety and with the vagus nerve: cholecystokinin (CCK), gastrin and insulin.

The third objective pursued was to assess the possible lesions caused by the mechanical action of the electrodes and the electrical activity of the parameters of stimulation on the vagus nerve, in its thoracic and abdominal trajectories, while also assessing the development of the lesions in time.

RESULTS AND DISCUSSION

Based on the results obtained with the stimulation of the vagus nerve -in our initial experimental study on rabbits-, we achieved a reduction in food intake and body weight, it was proposed to continue with the application of vagal nerve stimulation in the treatment of morbid obesity in a porcine model (figure 1), physiologically closer to humans, and determining, if not all, several of the mechanisms of action of food intake reduction in rabbits. The possible advantages offered by electromodulation as compared with bariatric surgery are based on the following: 1) this is a less aggressive and reversible technique, 2) the

surgery time is shorter and 3) the postoperative is more comfortable. It is possible that all of these circumstances would manifest themselves in post-operative recuperation that is more advantageous for the patient.

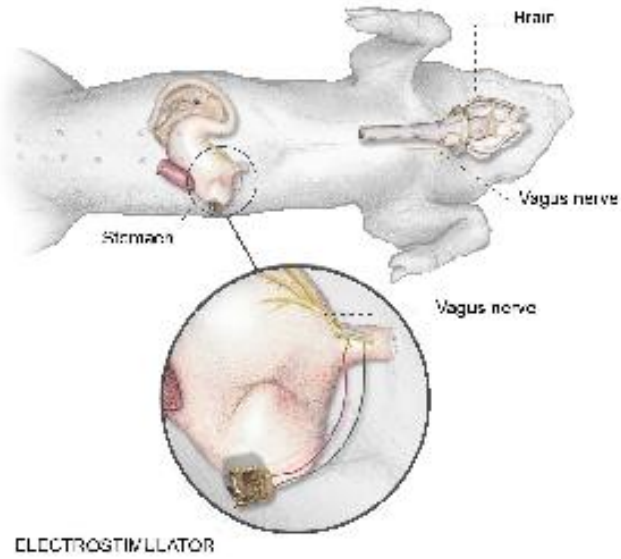

Figure 1. Electroestimulation localization in the porcine model.

Electrosimulation was applied to the vagus nerve by virtue of its strategic situation. The vagus nerve serves as a point of union between the gastrointestinal tract and the central nervous system. Therefore, the vagus nerve is linked to the centres of satiety, such as the hypothalamus and the area postrema. The neurons located in these areas translate the humour-related information from insulin and leptin [36-38], and regulate energy homeostasis, food intake and weight. These neurons stimulate the autonomic nervous system that regulates alimentary behaviour and metabolism [39]. The vagus nerve also transmits motor and sensory information from the digestive tract, in the second case.

As regards the results obtained by our research team, it should be stressed that in the experimental work on rabbits, the stimulation of the vagal trunk led to a 12% and 40% reduction in feed consumed and body weight, respectively, when the cathode was positioned cranial to the to the anode (Group B), while in the opposite position (Group A), there was an increase in weight and feed consumed [34,35]. Furthermore in another study in rabbits a non significant differences in food intake were seen in the preoperative period (15 days) between groups A and B (218.23±11.96 *vs* 244.78±11.40). Similarly, no significant difference was evidenced between pre and postoperative food intake measurements in group A (218.23±11.96 *vs* 246.39±40). The amount of food ingested by group B during the postoperative period (21 days), however, was significantly lower when compared to preoperative ingestion recordings (244.78±11.40 *vs* 191.43±13.04, p=0.01), with food consumed during the postoperative interval in this group representing 78.22 % of the preoperative interval food intake.

In this study heart rate (table 1), Biespectral index (BIS) (table 1) and systemic gastrin (table 2) were assessed, and their relationship with vagal nerve impulses studied in order to

determine whether the food intake decrease was related to afferent or efferent fibres involvement.

Regarding anaesthesia parameters, when comparing mean heart rate values before and after electroestimulator implantation and under general anaesthesia a significant decrease in this parameter was evidenced after implantation in group A (p=0.04) whereas this decrease did not reach statistical significance in group B (Table 1).

Table 1. Mean Biespectral index values under general anaesthesia (mean±SEM) variation in group A and B, before and during stimulation (0.5V, 0.5Hz and 10 miliseconds pulse width).

	Group	A	B
Heart rate	Prestimulation period (30 minutes)	248.13±2.70	229.93±7.22*
	Postestimulation period (30 minutes)	231.85±4.68#	220.81±3.03
BIS	Prestimulation period (30 minutes)	42.00±2.49	49.07±2.62
	Postestimulation period (30 minutes)	40.70±1.37	54.25±1.72*

(*) Statistical significance between groups.
(#)Statistical significance between periods.
Group A. Anode cranial to cathode. Stimulation parameters (0.5V, 0.5Hz, 10 milliseconds pulse width)
Group B. Cathode cranial to anode. Stimulation parameters (0.5V, 0.5Hz, 10 milliseconds pulse width)

In our opinion, the decrease observed in heart rate after electroestimulation implantation was due to vagus nerve manipulation, and not to the stimulation, as has been previously suggested by *Matheny and Shaar* [40]. Nevertheless it could also be possible that stimulating with the anode cranial to cathode as occurs in group A helps to decrease heart rate.

Although the bispectral index has been developed to obtain the degree of sedation and hypnosis, a positive relation between BIS index and metabolic activity of the central nervous system has been reported, with BIS values increasing with increasing cerebral cortex [41,42] or even limbic system metabolism.

BIS values obtained from group A did not present significant differences between preimplantation and postimplantation intervals (table 1), thus there is little evidence in this study that this kind of stimulation acts on the central nervous system. Group B's BIS recordings, however, augmented in the postimplantation period (table 1), with values greater than those obtained at the same period in the group A ($p<0.05$). Our findings suggest that the food intake reduction could be caused by the stimulation of central nervous nuclei involved with hunger and satiety. This finding can be considered logical, since 80% of the total abdominal vagus nerve fibers are afferent [1].

Systemic gastrin concentration measured over time evidenced an increase in group A and a decrease in group B (Table 2). Group A measurements were higher than group B in all cases. This difference, however, did not reach statistical significance.

We found no explanation to the greater gastrin baseline value obtained in group A. Special care was exerted during this study to control known factors that could change gastrin release, such as time of the day when the surgery was performed, sex, etc. All these variables were the same in both groups.

Table 2. Systemic gastrin values under general anaesthesia (mean±SEM) measured in groups A and B, before and during stimulation (0.5V, 0.5Hz and 10 miliseconds pulse width).

	Systemic gastrin (pmol/l)	
	Group A	Group B
T0	98.72±42.34	80.64±33.99
T1	106.66±46.95	79.94±34.37
T2	103.88±51.25	75.58±37.59
T3	108.04±42.30	66.05±31.70
T4	116.72±37.40	67.17±47.49

Group A. Anode cranial to cathode. Stimulation parameters (0.5V, 0.5Hz, 10 milliseconds pulse width)
Group B. Cathode cranial to anode. Stimulation parameters (0.5V, 0.5Hz, 10 milliseconds pulse width)
T0: Baseline. Immediately before vagal nerve dissection and intraoral test.
T1: Immediately after electrostimulation implantation.
T2: 15 minutes after T1.
T3: 15 minutes after T2.
T4: 15 minutes after T3.

The decrease in systemic gastrin observed on group B could be related to stimulation of the gastrin inhibiting system. Prior studies have reported that vagus nerve stimulation provokes an increase in gastrin concentration (43), whereas other investigators have published contradictory results. According to *Qvigstad G K et al* [44] vagotomy effects on gastrin release are inhibited by atropine, as opposed to the effect of atropine administration when the vagus is intact. These results, along with those reported by Debas et al [45], indicate that inhibitory and excitatory cholinergic mechanisms exist.

In our studies on porcine models, and unlike the results obtained in rabbits, the animals did not undergo changes in the quantity of feed consumed or in their body weight in the 20 days following the implantation of the electrostimulator [46], however changes were seen in the concentration of various hormones related to satiety and the vagus nerve (insulin, gastrin and cholecystokinin) [47]. The stimulation of the vagus nerve increased the basal concentration of insulin and reduced the post-prandial response to insulin. Also, in this study and in accordance with *Smith et al* [48], the group that was subjected to stimulation showed an increase in the concentration of gastrin throughout the period of the study. Finally, unlike *Lewis et al* [49] and in accordance with *Kim et al* [50], the stimulation of the vagus nerve increased the basal concentration of cholecystokinin.

The difference in the results we have described in rabbits and pigs, and in relation to the works published on the stimulation of the vagus nerve in controlling food intake, we mostly attribute it to the different stimulation protocol used.

Therefore, *Reddy et al* [31] and *Roslin et al* [32] applied different nerve stimulation parameters, the main reason behind the different results observed in our study. While we used

the same stimulation parameters as *Krolczyk et al* [33] in rats and *Sobocki et al* [34,35] in rabbits, without taking into account the fact that the diameter of the vagal trunk between species varied considerably, mostly in relation to the porcine species. As a result of the difference in diameter, the resistance varied from one nerve to another, and, together with the stimulation «of voltage» (we apply constant voltage), the intensity of the impulse transmitted was therefore different in the course of time. Therefore, different types of fibres were being stimulated, in relation to the direction in which the impulse was transmitted (afferent and efferent) and fibres with different functions, thereby leading to different effects.

As described by *Andrews et al* [51], the stimulation parameters are a fundamental factor in the selection of nerve fibres to be stimulated, the stimulation threshold is less in fast conduction fibres, and, on the other hand, the frequency of stimulation that follows slow conduction fibres will be lower than that of fast conduction fibres.

In parallel to the study of the variation in the food intake pattern and body weight pattern, morphological studies of the healthy vagus nerve were carried out, and of the nerve following stimulation. More specifically, the study involving rabbits showed that the stimulation caused lesions, according to the direction in which the impulse was transmitted. These were less serious in the group in which a reduction food intake was achieved (group B). These differences were significantly greater at the implantation site in group A when comparing inflammation (U Mann Whitney=4. Sig 0.045), fibrosis (U Mann Whitney=3. Sig 0.031) and total lesion score (U Mann Whitney=2. Sig. 0.024). This suggesting that this reduction was not due to the lesions of the vagus nerve, which would have a similar effect to that of a vagotomy, with the subsequent reduction in the speed at which the stomach empties.

In the study involving pigs [52], the magnitude of the lesions was lesser, and no sample showed signs of necrosis. One of the most important histopathological and macroscopic findings was the significant increase in the connective tissue of the vagus nerve, in the area where the electrodes were fitted. These findings were related to the damage caused by the mechanical effect. The mechanical damage caused a thickening of the epineurium, as a result of the accumulation of connective tissue in the portion of the nerve that came into contact with the electrodes. Although the functional significance is not entirely defined, in our opinion, as demonstrated by *Agnew et al* [53], this finding would seem to indicate that the impedance of the nerve varies with time. We believe that the increase in nerve tissue could lead to an increase in the stimulation threshold, which, as in the study conducted by *Rodriguez et al* [54] on the sciatic nerve, would happen 35-45 days after fitting the electrodes,

CONCLUSION

According to our experiments, it is essential to assess the implication of the extent of the stimulus on the effects on alimentary patterns and the mechanisms through which the stimulation of the vagus nerve reduces food intake. As with VNS, the active mechanisms through which this reduction in weight is achieved are as yet unknown, as are the possible side-effects, such as an increase gastric output, changes in gastric emptying, etc., as a result of prolonged stimulation.

It also have to be studied more deeply how the histological and functional characteristics of the nerve fibres vary with electrostimulation. It may happen, therefore, that in the time

course of the stimulation of the vagus nerve stops functioning, and therefore a non-continuous form of stimulation will have to be prepared, also with a view to reducing the effects of fibrosis or degeneration of the nerve. There is very little literature relating to histopathological lesions caused to the nerve during stimulation, including VNS, which has already been applied for several years.

In summary, further studies that need to be conducted involve long-term studies to assess tolerance, the effect on the nerve, the mechanisms of action and the side-effects.

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REFERENCES

[1] Evans DHL, Murray JG, *Histological and functional studies on the fibre composition of the vagus nerve of the rabbit.* J Anat 1954;88(3):320-337.

[2] Qian BF, El-Salhy M, Danielsson A, Shalaby A, Axelsson H. *Effects of unilateral cervical vagotomy on antral endocrine cells in mouse.* Histol Histopathol 1999;14:705-709.

[3] Morest DK. *Experimental study of the projections of the nucleus of the tractus solitarius and the area postrema in the cat.* J Comp Neur 1967:130(4);277-300.

[4] Norgren R. *Projections from the nucleus of the solitary tract in the rat*. Neurosci 1978;3:207-218.

[5] Ricardo JA, Kohn ET. *Anatomical evidence of direct projections from the nucleus of the solitary tract to the hypothalamus, amygdala, and other forebrain structures in the rat.* Brain Res 1978;153:1-26.

[6] Loewy AD, Burton H. *Nuclei of the solitary tract: efferent projections to the lower brain stem and spinal cord of the cat*. J Compo Neurol 1978;181:421-450.

[7] Saper CB, Loewy AD. *Efferent connections of the parabrachial nucleus in the rat.* Brain Res 1980;197:291-317.

[8] Berthoud HR, Jedrzejewska A, Bowley TL. *Simultaneous labelling of vagal innervation of the gut and afferent projections form the visceral forebrain with Dil injected into the dorsal vagal complex in the rat*. J Comp Neurol 1990;301:65-79.

[9] Holstege G. *Some anatomical observations on the projections from the hypothalamus to brainstem and spinal cord. An HRP and autoradiographic tracing study in the cat*. J Comp Neurol 1987;260:98-126.

[10] Higgins GA, Schwaber JS. *Somatostatinergic projections form the central nucleus of the amygdala to the vagal nuclei.* Peptides 1983;4:657-662.

[11] Asala SA, Bower AJ. *An electron microscope study of the vagus nerve composition*. Anat Embryol 1986;175:247-253.

[12] Cooper IS, Amin I, Gilman S. *The effect of chronic cerebellar stimulation upon epilepsy in man.* Transactions of American Neurological Association 1973;98:192-6.

[13] Bailey P, Bremer F. *A sensory cortical representation of the vagus nerve.* J Neurophysiol 1938;405-412.
[14] Zabara J. *Peripheral control of hypersynchronous discharge in epilepsy (Abstract).* Electroencephalogr Clin Neruophysiol 1985;61:162
[15] Zabara J. *Inhibition of experimental seizures in canines by repetitive vagal stimulation*. Epilepsia 1992;33:1005-12
[16] Penry JK, Dean JC. *Prevention of intractable partial seizures by intermittent vagal stimulation in humans*: Preliminary results. Epilepsia 1990;31:S40-S43.
[17] Ness TJ, Fillingim RB, Randich A, Backensto EM, Faught E. *Low intensity vagal nerve stimulation lowers human thermal pain thresholds.* Pain 2000;86:81-85.
[18] Clark KB, Krahl SE, Smith DC, Jensen RA. *Postraining unilateral vagal stimulation enhances retention performance in the rat*. Neurobiol Lear Mem 1995;63:213-6.
[19] (http://www.who.int).
[20] Rashid MN, Fuentes F, Touchon RC, Wehner PS. *Obesity and the risk for cardiovascular disease.* Prev Cardiol 2003;6(1):42-7.
[21] Scheen AJ. *Treatment of diabetes in patient with severe obesity*. Biomed Pharmocother 2000;54(2):74-79.
[22] Borys JM, Boute D. *Obstructive sleep apnoea syndrome: a frequent complication of obesity.* Biomed Pharmacother 1994;48(3-4):137-41.
[23] Brown P, Allen AR. *Obesity linked to some forms of cancer*. W V Med J 2002;98(6):271-2.
[24] Koplan JP, Dietz WH. *Caloric imbalance and public health policy*. J Am Med Ass 1999;282:1579-1581.
[25] Foley EF, Bentti PN, Borlase BC, Hollingshead J, Blackburn GL. *Impact of gastric restrictive surgery on hypertension in the morbid obese*. Am J Surg 1992;163:294.
[26] McDonald KG, Long S, Swanson MS. *The gastric bypass operation reduces the progression and mortality of non-insulin dependent diabetes mellitus*. J Gastrointest Surg 1997;1:213-220.
[27] Baltasar A, Bou R, Del Rio J. Cirugía bariátrica: *Resultados a largo plazo de la gastroplastia vertical anillada. ¿Una esperanza frustrada?*. Cir Esp 1997;62:175-179.
[28] Kriwanek S, Blauienseiner W, Lwbisch E, Beckerhinn P, Roka R. *Dietary changes after vertical banded gastroplasty.* Obes Surg 2000;10:37-40.
[29] Cigaina V, Saggioro A, Rigo V, Pinato GP, Ischia S. *Long term effects of gastric pacing reduce feed intake in swine.* Obes Surg 1996;6:250-253.
[30] Cigaina V. *Gastric pacing as therapy for morbid obesity: preliminary results*. Obes Surg 2002;12 Suppl 1:12S-16S.
[31] Reddy R, Horovita J, Roslin M. *Chronic bilateral vagus nerve stimulation changes eating behaviour resulting in weight loss in canine model*. Obes Res 1999;7(Suppl 1);82S.
[32] Roslin M, Kurian M. *The use of electrical stimulation of the vagus nerve to treat morbid obesity*. Epilepsy and Behav 2001;2:S11-6.
[33] Krolczyk G, Zurowski D, Sobocki J, Slowiaczek MP, Lasdkiewicz J, Matyja A, Zarasla K, Araska W, Thor PJ. *Effect of continuous microchip (MC) vagal neuromodulation on gastrointestinal function in rats.* J Physiol Pharmacol 2001;52(4):705-715.
[34] Sobocki J, et al. *Microchip vagal pacing reduces food intake and body mass*. Hepatogastroenterology 2001;48(42):1783-7.

[35] Sobocki J, et al. *The cybergut. An experimental study on permanent microchip neuromodulation for control of gut function*. Acta Chir Belg 2002;102:68-70.

[36] Ahrem B, Taborsky GJ. *The mechanism of vagal nerve stimulation of glucagon and insulin secretion in the dog.* Endocrinology 1986;118(4):1551-7.

[37] Holst JJ, Schwartz TW, Knuhtsen S, Jensen SL, Nielsen OV. *Autonomic nervous control of the endocrine secretion from the isolated, perfused pig pancreas*. J Auton Syst 1986 ;17(1):71-84.

[38] Ionescu E, Jeanrenaud B. *Effect of electrical stimulation of the vagus nerve on insulinemia and glycemia in Acomys cahirinus mice*. Endocrinology 1988;123(2):885-90.

[39] Bray GA, Inoue S, Nishizawa Y. *Hypothalamic obesity: autonomic hypothesis and the lateral hypothalamus.* Diabetologia 1981;20:366-67.

[40] Matheny RG, Shaar CJ. *Vagus nerve stimulation as a method of temporarily slow or arrest heart.* Ann Thoracic Surg 1997;63(Suppl6):S28-9.

[41] Kearse LA, Manberg P, DeBros F, Chamoun N, Sinali V. *Bispectral análisis of the electroencepahlogram during induction of anaesthesia may predict hemodynamic responses to laryngoscopy and intubation.* Electro and Clin Neurophysiol 1994;90:194-200.

[42] Alkire MT, Barker SJ, Haer RJ, Fallon JH, Jacobsen BS. Shah NK, et al. *Correlation of processed EEG variables with regional cerebral metabolism during propofol anesthesia*. Anesthesiology 995;83(3A):191.

[43] Olesen M, Holst JJ, Sottimano C, Nielsen. *Autonomic Nervous Control of fundic secretion of Somatostatin and antral secretion of gastrin and somatostatin in Pigs*. Digestion 1987;36:24-35.

[44] Qvigstad G, Bijorgaas M, Eide I, Sandvik AK, Woldum HL. *Vagal stimulation augments maximal (penta)gastrin stimulated acid secretion in human. Acta Physiol Scand 1999;165:277-281.*

[45] Debas HT, Hollinshead J, Seal A, Soon-Shiong P, Walsh JH. *Vagal control of gastrin release in the dog: pathways for stimulation and inhibition.* Surgery 1984;95(1):34-37.

[46] Díaz-Güemes I, Sánchez FM, Luis L, Sun F, Pascual S, Usón J. *Continuous Vagal nerve stimulation effect on gut-brain axis in swine*. Neuromodulation 2007;10(1):52-58.

[47] Díaz-Güemes I, et al. *Effect of subthreshold stimulation of vagal nerve on food intake pattern in swine.* 9th International Functional Electrical Stimulation Society Annual Conference. Bournemouth. UK. 5-9 september 2004.

[48] Smith CL, et al. *Control factors in the release of gastrin by direct electrical stimulation of the vagus.* Dig Dis 1975;20(1):13-22.

[49] Lewis LD, Williams JA. *Regulation od cholecystokinin secretion gby food hormones, and neural pathways in the rat.* Am J Physiol 1990;258:G512-8.

[50] Kim CK, et al. *Role of endogenous cholecistokinin on vagally induced pancreatic secretion in dogs.* Am J Physiol 1989;257:G944-9.

[51] Andrews PLR, Taylor TV. *An electrophysiological study of the posterior vagus nerve in man*. Clin Sci 1982;63:169-173.

[52] Díaz-Güemes Martín-Portugués I, Sánchez Margallo FM, Pascual Sánchez Gijón, Crisóstomo Ayala and Usón Gargallo J. *Histophatologic features of the vagus nerve alter electrical stimulation in swine.* Histology and Histopathology 2005;20(3):851-856.

[53] Agnew WF, McCreery DB. *Considerations for safety with chronically implanted nerve electrodes*. Epilepsia 1990;31 (Suppl 2):S27-32.

[54] Rodríguez FJ, et al. *Polymide cuff electrodes for peripheral nerve stimulation*. J Neurosci Methods 2000;98:105-118.

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Chapter 14

AMINO ACID-SENSING BY THE ABDOMINAL VAGUS

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ABSTRACT

Recent advancement in molecular biology in the field of taste perception has raised the possibility for ingested nutrients to be *tasted* in the upper gastrointestinal tract as well as tongue. Many works suggest that the individual 20 amino acid including glutamate can be detected by the vagus afferent within the duodenum. Recently, it was reveled that the rat gastric branch of the vagus nerve could specifically detect a non-essential amino acid, glutamate. The glutamate signaling could be transferred to the vagus nerve via mucosal chemical substances such as NO and serotonin. That leaded us to hypothesize that amino acid-sensing pathway exist in the gastric mucosa, like observed in the chemical sensing systems similar to the one functioning in the tongue and intestine. In this review, we summarized current status of gut amino acid-sensing research and possible significance of the amino acid induced visceral information in the body nutrient homeostasis.

Keywords: vagal afferent, serotonin (5-HT), cholesystokinin (CCK), amino acid, glutamate, visceral information, umami taste, nitric oxide (NO)

INTRODUCTION

Nutrient signal via afferent abdominal vagus induced vicero-viceral reflex and viceroy-somatic reflex, and regulated food intake, gastrointestinal motility, metabolic activity. In the stomach and the intestine, the dietary proteins are digested to amino acids and oligopeptides,

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and absorbed into blood within intestinal mucosa. Individual amino acid is sensed by the vagal afferents innervated into the gastrointestinal mucosa, portal vein and liver, and its nutrient information is transferred to the brainstem. So, the abdominal vagus is predominantly consisted of afferent fibers (the ratio of afferent/efferent fiber is about 10/1), so one might consider this a predominantly sensory nerve. Vagal cell bodies lie in the nodose ganglia and project centrally to the nucleus of the solitary tract (NTS) in the brainstem from where information is processed and relayed to higher brain areas (Schwartz, 2006 for review). Consequently, its signal regulates food intake, gastrointestinal motility, metabolic activity (Mei, 1985 for review; Schwartz, 2000 for review).

What amino acids are percepted within the gastrointestinal canal and its information is transferred to the vagal afferent in the individual organs? In the intestine, there are many reports about various amino acid- and oligopeptide-sensings, and is going to be revealed its mechanisms (Darcel et al., 2005; Mei, 1985 for review; Niijima, 2000; Niijima et al., 2005). Also, there are some reports about amino acid-sensing by hepatic vagal afferent (Niijima, 2000; Niijima et al., 2005; Tanaka et al., 1986, 1990). In the gastric vagal afferent, it has been known that the afferent nerve could detect only gastric distention but failed to catch the luminal nutrient information such as a carbohydrate and protein (Mathis et al., 1998). However, we discovered that the gastric vagal afferent was stimulated by the luminal glutamate (Niijima, 2000) and the stimulating effect was observed only glutamate among 20 amino acids, which compose dietary proteins (Uneyama et al., 2006a). We can know few evidence on the mucosal chemical transduction of the gut nutrient-sensing including amino acid-sensing is still unclear, except involvement of major gut hormone, cholecystokinin (CCK) and the monoamine serotonin (5-HT) (Zhu et al., 2001; Lal et al., 2001; Uneyama et al., 2006a), which activate specific receptors on primary afferent nerve terminals (Grundy, 2004 for review; Raybould, 2002 for review).

In this chapter, we summarized current status of gut amino acid-sensing research and discussed possible significance of the amino acid-induced visceral information in the body nutrient homeostasis via vago-vagal reflex. In addition, we summarized the property of vagal afferent response of the nutrient transmitter within the alimentary tract, CCK and 5-HT.

AMINO ACID-SENSING IN THE INTESTINE

In the intestine, most of dietary proteins are digested to peptides and amino acids by proteases, and absorbed into epithelial cell. So, abundant amino acids exist in the intestinal lumina after protein intake. There are many reports that intra-duodenal infusion of amino acid or oligopeptide changes the intestinal afferent activity. Sharma and Nasset (1962) showed an apparently increased mesenteric afferent activity in either the whole nerve or multifiber preparations of gastrointestinal tract following to amino acid infusions in the cat (Table 1). Using a unitary recording technique in the nodose ganglia, Jeannigros and colleague (1980, 1982) subsequently revealed in detail the afferent response of the vagus nerve to amino acid infusions in the cat small intestine. The report described many sensors sensitive to arginine, leucine and others (Table 1). Recently, we re-examined luminal amino acid sensitivity for the celiac vagal afferents in the rat (Table 1). Typical response of isotonic solution of each amino acid was shown in Figure1. Intra-duodenal infusion of alanine, arginine, leucine, lysine,

serine, tryptophan, valine and glutamate increased afferent activity of the celiac branch of the vagus nerve in the rat. In contrast to these amino acids, intra-duodenal application of cysteine, glycine, isoleucine, methionine, phenylalanine, proline or threonine led to depress the afferent nerve activity. In the rat, duodenal infusions of protein hydrolysates also increased mesenteric vagal afferent activity (Eastwood et al., 1998; Schwartz et al., 1998). Schwartz et al. (1998) revealed duodenal protein hydrolysates (peptone) stimulated the celiac afferents, indicating that amino acid- or oligopeptide-sensor might be existed in the rat duodenum.

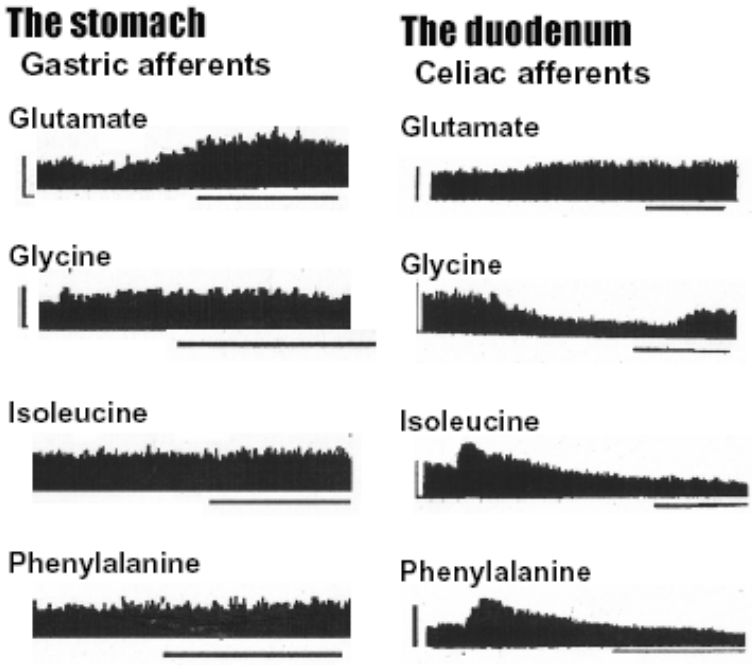

Figure 1. Abdominal vagal afferent responses to luminal amino acids. Representative recordings of gastric (right) and celiac (left) afferent discharges displayed as a sequential rate histogram (5 sec). Each vertical and horizontal bar indicates 100 impulses and 30 min. (Data were quoted from Uneyama et al. (2006b).)

CCK and 5-HT

It is well known that CCK and 5-HT activate specific receptors on primary vagal afferent nerve terminal (Grundy, 2004 for review; Raybould, 2002 for review). Intestinal vagal afferent of rat and ferret was activated by both exogenous CCK (Hillsley and Grundy, 1998a; Li et al., 2004; Richards et al., 1996; Schwartz et al., 1995) and exogenous 5-HT (Blackshaw and Grundy, 1993; Hillsley and Grundy, 1998a; Hillsley et al., 1998b). Electrophysiological evidence suggests that 5-HT and CCK act on distinct subpopulations of vagal mucosal afferent nerves of rat (Hillsley and Grundy, 1998a). The different response characteristics of 5-HT and CCK on vagal mucosal afferents, with a short burst to 5-HT and a more prolonged period of activity in response to CCK, were also observed in the ferret (Blackshaw and Grundy, 1990, 1993). In the celiac vagal nerve of rat, afferents responded to 5-HT in one of two way: a short latency, transient excitation mediated by 5-HT_3 receptors, or a delayed

onset, more prolonged effect that is unclear. (Uneyama et al., 2004). In the mesenteric afferent, the similar phenomena is observed and the secondary response is mediated by 5-HT_{2A} receptor (Hillsley et al., 1998b).

AMINO ACID-SENSING IN THE PORTAL VEIN AND LIVER

The amino acids which are absorbed in the intestine are conveyed to liver via portal vein. In the hepato-portal system, there are many reports that the hepatic branch of the vagal afferent activity was changed by various amino acids (Table 1). Intra-portal injection 10 mmol/L alanine, arginine, histidine, leucine, lysine, serine, tryptophan, valine and glutamate increased afferent activity of the hepatic branch of the vagau nerve (Niijima and Meguid, 1995; Niijima, 2000; Niijima et al., 2005; Tanaka et al., 1986, 1990). On the other hand, cysteine, glycine, isoleucine, methionine, phenylalanine, praline and threonine depressed afferent activity (Niijima et al., 2005; Saitou et al., 1993). Thus, the existence of various amino acids sensors might exist in the portal vein or liver as well as in the intestine.

Table 1. Individual amino acids change the vagus afferent activity.

Amino Acid	Gastric Afferent		Hepatic Afferent		Celiac Afferent			
	Rat	Ref.	Rat	Ref.	Rat	Ref.	Cat	Ref.
Alanine	NE	9	+	2, 4, 8	+	4	+ or NE	1
Arginine	NE	9	+	2, 4, 7	+	4	+	1
Asparagine	NE	9						
Asparatic Acid	NE	9						
Cysteine	NE	9	-	2, 4	-	4		
Glutamine	NE	9					+ or NE	1
Glutamate (MSG)	+	3, 9	+	3, 4	+	3, 4		
Glycine	NE	9	-	2, 4, 5	-	4	+	6
Histidine	NE	9	+	2, 4	+	4	+ or NE	1, 6
Isoleucine	NE	9	-	2, 4	-	4		
Leucine	NE	9	+	2, 4, 8	+	4	+	1
Lysine	NE	9	+	2, 4	+	4		
Methionine	NE	9	-	2, 4	-	4	+ or NE	1
Phenylalanine	NE	9	-	2, 4	-	4		
Proline	NE	9	-	2, 4	-	4		
Serine	NE	9	+	2, 4	+	4		
Threonine	NE	9	-	2, 4	-	4		
Tryptophan	NE	9	+	2, 4	+	4	+	1
Tyrosine	NE	9						
Valine	NE	9	+	2, 4	+	4	+	1

+ : activation. -: suppression. NE : no effect. When none is indicated, test has not been performed.

Ref. : Reference. 1. Jeanningros et al., 1982; 2. Niijima et al., 1995 ; 3. Niijima, 2000; 4. Niijima et al., 2005 ; 5. Saitou et al., 1993; 6. Sharma and Nasset, 1962 ; 7. Tanaka et al., 1986 ; 8. Tanaka et al., 1990 ; 9. Uneyama et al., 2006a.

CCK and 5-HT

On the hepatic vagal afferent, CCK and 5-HT also activate afferent activity. Activation of CCK_1 receptors increases hepatic vagal afferent activity of rat (Cox and Randich, 1997; Horn and Friedman, 2004). The vagal afferent response to exogenous CCK is atropine insensitive. 5-HT acting via $5\text{-}HT_3$ receptors increases hepatic vagal afferent activity of rat (Horn and Friedman, 2004).

AMINO ACID-SENSING IN THE STOMACH

It has been believed for a long time that the gastric vagal afferents can only detect the gastric distension but not catch each nutrient. Mathis et al. (1998) also reported that the rat gastric afferents are volume-dependently activated by intra-gastric liquid loading, but the intensity of the afferent activation was not changed by nutrient composition (saline, carbohydrate or protein). However, we firstly reported that glutamate evoked the visceral sensation from the stomach (Niijima, 2000). This report is very impressive to the gastric nutrient perception research because this data strongly suggest that chemical perception, especially amino acid-sensing system exist in the gastric mucosa. Interestingly, among 20 amino acids, the rat gastric afferents can be stimulated only by glutamate (Figure 1 and table 1). Thus, amino acid-sensing profiles of the gastric afferents seems to be quite different from the intestinal and the hepatic vagal afferent.

CCK and 5-HT

On the gastric vagal afferent, CCK and 5-HT also activate afferent activity. Activation of CCK_1 receptors by CCK increases gastric vagal afferent activity of rat (Davison and Clarke, 1988; Grundy et al., 1995; Kurosawa et al., 1997, 1999; Schwartz et al., 1991, 1993, 1994). The vagal afferent response to exogenous CCK is atropine insensitive. 5-HT acting via $5\text{-}HT_3$ receptors increases gastric vagal afferent activity of ferret (Andrews and Davidson, 1990) and rat (Uneyama et al., 2002, 2006a), and gastro-esophagal vagal afferent activity of mouse (Page et al., 2002). These results suggest that CCK and 5-HT are also neuroactive mediators in the stomach.

MUCOSAL MECHANISM OF THE GUT AMINO ACID SENSING BY THE ABDOMINAL VAGUS

As for the hypothesis regarding the gut chemical-sensing for nutrients, we can find the "intestinal sensor cell hypothesis", which was originally proposed at 1970s by Prof. Fujita at Niigata university school of medicine in Japan (Fujita et al., 1980). This hypothesis is that nutrient-sensing cells are distributed in the gastric antrum or duodenal mucosa, and when these cells are interacted with luminal nutrients, these sensor cells release hormone in an endocrine or paracrine manner, to transfer the luminal nutrient information to other organs

including brain via endocrine or vagal pathways. However, what cells carry the gut nutrient perception is unclear for a long time. In 1996, Höfer et al. suggested that taste cell-like cell which has a resemble character to taste cell in the oral cavity was distributed in the gastric and intestinal mucosa, and proposed the sensor cell is this taste-like cells.

After that, with the development of molecular biology of the taste research, several taste receptors sensing amino acids has been identified. Now we know metabotrophic glutamate receptors (mGluRs) such as mGluR1 and mGluR4, calcium sensing receptor (CasR), and a taste receptor (T1R1/T1R3 complex) linked with amino acid sensation on the tongue. These receptors expressed on the tongue might be candidate molecules for luminal amino acid sensors. San Gabriel et al. (2007) reported that mGluR1 is located in glandular stomach. The report suggests the existence of the possibility that glutamate is sensed by mGluR1 and conveys sensory information to vagal afferent nerves.

We can find several studies describing the signal transduction of the gut nutrient-sensing. A role for endogenous CCK acting at vagal CCK_1 receptor in response to intestinal nutrients has been suggested by Eastwood et al. (1998). They showed that pretreatment with devazepide (a specific CCK_1 receptor antagonist) blocks the mesenteric afferent excitation produced by jejunal exposure to a protein hydrolysate, casein (a potent secretagogue of CCK in the rat) (Lewis et al., 1990). In the intestine, a main process for the intestinal uptake of peptone is the proton-coupled oligopeptide transporter PepT1, a member of the family of peptide transporters found in all species from bacteria to humans (Adibi, 2003; Daniel, 2004). There is considerable evidence that the release of CCK by endocrine cells and the subsequent activation of CCK_1 receptor on vagal afferent nerves is a main pathway that mediates the ability of protein in the intestine to inhibit gastric emptying (Forster et al., 1990; Raybould, 1991). PepT1 is localized to the apical membrane of the enterocytes and is most highly expressed along the whole length of the small intestine (Adibi, 2003). PepT1 is a candidate in the sensory transduction process involved in the intestinal release of CCK and the subsequent increase in spontaneous activity of CCK-sensitive duodenal vagal afferents (Darcel et al., 2005).

Luminal carbohydrates stimulated 5-HT release from enterochromaffin cells in the duodenum and were sensed by the vagal afferents via 5-HT_3 receptor activation (Zhu et al., 2001). Luminal lipids also stimulate vagal afferents by releasing mucosal CCK and 5-HT from the duodenal mucosa as well (Lal et al., 2001). Thus CCK and 5-HT is major candidates for intrinsic transmitters in the duodenal nutrient-sensing.

How the luminal amino acid was sensed by the vagal afferent in the gastrointestinal mucosa? We firstly showed the mucosal chemical transduction cascade of a nonessential amino acid (glutamate) in the rat stomach with pharmacological approaches (Figure 2). Luminal perfusion with the local anesthesia lidocaine abolished the glutamate-evoked afferent activation, indicating that this response was chemical event within the gastric mucosa. The glutamate response was furthermore, blocked by depletion of 5-HT and inhibition of 5-HT_3 receptor or nitric oxide (NO) synthase enzyme. The afferent response was also mimicked by luminal perfusion with a NO donor, sodium nitroprusside. In addition, the NO donor-induced afferent activation was abolished by 5-HT_3 receptor blockade as well (Uneyama et al., 2006a). This strongly supports the possibility that inter-cellular communication between mucosal cells and vagus nerve using NO and 5-HT may exist in the rat gastric mucosa. Thinking of more than 90% of 5-HT throughout the body is localized in the enterochromaffin (EC) cells of the gastrointestinal mucosa, the physiological role of the mucosal 5-HT released

from EC cells is a paracrine substance to recognize specifically a luminal amino acid (glutamate) in the stomach, like reported in the duodenal glucose-sensing.

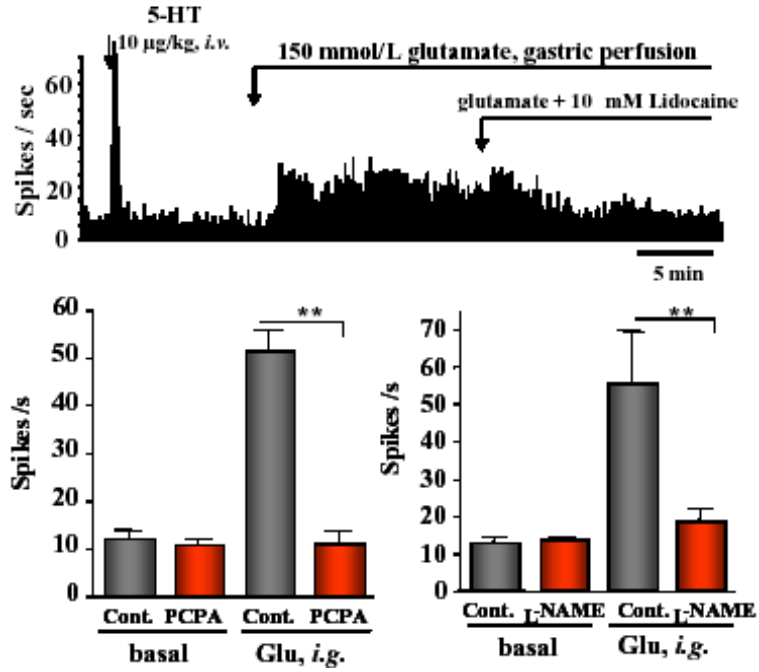

Figure 2. Mucosal chemical transduction in the gastric glutamate sensing. *Top:* Effect of luminal perfusion with a local anesthesia on the glutamate response. The perfusate was changed to the glutamate solution containing 10 mmol/L lidocaine 10 min after starting the luminal perfusion of 150 mmol/L glutamate. Each perfusate was applied at a flow rate of 1 mL/min. *Bottom left*; Effect of mucosal 5-HT depletion on the gastric glutamate response. PCPA (*p*-cholorophenylalanine) was intraperitoneally applied twice per day for 2 days at a dose of 200 mg/kg. *Bottom right;* Effect of nitric oxide synthase (NOS) blockade on the glutamate response. A NOS inhibitor, N(ω)-nitro-1-arginine methyl ester (L-NAME; 10 mg/kg) was intravenously applied 10 min before intra-gastric administration of 150 mmol/L glutamate. Each point and vertical bar represent mean ± SEM. (Data were modified from Uneyama et al. (2006a).)

PHYSIOLOGICAL SIGNIFICANCE OF THE LUMINAL AMINO ACID SENSING IN THE BODY NUTRITION HOMEOSTASIS

It is well known that the abdominal vagus is consisted from the afferent pathway which conveys the nutrient information from the abdominal organs to the brain, and the efferent pathways which conveys the information from the brain to these internal organs. The afferent signals transfer the information of food volume, osmotic pressure, pH, and the nutrient composition within the alimentary canal and the related organs (portal vein and liver). In the rat duodenum, the intestinal infusion of glutamate and lysine induced a reflex activation of gastric (Niijima, 1991, 2000; Niijima et al., 2005) and pancreatic efferents of the vagus (Niijima, 2000). In the hepato-portal system, intra-portal injection of arginine increased

afferent activity of the hepatic branch of the vagus nerve, slightly depressed the efferent activity of the pancreatic vagal branch, and increased the efferent activity of the pancreatic splanchnic branch (Tanaka et al., 1986). On the other hand, the intra-portal injection of glycine depressed afferent activity of the hepatic branch of the vagus nerve, but increased the efferent activity of the pancreatic vagal branch (Saitou et al., 1993), and depressed the efferent activity of the gastric vagal branch (Niijima et al., 2005). The intra-portal injection of glutamate and lysine induced a reflex activation of efferent gastric (Niijima, 2000; Niijima et al., 2005) and pancreatic branches of the vagus (Niijima, 2000). In the stomach, the intra-gastric infusion of glutamate induced a reflex activation of efferent gastric and pancreatic branches of the vagus (Niijima, 2000). Sensory perception by the abdominal vagus initiates and sustains the coordinated processes of gastrointestinal motility, circulation, absorption, exocrine and endocrine secretion, immunity, and satiation. Several studies including our group show that the activities of abdominal afferents are modulated by luminal or blood amino acids. These imply possible contribution of amino acids on the body nutrition homeostasis via vago-vagal reflex, especially in regulating food intake, digestion and nutrient metabolism. Indeed, the intra-gastric application of glutamate stimulated insulin secretion and increased the glucose availability in the glucose tolerance test (Bertrand et al., 1995), and supplementation of glutamate to the dietary foods enhanced the gastric secretion and motility in the dog and human (Uneyama et al., 2008). Amino acid-sensing by the abdominal vagus might regulate the gastrointestinal motility and exo- and endocrines to maintain the body nutrient homeostasis.

CONCLUSION

In this chapter, we summarized recent research on the gut amino acid perception by the abdominal vagus. As a result, it was estimated that amino acids are detected by the three major branches of the abdominal vagus (gastric, celiac and hepato-portal branches) and in turns, evoke the activation of vagal efferents innervated into the abdominal organs. So, we believe that the complete elucidation for the physiological role of the gut amino acid-sensing may lead to a better understanding of the control of digestion, absorption and metabolism after protein intake. Along this line of research, the knowledge in the presence of specific regulatory mechanisms (receptors and signal transductions) in the amino acid-sensing by the abdominal vagus will open the door for a new horizon in nutrition and medicine.

REFERENCES

Adibi, S.A. (2003). *Regulation of expression of the intestinal oligopeptide transporter (Pept-1) in health and disease*. Am. J. Physiol., 285, G779-G788.

Andrews, P.L.E. & Davidson, H.I.M. (1990). *Activation of vagal afferent terminals by 5-hydroxytryptamine is mediated by 5-HT_3 receptor in the anaesthetized ferret*. J. Physiol., 422, 92P.

Bertrand, G., Puech, R., Loubatieres-Mariani, M.M. & Bockaert, J. (1995). *Glutamate stimulates insulin secretion and improves glucose tolerance in rats.* Am. J. Physiol. 269, E551-E556.

Blackshaw, L.A. & Grundy, D. (1990). *Effects of cholecystokinin (CCK-8) on two classes of gastroduodenal vagal afferent fibre*. J. Auton. Nerv. Syst., 31, 191-201.

Blackshaw, L.A. & Grundy, D. (1993). *Effects of 5-hydroxytryptamine on discharge of vagal mucosal afferent fibres from the upper gastrointestinal tract of the ferret*. J. Auton. Nerv. Syst., 45, 41-50.

Cox, J.E. & Randich, A. (1997). *CCK-8 activates hepatic vagal C-fiber afferents.* Brain Res., 776, 189-194.

Daniel, H. (2004). *Molecular and integrative physiology of intestinal peptide transport.* Annu. Rev. Physiol., 66, 361-384.

Davison, J.S. & Clarke, G.D. (1988). *Mechanical properties and sensitivity to CCK of vagal gastric slowly adapting mechanoreceptors*. Am. J. Physiol., 255, G55-G61.

Darcel, N.P., Liou, A.P. Tomé, D. & Raybould, H.E. (2005). *Activation of vagal afferents in the rat duodenum by protein digests requires PepT1*. J. Nutr., 135, 1491-1495.

Eastwood, C., Maubach, K., Kirkup, A.J. & Grundy, D. (1998). *The role of endogenous cholecystokinin in the sensory transduction of luminal nutrient signals in the rat jejunum*. Neurosci. Lett., 254, 145-148.

Forster, E.R., Green, T.,Elliot, M., Bremner, A. & Dockray, G.J. (1990). *Gastric emptying in rats: role of afferent neurons and cholecystokinin.* Am. J. Physiol., 258, G552-G556.

Fujita, T., Kobayashi, S. & Yui, R. (1980). *Paraneuron concept and its current implications.* Adv. Biochem. Psychopharmacol., 25, 321-325.

Grundy, D. Bagaev, V. & Hillsley, K. (1995). *Inhibition of gastric mechanoreceptor discharge by cholecystokinin in the rat.* Am. J. Physiol., 268, G355-G360.

Grundy, D. (2004). What activates visceral afferents? Gut, 53 (Suppl. II), ii5-ii8.

Hillsley, K. & Grundy, D. (1998a). *Sertonin and cholecystokinin activate different populations of rat mesenteric vagal afferents*. Nerosci. Lett., 255, 63-66.

Hillsley, K., Kirkup, A. & Grundy, D. (1998b). *Direct and indirect actions of 5-hydroxytryptamine on the discharge of mesenteric afferent fibres innervating the rat jejunum*. J. Physiol., 506, 551-561.

Höfer, D., Püschel, B. & Drenckhahn, D. (1996). *Taste receptor-like cells in the rat gut identified by expression of alpha-gustducin*. Proc. Natl. Acad. Sci. U S A, 93, 6631-6634.

Horn, C.C. & Friedman, M.I. (2004). *Separation of hepatic and gastrointestinal signals from the common "hepatic" branch of the vagus*. Am. J. Phsiol., 287, R120-R126.

Jeanningros, R. & Mei, N. (1980). *Données préliminaires sur la réponse des chémorécepteurs intestinaux acides aminés.* Reprod. Nutr. Dévelop., 20, 1615-1619.

Jeanningros, R. (1982). *Vagal unitary responses to intestinal amino acid infusions in the anesthetized cat: a putative signal for protein induced satiety*. Physiol. Behav., 28, 9-21.

Kurosawa, M., Uvnäs-Moberg, K., Miyasaka, K. & Lundeberg, T. (1997). *Interleukin-1 increases activity of the gastric vagal afferent nerve partly via stimulation of type A CCK receptor in anethtized rats.* J. Auton. Nerv. Syst., 62, 72-78.

Kurosawa, M., Bucinskaite, V., Taniguchi, T., Miyasaka, K., Funakoshi, A. & Lundeberg, T. (1999). *Response of the gastric vagal afferent activity to cholecystokinin in rats lacking type A cholecystokinin receptors*. J. Auton. Nerv. Syst., 75, 51-59.

Lal, S., Kirkup, A.J., Brunsden, A.M., Thompson, D.G. & Grundy, D. (2001). *Vagal afferent responses to fatty acids of different chain length in the rat.* Am. J. Physiol., 281, G907-G915.

Lewis, L.D. & Williams, J.A. (1990). *Regulation of cholecystokinin secretion by food, hormones, and neuronal pathways in the rat.* Am. J. Physiol., 258, G512-G518.

Li, Y., Wu, X.Y. & Owyang, C. (2004). *Serotonin and cholecystokinin synergistically stimulate rat vagal primary afferent neurones*. J. Physiol., 559, 651-662.

Mathis, C., Moran, T.H. & Schwartz, G.J. (1998). *Load-sensitive rat gastric vagal afferents encode volume but not gastric nutrients.* Am. J. Physiol., 274, R280-R286.

Mei, N. (1985). *Intestinal chemosensitivity*. Physiol. Rev., 65, 211-237.

Niijima, A. (1991). *Effects of oral and intestinal stimulation with umami substance on gastric vagus activity*. Physiol. Behav, 49, 1025-1028.

Niijima, A. & Meguid, M.M. (1995). *An electrophysiological study on amino acid sensors in the hepato-portal system in the rat.* Obes. Res., 3 (Suppl. 5), 741s-745s.

Niijima, A. (2000). *Reflex effects of oral, gastrointestinal and hepatoportal glutamate sensors on vagal nerve activity*. J. Nutr., 130, 971S-973S.

Niijima, A., Torii, K. & Uneyama, H. (2005). *Role played by vagal chemical sensors in the hepato-portal region and duodeno-intestinal canal: an electiophysiological study.* Chem. Senses, 30 (Suppl. 1), i178-i179.

Page, A.J., Martin, C.M. & Blackshaw, L.A. (2002). *Vagal mechanoreceptors and chemoreceptors in mouse stomach and esophagus*. J. Neurophysiol., 87, 2095-2103.

Raybould, H.E. (1991). *Capsaicin-sensitive vagal afferents and CCK in inhibition of gastric motor function induced by intestinal nutrients*. Peptides, 12, 1279-1283.

Raybould, H.E. (2002). *Visceral perception: sensory transduction in visceral afferents and nutrients.* Gut, 51 (Suppl. I), i11-i14.

Richards, W., Hillsley, K., Eastwood, C. & Grundy, D. (1996). *Sensitivity of vagal mucosal afferents to cholecystokinin and its role in afferent signal transduction in the rat*. J. Physiol., 497, 473-481.

Saitou, S., Tanaka, K., Inoue, S., Takamura, Y. & Niijima, A. (1993). *Glycine sensor in the hepato-portal system and their reflex effects on pancreatic efferents in the rat*. Neurosci. Lett., 149,12-14.

San Gabriel, A.M., Maekawa, T., Uneyama, H., Yoshie, S. & Torii, K. (2007). *mGluR1 in the fundic glands of rat stomach*. FEBS Letters, 581, 1119-1123.

Schwartz, G.J., McHugh, P.R. & Moran, T.H. (1991). *Integration of vagal afferent responses to gastric loads and cholecystokinin in rats.* Am. J. Physiol., 261, R64-R69.

Schwartz, G.J., McHugh, P.R. & Moran, T.H. (1993). *Gastric loads and cholecystokinin synergistically stimulate rat gastric vagal afferents*. Am. J. Physiol., 265, R872-R876.

Schwartz, G.J., McHugh, P.R. & Moran, T.H. (1994). *Pharmacological dissociation of responses to CCK and gastric loads in rat mechanosensitive vagal afferents*. Am. J. Physiol., 267, R303-R308.

Schwartz, G.J., Tougas, G. & Moran, T.H. (1995*). Integration of vagal afferent responses to duodenal loads and exogenous CCK in rats.* Peptides, 16, 707-711.

Schwartz, G.J. & Moran, T.H. (1998). *Duodenal nutrient exposure elicits nutrient-specific gut motility and vagal afferent signals in rat*. Am. J. Physiol., 274, R1236-R1242.

Schwartz, G.J. (2000). *The role of gastrointestinal vagal afferents in the control of food intake: current prospects.* Nutrition, 16, 866-873.

Schwartz, G.J (2006). *Integrative capacity of the caudal brainstem in the control of food intake.* Philos. Trans. R. Soc. Lond. B. Biol. Sci., 361, 1275-1280.

Sharma, K.N. & Nasset, E.S. (1962). *Electrical activity in mesenteric nerves after perfusion of gut lumen.* Am. J. Physiol., 202, 725-730.

Tanaka, K., Inoue, S., Takamura, Y., Jiang, Z.Y. & Niijima, A. (1986). *Arginine sensors in the hepato-portal system and their reflex effects on pancreatic effrents in the rat.* Neurosci. Lett., 72, 69-73.

Tanaka, K., Inoue, S., Nagase, H., Takamura, Y., & Niijima, A. (1990). *Amino acid sensors sensitive to alanine and leucine exist in the hepato-portal system in the rat*. J. Auton. Nerv. Syst., 31, 41-46.

Uneyama, H, Niijima, A., Tananaka, T. & Torii, K. (2002). *Receptor subtype specific activation of the rat gastric vagal afferent fibers to serotonin.* Life Sci., 72, 415-423.

Uneyama, H., Tanaka, T. & Torii, K. (2004). *Gut nutrient sensing by the abdominal vagus.* Nippon Yakurigaku Zasshi, 124, 210-218.

Uneyama, H., Niijima, A., San Gabriel, A. & Torii, K. (2006a). *Luminal amino acid sensing in the rat gastric mucosa.* Am. J. Physiol., 291, G1163-G1170.

Uneyama, H., Niijima, A., San Gabriel, A. Tanaka, T. & Torii, K. (2006b). *Amino acids-sensing in the rat alimentary tract.* Jpn. J. Taste Smell Res., 13, 411-414.

Uneyama, H., San Gabriel, A., Kawai, M., Tomoe, M. & Torii, K. (2008). *Physiological role of dietary free glutamate in the food digestion.* Asia Pac. J. Clin. Nutr., 17 (Suppl 1), 372-375.

Zhu, J.X., Zhu, X.Y., Owyang, C. & Li, Y. (2001). *Intestinal serotonin acts as a paracrine substance to mediate vagal signal transmission evoked by luminal factors in the rat*. J. Physiol., 530, 431-442.

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Chapter 15

PROTEOMICS HELPS TO ELUCIDATE THE CAUSES OF SCHIZOPHRENIA

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ABSTRACT

Schizophrenia is a chronic, debilitating psychotic mental disorder that affects approximately 1% of the worldwide population. It is characterized by negative and positive symptoms, resulting from biochemical and environmental factors. Despite remarkable advances achieved in the treatment of schizophrenia as well as the growing advances in molecular diagnosis studies, the biochemical basis of the disease is not completely understood and no biomarkers for molecular diagnosis are avaiable to date.

Despite the comparative proteome analyses of healthy and diseased samples has been extensively used to discover biomarkers, a detailed and careful interpretation of such data may provide a picture of the biochemical integrated systems which will help the comprehension of pathological states, treatments and diagnosis.

The proteome analysis of schizophrenia brain tissues compared to healthy controls has revealed differentially expressed proteins that, not only serve as potential biomarkers, but also compose a scenario that can lead to the comprehension of the dysfunction of neural transmission in schizophrenia. This chapter will show these potential protein role players in schizophrenia pathogenesis.

1. INTRODUCTION

1.1 Schizophrenia

Schizophrenia (SCZ) is a chronic, debilitating psychotic mental disorder that affects about 1 percent of the worldwide population (Freedman, 2003). SCZ is characterized by a series of positive symptoms such as psychomotor agitation, persistently bizarre behavior,

motor excitement, auditory hallucinations, delusions and thought disorders. Likewise there are negative symptoms including psychomotor retardation, avolition, apathy, anhedonia, attentional impairment, and decreased emotional expression (Ananth et al., 1991). SCZ symptoms generally appear in young adulthood and, once present, usually persist for the entire patient's lifetime (Sawa and Snyder, 2002).

SCZ is a multifactorial disorder that includes neurodevelopmental aspects, strong genetic components and important environmental factors (Freedman, 2003), whose causes are not completely understood. The disease diagnosis is essentially clinical according to DSM-IV (Diagnostic and Statistical Manual of Mental Disorders). Besides some macroscopic brain alterations observed in brain scans of SCZ patients, there are no biochemical markers available.

1.2 Proteomics and Biomarkers

Proteomics can be defined as a young science that arose from the term "Proteome", described in 1995 (Wilkins et al. 1996, 1997) and defined as "the total set of expressed proteins by a genome, cell, tissue or organism at a given time under a given condition". Nowadays, the proteomics science is much more complex and complete than only identifying a complete set of expressed proteins: the characterization of these protein sets is also part of proteomics. Protein-protein interactions, protein structure, protein function and activity and protein translocations are part of this characterization. Despite this broad array of definitions that proteomics cover, the identification of differentially expressed proteins of a cell, tissue or organism under distinct situations (i.e., pathological states) is the most used and widespread proteomic tool.

Despite being young, this science of 14-year-old is likewise mature, since it is possible to find in the PubMed database 21,759 deposited articles using the keyword "proteomics" (September 14th 2009). Finally, proteomics can be considered then a young-adult science that has revealed a world of answers in the universe of questions[1].

The use of proteomics for biomarker discover has been extensively used to investigate pathological states. Many articles have claimed disease biomarkers too early since diverse validation experiments in a larger set of samples are necessary to establish some differentially expressed protein as a biomarker. Moreover, diseases might not have a single biomarker (Mischak et al., 2007). Most likely, a disease might have a set of biomarkers. And these sets of biomarkers might be overlapped in different disorders, which make the biomarker discoverer more complicated.

However, the generated data in biomarker discovery research is a considerable way to expand the proteomics' world of answers, since revealing the set of differentially expressed proteins makes it possible to dig deeper into possible direct or/and indirect protein interactions, which might lead to an explanation and comprehension of pathological states independently of biomarker discovery. Beyond that, this data also provide the foundation for further research on the characterization of specific proteins related to disease.

[1] From now on in this chapter, proteomics will be considered a tool to identify differentially expressed proteins (the most used form) in pathological states.

1.3 Proteomics and Schizophrenia

The lack of SCZ molecular diagnosis has driven the proteomics researchers to look for SCZ protein biomarkers. Most of the published articles that have studied the differentially expressed proteins in the brain tissue of SCZ patients were focused on biomarker discovery. However, all these articles have revealed lists of differentially expressed proteins that could be useful also to the elucidation of the disease mechanisms. Some revealed proteins in these articles are in line with previous evidence and some are completely new in SCZ research. Here we will present the proteomics studies in SCZ brain tissue which could shed light on the comprehension of the disease.

2. PROTEOMICS COLLABORATING TO REVEAL THE CAUSES OF SCHIZOPHRENIA

Proteomics studies in SCZ brain tissue have revealed proteins that are involved in crucial processes previously observed in the disease as well as proteins that have never been related to SCZ. The advantage is that proteomics is able to identify not only the involved biochemical pathways but also the real players inside the SCZ pathobiology scenario.

2.1 Dysfunction of Energy Metabolism

SCZ is the result of selective pressure under the brain up to the limit of its metabolic capabilities during the evolutive development of the human cognitive abilities. This is the hypothesis that Khaitovich et al. (2008) defended based on their data about the evolution of the human brain to explain the causes of SCZ. This valuable work validates all the findings made by SCZ proteomics experiments about the dysfunction of the energy metabolism.

More than 95% of the energy requirement in the cells is produced in mitochondria, where the energy processing happens. The brain, which requires a high energy demand, has consequently an increased amount of mitochondria. Moreover, the regulation of energy production processes are very sensitive because of their interlaced and complex web of interaction. Thus, alterations in the concentration of mitochondrial enzymes would necessarily lead to an altered energy production, affecting the whole mitochondrial metabolism.

Most of SCZ proteome reports have revealed the altered players in the energy metabolism in pathways such as glycolysis through the enzymes aldolase, enolase, hexokinase, glyceraldehyde 3-phosphate dehydrogenase, phosphoglycerate mutase and triosephosphate isomerase; Krebs Cycle through the enzymes aconitase and malate dehydrogenase; and oxidative phosphorylation through several ATPases subunits (Prabakaran et al. 2004; Clark et al. 2006; Sivagnanasundaram et al., 2007; Martins-de-Souza et al., 2009; Martins-de-Souza et al., 2009a; Martins-de-Souza et al., 2009b; Martins-de-Souza et al., 2009c).

Despite the researchers being unanimous about the involvement of energy metabolism in SCZ, the major discussion is about whether the alterations found and described are causatives

or consequences of the disease. Some evidences propose that the energy metabolism dysregulation is a consequence of oxidative stress events (reviewed in Yao et al., 2001). Other evidence shows causative aspects such as Ben-Shachar who reported in 2002 the altered morphology of mitochondria in size and density in SCZ patients. Ben-Shachar and Laifenfeld reviewed in 2004 that mitochondrial dysfunction triggers memory deficits, hippocampal dysfunction and synapse malfunctioning leading to disturbed neuronal plasticity in SCZ. Moreover, dysfunction in mitochondria leads to alterations in calcium concentrations and reactive oxygen species, which can trigger brain tissue damage processes. The players of these processes were identified by proteomics.

2.2 The Role of the Oligodendrocytes

Among many functions such as the regulation of the microenvironment around neurons (Ludwin, 1997), and the stability of brain tissue (Lunn et al., 1997), the main role of oligodendrocytes is the insulation of the neuronal axons through the formation of a myelin sheath exclusively in the central nervous system. The oligodendrocytes can be classified as satellite oligodendrocytes or myelinating oligodendrocytes. There are four types of myelinating oligodendrocytes accordingly to their myelinization capacities (Butt et al., 1995), and they can differ morphologically, also according to their maturation states (Bunge, 1968; Mori and Leblond, 1970).

The traditional and well stated SCZ dopaminergic hypotheses that have driven SCZ studies in the last decades (reviewed in Harrison, 1999) have shared space recently with the hypothesis of oligodendrocytes' role in SCZ pathogenesis, because of the consistent results from brain imaging, brain tissue histology and transcriptomics microarray-based studies (reviewed in Segal et al., 2007).

Proteomics studies have reinforced the oligodendrocytes/myelin hypothesis since the differential expression of indispensable proteins to oligodendrocytes and myelinization metabolism roles had been reveled in brain tissue.

Myelin basic protein (MBP) is the major constituent of myelin sheath of oligodendrocytes and Schwann cells in the nervous system, while Myelin oligodendrocyte glycoprotein (MOG) is a membrane protein localized in oligodentrocyte and myelin sheaths surface that plays a role in the central nervous system development and synaptic transmission. Both proteins that play pivotal roles in the oligodendrocyte metabolism were found downregulated in dorsolateral prefrontal cortex (DLPFC) and the anterior temporal lobe (ATL) (Martins-de-Souza et al., 2009, Martins-de-Souza et al., 2009a, Martins-de-Souza et al., 2009b) as well as described in previous transcriptomics studies (Tkachev et al., 2003). MOG gene is localized in a susceptibility locus to SCZ. Variations in MBP quantities and mylienation have been shown in SCZ brain tissue by immunocytochemistry (Chambers and Perrone-Bizzozero, 2004).

2',3'-cyclic-nucleotide 3'-phosphodiesterase (CNP) was found downregulated by proteomics in prefrontal cortex (PFC), ATL and DLPFC (Prabakaran et al., 2004, Martins-de-Souza et al., 2009, Martins-de-Souza et al., 2009a), confirming previous transcriptomics findings (Hakak et al. 2001; Tkachev et al. 2003; Aston et al. 2004; Katsel et al. 2005; Dracheva et al. 2006; McCullumsmith et al. 2007). CNP is a known marker of myelin-forming cells and has an indispensable role in oligodendrocyte function.

Roles of ERM-like protein (ERMN) were recently predicted and described, such as cytoskeletal arrangements during the advanced myelinogenesis, stability of the myelin sheath and maturation of oligodendrocytes. ERMN protein was found downregulated in ATL (Martins-de-Souza et al., 2009a) and upregulated in DLPFC (Martins-de-Souza et al., 2009) and has never been related with psychiatric disorders before these proteomics reports.

These proteomics findings are in line with previous data regarding the disturbed myelinization in SCZ through the identification of the real players of this dysfunction. Beyond that, proteomics is speculating that SCZ might be a degenerative disease or at least a disease with traces of degeneration, similarly to multiple sclerosis (MS), which partially shares the set of differentially expressed myelin-related proteins.

2.3 Dysregulation of Neurotransmission

The malfunctioning of the neurotransmitter system in patients with SCZ is well known and well described. However, not all the players which cause this malfunctioning are known. Proteomics studies have identified these protein players, which might be potential targets to therapies and treatments.

Clark et al. (2006) described the differential expression of the synaptic proteins SNAP-25 and serine/threonine protein phosphatase 1 (PP1) and N-ethylmaleimide sensitive factor (NSF) in anterior cingulate cortex (ACC). NSF, that is indispensable to vesicle-mediated transport, was previously found differentially expressed in SCZ PFC (Prabakaran et al., 2004). PP1 has a role in long-term synaptic plasticity and SNAP-25 regulatates neurotransmitter release and synaptic function and is involved in vesicle docking and membrane fusion.

Recently, the differential expression of synapse-related proteins were revealed in SCZ DLPFC and Wernicke's Area (Pennington et al. 2008; Martins-de-Souza et al., 2009; Martins-de-Souza et al., 2009b; Martins-de-Souza et al., 2009c) such as Dihydro-yrimidinase-related proteins (DPYSL) which acts in axon guidance and cell migration; members of the Septin family which has roles in the cell cycle and membrane trafficking; Dynamin 1 (DNM1) which is involved in vesicular trafficking processes; and Protein kinase C (PKC) and 14-3-3 proteins which are involved in diverse cellular signaling pathways. The differential expression of these proteins may lead to an abnormal synaptic circuitry in SCZ brains through the neurotransmitters dopamine, glutamate and GABA.

2.4 Involvement of Immune System

Since long time, SCZ has been described as an immune system disease. Recently, the altered concentration of cytokines and interferons in SCZ brain tissues have reinforced the role of the immune system in SCZ pathobiology (reviewed in Strous and Shoenfeld, 2006; Müller and Schwarz, 2008).

Proteomics studies also identified the role players of the immune system differentially expressed in SCZ brain tissues. Our group (Martins-de-Souza et al., 2009) has shown in DLPFC the differential expression of Signal-regulatory protein alpha (SIRPA), responsible for regulating neutrophil transmigration (Liu et al., 2002), B lymphocyte recruitment (Yoshida et al., 2002) and destruction of host cells in autoimmune diseases (Oldenborg, 2004)

through CD47 binding. We also found the differential expression of and Signal regulatory protein beta 1 (SIRPB1) that regulates neutrophil transepithelial migration and plays an important role in the response to inflammatory stimuli (Liu et al., 2005). Moreover, we found regulated the Peroxiredoxin 2 (PRDX2), which contributes to CD8(+) T-cell activity (Memon et al., 2005), and Plectin 1 (PLEC1—upregulated: 1.68x) that, next to other functions, is a regulator of T lymphocyte cytoarchitecture (Brodin et al., 2000).

Finally, a number of oligodendrocyte proteins that were revealed differentially expressed in SCZ brain tissue are part of biochemical processes regulated by immune system, such as MBP, MOG, CNP and proteolipid protein 1 (PLP).

2.5 Disturbed Calcium Homesostasis

Calcium (Ca^{+2}) is a key metabolite in SCZ since it is directly involved in neurotransmitter signalization and processing. Moreover, Ca^{+2} plays a pivotal role in the function of dopamine receptors D1 and D2 (Bergson et al. 2003) supporting the dopaminergic hypothesis of SCZ.

We identified a number of Ca^{+2}-related proteins in SCZ ATL and DLPFC (Martins-de-Souza et al., 2009; Martins-de-Souza et al., 2009a) such as the intracellular Ca^{2+} sensor calmodulin (CALM) and Calcineurin (CaN), which plays key neuronal functions and regulates dopaminergic and glutamatergic neurotransmission. The concomitant differential expression of plasma membrane calcium-transporting ATPase 4 (PMCA-4) and MBP in SCZ ATL supports Fu et al. (2007) findings about demyelination. PMCA-4 differential expression might promotes a higher Ca^{2+} influx that might result in PLA2 Ca^{2+}-dependent stimulation (Gattaz et al. 1990) that would lead to myelin degradation as a final consequence. Moreover, the PLA2 Ca^{2+}-dependent stimulation may lead to the dysfunction of the phospholipids metabolism, widely observed in SCZ (reviewed in du Bois et al. 2005).

2.6 Newly Described Proteins in Schizophrenia and Proteins with Unknown Function

Several SCZ proteome reports have shown proteins that have never been related to the disease. In the analysis of SCZ ATL (Martins-de-Souza et al., 2009), we found regulated the proteins phosphatidylethanolamine-binding protein 1 (PEBP1), aggregan core protein (AGC) and hyaluronan and proteoglycan link protein 2 (HAPLN2) that have never been related to SCZ before. In our DLPFC analysis (Martins-de-Souza et al., 2009) we found that 10.7% of the differentially expressed proteins have no defined biological function such as CGI-38 brain specific protein, Septin 4, Hypothetical protein MGC29649, Reticulon 4, L1 cell adhesion molecule, LanC lantibiotic synthetase component C like, EF hand domain family member and WD repeat protein 1. These proteins could have an important role in the SCZ pathogenesis that is not known yet. Thus, further analysis needs to be done.

3. CONCLUSIONS

I strongly defend that the data generated by the comparative proteome analyses of pathological states should be seen more accurately, since proteomics is not only a tool for biomarker discover. The lists of differentially expressed proteins contain much more information than potential biomarkers and such information needs to be explored.

Regarding SCZ, which mechanisms are not completely understood, the proteomics role goes beyond biomarkers discovery, even though this is an important issue. Many theories to explain this pathogenesis might be proposed if we group the differentially expressed proteins revealed by proteomics studies. In figure 1 there is a possible causative-consequence theory that may drive other experiments and other interpretation of SCZ. Beyond that, these differentially expressed proteins might be targets to therapeutic studies and drug discovery to be explored.

Proteomics is helping the comprehension of the pathological mechanisms of SCZ through the identification of differentially expressed proteins. These data has driven new theories leading us to the road of comprehension, together with other knowledge areas.

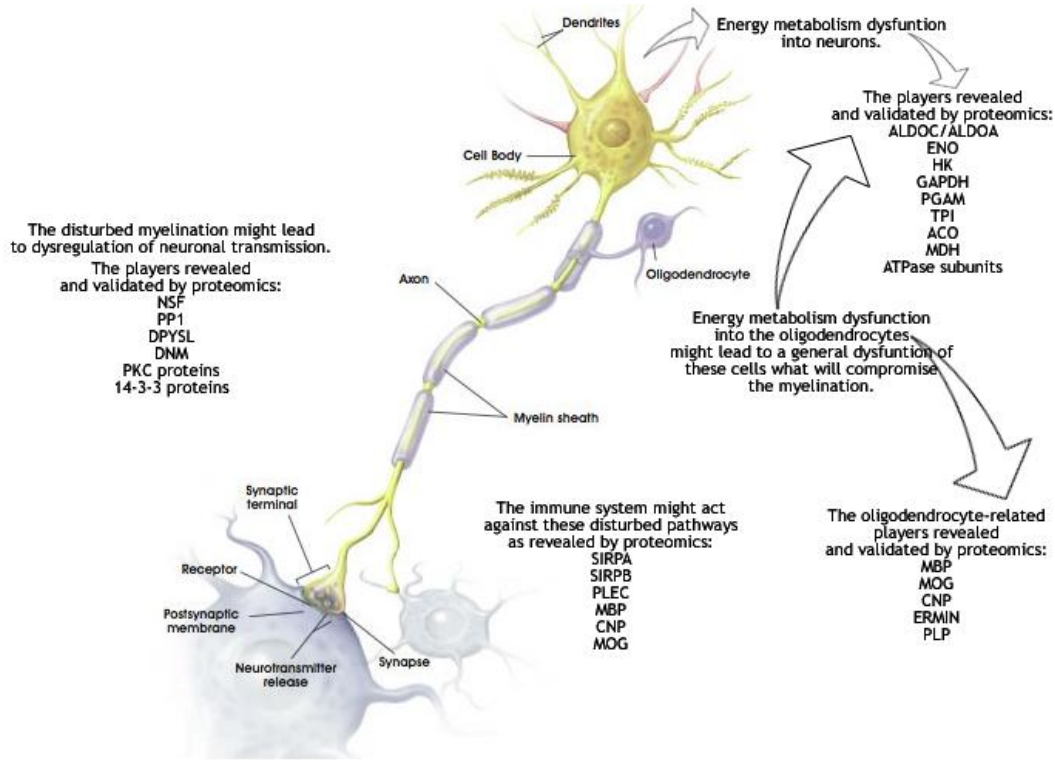

Figure 1. Speculative hypothesis for schizophrenia accordingly proteomics data. (This is a modified version of the figure http://stemcells.nih.gov/staticresources/info/scireport/images/figure81.jpg)

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REFERENCES

[1] Freedman R. *Schizophrenia*. N Engl J Med. 2003 Oct 30;349(18):1738-49.

[2] Ananth J, Djenderdjian A, Shamasunder P, Costa J, Herrera J, Sramek J. *Negative symptoms: psychopathological models*. J Psychiatry Neurosci 1991; 16:12-18

[3] Sawa A, Snyder SH Schizophrenia: *Diverse Approaches to a Complex Disease*. Science. 2002 296: 692-695.

[4] American PA. *Diagnostic and Statistical Manual of Mental Disorders*, 4th edn. American Psychiatric Association: Washington DC, 1994

[5] Wilkins MR, Sanchez JC, Gooley AA, Appel RD, Humphery-Smith I, Hochstrasser DF, Williams KL. *Progress with proteome projects: why all proteins expressed by a genome should be identified and how to do it*. Biotechnol Genet Eng Rev. 1996;13:19-50.

[6] Wilkins MR, Pasquali C, Appel RD, Ou K, Golaz O, Sanchez JC, Yan JX, Gooley AA, Hughes G, Humphery-Smith I, Williams K L, Hochstrasser DF. *From proteins to proteomes: large scale protein identification by two-dimensional electrophoresis and amino acid analysis*. Biotechnology (N Y). 1996 Jan;14(1):61-5.

[7] Williams KL, Hochstrasser DF *Introduction to the proteome*. In: Proteome Research: New Frontiers in Functional Genomics (Wilkins MR, Williams KL, Apple RD, Hochstrasser DF. eds.), Springer, 1997, pp. 1-12.

[8] Mischak H, Apweiler R, Banks RE, Conaway M, Coon J, Dominiczak A, Ehrich JHH, Fliser D, Girolami M, Hermjakob H, Hochstrasser D, Jankowski J, Julian BA, Kolch W, Massy ZA, Neususess C, Novak J, Peter K, Rossing K, Schanstra J, Semmes OJ, Theodorescu D, Thongboonkerd V, Weissinger EM, Van Eyk JE, Yamamoto T. *Clinical proteomics: A need to define the field and to begin to set adequate standards*. Proteomics Clin. Appl. 2007; 1, (2): 148-156.

[9] Khaitovich P, Lockstone HE, Wayland MT, Tsang TM, Jayatilaka SD, Guo AJ, Zhou J, Somel M, Harris LW, Holmes E, Pääbo S, Bahn S. *Metabolic changes in schizophrenia and human brain evolution.* Genome Biol. 2008;9(8):R124.

[10] Prabakaran S, Swatton JE, Ryan MM, Huffaker SJ, Huang JT, Griffin JL, Wayland M, Freeman T, Dudbridge F, Lilley KS, Karp NA, Hester S, Tkachev D, Mimmack ML, Yolken RH, Webster MJ, Torrey EF, Bahn S. *Mitochondrial dysfunction in schizophrenia: evidence for compromised brain metabolism and oxidative stress*. Mol Psychiatry. 2004 Jul;9(7):684-97, 643.

[11] Clark D, Dedova I, Cordwell S, Matsumoto I, *A proteome analysis of the anterior cingulate cortex gray matter in schizophrenia*. Mol Psychiatry. 11(2006), pp. 459-470.

[12] Sivagnanasundaram S, Crossett B, Dedova I, Cordwell S, Matsumoto I. *Abnormal pathways in the genu of the corpus callosum in schizophrenia pathogenesis: a proteome study*. Proteomics clin. appl. 2007: 1(10): 1291-1305.

[13] Martins-de-Souza D, Gattaz WF, Schmitt A, Rewerts C, Maccarrone G, Dias-Neto E, Turck CW. *Prefrontal cortex shotgun proteome analysis reveals altered calcium homeostasis and immune system imbalance in schizophrenia*. Eur Arch Psychiatry Clin Neurosci. 2009 Apr;259(3):151-63.

[14] Martins-de-Souza D(a), Gattaz WF, Schmitt A, Rewerts C, Marangoni S , Novello JC, Maccarrone G, Turck CW, Dias-Neto E. *Alterations in oligodendrocyte proteins, calcium homeostasis and new potential markers in schizophrenia anterior temporal lobe are revealed by shotgun proteome analysis.* J Neural Transm. 2009 Mar;116(3):275-89.

[15] Martins-de-Souza D(b), Gattaz WF, Schmitt A, Maccarrone G, Hunyadi-Gulyás E, Eberlin MN, Souza GHMF, Marangoni S , Novello JC, Turck CW, Dias-Neto E. *Proteomic analysis of dorsolateral pre-frontal cortex indicates the involvement of cytoskeleton, oligodendrocyte, energy metabolism and new potential markers in schizophrenia.* J Psychiatr Res. 2009; 43:978-986.

[16] Martins-de-Souza D(c), Gattaz WF, Schmitt A, Novello JC, Turck CW, Marangoni S , Dias-Neto E. *Proteome analysis of schizophrenia patients Wernicke's area reveals an energy metabolism dysregulation*. BMC Psychiatry. 2009 Apr 30;9(1):17.

[17] Yao JK, Reddy RD, van Kammen DP. *Oxidative damage and schizophrenia: an overview of the evidence and its therapeutic implications*.CNS Drugs. 2001;15(4):287-310.

[18] Ben-Shachar D. *Mitochondrial dysfunction in schizophrenia: a possible linkage to dopamine.* J Neurochem. 2002 Dec;83(6):1241-51.

[19] Ben-Shachar D, Laifenfeld D. *Mitochondria, synaptic plasticity, and schizophrenia*. Int Rev Neurobiol. 2004;59:273-96.

[20] Ludwin SK. *The pathobiology of the oligodendrocyte*. J Neuropathol Exp Neurol. 1997 Feb;56(2):111-24.

[21] Lunn KF, Baas PW, Duncan ID. *Microtubule organization and stability in the oligodendrocyte.* J Neurosci. 1997 Jul 1;17(13):4921-32.

[22] Butt AM, Ibrahim M, Ruge FM, Berry M. *Biochemical subtypes of oligodendrocyte in the anterior medullary velum of the rat as revealed by the monoclonal antibody Rip.* Glia. 1995 Jul;14(3):185-97.

[23] Bunge RP. *Glial cells and the central myelin sheath*. Physiol Rev. 1968 Jan;48(1):197-251.

[24] Mori S, Leblond CP. *Electron microscopic identification of three classes of oligodendrocytes and a preliminary study of their proliferative activity in the corpus callosum of young rats.* J Comp Neurol. 1970 May;139(1):1-28.

[25] Harrison PJ. *The neuropathology of schizophrenia. A critical review of the data and their interpretation*. Brain. 1999 Apr;122 (Pt 4):593-624.

[26] Tkachev D, Mimmack ML, Ryan MM, Wayland M, Freeman T, Jones PB, Starkey M, Webster MJ, Yolken RH, Bahn S. *Oligodendrocyte dysfunction in schizophrenia and bipolar disorder*. Lancet. 2003 Sep 6;362(9386):798-805.

[27] Chambers JS, Perrone-Bizzozero NI. *Altered myelination of the hippocampal formation in subjects with schizophrenia and bipolar disorder*. Neurochem Res. 2004 Dec;29(12):2293-302.

[28] Hakak Y, Walker JR, Li C, Wong WH, Davis KL, Buxbaum JD, Haroutunian V, Fienberg AA. *Genome-wide expression analysis reveals dysregulation of myelination-related genes in chronic schizophrenia*. Proc Natl Acad Sci U S A. 2001 Apr 10;98(8):4746-51.

[29] Aston C, Jiang L, Sokolov BP. *Microarray analysis of postmortem temporal cortex from patients with schizophrenia.* J Neurosci Res. 2004 Sep 15;77(6):858-66.

[30] Katsel P, Davis KL, Haroutunian V. *Variations in myelin and oligodendrocyte-related gene expression across multiple brain regions in schizophrenia: a gene ontology study*. Schizophr Res. 2005 Nov 15;79(2-3):157-73..

[31] Dracheva S, Davis KL, Chin B, Woo DA, Schmeidler J, Haroutunian V. *Myelin-associated mRNA and protein expression deficits in the anterior cingulate cortex and hippocampus in elderly schizophrenia patients.* Neurobiol Dis. 2006 Mar;21(3):531-40.

[32] McCullumsmith RE, Gupta D, Beneyto M, Kreger E, Haroutunian V, Davis KL, Meador-Woodruff JH. *Expression of transcripts for myelination-related genes in the anterior cingulate cortex in schizophrenia*. Schizophr Res. 2007 Feb;90(1-3):15-27.

[33] Pennington K, Beasley CL, Dicker P, Fagan A, English J, Pariante CM, Wait R, Dunn MJ, Cotter DR. *Prominent synaptic and metabolic abnormalities revealed by proteomic analysis of the dorsolateral prefrontal cortex in schizophrenia and bipolar disorder*. Mol Psychiatry. 2008 Dec;13(12):1102-17.

[34] Strous RD, Shoenfeld Y. *Schizophrenia, autoimmunity and immune system dysregulation: a comprehensive model updated and revisited*. J Autoimmun. 2006 Sep;27(2):71-80.

[35] Müller N, Schwarz MJ. *A psychoneuroimmunological perspective to Emil Kraepelins dichotomy: schizophrenia and major depression as inflammatory CNS disorders*. Eur Arch Psychiatry Clin Neurosci. 2008 Jun;258 Suppl 2:97-106.

[36] Liu Y, Buhring HJ, Zen K, Burst SL, Schnell FJ, Williams IR, Parkos CA. *Signal regulatory protein (SIRPalpha), a cellular ligand for CD47, regulates neutrophil transmigration*. J Biol Chem 2002; 277(12):10028–10036

[37] Yoshida H, Tomiyama Y, Oritani K, Murayama Y, Ishikawa J, Kato H, Miyagawa Ji J, Honma N, Nishiura T, Matsuzawa Y. *Interaction between Src homology 2 domain bearing protein tyrosine phosphatase substrate-1 and CD47 mediates the adhesion of human B lymphocytes to nonactivated endothelial cells*. J Immunol. 2002;168(7):3213-3220.

[38] Oldenborg PA. *Role of CD47 in erythroid cells and in autoimmunity*. Leuk Lymphoma.2004;45(7):1319-1327.

[39] Liu Y, Soto I, Tong Q, Chin A, Buhring HJ, Wu T, Zen K, Parkos CA. *SIRPbeta1 is expressed as a disulfide-linked homodimer in leukocytes and positively regulates neutrophil transepithelial migration.* J Biol Chem. 2005;280(43):36132-36140.

[40] Memon AA, Chang JW, Oh BR, Yoo YJ. *Identification of differentially expressed proteins during human urinary bladder cancer progression*. Cancer Detect Prev. 2005;29(3):249-255.

[41] Brodin L, Low P, Shupliakov O. *Sequential steps in clathrin-mediated synaptic vesicle endocytosis.* Curr. Opin. Neurobiol. 2000;10:312–320.

[42] Bergson C, Levenson R, Goldman-Rakic PS, Lidow MS. *Dopamine receptor-interacting proteins: the Ca(2+) connection in dopamine signaling.* Trends Pharmacol Sci. 2003 Sep;24(9):486-92.

[43] Fu Y, Wang H, Huff TB, Shi R, Cheng JX. *Coherent anti-Stokes Raman scattering imaging of myelin degradation reveals a calcium-dependent pathway in lyso-PtdCho-induced demyelination.* J Neurosci Res. 2007 Oct;85(13):2870-81.

[44] Gattaz WF, Hübner CV, Nevalainen TJ, Thuren T, Kinnunen PK. *Increased serum phospholipase A2 activity in schizophrenia: a replication study.* Biol Psychiatry. 1990 Sep 15;28(6):495-501.

[45] du Bois TM, Deng C, Huang XF. Membrane phospholipid composition, alterations in neurotransmitter systems and schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry. 2005 Jul;29(6):878-88.

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Chapter 16

IS NEURALGIA A TRANSCRIPTIONAL CHANNELOPATHY?

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ABSTRACT

Neuralgia, is a symptom of some neurological disorders and can be found at any age. It is characterized by paroxysmal and lancinating pain that follows the path of the affected nerve. It can be spontaneous or may be triggered by any type of stimuli. This pain is usually brief but may be severe, pain-free intervals being common. This symptom is the main characteristic of some neurological entities and, due to its presence and importance, these diseases are thereby known as trigeminal neuralgia, glossopharyngeal neuralgia or postherpetic neuralgia.

Neuralgia is caused by irritation or nerve damage arising from inflammation, trauma, surgery, compression by adjacent structures such as tumors, infection and chemical or physical irritation of a nerve, even though the cause remains unknown in most cases.

This chapter presents the evidence sustaining the hypothesis that neuralgia is a clinical expression of a transcriptional channelopathy. This will help (in the near future) in designing new drugs orientated towards such target and lead to advances in diagnosing and treating patients who are affected by this important symptom.

Neuralgia is a positive symptom for some neurological diseases which are characterised by a shooting paroxysmal pain, being spontaneous or triggered by a stimulus, and irradiated in the distribution of a nerve.

The International Association for the Study of Pain (IASP) defines neuralgia as being, "pain arising in the distribution of a nerve or nerves,"[1] and considers that the term should not be reserved for paroxysmal pains. However, there is no consensus regarding the topic; for some it is a painful disorder of the nerves[2], whilst others define it as being an intense or aching pain occurring along the course or distribution of a peripheral or cranial nerve[3] and others yet use it as a synonym for neuropathic pain[4] in spite of there being neuropathic pains which do not manifest themselves with the aforementioned characteristics[5].

Neuralgic pain is usually episodic, brief but severe in its intensity. This symptom is the main characteristic of some neurological entities which are thus named in this way (i.e. trigeminal neuralgia, glossopharyngeal neuralgia or postherpetic neuralgia).

Neuralgia has a varied aetiology; it can be caused by inflammation, traumatism (including surgery), irritation by chemical substances, nerves being compressed by adjacent structures (e.g. by tumours) and infection. Its pathophysiology still remains controversial.

This chapter presents evidence sustaining the hypothesis that neuralgia (understood as being a shooting/shock-like paroxysmal pain) is a clinical expression of a transcriptional channelopathy.

Channelopathies are clinical entities produced by alteration in the function of a determined ionic channel and become manifest by the presence of varied signs and symptoms.[6] Three types of channelopathy have been identified to date:

a) Genetic ones in which the alteration is produced by mutations of the channel-forming proteins, such as sodium 1.1 channel mutation causing generalised epilepsy with febrile seizure plus type 2 (GEFS+2);
b) Autoimmune ones caused by antibodies against some of the channel proteins, such as the Lambert Eaton syndrome produced by antibodies against the presynaptic calcium channel protein; and
c) Transcriptional ones, being channelopathies which are acquired due to changes in the expression of channel protein non-mutated genes; this is gone into in more depth later on since there is evidence that channelopathies of this type are possibly implicated in producing paroxysmal neuropathic pain.[6]

Voltage-gated sodium channels (VGSC). These consist of an alpha subunit (~260 kDa) forming the pore and one or more beta subunits (33-36 kDa). The alpha subunit has four homologous domains (I-IV) and each of them has six transmembrane segments (S1-S6). The pore wall is determined by a short segment between S5 and S6 (Figure 1).

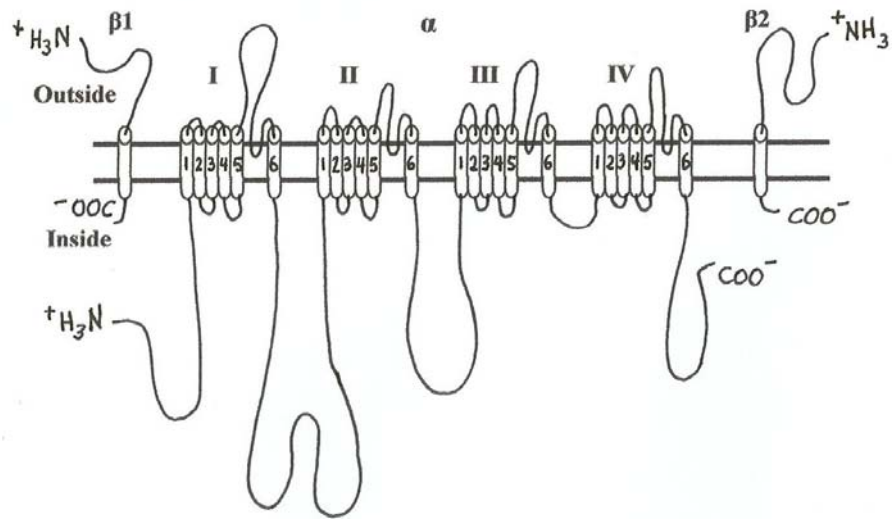

Figure 1. Sodium channel (VGSC)

Nine VGSC isoforms have been described in humans to date, all of them belonging to a single family ($Na_v1.1$ to $Na_v1.9$). It has been well established that activating voltage-gated sodium channels in axons produces the rapid regenerative upstroke of an action potential.[7]

Tetrodotoxin (TTX) has been used experimentally for blocking these channels. $Na_v1.8$ and $Na_v1.9$ require very high TTX doses for being blocked, meaning that they are known as TTX-resistant (TTX-R) channels, whilst the other isoforms are classified as being TTX-sensitive (TTX-S).[8]

Changes in the expression of some VGSC in rat experimental models have been identified in dorsal root ganglion (DRG) sensory neurons following nerve lesions. Researchers have related such changes to neuropathic pain by evaluating behavioural responses in the animals being studied.[9,10,11,12]

Waxman *et al*., (1994)[13], Dib-Hajj *et al*., (1996)[14] and Black *et al*., (1999)[15] have shown $Na_v1.3$ channel mRNA upregulation in DRG neurons following sciatic nerve axotomy in rats. Dib-Hajj *et al*., (1999)[16] produced similar results in a chronic constriction injury (CCI) model, but such results have not been reproduced following dorsal rhizotomy.[15] $Na_v1.3$ is a sodium channel (TTX-S) which is expressed in developing neurons but is scarce in mature neurons.[17] It has the fastest kinetics[8] of all VGSC, responding to sub-threshold stimuli with persistent depolarisation and has short inactivation. Cellular excitability thus becomes increased, as do the neurons' ability to maintain high frequency discharges,[18] thereby making them good candidates for producing ectopic foci.

$Na_v1.8$ is a sodium channel isoform (TTX-R) which is exclusively expressed in sensory neurons and has higher levels in small-diameter nociceptive DRG neurons.[10] Dib-Hajj *et al*., (1996)[14] demonstrated down-regulation of transcripts for the $Na_v1.8$ channel in spinal sensory neurons following axotomy of the sciatic nerve in rats. Dib-Hajj *et al*., (1999)[16] used the CCI model and also found transcript down-regulation for the $Na_v1.8$ channel. Sleeper *et al*., (2000)[19] produced similar results following sciatic nerve axotomy in rats, but not after dorsal root lesion (rhizotomy).

It seems that the above does not explain $Na_v1.8$ participation in producing neuropathic pain; however, other investigators have found $Na_v1.8$ up-regulation after axotomy in these particular neurons.[20] On the other hand, Gold *et al*.,[21] and Novakovic *et al*.,[22] have demonstrated this channel's redistribution (in neuropathic conditions) in uninjured neighbouring axons and Lai *et al*.,[23] managed to revert allodynia and hyperalgesia in a model of neuropathic pain in rats by intrathecal administration of specific antisense oligodeoxynucleotides (ODN) to $Na_v1.8$. This has supported the hypothesis that $Na_v1.8$ expression is fundamental for the manifestation of patterns of neuropathic pain and could play an important role in the process of central sensitisation.

$Na_v1.9$ is another sodium channel isoform (TTX-R) which is expressed in high levels in small DRG neurons. Some studies have shown down-regulation of this channel in different models of neuropathic pain in rats,[12,16,19] but there is no overall evidence for assigning it a role in producing neuropathic pain.[24,25,26]

Coward *et al*.,[27] first studied the presence of $Na_v1.8$ and $Na_v1.9$ in the peripheral nerves of patients suffering peripheral nerve lesions, including traumatic lesions of the brachial plexus, distal limb neuromas and sural nerve neuropathies, all of them being accompanied by persistent pain, hyperalgesia and allodynia. The researchers found a marked increase in immunoreactivity for $Na_v1.8$ but not for $Na_v1.9$.

On the other hand, England *et al*.,[28] found that sodium channels became abnormally accumulated in the neuromas of 16 patients suffering painful neuromas by using immunocytochemistry, dense immunolocalisation and radioimmunoassay techniques.

The aforementioned evidence sustains the hypothesis that transcriptional VGSC channelopathy is fundamental in producing neuralgia and could explain the abnormal sensory phenomena characteristic of neuropathic pain.

Speculation has been raised for some years now about the participation of some subtype of voltage-gated calcium channel (VGCC) in producing neuropathic pain.[29,30] Such possibility has increased with the satisfactory results obtained from using antiepileptic gabapentin. However, future experimental results are needed for confirming this possibility.

It can thus be said that neuralgia is an important symptom which is difficult to treat in a good number of cases of neuropathic pain. Even though there is a lack of unanimity in existing studies regarding whether some symptoms such as shooting/shock-like pains are characteristic of this entity,[31] evidence from both experimental and human models has led to supporting the hypothesis that some VGNC isoforms' transcriptional channelopathy (especially $Na_v1.3$ and $Na_v1.8$) plays a role in initiating and maintaining neuralgia. It is hoped that advances in designing drugs aimed at this objective will bring the hope of treatment and better quality of life for patients affected by this pathology.

REFERENCES

[1] Merskey H, Bogduk N, editors. *Classification of chronic pain*. 2nd edition. Seattle: IASP Press; 1994.

[2] *http://en.wikipedia.org/wiki/Neuralgia*

[3] *http://www.ncbi.nlm.nih.gov/sites/entrez*

[4] Oaklander AL. *Mechanisms of pain and itch caused by herpes zoster (shingles).* J Pain 2008;9(1):S10-8.

[5] Attal N et al., *Neuropathic pain: Are there distinct subtypes depending on the aetiology or anatomical lesion?* Pain (2008), doi:10.1016/j.pain.2008.01.006

[6] Waxman SG. *Transcriptional channelopathies: an emerging class of disorders*. Nat Rev Neurosci 2001;2(9):652-9.

[7] Hille B. 2001. *Ion channels of excitable membranes*. 3rd ed. Sunderland, MA: Sinauer Associates, Inc.

[8] Aurilio C, Pota V, Pace MC et al. *Ionic channels and neuropathic pain: physiopathology and applications.* J Cell Physiol 2008;215:8-14.

[9] Black JA, Dib-Hajj S, McNabola K, et al. *Spinal sensory neurons express multiple sodium channel alpha-subunit mRNAs*. Brain Res Mol Brain Res 1996;43(1-2):117-31.

[10] Akopian AN, Sivilotti L, Wood JN. *A tetrodotoxin-resistant voltage-gated sodium channel expressed by sensory neurons.* Nature 1996;379:257-62.

[11] Sangameswaran L, Delgado SG, Fish LM, et al. *Structure and function of a novel voltage-gated, tetrodotoxin-resistant sodium channel specific to sensory neurons*. J Biol Chem 1996;271(11):5953-6.

[12] Dib-Hajj SD, Tyrrell L, Black JA et al. *NaN, a novel voltage-gated Na channel, is expressed preferentially in peripheral sensory neurons and down-regulated after axotomy.* Proc Natl Acad Sci 1998;95:8963-8.

[13] Waxman SG, Kocsis JD, Black JA. *Type III sodium channel mRNA is expressed in embryonic but not adult spinal sensory neurons, and is re-expressed following axotomy*. J Neurophysiol 1994;72(1):466-70.

[14] Dib-Hajj S, Black JA, felts P et al. *Down-regulation of transcripts for Na channel α-SNS in spinal sensory neurons following axotomy*. Proc Natl Acad Sci 1996;93:14950-4.

[15] Black JA, Cummins TR, Plumpton C et al. *Upregulation of a silent sodium channel after peripheral, but not central, nerve injury in DRG neurons*. J Neurophysiol 1999;82: 2776-85.

[16] Dib-Hajj SD, Fjell J, Cummins TR et al. *Plasticity of sodium channel expression in DRG neurons in the chronic constriction injury model of neuropathic pain*. Pain 1999;83:591-600.

[17] Beckh S, Noda M, Lübbert H, et al. *Differential regulation of three sodium channel messenger RNAs in the rat central nervous system during development.* EMBO J 1989;8(12):3611-6.

[18] Benarroch EE. *Sodium channels and pain*. Neurology 2007;68:233-6.

[19] Sleeper AA, Cummins TR, Dib-Hajj S et al. *Changes in expression of two tetrodotoxin-resistant sodium channels and their currents in dorsal root ganglion neurons after sciatic nerve injury but not rhizotomy.* J Neurosci 2000;20(19):7279-89.

[20] Abdulla FA, Smith PA. *Changes in Na^+ channel currents of rat dorsal root ganglion neurons following axotomy and axotomy-induced autonomy*. J Neurophysiol 2002;88:2518-29.

[21] Gold MS, Weinreich D, Kim CS, et al. *Redistribution of Na_v 1.8 in uninjured axons enables neuropathic pain.* J Neurosci 2003;23(1):158-66.

[22] Novakovic SD, Tzoumaka E, McGivern JG et al. *Distribution of the tetrodotoxin-resistant sodium channel PN3 in rat sensory neurons in normal and neuropathic conditions*. J Neurosci 1998;18(6):2174-87.

[23] Lai J, Gold MS, Kim CS, et al. *Inhibition of neuropathic pain by decreased expression of tetrodotoxin-resistant sodium channel*, NaV1,8. Pain 2002;95:143-52.

[24] Lai J, Porreca F, Hunter JC, et al. *Voltage-gated sodium channels and hyperalgesia.* Annu Rev Pharmacol Toxicol 2004;44:371-97.

[25] Hargus NJ, Patel MK. *Voltage-gated Na+ channels in neuropathic pain*. Expert Opin Investig Drugs 2007;16(5):635-46.

[26] Cummins TR, Sheets PL, Waxman SG. *The roles of sodium channels in nociception: implications for mechanisms of pain.* Pain 2007;131:243-57.

[27] Coward K, Plumpton C, Facer P, et al. *Immunolocalization of SNS/PN3 and NaN/SNS2 sodium channel in human pain states.* Pain 2000;85:41-50.

[28] England JD, Happel LT, Kline DG, et al. *Sodium channel accumulation in humans with painful neruomas.* Neurology 1996;47:272-6.

[29] Chaplan SR, Pogrel JW, Yaksh TL. *Role of voltage-dependent calcium channel subtypes in experimental tactile allodynia.* J Pharmacol Exp Ther 1994;269:1117-23.

[30] Chaplan SR. *Neuropathic pain: role of voltage-dependent calcium channels*. Reg Anesth Pain Med 2000;25 (3):283-5.

[31] Jensen TS, Baron R. *Translation of symptoms and signs into mechanisms in neuropathic pain:* Pain 2003;102:1-8.p

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