IntechOpen

Autonomic Nervous System

Edited by Pavol Svorc

AUTONOMIC NERVOUS SYSTEM

Edited by **Pavol Svorc**

Autonomic Nervous System
http://dx.doi.org/10.5772/intechopen.73119
Edited by Pavol Svorc

Contributors

Pavol Svorc, Alexandra Proshchina, Yuliya Krivova, Valeriy Barabanov, Sergey Saveliev, Olga Leonova, John Kingma, Denys Simard, Jacques Rouleau, Pamela Lein, Vidya Chandrasekaran, Ângela Leal, Isabel Rocha, Mafalda Carvalho, Hélder Mota-Filipe, Manuel Víctor López-González, Marc Stefan Dawid Milner, Marta González-García, Amelia Díaz-Casares

Notice

First published in London, United Kingdom, 2018 by IntechOpen
eBook (PDF) Published by IntechOpen, 2019
IntechOpen is the global imprint of INTECHOPEN LIMITED, registered in England and Wales, registration number: 11086078, The Shard, 25th floor, 32 London Bridge Street
London, SE19SG – United Kingdom
Printed in Croatia

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library

Additional hard and PDF copies can be obtained from orders@intechopen.com

Autonomic Nervous System
Edited by Pavol Svorc

p. cm.

Print ISBN 978-1-78984-192-3

Online ISBN 978-1-78984-191-6

eBook (PDF) ISBN 978-1-83881-720-6

Meet the editor

Pavol Svorc, Associate Professor of Normal and Pathological Physiology, Doctor of Natural Sciences, and Doctor of Philosophy, works at the Faculty of Medicine of Šafarik's University, Košice. From 2011 until 2014 he was Head of the Institute of Physiology and Pathophysiology, Faculty of Medicine, University of Ostrava, Czech Republic. He is a member of the committee for Human Physiology and Pathophysiology at the Jessenius Faculty of Medicine in Martin and of the committee for Neuroscience at the Faculty of Medicine in Ostrava. Currently, he is visiting Associate Professor at the Faculty of Medicine in Ostrava, Ostrava University. His research focuses on the chronobiology of the cardiovascular system, autonomic nervous system, respiratory system, and anesthesia. He has authored several international journal articles and book chapters and participated in 126 conferences and workshops worldwide.

Contents

Preface

The autonomic nervous system comprises one of the most important involuntary control mechanisms modulating the function of the visceral organs.

This book consists of six chapters. The introductory chapter provides a general description of the autonomic nervous system, and the extent to which it is addressed, for example, in medical faculties.

The ensuing chapters review and analyze in detail some of the specifics and the role of the autonomic nervous system in the control of vital functions. Particularly interesting is the review article by López-González, González-García, and Dawid-Milner, titled "A5 and A6 Noradrenergic Cell Groups: Implications for Cardiorespiratory Control," regarding noradrenergic cell groups at the level of the spinal cord controlling the cardiorespiratory system. These projections play a key role in the modulation of all antinociceptive and autonomic responses elicited by painful or threatening situations. The A6 noradrenergic cell group may have the most significant effect on somatosensory transmission, and the A5 group on sympathetic function.

The shape of dendrites influences the propagation and integration of postsynaptic potentials and determines presynaptic convergence. Dendritc shape is correlated with tonic activity, while aberrant dendritic morphology is associated with disease. There is, therefore, significant interest in understanding how dendritic morphology is regulated in these neurons. In the review article by Chandrasekaran and Lein, titled "Regulation of Dendritogenesis in Sympathetic Neurons," the role of target-derived nerve growth factor in regulating the size of the dendritic arbor of sympathetic neurons *in vivo* is described. In addition, the authors present their own *in vitro* experimental results, which suggest that there are other factors, such as bone morphogenetic proteins, that trigger cultured sympathetic neurons to extend a dendritic arbor comparable with their *in vivo* counterparts.

Clinical practice may benefit from the article by Kingma, Simard, and Rouleau, titled "Autonomic Nervous System and Neurocardiac Physiopathology," in which the effect of autonomic neural dysfunction in arrhythmogenesis is analyzed in detail. Disorders within the autonomic nervous system contribute to pathogenesis of organ injury, comorbidities, and may even impact survival. Improved comprehension of modifications within the cardiac/neuro axis at the molecular, cellular, organ, and whole-body levels is critical for the development of therapeutic strategies.

The review article by Proshchina et al., titled "Development of Human Pancreatic Innervation," addresses human pancreatic innervation, which is of particular interest due to its possible role in the pathogenesis of such diseases as diabetes mellitus, pancreatitis, and

pancreatic cancer. It has been suggested that pancreatic autonomic innervation plays an important role, not only in the regulation of endocrine and exocrine activity, but also in normal islet morphogenesis.

The association between inflammation and common human diseases remains an unsolved mystery in contemporary biology and medicine. Inflammation, as a response to infection, impacts different parts of the nervous system. In fact, recent studies have indicated that systemic inflammation can be attenuated by autonomic nerve fibers. The article by Leal et al., titled "Inflammation and the Autonomic Function," analyzes the general autonomic mechanisms controlling inflammatory responses in several conditions, including burn processes, rheumatoid arthritis, and obesity, with a special focus on the inflammatory processes associated with sepsis.

This book may stimulate interest in many researchers who could use this information to advance their research towards a better understanding of autonomic regulatory mechanisms.

Pavol Svorc, Assoc. Prof., Dr., Ph.D.
Department of Physiology
Faculty of Medicine
Safarik's University
Košice, Slovak Republic

Section 1

Autonomic Nervous System

Chapter 1

Introductory Chapter: Autonomic Nervous System - What We Know About It

Pavol Svorc

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.81026

1. Introduction

The nervous system captures and processes stimuli acting on an organism and provides the means for an adequate response. It provides neural control that is faster than hormonal pathways and is, therefore, more suitable for transmitting information that requires a rapid, coordinated response. The sensory, somatic, and autonomic parts of the nervous system have been extensively studied. What is the physiology of the autonomic nervous system and what do we teach students about this system in medical faculties?

The autonomic (vegetative) nervous system is an involuntary system that primarily controls and modulates the functions of the visceral organs. Similarly, through the control of somatic functions, a relatively large part of autonomic regulation is controlled through the reflex arc. The autonomic nervous system innervates the smooth muscles of vessels, digestive system, bladder and urethra, lower airways, cardiac muscle, sweat and lacrimal glands, and adrenal medulla. The autonomic nervous system has three branches: sympathetic, parasympathetic, and enteric [1–4]. In many cases, the sympathetic and parasympathetic nervous systems have "opposite" actions, in which one system activates and the other inhibits a physiological response. The current view is that the sympathetic nervous system is a "quick response mobilizing system" and the parasympathetic nervous system is a "more slowly activated inhibitory system."

The enteric—or intrinsic—nervous system is one of the main divisions of the autonomic nervous system and consists of a network of neurons that manage the functions of the gastrointestinal tract [5]. It is capable of acting independently of the sympathetic and parasympathetic nervous systems; however, it may be modulated by sympathetic and parasympathetic activity. The main components are the plexus myentericus (Auerbach), which mainly influences motility, and the plexus submucosus (Meissner), which is responsible for glandular secretions [6]. The enteric nervous system has also been referred to as the "second brain" [7].

2. Composition

The composition of the efferent pathway is the same for both the sympathetic and parasympathetic divisions. It consists of two types of neurons:

- The first type is located in the brain stem or spinal cord and is referred to as *preganglionic* neurons.
- The second type is located in the ganglia, or in the body itself, and is referred to as *postganglionic* neurons.

3. Parasympathetic division

From an anatomical perspective, the parasympathetic division is the craniosacral component of the autonomic nervous system. This system is the primary mechanism that controls "rest and digest." The parasympathetic part is dominant in rest conditions, especially when an organism progresses from states of energetic exacting stress to a rest state.

Output	Preganglionic	Ganglion	Postganglionic	Effector
Cranial part				
Ncl. Edinger-Westphal	N. oculomotorius (III. cranial nerve) and its ramus inferior	Ggl. ciliare	Nn. ciliares breves	M. sphincter pupillae (miosis) and *M. ciliaris* (accommodation)
Ncl. salivatorius superior	N. facialis (VII. cranial nerve) and branch of n. petrosus major	Ggl. pterygopalatinum	Nn. pterygopalatini N. zygomaticus N. lacrimalis	Tear gland
	N. facialis (VII. cranial nerve), chorda tympani, and n. lingualis	Ggl. submandibulare	Nn. lingualis	Submandibular and sublingual salivary glands
Ncl. salivatorius inferior	N. glossopharyngeus (IX. cranial nerve) and branches of n. petrosus minor and n. tympanicus	Ggl. oticum	N. auriculotemporalis	Parotid gland
Ncl. dorsalis n. vagi	N. vagi (X. cranial nerve)	Intramural ganglia in the heart and in the respiratory and digestive systems	N. vagi (X. cranial nerve)	
Sacral part				
Ncl. intermediolateralis			Plexus hypogastricus inferior	

http://dx.doi.org/10.5772/intechopen.81026

4. Sympathetic division

From an anatomical perspective, the sympathetic division represents the thoracolumbal component of the autonomic nervous system. This system is the primary mechanism that controls the "fight-or-flight" response. The sympathetic part is dominant in stressful situations, especially when an organism prepares for situations associated with high-energy output.

Axons of neurons C8–L3 (nucleus [ncl.] intermedial and ncl. intermediolateralis) leave the spinal cord by the ventral roots of the rami *communicantes albi* and enter the sympathetic trunk. In this part, most neural connetions are placed. Only a part of the neuron is interconnected in the prevertebral ganglia. Ganglionic fibers proceed to the organs either through the *rami viscerales* (from the sympathetic trunk) or through the *rami communicantes grisey* and further by sensory neurons to the periphery (especially the skin). Fibers from the rami viscerales proceed most often periarteriorlarly.

5. Neurotransmitters and receptors of the autonomic nervous system

Acetylcholine binds to two types of membrane receptor: muscarinic and nicotinic. Muscarinic receptors are located on the membranes of effector cells, between the terminals of the postganglionic parasympathetic and sympathetic cholinergic fibers and effector organs. Their activation exhibits a slower excitatory effect. Nicotinic receptors are localized to the membranes of ganglionic parasympathetic and sympathetic neurons, and their activation exhibits a rapid depolarization-excitatory effect on ganglionic neurons.

Noradrenaline is a neurotransmitter of the sympathetic part of the autonomic nervous system. It binds to two types of membrane receptors: α-adrenergic and β-adrenergic. The results of the combinations are different responses of the effector organs. For example, stimulation of α-receptors on vessel smooth muscle induces vasoconstriction, while stimulation of β-receptors of bronchial smooth muscle induces bronchodilatation.

There are inhibitory and excitatory synapses between neurons. Relatively recently, the third subsystem of neurons, known as non-adrenergic, non-cholinergic transmitters (because they use nitric oxide as a neurotransmitter), has been described and found to be integral to autonomic function, particularly in the gut and lungs [8].

6. Mechanism of action

α_1-Receptors are found in the vascular smooth muscle of the skin and splanchnic region, in the sphincters of the gastrointestinal tract and bladder, and in the radial muscle of the iris:

1. The α_1-receptor is embedded in the cell membrane, where it is coupled via a G_q protein to phospholipase C. In the inactive state, the α_q subunit of the heterotrimeric G_q protein is bound to GDP.
2. When an agonist, such as noradrenaline, binds to the α_1-receptor, a conformational change occurs in the α_q subunit of the G_q protein that has two effects: GDP is released from the α_q subunit and replaced by GTP, and the α_q subunit (with GTP attached) detaches from the rest of the G_q protein.
3. The α_q-GTP complex migrates within the cell membrane and binds to and activates phospholipase C. Intrinsic GTPase activity then converts GTP back to GDP, and the α_q subunit returns to the inactive state (not shown).
4. Activated phospholipase C catalyzes the liberation of diacylglycerol and IP_3 from phosphatidylinositol 4,5-diphosphate. The IP_3 that is generated causes the release of Ca^{2+} from intracellular stores in the endoplasmic or sarcoplasmic reticulum, resulting in an increase in intracellular Ca^{2+} concentration. Together, Ca^{2+} and diacylglycerol activate protein kinase C, which in turn phosphorylates proteins. These phosphorylated proteins execute the final physiological actions, such as contraction of smooth muscle.

α_2-Receptors are less common than α_1-receptors; they are found in the walls of the gastrointestinal tract and in presynaptic adrenergic nerve terminals:

1. The agonist (noradrenaline) binds to the α_2_receptor, which is coupled to adenyl cyclase by an inhibitory G protein (G_i).
2. When noradrenaline is bound, G_i protein releases GDP and binds to GTP, and the α_i subunit dissociates from the G protein complex.
3. The α_i-subunit then migrates in the membrane and binds to and inhibits adenyl cyclase. As a result, cAMP levels decrease, producing the final physiological action. For example, activation of α_2-receptors in the wall of the gastrointestinal tract causes relaxation.

β_1-Receptors are prominent in the heart (increase in activity), in the saliva glands (increase in secretion), in the adipose tissue, and in the kidney (where they promote renin secretion).

β_2-Receptors are found in the vascular smooth muscle of skeletal muscle, in the walls of the gastrointestinal tract and bladder, and in the bronchioles. The activation of β_2-receptors in these tissue leads to relaxation or dilatation:

1. β_2-Receptors are embedded in the cell membrane. They are coupled, via a G_S protein, to adenylyl cyclase. In the inactive state, the α_S-subunit of the G_S-protein is bound to GDP.
2. When an agonist, such as noradrenaline, binds to the β_2-receptor, a conformational change occurs in the α_S-subunit. This change has two effects: GDP is released from the α_S-subunit and replaced by GTP, and the activated α_S-subunit detaches from the G protein complex.
3. The α_S-GTP complex migrates within the cell membrane and binds to and activates adenylyl cyclase. GTPase activity converts GTP back to GDP, and the α_S subunit is returned to its inactive state.

4. Activated adenylyl cyclase catalyzes the conversion of ATP to cAMP, which serves as the second messenger. cAMP, via the steps involving activation of protein kinases, initiates the final physiological actions.

Nicotinic receptors are found in several important locations: on the motor end plate of skeletal muscle, on all postganglionic neurons of both the sympathetic and parasympathetic nervous systems, and on the chromaffin cells of the adrenal medulla:

1. The nicotinic receptor for acetylcholine is an ion channel for Na^+ and K^+. The receptor has five subunits: two α, one β, one δ, and one γ. These five subunits form a funnel around the mouth of a central core. When no acetylcholine is bound, the channel is closed.

2. When acetylcholine is bound to each of the two α-subunits, a conformational change occurs in all of the subunits, resulting in opening of the central core of the channel. When the core of the channel opens, Na^+ and K^+ flow down their respective electrochemical gradients.

Muscarinic receptors are located in all of the effector organs of the parasympathetic nervous system: in the heart, gastrointestinal tract, bronchioles, bladder, and male sex organs. These receptors also are found in certain effector organs of the sympathetic nervous system, specifically, in sweat glands:

1. Some muscarinic receptors have the same mechanism of action as α_1-adrenoreceptors. In these cases, binding of acetylcholine to the muscarinic receptor causes dissociation of the α-subunit of the G protein, activation of phospholipase C, and production of IP_3 and diacylglycerol. IP_3 releases stored Ca^{2+}, and increased intracellular Ca^{2+} with diacylglycerol produces tissue-specific physiological actions.

2. Other muscarinic receptors alter physiological processes via direct action of the G protein. In these cases, no other second messenger is involved. For example, muscarinic receptors in the cardiac sinoatrial node, when activated by Ach, produce activation of a G_i-protein and release of the α_I-subunit, which binds directly to the K^+ channel of the sinoatrial node. When the α_I-subunit binds to K^+ channels, the channels open, slowing the rate of depolarization of the sinoatrial node, and decreasing heart rate.

7. Autonomic nervous system: autonomic centers

Centers of the autonomic nervous system are regarded to be integrators of responses to internal and external stimuli that are related to the control of autonomic functions. From this perspective, there are probably no autonomic centers in the spinal cord, although all sympathetic and sacral parasympathetic fibers extend outward from the spinal cord. It is not clear whether there are centers controlling and coordinating the activities of the relevant parts of the autonomic nervous system or whether they are only peripheral centers. Similarly, the importance of the brain cortex, which is involved in the control of autonomic functions, lies in the integration and generation of conditioned reflexes associated with the autonomic nerves.

Regarding the *brainstem* and *hypothalamus*. Reticular formation is responsible for the regulation of the cardiovascular and respiratory systems and is the center of some autonomic reflexes. The cardiovascular center includes the following structures:

- The ncl. dorsalis n. vagi is the source of vagal parasympathetic afferentation.
- The pressoric area is located on both sides of the dorsolateral part of the reticular formation. Increased activity leads to an increase in blood pressure. Sympathetic preganglionic neurons innervating the heart, blood vessels, and juxtaglomerular apparatus are efferent pathways from this center.
- The depressoric area is located in the ventromedial part of both sides of the reticular formation. Increased activity leads to decrease in blood pressure and, reciprocally, is connected to the pressoric area.
- The respiratory center is functionally situated in the autonomic centers because it affects the spinal motor neurons controlling breathing movements through the autonomic respiratory rhythm generator and inspiration rhythm.
- The autonomic reflexes are associated with input and processing of food. It is a reflex encompassing sucking, swallowing, salivation, secretion of gastric and pancreatic juices, and vomiting.

8. Hypothalamus

The function of the hypothalamus is highly complex; in fact, there is no important activity in the body that is not regulated in some way by the hypothalamus.

8.1. Center of hunger and satiety

The satiety center is located near the regulatory centers for secretion of hormones and endocrine processes in the body. The center of hunger is located near the center of satiety. Hunger is a feeling (unconditioned reaction of the body) caused by the lack of food. It is an important signal and one that prompts the body about the need for food intake and the energy from it. Hunger occurs when blood glucose levels fall below a certain level. The need for food intake is also influenced by signals from the digestive system and, by the action of certain hormones, state of mind and/or state of attention, among others, may play a role. Feelings of hunger vary among individuals, with different speeds and intensities, and are tolerated differently—some tolerate hunger well, while in others, it is associated with mood changes manifesting as irritability or fractiousness. Prolonged starvation leads to elimination of psychological barriers and principles (e.g., cannibalism from situational emergency), with hallucinations or paranoia.

8.2. Control of food intake

It is assumed that the information from the periphery (sensory inputs from the digestive tract, including gustatory afferentation) is guided into the ncl. arcuatus in the hypothalamus, where

there are projections into the lateral part of the hypothalamus: ncl. paraventricularis and ncl. dorsomedialis. All of these structures contain two types of neurons: orexigenic, which synthesize substances, of which higher levels correlate with increased ingestion of food and activate the ncl. ventromedialis, and anorexigenic, which synthesize substances, of which higher levels correlate with reduced intake of food.

8.3. Center of thirst

Thirst is the body's response to a lack of fluids, and there are two types of dehydration. The first type is the shortage of water (mostly in well-trained athletes, who secrete thin "water" sweat). In this case, the blood is concentrated, but only briefly, because water from the intercellular spaces immediately starts to flow into the blood, resulting in increases in salt concentration in the extracellular fluid. In response, water from cells moves into the intercellular spaces and, thus, results in partial dehydration. The second type of dehydration is not only the loss of water but also a large amount of salts (untrained athletes secrete dense "salty" sweat), which are mainly in the blood and in the extracellular fluids. This usually results in only a slight increase in the concentration of ions (salts) in the extracellular fluid. In this type of dehydration, water content in the cells remains stable; however, the amount of circulating blood and intercellular fluid is reduced.

The ncl. paraventricularis contains cells that are in the contact with blood flow and cerebrospinal fluid and respond either by initiating thirst or, conversely, by initiating the urge to urinate. Stimuli are from osmoreceptors (on cue from increases in osmotically active substances in the extracellular fluid), from the renin-angiotensin system (decreased plasma volume; greater concentrations of angiotensin II elevate blood pressure and cause the feeling of thirst) and baroreceptors (decrease in plasma volume). If there is a fluid deficiency in the body, the pressure in the veins is small, and blood becomes too "dense."

8.4. Control of body temperature

The preoptic area in the hypothalamus is responsible for monitoring body temperature and for reactions to increases in temperature. Extreme increases in temperature are apparent when this area is injured or damaged. The area hypothalamica posterior contains neurons that do not directly monitor body temperature; however, they react to the information from peripheral and central thermoreceptors and activate output functions of thermoregulation. Output functions of thermoregulation are concentrated on the maintenance of adequate body temperature and protection of the organism against hypothermia.

8.5. Control of the endocrine glands

Control through the hypothalamic-hypophyseal tract (ncl. paraventricularis and ncl. supraopticus—antidiuretic hormone and oxytocin) and hypothalamic sympathetic fibers influences adrenaline and noradrenaline secretion. The hypothalamus also controls the secretory activity of the anterior pituitary gland through the release of liberins and inhibitory (inhibins) factors.

8.6. Relationship with sexual function

The hypothalamus has an association with all sexual activities including sexual development, the menstrual cycle, ovulation, erection, copulation, ejaculation, pregnancy, birth, lactation, and sexual urges and behavior. Injury to the anterior hypothalamus results in disordered libido, while injury to the posterior hypothalamus results in increased sexual urges.

8.7. Control of emotions

Emotions are psychological processes that involve subjective experiences of comfort and discomfort linked to physiological changes (changes in heart rate and respiratory rate), motor manifestations (mimics, gesticulation), change readiness, and concentration. Emotions induce and influence other psychological processes. Hypothalamic nuclei, together with the anterior nuclei of the thalamus and cingulate gyrus, form the Papez circuit, which is an important part of the limbic system. They represent a very close structural relationship and, thus, represent the basis for the formation of autonomic manifestations of emotion.

8.8. Control of biological rhythms

Rhythmic activity is generated by the ncl. suprachiasmaticus. Rhythmic hypothalamic processes extend into practically all other functions of the hypothalamus as sympathetic tone, hormone secretion, regulation of temperature, intake of food and fluids, sexual function, emotion, and immune processes.

Other relationships include relation to sleep (sleep center in the anterior hypothalamus and center of wakefulness in the posterior hypothalamus), immunity (mediated by changes in the production of hormones [glucocorticoid production]), and changes in the tone of the autonomic nervous system. Sympathetic-immune interactions particularly affect the secondary lymphoid organs (spleen, lymph nodes) and are believed to increase preparedness for escape/attack. Relation to memory (Papez's circuit—transmission of short-term to long-term memory), complex behavior (motivations, emotions), control of metabolism (through control of the endocrine glands—secretion of adrenaline, adrenocorticotropic hormone, etc.), sensory function and relation to the motor system ninvoluntary movements, extrapyramidal tract, basal ganglia).

9. Clinical practice

Disorders of the autonomic nervous system result in relatively serious neurological conditions. For example, excessive activation of the sympathetic nervous system by emotions, painful stimuli, and drops in blood pressure, such as hemorrhagic shock or hypoglycemia, trigger a prepared stress response from the body. Chronically increased sympathetic activity (sleep deprivation and social insecurity, among others) can lead to psychosomatic disorders

such as hypertension, type 2 diabetes mellitus, and/or gastric ulceration. Hypothalamic disorders can cause damage to thermoregulation, circadian rhythms, insomnia, the menstrual cycle, premature maturation, growth disturbances, eating disorders (aphagia and subsequent anorexia, hyperphagia may develop), or hormone production disorders [6].

Therefore, proper and early diagnosis of autonomic nervous system disorders forms the basis of successful treatment. Symptoms that indicate autonomic system disorders include sweating, digestive disorders, dizziness, changes in heart rate, or urinary problems. Objectively, the autonomic nervous system can be investigated using classical and special methods. Classical methods include, in particular, the examination of cardiovascular reflexes, Valsalva maneuver, orthostatic test, or deep breathing. These tests do not, however, evaluate the extent of dysfunction [9]. Currently, a special method for examining autonomic nervous system activity involves the measurement of heart rate variability. This is a parameter that reflects the current functional state of the autonomic nervous system. In recent years, heart rate variability measurement has also attracted attention outside of research in everyday clinical and outpatient practice and in health promotion [10]. Parameters of heart rate variability are able to provide information about the proportion of sympathetic and parasympathetic components with respect to respiration or thermoregulation. Heart rate is also affected by many other factors that can increase sympathetic tone, for example, male sex, younger age, and violent emotions. Female sex, older age, or good physical condition may be involved in reducing heart rate. Heart rate variability determination is performed using time or spectral analysis methods.

From this perspective, detailed study of the functions and mechanisms of the autonomic nervous system is important and necessary.

Author details

Pavol Svorc

Address all correspondence to: pavol.svorc@upjs.sk

Department of Physiology, Faculty of Medicine, Safarik University, Kosice, Slovak Republic

References

[1] Langley JN. The Autonomic Nervous System. Part 1. Cambridge: W. Heffer; 1921

[2] Jänig W. Integrative Action of the Autonomic Nervous System: Neurobiology of Homeostasis (Digitally Printed Version. ed.). Cambridge: Cambridge University Press; 2008. p.13. ISBN: 978052106754-6

[3] Furness J. Enteric nervous system. Scholarpedia. 2007;**2**(10):4064. DOI: 10.4249/scholarpedia.4064. Archived from the Original on 8 October 2017

[4] Willis WD. The autonomic nervous system and its central control. In: Berne, RM. Physiology. 5th ed. St. Louis, MO: Mosby; 2004. ISBN 0323022251

[5] Furness JB. The Enteric Nervous System. John Wiley & Sons, e-book; 2008. pp. 35-38. ISBN: 978-1-4051-7344-5

[6] Šlamberová R. Fyziologie a patofyziologie autonomního nervového systému. In: Rokyta R et al., editors. Fyziologie a Patologická Fyziologie pro Klinickou Praxi. Praha: Grada Publishing; 2015. pp. 481-487. ISBN: 978-80247-9902-5 (PDF); 978-80-247-4867-2 (Print) (in Czech)

[7] Pocock G, Richards C. Human Physiology The Basis of Medicine. Third ed. Oxford University Press; 2006. p. 63. ISBN: 978-0-19-856878-0

[8] Belvisi MG, Stretton DC, Yacoub M, Barnes PJ. Nitric oxide is the endogenous neurotransmitter of bronchodilator nerves in humans. European Journal of Pharmacology. 1992; **210**(2):221-222. DOI: 10.1016/0014-2999(92)90676-U. PMID 1350993

[9] Opavský J, Salinger J. Vyšetrovacie metódy dysfunkcie autonómneho nervového systému —Prehľad pre potreby klinické praxe. Non-Invasive Cardiology. 1995;**4**:139-153. (in Slovak language)

[10] Sammito S, Sammito W, Bockelmann I. The circadian rhythm of heart rate variability. Biological Rhythm Research. 2016;**47**(5):717-730. DOI: 10.1080/09291016.2016.1183887

Section 2

Autonomic Nervous System and Digestion

Chapter 2

Development of Human Pancreatic Innervation

Alexandra E. Proshchina, Yuliya S. Krivova, Olga G. Leonova, Valeriy M. Barabanov and Sergey V. Saveliev

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.77089

Abstract

Human pancreatic innervation is of particular interest due to its possible role in the pathogenesis of such diseases as diabetes mellitus, pancreatitis and pancreatic cancer. Despite the clinical importance, data concerning pancreatic innervation during human ontogeny and in various disorders are very limited. In this chapter, we present a review on human pancreatic autonomic innervation on the basis of the literature data and our previous results. Special attention is paid to the innervation of the endocrine pancreas. Gradual branching of neural network was seen during human pancreatic development. Innervation of the foetal pancreas is more abundant than in adults. In agreement with previous observations, we have revealed a close integration and similarity between endocrine cells and nervous elements in the developing human pancreas. Moreover, simultaneous interactions between the nervous system components, epithelial cells and endocrine cells were detected in the pancreas during prenatal human development. It has been suggested that pancreatic innervation plays an important role not only in regulation of endocrine and exocrine activity but also in normal islet morphogenesis.

Keywords: pancreatic innervation, islets of Langerhans, human development, sympathetic system, parasympathetic system

1. Introduction

The pancreas of most vertebrates is an organ that combines both endocrine and exocrine functions. Functions of the exocrine pancreas are the synthesis, accumulation and secretion of digestive enzymes (protease, amylase, lipase and nucleases) and preferment (elastase, procarboxypeptidase, trypsinogen, pepsinogen, deoxyribonuclease and ribonuclease). The main

function of the endocrine pancreas is regulation of carbohydrate metabolism. Specialised endocrine cells are grouped in units called pancreatic islets or islets of Langerhans. Islets of mammals (including humans) contain four major types of endocrine cells: beta cells secreting insulin, alpha cells secreting glucagon, delta cells secreting somatostatin and PP cells that synthesise pancreatic polypeptide [1]. Recently, another type of pancreatic endocrine cells was described—ghrelin-containing cells (epsilon cells) [2]. Pancreatic innervation is of interest due to its role in the pathogenesis of some diseases including chronic pancreatitis, pancreatic cancer and type 1 diabetes. Pain is the dominant clinical symptom in the majority of cases (73–93%) in patients with pancreatic cancer and pancreatitis. At the same time, the aetiology and pathogenesis of pain in chronic pancreatitis and pancreatic cancer are still unclear and are the subject of numerous studies [3].

In experiments on rodents (mice and rats) and cell cultures, it was indicated that nerve fibres and glial cells located in pancreatic islets may be the first target of autoimmune attack in type 1 diabetes [4–7]. Recently, there were reports of involvement of the peripheral nervous system in the pathogenesis of types 1 and 2 diabetes in humans [8, 9]. Moreover, the participation of the nervous system in the regulation of maturation, level of proliferation and number of insulin-producing beta cells, both in prenatal pancreatic development and in the postnatal period, was indicated in a number of experimental studies. Therefore, detailed information about the innervation of the endocrine pancreas is needed for understanding the mechanisms of beta cell pool renewal.

The pancreas is well innervated by the autonomic nervous system in various mammalian species [3, 10–15]. Rich innervation of the blood vessels and the exocrine part of the pancreas as well a more abundant innervation of the islets compared with the surrounding acinar part was detected already in the early studies [16, 17].

Connections between neurons are usually studied using anterograde and retrograde labelling of pathways. Pancreatic innervation was studied in various animal species using different tracing methods involving viruses, cholera toxin B, horseradish peroxidase, True Blue or DiI. It is believed that nerve fibres enter (and exit) in the pancreas as a part of neurovascular trunks. Within the pancreas, they also pass along the blood vessels and terminate (or, conversely, begin) near to the capillary wall and endocrine cells [18]. At the same time, they do not form classical synapses with target cells, but release neurotransmitters into the intercellular space, thus affecting more than one target simultaneously (i.e. they are enpassant synapses) [14]. Using retrograde labelling, the connection of pancreatic innervation with the central parasympathetic and sympathetic neurons in the brain stem, midbrain, hypothalamus and forebrain was shown [19–21]. Some of these brain centres are involved in monitoring of food intake or circadian rhythms, and it would be logical to assume that they send signals to the pancreas to adapt the digestive ferments and pancreatic hormone secretion to behavioural status. However, the central regulation of these processes has not yet been sufficiently studied [14].

In the pancreas, nerve endings were shown around blood vessels, as well as pancreatic acinar, ductal and endocrine cells, using immunohistochemistry and electron microscopy [17, 18]. Four types of plexuses (perivascular, periductal, periacinar and peri-insular) have been

identified in the mouse pancreas [18]. Similar data were obtained in studies on the pancreas of the rat and nutria [22, 23]. One of the most interesting features of the mammalian pancreas is that endocrine cells may form highly organised complexes with structures of the nervous system, so-called neuro-insular complexes (NICs). The structure of NIC in the human pancreas has not been studied in detail since their first description by van Campenhout [24] and Simard [25]. Fujita described two types of NIC, which he observed in the foetal and adult pancreas of the dog, cat and rabbit [26]. Some of the pancreatic ganglia contained endocrine cells forming NIC type I (NIC I). In NIC type II (NIC II), endocrine cells lie on the surface of, or even in the midst of, the nerve bundle. However, the distinction between these two types of complexes is conditional because there is an intermediate type of complex in which islets associate with nerve cells and nerve fibres simultaneously. Thus, in the pancreas, endocrine islets are closely associated with a dispersed neural network, which consists of autonomic nerves including sympathetic, parasympathetic and sensory nerves. Unfortunately, because of depth limitations in microscopy, this network cannot be easily portrayed by standard microtome-based two-dimensional (2D) histology. The systematic development of three-dimensional (3D) islet neurohistology has provided insight into neural-islet regulatory mechanisms and the role of neural tissue remodelling in the development of diabetes [27–29].

In addition, endocrine cells of pancreatic islets are similar to nervous cells in some biochemical and physiological characteristics. Some proteins expressed in endocrine cells of pancreatic islets are also specific to the nervous system: S100, GFAP (glial fibrillary acidic protein), GAD (glutamic acid decarboxylase), TH (tyrosine hydroxylase), NPY (neuropeptide Y), NSE (neuron-specific enolase) and others [6, 7, 30–32]. Moreover, a number of transcription factors that are characteristic of the nervous system, such as Ngn3 (neurogenin3), BETA2/NEUROD, etc., are expressed during the differentiation of pancreatic endocrine cells [33–35]. The cells of the endocrine pancreas are classified as cells of a dispersed (diffuse) endocrine epithelial system. The cells of the dispersed endocrine system are a part of the so-called APUD (amine precursor uptake and decarboxylation) system [36]. These cells have the combined ability to the capture and deposit amine precursors and synthesise biogenic amines. The obvious similarity between the pancreatic endocrine cells and nerve tissue leaves the issue of its causes open to discuss.

The precise innervation patterns of islets are unknown, particularly in humans [37]. Every year reviews are published, in which morphology and function of pancreatic innervation are discussed (see for review [10, 11, 14, 15, 38–40]). However, the nature and distribution of the nervous system structures in the pancreas were studied mainly in rodents. Interspecies differences in the structure and innervation of the pancreas between humans and experimental animals (mice and rats) are quite large. In humans, the pancreas is a compact organ, while in rodents it is treelike, distributed over the mesentery of the small intestine. Therefore, it is impossible to automatically transfer the data obtained on experimental animals to humans.

In addition, knowledge about the dynamics of innervation during ontogenesis and in various diseases of the pancreas is very limited. Single studies are devoted to the formation of innervation in prenatal human development (mainly in the last century, without the use of modern methods). Therefore, the fine details of pancreatic innervation (such as the distribution of

sympathetic and parasympathetic fibres and the formation of neuro-insular complexes) in human ontogenesis are insufficiently studied. This is mainly due to the inaccessibility of the material and to a number of technical difficulties, including the quality of pancreatic autopsy samples due to the activity of enzymes of the exocrine part [40].

However, over the past 10 years, different groups of researchers have made significant progress in the study of the peculiarities of innervation in rodents. The most attention was paid to the influence of the nervous system on the endocrine pancreas. It has been shown that both sympathetic and parasympathetic nervous systems affect postnatal development of the endocrine pancreas and its plasticity in adult animals [9, 41]. For example, after vagotomy there was a decrease in insulin-containing cell proliferation in mice and rats [42]. The important role of the sympathetic innervation for the formation of islet cytoarchitecture and their functional maturation during development was also shown [43].

Thanks to recent progresses in the field of islet research (including the study of isolated islets, in thick slices and in vivo), a number of issues concerning the structure and functions of pancreatic innervation have been clarified (see, e.g. [44–47]). In this chapter, we summarise the literature data and our previous results concerning the morphological organisation of autonomic innervation in the human foetal and adult pancreas. We also discuss the possible role of the close integration between the nervous system and epithelial and endocrine cells in the development of the endocrine pancreas.

2. Sources of pancreatic innervation

The pancreas is innervated by sympathetic and parasympathetic nerve fibres [11, 13]. The literature data indicate poor innervation of adult human pancreatic islets in comparison with rodents [44, 48–50]. At the end of the twentieth century, pancreatic innervation by postganglionic adrenergic and cholinergic fibres was intensively studied (for references, see [51]). Single nerve cells and nerve ganglia, both myelinated and unmyelinated nerve fibres of various diameters, have been detected in the human pancreas [23, 37, 48, 49]. In a simplified form, it can be considered that pancreatic sympathetic innervation is effected by the fibres of the ventral trunk and the parasympathetic innervation by the vagus nerve.

2.1. Efferent sympathetic fibres

Bodies of neurons, which form the efferent preganglionic sympathetic nerve fibres, are localised in the thoracic and upper lumbar segments of the spinal cord (T5–L1) [37, 52] or, according to some literature, in C8–L3 [21, 53]. Myelinated axons of these cells leave the ventral roots of the spinal cord and terminate on the bodies of neurons that lie in the ganglia of the paravertebral sympathetic chain, or pass through this chain via the n. splanchnicus to the celiac (*celiac*) and superior mesenteric (*mesenteric*) ganglia, and then terminate on neurons localised in these ganglia [54, 55]. The preganglionic fibres of the sympathetic system secrete acetylcholine (Ach). Postganglionic nerve fibres go to the pancreas, where they secrete norepinephrine, which binds to α and β adrenergic receptors and the neuropeptides galanin and NPY (neuropeptide Y) [10, 11, 53, 56].

In humans, the body and tail of the pancreas are innervated by nerve fibres originating from the ventral plexus and accompanying two arteries: the splenic artery and the transverse artery of the pancreas. The pancreatic head receives the largest number of nerve fibres [57, 58].

In the exocrine pancreas, sympathetic axons contact mostly with intrapancreatic ganglia, blood vessels and ducts. In mice, the innervation of the exocrine part is less pronounced than in humans. The major nerves run along the interlobular arteries and form the peri-insular plexus [18]. At the same time, in mice axons of sympathetic nerves contact alpha cells, while contact with beta cells is not found [44]. The axons of sympathetic nerves also innervate smooth muscle cells and pericytes of blood vessels and perivascular space, forming the so-called sympathetic neurovascular complex. In humans, sympathetic fibres innervate smooth muscle cells and pericytes and rarely contact directly with the endocrine cells. Apparently, the effects of the sympathetic innervation are likely mediated through indirect effects on local blood flow within the islet microcirculation [44, 59].

2.2. Efferent parasympathetic fibres

The bodies of the neurons forming the parasympathetic preganglionic nerve fibres lie in the dorsal motor nucleus of the n. vagus (X) [60–62] and, possibly, in the *nucleus ambiguus* [11–13]. Both of these nuclei are under the control of the hypothalamus. Preganglionic parasympathetic fibres are directed to the pancreas as a part of the vagus nerve branches. In the pancreas, parasympathetic fibres terminate on the bodies of parasympathetic neurons lying in intrapancreatic ganglia [38, 63]. These ganglia contain from 3 to 30 neurons and are usually located in intralobular connective tissue, within lobules or in close proximity to islets [13, 27, 29]. It is also important that these ganglia receive input not only from the parasympathetic nervous system but also from the sympathetic nervous system, as well as fibres from other intrapancreatic ganglia and also from the *myenteric plexus* [13]. Parasympathetic fibres are also involved in the formation of nerve plexuses around the arteries and mingle with sympathetic fibres.

Preganglionic parasympathetic fibres secrete acetylcholine (Ach), which binds to nicotine receptors on the membranes of neurons [53]. Short, unmyelinated postganglionic fibres terminate on the epithelial cells of acini and ducts, smooth muscle cells and islet cells. Postganglionic parasympathetic fibres release several neurotransmitters (Ach (acetylcholine) and NO (nitric oxide)) and neuropeptides (VIP (vasoactive intestinal peptide), GRP (gastrin-releasing peptide) and PACAP (pituitary-activating adenyl cyclase polypeptide)) [10, 11, 13, 56]. Postganglionic nerve fibres perform their functions mainly via Ach by binding to muscarinic receptors found, in particular, in the endocrine cells of the islets [12, 53]. In mice, postganglionic parasympathetic nerve fibres innervate all types of islets cells [10, 11, 44]. Recently, it was found that parasympathetic islet innervation in humans differs from that in mice: first, it was shown that only a small number of fibres penetrate inside the islets (most of the axons terminate in the exocrine part of the pancreas) [44], and, secondly, it was recently shown that stimulation with Ach mostly stimulates beta and delta cells, whereas alpha cells react to a lesser extent [64]. Interestingly, alpha cells themselves may be the primary source of Ach in human islets [45]. Apparently, in human islets, this classical neurotransmitter regulates the activity of other cell types in a paracrine manner. However, now, this concept is again under revision thanks recently to the work of Tang et al. [29].

2.3. The afferent fibres

In the pancreas, there are afferent (sensory) nerve fibres in addition to efferent sympathetic and parasympathetic innervation [10–12, 53, 54]. Bundles of sensory nerve fibres leave the pancreas and follow the sympathetic (*n. splanchnicus*) and vagus nerves. The bodies of sensory sympathetic neurons are localised in the ganglia of the dorsal roots in the spinal cord, mainly at the level of the lower thoracic segments (the so-called spinal afferents) projected on interneuron plates I and IV [52, 65]. For the parasympathetic system, the bodies of afferent neurons are localised in the ganglion nodosum, sending information to the nucleus of tractus solitarii [12, 54]. The neurotransmitters of the sensory nerve fibres are CGRP (calcitonin gene-related peptide) and SP (substance P). Most sympathetic and parasympathetic afferent nerves are sensitive to capsaicin [14]. Capsaicin (vanillin) receptors mainly transmit pain information [66]. In addition, Pacinian corpuscles were described in the pancreas of various mammalian species. The suggested function of this receptor is to transmit information about pressure and vibration stimuli. In the human pancreas, they were discovered in the early twentieth century [67]. Despite this fact being presented in many histology textbooks, in the modern literature, only three cases of these findings (all in pancreatic cancer) were described [67, 68]. In our research, we have studied pancreatic autopsies of 42 foetuses and neonates aged from the 10th to 40th week of gestation and of 65 adults, 18 of whom suffered from diabetes mellitus type 2. In total, more than 1000 sections were investigated. However, Pacinian corpuscles are a rare finding in the human pancreas: we were able to detect Pacinian corpuscles only in one pancreatic section of a newborn with diagnosed diabetic fetopathy. Thus, Pacinian corpuscles do not appear to play a significant role in the sensory innervation of the human pancreas.

2.4. Enteric nervous system

In some studies on pancreatic innervation, it is assumed that the pancreas is innervated not only by extrinsic efferent and afferent nerves but also by intrinsic enteric neurons of the so-called enteric nervous system (ENS) [12, 69]. The ENS controls the motor, secretion and other functions of the gastrointestinal tract and is closely related with the diffuse endocrine system [70]. Enteric ganglia have some morphological and functional differences from sympathetic and parasympathetic ganglia:

1. The ENS performs complex integrative functions independently of higher nerve centres.
2. In the ENS, a large number of various neurotransmitters, many of which are characteristic of the central nervous system, are produce.
3. Unlike other autonomous ganglia, enteric ganglia do not contain connective tissue and blood vessels. Enteric ganglia are demarcated from the surrounding tissue of the so-called blood-ganglionic barrier, similar to the blood–brain barrier. It is insufficiently studied, and not all researchers agree with its existence.
4. Glial cells of enteric ganglia are similar in morphology, cell markers and functions with astrocytes of the CNS.

The complex structure of the enteric nervous system, containing a variety of morphological and functional types of neurons and their neurotransmitters, allows the ENS to perform complex reflex acts, some of which are implemented autonomously and some in interaction with the central nervous system and other parts of the autonomous nervous system. Intrapancreatic ganglia are connected with autonomous ganglia in the intestinal nerve plexus [71–73]. Neurotransmitters for neurons of these ganglia are, among others, serotonin and nitric oxide (NO) [73]. However, according the dominant viewpoint, intramural pancreatic neurons belong to the parasympathetic system.

3. Functional role of pancreatic innervation

As was mentioned earlier, the pancreas combines exo- and endocrine functions, secreting digestive enzymes and hormones, which regulate glucose homeostasis. The nervous system regulates the activity of both the endocrine and exocrine pancreas. However, it is problematic to separate the innervation of the pancreatic endocrine part from the innervation of the exocrine, since the tracing method used for this purpose belongs to the pancreas as a whole. In addition, the activity of both endocrine and exocrine parts of the pancreas depends on food intake. Therefore, it is not surprising that the cephalic phase has been described for both pancreatic parts. Although the stimulation of the ventromedial hypothalamus and efferent sympathetic and parasympathetic neurons affects the secretion of islet hormones (see below), it is unknown whether this stimulation is direct through axons innervating the islet or indirect by activating other organs, which affect insulin and glucagon secretion [14]. Moreover, it is very difficult to separate the nervous system effects from other (e.g. humoral) influences.

So, in the laboratory of I.P. Pavlov, in 1895, I.L. Dolinsky conducted an experiment in which he established that acid injection into the duodenum causes a release of pancreatic juice [74]. In 1901, British physiologists William Baileys and Ernest Starling concluded that there is some substance released by the duodenum that stimulates secretion by the pancreas. In the following year, 1902, this substance was discovered and named secretin. Secretin was the first such "chemical messenger" identified. This type of substance is now called a hormone.

At the same time, in the classic studies of I. P. Pavlov with M. A. Afanasiev, the nervous mechanism of pancreatic secretion was found. In the work "On secretory nerves of the pancreas" (1877), they showed that vagus nerve stimulation causes pancreatic secretion. Moreover, I. P. Pavlov with his colleagues detected that imaginary feeding in animals with chronic pancreatic fistula causes an abundant release of pancreatic juice. Later, this was confirmed by the studies of K. M. Bykov and G. M. Davydov in patients with pancreatic fistula. An abundant pancreatic juice released by this patient occurred while talking about delicious food [74]. However, pancreatic juice obtained after vagus nerve stimulation is released in a small quantity and is rich in proteins and enzymes, whereas after the secretin injection, it contains little proteins and enzymes and is released in large quantities [74]. It should be noted that both these factors (nervous and humoral) act simultaneously and synergistically.

Currently, it is considered that efferent sympathetic nerve fibres indirectly inhibit the release of enzymes of the exocrine pancreas by suppressing the stimulating effects of ganglia and constriction of vessels (vasoconstriction), thereby reducing blood flow [13, 59]. The stimulation of short, unmyelinated postganglionic parasympathetic fibres increases release from secretory cells of the exocrine pancreas and ducts causing vasodilation [13, 57].

The autonomous nervous system also regulates hormone release in the endocrine pancreas, thereby affecting glucose metabolism [10, 11, 14, 53]. Many various chemical factors affect insulin and glucagon expression. Auto-, juxta-, para- and endocrine ways potentially regulate secretion of islet hormones. Since the classical studies of Claude Bernard, which showed that injection into the floor of the fourth ventricle causes hyperglycemia, the involvement of the nervous system in the regulation of pancreatic endocrine function and metabolic control has been shown in many studies. It is, therefore, rather difficult to separate one effect from the other [14, 53].

The cellular architecture of islets affects paracrine regulation and synchronises the release of insulin [75]. All pancreatic islets secrete hormones consistently, with an approximately 5-min interval [76]. In order to create this secretion pattern, the activity of insulin-containing beta cells must be consistent both within the individual islet and between the islets [14]. At the same time, the secretory activity of other islets endocrine cells, such as glucagon-secreting alpha cells that have opposite effects on glucose homeostasis, should be consistent with the activity of beta cells. Thanks to this interaction, endocrine cells can simultaneously send signals regulating the effective delivery of islet hormones into the circulatory system and, ultimately, to the liver, regulating the maintenance of glucose homeostasis [76].

However, the islets of Langerhans are a part of a complex coherent system. They are also exposed to humoral factors such as circulating plasma hormones (e.g. epinephrine). The brain also regulates the secretion of islet hormones via the autonomic nervous system [14]. Thus, in works by Akmaev et al. [19], it was shown that the hypothalamus is able to stimulate insulin secretion from beta cells of pancreatic islets along the nerve pathway, which was named "paraventricular-vagal." This pathway starts from small neurons of the paraventricular nucleus (PVN) of the hypothalamus, synaptically switches in the medulla oblongata to neurons of the dorsal nucleus of the vagus nerve and reaches the pancreatic islets in the composition of the vagus nerve. In this pathway, beta cells receive stimulating signals. Inhibitory signals come from neurons by a humoral way: PVN neurons secrete corticotropin-releasing hormone, which stimulates the secretion of adrenocorticotropic hormone in the pituitary gland that induces the secretion of glucocorticoids in the adrenal cortex. Glucocorticoids inhibit insulin release from beta cells. This kind of double control, according to the authors, is typical for the regulation of endocrine functions. Recently, there has been data that significantly complements this concept: various areas of the hypothalamus have different effects on the secretion of insulin and/or glucagon [77]. So, a detailed study of this system is needed to further identify both neurons and functionally related projections of the central nervous system regulating islet functions.

For most species studied, it is characteristic that nerve fibres are localised mainly at the periphery of the pancreatic islets, forming a peri-insular nervous network [17]. Only single

nerve fibres are detected within islets. The bodies of ganglion neurons are also rarely localised in the pancreatic islets and may be in direct contact with endocrine cells [17, 27, 29, 78, 79].

It is believed that autonomic innervations indirectly affect the release of insulin in the cephalic phase during food intake and also take part in the increase of glucagon and decrease of insulin release by sympathetic stimulation [10, 80]. Stimulation of the splanchnic nerve increases the release of glucagon and reduces the release of insulin and somatostatin from endocrine cells of the pancreas [12, 14, 15]. Sympathetic nerves are also believed to be involved in islet response for hypoglycemia, which includes increased glucagon secretion and inhibition of insulin secretion. The general sympathetic effect is expressed by reducing the insulin concentration in plasma (by increasing the concentration of catecholamines that inhibit insulin secretion) [10, 11].

Parasympathetic nerves are responsible for the early phase of insulin secretion, including the cephalic phase (i.e. insulin secretion, which occurs during anticipation of eating). In general, parasympathetic stimulation is believed to increase the release of insulin, glucagon, somatostatin and pancreatic polypeptide in many different species (for review, see [10, 11, 14, 15]).

Sensory nerves are also involved in the regulation of hormone secretion by endocrine cells [11]. Following chemical destruction of sensory nerves (capsaicin treatment) in mice, there is an increase in insulin secretion in response to glucose compared to control [81].

In conclusion, it should be added that pancreatic innervation is insufficiently studied, especially in humans [40, 44]. Interestingly, the innervation of the islets is very plastic: it has been shown that islets transplanted into the portal vein of diabetic rats were reinnervated by the nerves of the liver [82]. This makes it necessary to further study the role of innervation in the regulation of glucose homeostasis and plasticity of the endocrine part of the pancreas.

4. Pancreatic innervation during prenatal development

Despite the clinical importance, data concerning pancreatic innervation during human ontogeny and in diseases are very limited [37]. Such studies have been performed on rodents and mostly concern the sympathetic innervation [43, 55, 83]. The embryonic sources of neural elements are fibres of the vagus (*n. vagus*) and splanchnic nerves (*n. splanchnicus*) growing into the developing pancreas and neurons that differentiate from the neural crest cells migrating to the pancreas. Sympathetic fibres innervate the developing mouse pancreas starting from the 15th day of embryonic development (E14.5) [43]. Consequently, the degree of sympathetic innervation increases until 20 days of postnatal development (P20) [55]. The development of the pancreatic sympathetic innervation depends on nerve growth factor (NGF) [43].

The human pancreas receives extensive innervation, showing peculiar growth dynamics during gestation [37]. Ingrowths of nerves in the human pancreas start at 6 weeks of development. Further morphogenesis of pancreatic innervation is characterised by the increase of sources of innervation and degree of nervous element differentiation [84, 85]. Large bundles of nerve fibres and groups of poorly differentiated neurons are found in the human pancreas

starting from the 8th week of development. At the end of the 9th week, the pancreas is innervated from almost all sources, characteristic of adults (celiac plexus, superior mesenteric plexus and posterior vagal trunk) [85]. In 1940, it was shown that pancreatic nerve cells migrate from the solar plexus and from ganglia located in the wall of the duodenum and along the branches of the vagus nerve (mainly right). At the same time, neuroblasts were detected in the pancreas of 20-week-old foetuses. Moreover, even in newborns pancreatic nerve cells were neuroblastic [86].

The gradual branching of the vascular and neural networks is observed in the human pancreatic development. Primitive free nerve endings are detected starting from the 12th week of development. In an immunohistochemical study of pancreatic innervation development in human foetuses, two peaks of increase in the number of structures of the nervous system in the head of the gland were revealed at the 14th and 22th weeks. In the pancreatic body and tail, the number of nerve structures increases from the 20th week [37]. By 30–32 weeks of development, the density of nerve endings is reduced compared to previous periods [85]. The innervation of pancreatic islets in humans is formed from the 14 to 15th weeks of the development. It differs from experimental mammals (rodents): the development of pancreatic islet innervation in rodents (mouse, Mongolian gerbil and golden hamster) is observed in the first weeks after birth [83, 87, 88].

Our study was performed on a collection of pancreatic autopsies, which allows us to explore the features of intrapancreatic innervation directly in humans using a variety of methods: classical histology; immunohistochemistry; light, fluorescent and confocal microscopy; morpho- and stereometry; statistical analysis; 3D histology; and computer reconstruction. The study was performed on 50 pancreatic autopsies of foetuses from the 10th to 40th gestational week (g.w.). Foetal pancreatic autopsies were divided into four groups according to the classification of the foetal period: pre-foetal period (10–12 g.w.), early foetal period (13–20 g.w.), middle foetal period (21–28 g.w.) and late foetal period (29–40 g.w.). A panel of antibodies for nervous system proteins (chromogranin A, neuron-specific enolase (NSE), neural cell adhesion molecule (NCAM), synaptosomal-associated protein of 25 kDa (SNAP-25, peripherin, S100 protein and neuron-specific class III β-tubulin), endocrine cell hormones (insulin, glucagon and somatostatin) and epithelial cells (cytokeratin 19 (CK19)) were used in this work [89, 90]. We generated new data concerning the spatio-temporal distribution of the innervation in the human pancreas during prenatal development.

In the pre-foetal period (10–12 g.w.), large weakly branched bundles of nerve fibres and nerve ganglia were detected already at the 10th week of gestational development using antibodies to NSE, NCAM and neuron-specific β-III tubulin (**Table 1**). The largest bundles of nerve fibres were detected in the dense peri-pancreatic mesenchyme, and the group of neurons and bundles of nerve fibres of smaller diameter were located in the loose mesenchyme between pancreatic ducts (**Figure 1a**). A network of fine nerve fibres was not developed. In some cases, bundles of nerve fibres were found near large vessels. Nerve ganglia in the pancreas of 10–12 week foetuses were small groups of cells.

Starting from 12 weeks, cells immunopositive for antibodies to S100 protein were found in nervous system structures. Localisation of neuromarkers was different. In the nerves, NSE-positive

Markers	NSE	NCAM	Neuron-specific β-III tubulin	S100 protein	Chromogranin A	SNAP-25	Peripherin
Nerve fibres and ganglions	10 weeks	10 weeks	10 weeks	12 weeks	14 weeks (weak staining)	14 weeks	14 weeks
Endocrine cells	12 weeks	14 weeks	14 weeks	15–16 weeks (some islets cells)	12 weeks	16 weeks	—

Table 1. Appearance of immunopositive reactions to neural proteins in the developing human pancreas.

fibres formed the core, while small S100-positive cells surrounded them. The ganglionic cells were NSE-positive, and the small cells surrounding them S100-positive. The bodies of ganglion neurons were immunonegative to S100, that is, the positive reaction to S100 protein was observed in satellite cells of intrapancreatic ganglia and in Schwann cells of nerve fibre bundles, while NSE was detected in neuronal bodies and processes. In addition, NSE- and chromogranin A-positive endocrine cells were first found in 12-week foetuses (**Table 1**).

The formation of the human pancreatic islets starts only at 12 weeks of development. In the pre-foetal period, only contacts between single endocrine cells or small groups and fine nerve fibres were detected, and classical NIC I and NIC II were not found. At gestational week 10 (postconception week 8), thickening of the ductal epithelial layer was found, in which endocrine cells were concentrated forming "buds" on pancreatic ducts. As development proceeds, buds containing different types of endocrine cells separate from the ducts forming small clusters or mantle-type islets. In our studies, contacts between the structures of the nervous system and epithelial cells of primitive ducts were detected in the foetal pancreas at early stages of development (10–13 weeks) before the formation of islets.

The formation of the pancreatic lobules begins in the early foetal period, from 13 weeks. At the same time, active formation of the islets of Langerhans and innervation of the endocrine part starts (**Figure 1b**). Nervous system of the pancreas of 14–15 week foetuses becomes more branched in comparison with 10–12 weeks of development. Large bundles of nerve fibres are localised in the connective tissue of gland's capsule. Smaller nerves pass into the interlobular connective tissue separately or along the blood vessels. Nerve fibres and ganglia are first found within the lobules. At the 16th week of development, the nervous apparatus of the pancreas is presented by bundles of nerve fibres of different diameters and nerve ganglia, which are located in the interlobular connective tissue and within the lobules. The nerve fibres connecting two nerve ganglia were found in 14–15 week foetuses, i.e. the first clearly detected integration of the nervous system structures was shown.

Localisation of antigens in the structures of the nervous system was also similar with the pre-foetal period. In addition, the immunopositive cells for chromogranin A, SNAP-25 and peripherin were detected in the nerve fibres and ganglia starting from 14 to 15 weeks of the development (**Table 1**). SNAP-25, NCAM, NSE, peripherin and neuron-specific β-III tubulin

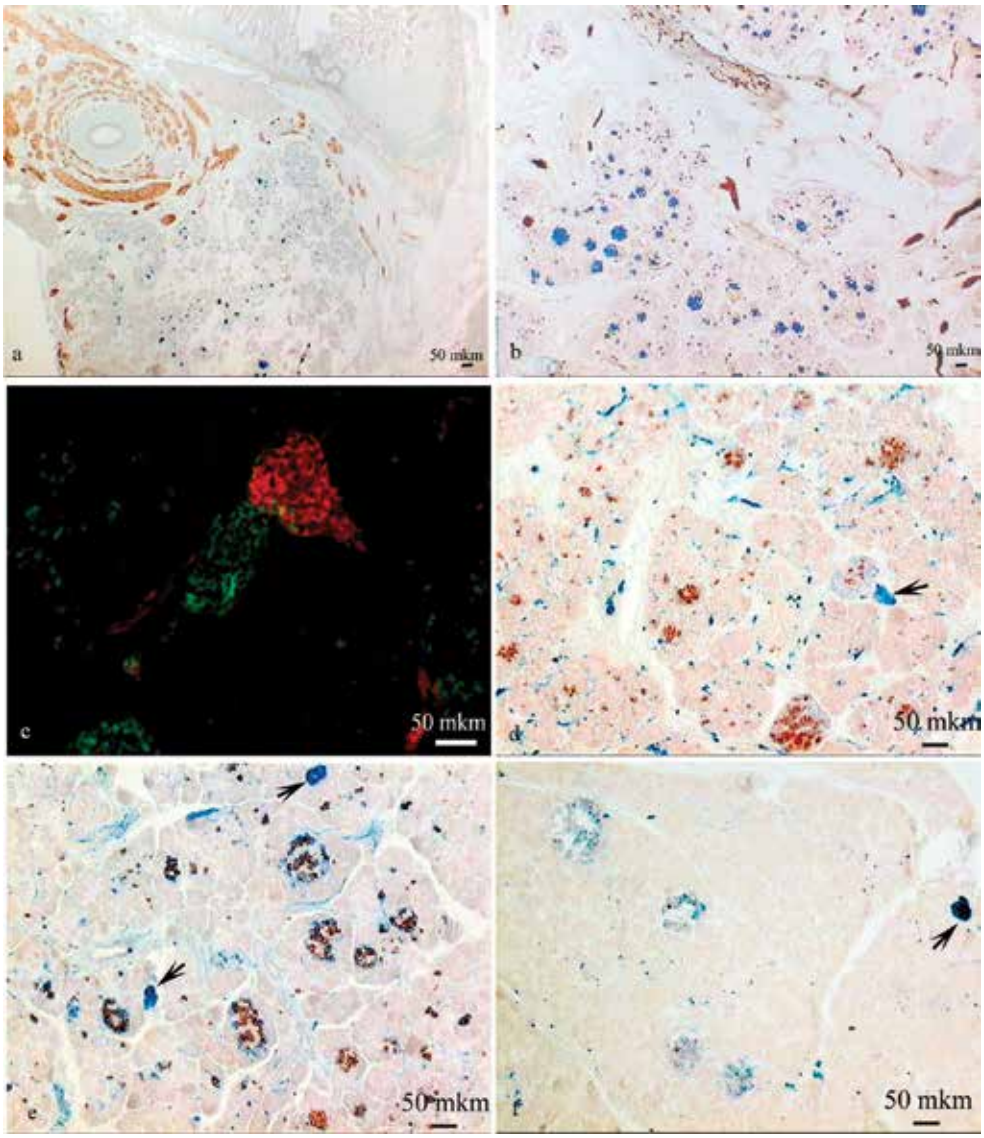

Figure 1. Spatio-temporal distribution of the nervous system structures in the human pancreas during ontogenesis. (a, b, d–f) double immunohistochemistry on the pancreatic slices of foetuses ((a) 12 g.w., (b) 16 g.w., (d) 28 g.w.), child ((e) 3 months) and adult ((f) 88 years): (a, b) insulin (blue) + S100 (red), (d, e) insulin (red)+ NSE (blue) and (f) glucagon (red) + NSE (blue). Arrows indicate some ganglia. (c) Stack of serial immunofluorescence images of NIC in the foetal pancreas (20 g.w.) (sum thickness of slices 90 mkm): Glucagon (green) + S100 (red).

were detected in bundles of nerve fibres of different diameters and the bodies of neurons in human foetuses. However, there were fine nerve fibres located in the acinar parenchyma that were immunonegative for peripherin but reacted with other markers in all investigated cases. This suggests that nerve fibres of the human pancreas differ according to the set of expressed proteins. In addition, positive immunostaining for NCAM and neuron-specific β-III tubulin was observed in endocrine cells starting from 14 weeks of development,

while SNAP-25-positive endocrine cells were detected only from 16 weeks of development. Immunopositivity to antibodies against S100 protein was found only in some islet cells starting from 15 to 16 weeks of development (**Table 1**).

The contacts of nerves fibres with endocrine cells were detected starting from 12 weeks of development. Already in the early foetal period, it was possible to identify NIC I (single insulin- or glucagon-containing cells in ganglia (Supplementary Video 1) or ganglia associated with the islets) and NIC II (single endocrine cells in the nerve (Supplementary Video 2), nerve endings associated with single endocrine cells or with the islets) and make their 3D reconstruction. The analysis of three-dimensional reconstructions allowed us to show ganglia associated with two islets at once, islets associated simultaneously with two ganglia, and NIC of mixed (intermediate) type [91]. Moreover, in the foetal pancreas, starting from 13 weeks, we showed simultaneously neuro-insular complexes and contacts between the structures of nervous system and epithelial cells located in ducts as well as in cell clusters that were often connected with the ducts. Based on these findings, we suggested that the development of neuro-insular complexes may be due to integration between the structures of the nervous system and epithelial progenitors at the initial stages of islet formation. Furthermore, endocrine cells are supposed to migrate along nerve fibres from the ducts, small clusters of endocrine cells and islets to the other islets, which are located a distance from pancreatic ducts, due to exocrine pancreatic growth, thus increasing their pool of endocrine cells. We suppose that the mechanism of pancreatic islet formation is similar to the formation of some peripheral analysers.

The pattern of immunoreactivity of neural markers during the middle (21–28 g.w.) and late foetal periods is similar to those in the early foetal period. In the middle of the foetal period, the density of pancreatic innervation is higher than in the early foetal period (**Figure 1c**, **d**). Despite increasing the size of pancreatic lobules and more sparse distribution of large and medium bundles of nerve fibres, the network of fine nerve fibres gradually branch and become denser. However, during late foetal and neonatal development, this network is much sparser (**Figure 1e**). This is due to the increase in the size of lobules. However, at all stages of human prenatal development, density of distribution of the nervous system structures is higher than in adults (**Figure 1f**). The density of NIC distribution also gradually decreases at birth. Our quantitative data indicate that the largest number of NIC I was observed in the early and middle foetal periods, during the active morphogenesis of pancreatic islets, whereas at birth (in the late foetal period) and in the adult, NIC II became more prevalent [91]. During the middle and late foetal periods, the nervous system components also contact epithelial cells located in ducts or in clusters outside the ductal epithelium and form complexes with separate epithelial cells. We observed CK19-positive cells inside the ganglia and nerve bundles, which were located separately or integrated within the islets [90].

In this study, our previous data were confirmed and refined [89] that the formation of the nervous system in the development of human pancreas can be divided into three stages. In the pre-foetal period, the nervous apparatus of the pancreas is represented by slightly branched bundles of nerve fibres and nerve ganglia. However, the structures of the nervous system differ from the late foetuses and adults by antigenic composition. Expression of various neural proteins does not begin simultaneously in the foetal pancreas.

The second stage of development of the nervous apparatus of the pancreas (during the early and middle foetal periods) is characterised by gradual branching of the neural network and formation of connections between the structures of the nervous system and exocrine and endocrine parts. In the early foetal period, nerve fibres gradually branch, nerve fibres and nerve ganglia appear localised between the acini, and a network of fine nerve fibres starts to form. In the later stages of development, the distribution of neural structures (nerve fibres, nerve ganglia and parenchymal network of fine nerve fibres) become sparser with increase in the size of the pancreas. Thus, innervation of the pancreas at this stage of development gradually becomes similar to the distribution structures of the nervous system in the adult pancreas.

In our studies, we demonstrated close integration between the structures of the nervous system and endocrine cells in the human pancreas, which were more frequently observed during prenatal development. Thus, a dense network is formed in the developing human pancreas, in which the structures of the nervous system are associated with the islets of Langerhans. The close relationship between developing islets and structures of the nervous system suggests that neuroendocrine interactions can influence not only the secretion of hormones but also to participate in the morphogenesis of the islets, presumably due to the participation in migration of endocrine cells from ducts to islets. Understanding the role of NICs in islet formation can lead to new approaches to understanding the mechanisms and treatment of diabetes.

5. Conclusions

Thus, our knowledge about the peripheral nervous system in the human pancreas is limited. Importantly, human islet development has not been examined for the presence of classical markers of the parasympathetic and sympathetic nervous systems. Furthermore, the exact location where neuronal axons terminate within the human islets in adults was not shown until recently.

However, the human pancreas is abundantly innervated during the gestational period. The value of such an abundant innervation of the pancreas and pancreatic islets, in particular, in human development is not clear. The observed differences between the nervous apparatus of foetuses and adults may have functional significance for pancreatic morphogenesis. Interestingly, some authors have described similar dynamics of innervation development in other internal human organs. The close relationship between the nervous and endocrine systems makes it necessary to further study the role of innervation in the plasticity of the endocrine pancreas both during formation of endocrine function and disorders of carbohydrate metabolism.

Acknowledgements

This work was supported by the Russian Foundation for Basic Research, project no. 18-015-00146.

Conflict of interest

The authors declare no competing interests.

Author details

Alexandra E. Proshchina[1]*, Yuliya S. Krivova[1], Olga G. Leonova[2], Valeriy M. Barabanov[1] and Sergey V. Saveliev[1]

*Address all correspondence to: proshchina@yandex.ru

1 Research Institute of Human Morphology, Moscow, Russia

2 Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russia

References

[1] Veld In't P, Marichal M. Microscopic anatomy of the human islet of Langerhans. In: Islam MS, editor. The Islets of Langerhans. Netherlands: Springer; 2010. pp. 1-19

[2] Wierup N, Sundler F, Heller RS. The islet ghrelin cell. Journal of Molecular Endocrinology. 2013;**52**(1):R35-R49. DOI: 10.1530/JME-13-0122

[3] Lindsay TH, Halvorson KG, Peters CM, Ghilardi JR, Kuskowsk MA, Wong GY, et al. A quantitative analysis of the sensory and sympathetic innervation of the mouse pancreas. Neuroscience. 2006;**137**(4):1417-1426. DOI: 10.1016/j.neuroscience.2005.10.055

[4] Mei Q, Mundinger TO, Lernmark A, Taborsky GJ Jr. Early, selective, and marked loss of sympathetic nerves from the islets of BioBreeder diabetic rats. Diabetes. 2002;**51**(10):2997-3002. DOI: 10.2337/diabetes.51.10.2997

[5] Razavi R, Chan Y, Afifiyan FN, Liu XJ, Wan X, Yantha J, Tsui H, Tang L, Tsai S, Santamaria P, Driver JP, Serreze D, Salter MW, Dosch HM. TRPV1+ sensory neurons control beta cell stress and islet inflammation in autoimmune diabetes. Cell. 2006;**127**(6):1123-1135. DOI: 10.1016/j.cell.2006.10.038

[6] Tsui H, Winer S, Chan Y, Truong D, Tang L, Yantha J, et al. Islet glia, neurons, and beta cells. Annals of the New York Academy of Sciences. 2008;**1150**(1):32-42. DOI: 10.1196/annals.1447.0330

[7] Winer S, Tsui H, Lau A, Song A, Li X, Cheung RK, et al. Autoimmune islet destruction in spontaneous type 1 diabetes is not beta-cell exclusive. Nature Medicine. 2003;**9**(2):198-205. DOI: 10.1038/nm818

[8] Mundinger TO, Mei Q, Foulis AK, Fligner CL, Hull RL, Taborsky GJ. Human type 1 diabetes is characterized by an early, marked, sustained and islet-selective loss of sympathetic nerves. Diabetes. 2016;**65**(8):2322-2330. DOI: 10.2337/db16-0284

[9] Thorens B. Neural regulation of pancreatic islet cell mass and function. Diabetes, Obesity & Metabolism. 2014;**16**(S1):87-95. DOI: 10.1111/dom.12346

[10] Ahren B. Autonomic regulation of islet hormone secretion–Implications for health and disease. Diabetologia. 2000;**43**(4):393-410. DOI: 10.1007/s001250051322

[11] Ahrén B. Islet nerves in focus—Defining their neurobiological and clinical role. Diabetologia. 2012;**55**(12):3152-3154. DOI: 10.1007/s00125-012-2727-6

[12] Gilon P, Henquin JC. Mechanism and physiological significance of the cholinergic control of pancreatic b-cell function. Endocrine Reviews. 2001;**22**(5):565-604. DOI: 10.1210/er.22.5.565

[13] Love JA, Yi E, Smith TG. Autonomic pathways regulating pancreatic exocrine secretion. Autonomic Neuroscience. 2007;**133**(1):19-34. DOI: 10.1016/j.autneu.2006.10.001

[14] Rodriguez-Diaz R, Caicedo A. Novel approaches to studying the role of innervation in the biology of pancreatic islets. Endocrinology and Metabolism Clinics of North America. 2013;**42**(1):39-56. DOI: 10.1016/j.ecl.2012.11.001

[15] Dolenšek J, Rupnik MS, Stožer A. Structural similarities and differences between the human and the mouse pancreas. Islets. 2015;**7**(1):e1024405. DOI: 10.1080/19382014.2015.1024405

[16] Coupland RE. The innervation of pancreas of the rat, cat and rabbit as revealed by the cholinesterase technique. Journal of Anatomy. 1958;**92**(1):143-149

[17] Sunami E, Kanazawa H, Hashizume H, Takeda M, Hatakeyama K, Ushiki T. Morphological characteristics of Schwann cells in the islets of Langerhans of the murine pancreas. Archives of Histology and Cytology. 2001;**64**(2):191-201. DOI: 10.1679/aohc.64.191

[18] Ushiki T, Watanabe S. Distribution and ultrastructure of the autonomic nerves in the mouse pancreas. Microscopy Research and Technique. 1997;**37**(5-6):399-406. DOI: 10.1002/(SICI)1097-0029(19970601)37:5/6<399::AID-JEMT4>3.0.CO;2-9

[19] Akmaev IG. Neuroimmunoendocrine aspects of the pathogenesis of diabetes mellitus [article in Russian]. Diabetes Mellitus. 2005;**3**:8-12

[20] Beall C, Ashford ML, McCrimmon RJ. The physiology and pathophysiology of the neural control of the counterregulatory response. American Journal of Physiology. Regulatory, Integrative and Comparative Physiology. 2012;**302**(2):R215-R223. DOI: 10.1152/ajpregu.00531.2011

[21] Osundiji MA, Evans ML. Brain control of insulin and glucagon secretion. Endocrinology and Metabolism Clinics of North America. 2013;**42**(1):1-14. DOI: 10.1016/j.ecl.2012.11.006

[22] Chumasov EI, Petrova ES, Korzhevskii DE. Distribution and structural organization of the autonomic nervous apparatus in the rat pancreas (an immunohistochemical

study). Neuroscience and Behavioral Physiology. 2012;**42**(8):781-788. DOI: 10.1007/s11055-012-9635-6

[23] Krivova IS, Barabanov VM, Savel'eva ES, Savel'ev SV. Neuroendocrine complexes in the pancreas of nutria (*Myocastor coypus*) (an immunohistochemical study) [article in Russian]. Morfologiia. 2009;**135**(3):59-62

[24] Van Campenhout E. Contributions a l'etude de l'histogenese du pancreas, chez quelques mammiferes. Les complexes sympathico-insulaires. [Contributions to the study of the histogenesis of the pancreas in some mammals. The sympathico-insular complex]. Archives de Biologie. 1927;**37**:121-171

[25] Simard LC. Les complecses neuro-insulaires. [The neuro-insular complex]. Archives d'anatomie microscopique. 1937;**33**:49-64

[26] Fujita T. Histological studies on the neuro-insular complex in the pancreas of some mammals. Zeitschrift fur Zellforschung und Mikroskopische Anatomie. 1959;**50**(1):94-109. DOI: 10.1007/bf00342656

[27] Tang SC, Shen CN, Lin PY, Peng SJ, Chien HJ, Chou YH, Chamberlain CE, Pasricha PJ. Pancreatic neuro-insular network in young mice revealed by 3D panoramic histology. Diabetologia. 2018;**61**(1):158-167. DOI: 10.1007/s00125-017-4408-y

[28] Chien HJ, Peng SJ, Hua TE, Kuo CH, Juang JH, Tang SC. 3-D imaging of islets in obesity: Formation of the islet-duct complex and neurovascular remodeling in young hyperphagic mice. International Journal of Obesity. 2015;**40**(4):685-697. DOI: 10.1038/ijo.2015.224

[29] Tang SC, Baeyens L, Shen CN, Peng SJ, Chien HJ, Scheel DW, Chamberlain CE, German MS. Human pancreatic neuro-insular network in health and fatty infiltration. Diabetolo gia. 2018;**61**(1):168-181. DOI: 10.1007/s00125-017-4409-x

[30] Von Dorsche HH, Falt K, Hahn HJ, Reiher H. Neuron-specific enolase (NSE) as a neuroendocrine cell marker in the human fetal pancreas. Acta Histochemica. 1989;**85**(2):227-228. DOI: 10.1016/s0065-1281(89)80073-x

[31] Kim J, Richter W, Aanstoot HJ, Shi Y, Fu Q, Rajotte R, et al. Differential expression of GAD65 and GAD67 in human, rat, and mouse pancreatic islets. Diabetes. 1993;**42**(12):1799-1808. DOI: 10.2337/diabetes.42.12.1799

[32] Teitelman G, Alpert S, Polak JM, Martinez A, Hanahan D. Precursor cells of mouse endocrine pancreas coexpress insulin, glucagon and the neuronal proteins tyrosine hydroxylase and neuropeptide Y, but not pancreatic polypeptide. Development. 1993;**118**(4):1031-1039

[33] Schwitzgebel VM, Scheel DW, Conners JR, Kalamaras J, Lee JE, Anderson DJ, Sussel L, Johnson JD, German MS. Expression of neurogenin3 reveals an islet cell precursor population in the pancreas. Development. 2000 Aug;**127**(16):3533-3542

[34] Bonal C, Herrera PL. Genes controlling pancreas ontogeny. The International Journal of Developmental Biology. 2008;**52**(7):823-835. DOI: 10.1387/ijdb.072444cb

[35] Naya FJ, Huang HP, Qiu Y, Mutoh H, DeMayo FJ, Leiter AB, Tsai MJ. Diabetes, defective pancreatic morphogenesis, and abnormal enteroendocrine differentiation in BETA2/

neuroD-deficient mice. Genes & Development. 1997 Sep 15;**11**(18):2323-2334. DOI: 10.1101/gad.11.18.2323

[36] Pearse AG, Polak JM. Neural crest origin of the endocrine polypeptide (APUD) cells of the gastrointestinal tract and pancreas. Gut. 1971;**12**(10):783-788. DOI: 10.1136/gut.12.10.783

[37] Amella C, Cappello F, Kahl P, Fritsch H, Lozanoff S, Sergi C. Spatial and temporal dynamics of innervation during the development of fetal human pancreas. Neuroscience. 2008;**154**(4):1477-1487. DOI: 10.1016/j.neuroscience.2008.04.050

[38] Cerf ME. Islet organogenesis, angiogenesis and innervation. Cell Biology International. 2011;**35**(11):1065-1078. DOI: 10.1042/CBI20100780

[39] Pan FC, Brissova M. Pancreas development in humans. Current Opinion in Endocrinology, Diabetes, and Obesity. 2014;**21**(2):77-82. DOI: 10.1097/MED.0000000000000047

[40] Abdulreda MH, Rodriguez-Diaz R, Cabrera O, Caicedo A, Berggren PO. The different faces of the pancreatic islet. Advances in Experimental Medicine and Biology. 2016;**938**:11-24. DOI: 10.1007/978-3-319-39824-2_2

[41] Kiba T. Relationships between the autonomic nervous system and the pancreas including regulation of regeneration and apoptosis: Recent developments. Pancreas. 2004;**29**(2):e51-e58. DOI: 10.1097/00006676-200408000-00019

[42] Lausier J, Diaz WC, Roskens V, Larock K, Herzer K, Fong CG, et al. Vagal control of pancreatic beta-cell proliferation. American Journal of Physiology. Endocrinology and Metabolism. 2010;**299**(5):E786-E793. DOI: 10.1152/ajpendo.00202.2010

[43] Borden P, Houtz J, Leach SD, Kuruvilla R. Sympathetic innervation during development is necessary for pancreatic islet architecture and functional maturation. Cell Reports. 2013;**4**(2):287-301. DOI: 10.1016/j.celrep.2013.06.019

[44] Rodriguez-Diaz R, Abdulreda MH, Formoso AL, Gans I, Ricordi C, Berggren PO, et al. Innervation patterns of autonomic axons in the human endocrine pancreas. Cell Metabolism. 2011;**14**(1):45-54. DOI: 10.1016/j.cmet.2011.05.008

[45] Rodriguez-Diaz R, Dando R, Jacques-Silva MC, Fachado A, Molina J, Abdulreda MH, Ricordi C, Roper SD, Berggren PO, Caicedo A. Alpha cells secrete acetylcholine as a non-neuronal paracrine signal priming beta cell function in humans. Nature Medicine. 2011;**17**(7):888-892. DOI: 10.1038/nm.2371

[46] Chiu YC, Hua TE, Fu YY, Pasricha PJ, Tang SC. 3-D imaging and illustration of the perfusive mouse islet sympathetic innervation and its remodelling in injury. Diabetologia. 2012;**55**(12):3252-3261. DOI: 10.1007/s00125-012-2699-6

[47] Stožer A, Dolenšek J, Rupnik MS. Glucose-stimulated calcium dynamics in islets of Langerhans in acute mouse pancreas tissue slices. PLoS One. 2013;**8**(1):e54638. DOI: 10.1371/journal.pone.0054638

[48] Fink T, Di Sebastiano P, Bochlerj M, Beger HG, Weihe E. Growth-associated protein-43 and protein gene-product 9,5 innervation in human pancreas: Changes in chronic pancreatitis. Neuroscience. 1994;**63**(1):249-266. DOI: 10.1016/0306-4522(94)90020-5

[49] Castorina S, Romeo R, Marcello MF. Immunohistochemical study of intrinsic innervation in the human pancreas. Bollettino della Società Italiana di Biologia Sperimentale. 1996;**72**(1-2):1-7

[50] Pour PM, Saruc M. The pattern of neural elements in the islets of normal and diseased pancreas and in isolated islets. Journal of the Pancreas: JOP. 2011;**12**(4):395-403

[51] Böck P. Fine structure of the neuro-insular complex type II in the cat. Archivum Histologicum Japonicum. 1986;**49**(2):189-197. DOI: 10.1679/aohc.49.189

[52] Furuzawa Y, Ohmori Y, Watanabe T. Anatomical localization of sympathetic postganglionic and sensory neurons innervating the pancreas of the cat. The Journal of Veterinary Medical Science. 1996 Mar;**58**(3):243-248

[53] Brunicardi FC, Shavelle DM, Andersen DK. Neural regulation of the endocrine pancreas. International Journal of Pancreatology. 1995;**18**(3):177-195. DOI: 10.1007/BF02784941

[54] Salvioli B, Bovara M, Barbara G, De Ponti F, Stanghellini V, Tonini M, et al. Neurology and neuropathology of the pancreatic innervation. Journal of the Pancreas: JOP. 2002;**3**(2):26-33

[55] Cabrera-Vasquez S, Navarro-Tableros V, Sanchez-Soto C, Gutierrez-Ospina G, Hiriart M. Remodelling sympathetic innervation in rat pancreatic islets ontogeny. BMC Developmental Biology. 2009;**9**(1):34. DOI: 10.1186/1471-213x-9-34

[56] Myojin T, Kitamura N, Hondo E, Baltazar ET, Pearson GT, Yamada J. Immunohistochemical localization of neuropeptides in bovine pancreas. Anatomia, Histologia, Embryologia. 2000 Jun;**29**(3):167-172. DOI: 10.1046/j.1439-0264.2000.00257.x

[57] Tiscornia OM. The neural control of exocrine and endocrine pancreas. The American Journal of Gastroenterology. 1977 Jun;**67**(6):541-560

[58] Yi SQ, Miwa K, Ohta T, Kayahara M, Kitagawa H, Tanaka A, Shimokawa T, Akita K, Tanaka S. Innervation of the pancreas from the perspective of perineural invasion of pancreatic cancer. Pancreas. 2003;**27**(3):225-229

[59] Almaça J, Weitz J, Rodriguez-Diaz R, Pereira E, Caicedo A. The pericyte of the pancreatic islet regulates capillary diameter and local blood flow. Cell Metabolism. 2018;**27**(3):630-644.e4. DOI: 10.1016/j.cmet.2018.02.016

[60] Rinaman L, Miselis RR. The organization of vagal innervation of rat pancreas using cholera toxin-horseradish peroxidase conjugate. Journal of the Autonomic Nervous System. 1987;**21**(2-3):109-125

[61] Chen XH, Itoh M, Sun W, Miki T, Takeuchi Y. Localization of sympathetic and parasympathetic neurons innervating pancreas and spleen in the cat. Journal of the Autonomic Nervous System. 1996;**59**(1-2):12-16

[62] Browning KN, Coleman FH, Travagli RA. Characterization of pancreas-projecting rat dorsal motor nucleus of vagus neurons. American Journal of Physiology. Gastrointestinal and Liver Physiology. 2005 May;**288**(5):G950-G955. DOI: 10.1152/ajpgi.00549.2004

[63] Berthoud HR, Powley TL. Morphology and distribution of efferent vagal innervation of rat pancreas as revealed with anterograde transport of Dil. Brain Research. 1991;**553**(2):336-341

[64] Molina J, Rodriguez-Diaz R, Fachado A, Jacques-Silva MC, Berggren PO, Caicedo A. Control of insulin secretion by cholinergic signaling in the human pancreatic islet. Diabetes. 2014;**63**(8):2714-2726. DOI: 10.2337/db13-1371

[65] Won MH, Park HS, Jeong YG, Park HJ. Afferent innervation of the rat pancreas: Retrograde tracing and immunohistochemistry in the dorsal root ganglia. Pancreas. 1998;**16**(1):80-87

[66] Szallasi A, Blumberg PM. Vanilloid (capsaicin) receptors and mechanisms. Pharmacological Reviews. 1999;**51**(2):159-212

[67] Standop J, Ulrich A, Schneider MB, Andrén-Sandberg A, Pour PM. Pacinian corpuscle in the human pancreas. Pancreas. 2001;**23**(1):36-39

[68] García-Suárez O, Calavia MG, Pérez-Moltó FJ, Alvarez-Abad C, Pérez-Piñera P, Cobo JM, Vega JA. Immunohistochemical profile of human pancreatic pacinian corpuscles. Pancreas. 2010;**39**(3):403-410. DOI: 10.1097/MPA.0b013e3181bc0372

[69] Watari N. Fine structure of nervous elements in the pancreas of some vertebrates. Zeitschrift für Zellforschung und Mikroskopische Anatomie. 1968;**85**(3):291-314

[70] Khochanskij DN, Makarova OV. Current views on the structure and function of enteric nervous system [article in Russian]. Morphological Newsletter. 2015;**1**:106-117

[71] Kirchgessner AL, Gershon MD. Innervation of the pancreas by neurons in the gut. The Journal of Neuroscience. 1990;**10**(5):1626-1642

[72] Kirchgessner AL, Pintar JE. Guinea pig pancreatic ganglia: Projections, transmitter content, and the type-specific localization of monoamine oxidase. The Journal of Comparative Neurology. 1991;**305**(4):613-631

[73] Love JA, Szebeni K. Morphology and histochemistry of the rabbit pancreatic innervation. Pancreas. 1999;**18**(1):53-64

[74] Shelagurov AA. Pancreatitis [in Russian]. Moscow: Medicine; 1967. 360 p

[75] Halban PA, Wollheim CB, Blondel B, Meda P, Niesor EN, Mintz DH. The possible importance of contact between pancreatic islet cells for the control of insulin release. Endocrinology 1982;**111**(1):86-94

[76] Tengholm A, Gylfe E. Oscillatory control of insulin secretion. Molecular and Cellular Endocrinology. 2009;**297**(1-2):58-72. DOI: 10.1016/j.mce.2008.07.009

[77] Rosario W, Singh I, Wautlet A, Patterson C, Flak J, Becker TC, et al. The brain to pancreatic islet neuronal map reveals differential glucose regulation from distinct hypothalamic regions. Diabetes. 2016;**65**(9):2711-2723. DOI: 10.2337/db15-0629

[78] Kobayashi S, Fujita T. Fine structure of mammalian and avian pancreatic islets with a special reference to D-cells and nervous elements. Zeitschrift für Zellforschung und Mikroskopische Anatomie. 1969;**100**(3):340-363. DOI: 10.1007/bf00571491

[79] Donev S. Ultrastructural evidence for presence of a glial sheath investing the islets of Langerhans in the pancreas of mammals. Cell and Tissue Research. 1984;**237**(2):343-348. DOI: 10.1007/bf00217154

[80] Gilliam LK, Palmer JP, Taborsky GJ Jr. Tyramine-mediated activation of sympathetic nerves inhibits insulin secretion in humans. The Journal of Clinical Endocrinology and Metabolism 2007;**92**(10):4035-4038. DOI: 10.1210/jc.2007-0536.

[81] Karlsson S, Scheurink AJ, Steffens AB, Ahrén B. Involvement of capsaicin-sensitive nerves in regulation of insulin secretion and glucose tolerance in conscious mice. The American Journal of Physiology. 1994;**267**(4 Pt2):R1071-R1077

[82] Gardemann A, Jungermann K, Grosse V, Cossel L, Wohlrab F, Hahn HJ, Blech W, Hildebrandt W. Intraportal transplantation of pancreatic islets into livers of diabetic rats. Reinnervation of islets and regulation of insulin secretion by the hepatic sympathetic nerves. Diabetes. 1994;**43**(11):1345-1352

[83] Burris R, Hebrok M. Pancreatic innervation in mouse development and β-cell regeneration. Neuroscience. 2007;**150**(3):592-602. DOI: 10.1016/j.neuroscience.2007.09.079

[84] Pervushin VI, Stavrova NP. Comparison between the development of the nervous apparatus and parenchymatous units of the pancreas in human embryogenesis [article in Russian]. Arkhiv Anatomii, Gistologii i Émbriologii. 1973;**65**(8):68-74

[85] Bashkin AD. Development of the neural apparatus of the human pancreas in prenatal ontogeny [article in Russian]. Arkhiv Anatomii, Gistologii i Émbriologii. 1988;**94**(2):13-18

[86] Jabotinsky YM. Normal and pathological morphology of vegetative ganglia [in Russian]. Moscow: AMS USSR; 1953. 291 p

[87] Cegrell L. The postnatal occurrence of biogenic monoamines in pancreatic islets of golden hamsters. Acta Endocrinologica. 1975;**78**(2):289-293

[88] Thomas NW, Findlay JA. Innervation of the endocrine pancreas in the mongolian gerbil (*Meriones unguiculatus*). In: Coupland RE, Forssmann WG, editors. Peripheral Neuroendocrine Interactions. New York: Springer Verlag; 1978. pp. 134-143

[89] Krivova YS, Proshchina AE, Barabanov VM, Saveliev SV. Development of the islets of Langerhans in the human fetal pancreas. In: Satou A, Nakamura H, editors. Pancreas: Anatomy, Diseases and Health Implications. Nova Science Publishers: NY; 2012. pp. 53-88

[90] Krivova Y, Proshchina A, Barabanov V, Leonova O, Saveliev S. Structure of neuro-endocrine and neuro-epithelial interactions in human foetal pancreas. Tissue & Cell. 2016;**48**(6):567-576. DOI: 10.1016/j.tice.2016.10.005

[91] Krivova YS, Proshchina AE, Barabanov VM, Saveliev SV. Neuro-Insular Complexes in the Human Pancreas. In: Seicean A, editor. Challenges in Pancreatic Pathology. Rijeka, Croatia: InTech; 2017. pp. 3-17. DOI: 10.5772/65059

Section 3

Autonomic Nervous System and Implications in Clinical Practice

Chapter 3

Autonomic Nervous System and Neurocardiac Physiopathology

John G. Kingma, Denys Simard and
Jacques R. Rouleau

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.77087

Abstract

The autonomic nervous system regulates multiple physiological functions; how distinct neurons in peripheral autonomic and intrathoracic ganglia communicate remains to be established. Increasing focus is being paid to functionality of the neurocardiac axis and crosstalk between the intrinsic nervous system and diverse organ systems. Current findings indicate that progression of cardiovascular disease comprises peripheral and central aspects of the cardiac nervous system hierarchy. Indeed, autonomic neuronal dysfunction is known to participate in arrhythmogenesis and sudden cardiac death; diverse interventions (pharmacological, non-pharmacological) that affect neuronal remodeling in the heart following injury caused by cardiovascular disease (congestive heart failure, etc.) or acute myocardial infarction are being investigated. Herein we examine recent findings from clinical and animal studies on the role of the intrinsic cardiac nervous system on regulation of myocardial perfusion and the consequences of cardiac injury. We also discuss different interventions that target the autonomic nervous system, stimulate neuronal remodeling and adaptation, and thereby optimize patient outcomes.

Keywords: autonomic nervous system, sympathetic, parasympathetic nerves, intrinsic cardiac neurons, intrinsic cardiac nervous system, ischemia, arrhythmias

1. Introduction

Physiological functions (i.e. muscle contraction, glandular function, visceral activity, nerve impulses, etc.) of the body are controlled by the autonomic nervous system (ANS). Innervation to the heart is consistent among species [1–3]; the ANS comprises central, intrathoracic extracardiac and intrinsic cardiac components (see review by Hanna et al. [4]). The sympathetic and parasympathetic systems interact to stimulate energy expenditure under conditions of stress or return the

body to a restful state; these systems comprise pathways that include preganglionic and postganglionic neurons (activated by endogenous chemical neurotransmitters). Increasing attention focuses on the complex anatomy and function of the cardiac neuraxis; how diverse populations of neurons in peripheral autonomic and intrathoracic ganglia communicate with each other and between different organ systems remains the subject of ongoing investigation. Treatment strategies that modulate the ANS are being developed and tested in the setting of cardiac dysfunction, arrhythmias and sudden death with the objective of stimulating or maintaining cardiovascular function. Improved mechanistic understanding of changes that occur within the nervous system hierarchy during pathogenesis of cardiac disease is therefore essential. This chapter examines current scientific literature on the effects of ischemia on the cardiac nervous system; the role of intrinsic cardiac neurons on regulation of myocardial blood flow, cardiac function, pathogenesis of nerve and myocardial tissue injury is discussed. For this review, clinical and basic science reports were searched on MEDLINE, Google Scholar and PubMed with the keywords intrinsic cardiac nervous system (ICNS), ischemia, reperfusion, cellular protection, myocardium, nerves and combinations thereof. In addition, we referred to data from our own research.

2. Cardiac nervous system

The sympathetic (adrenergic) component of the ANS stimulates cardiac conduction and myocardial cells; on the other hand, the parasympathetic (cholinergic) nervous system exerts an inhibitory influence [5, 6]. Regulation of cardiac performance by the ANS involves modulation of heart rate (positive chronotropy), increases in cardiac contractility (positive inotropy) and cardiac relaxation (positive lusitropy), decreased venous capacitance plus constriction of resistance and cutaneous vessels [7].

Sympathetic cardiac nerves originate from stellate, superior, middle cervical and thoracic ganglia [8]; postganglionic sympathetic neurons project efferent axons to the heart [9]. Parasympathetic nerves develop from the cardiac component of the cranial neural crest; preganglionic neurons access to the heart occurs via the vagus nerves [10, 11]. Cardiac ganglia are located in epicardial fat, in ganglionated plexi adjacent to the major cardiac vessels and in the ventricular wall [12–14]. ANS neurons are classified by chemical phenotyping; cholinergic and adrenergic populations of ganglionic cardiac neurons are readily found in cardiac ganglia [15–17]. Sensory neurons, interneurons and sensory fibers that develop from the *nucleus ambiguus* [18–20] likely play a role in pathogenesis of cardiac disease. In fact, activation of the neuroendocrine system is considered central to pathogenesis of cardiac disease; excess sympathetic activation promotes cardiovascular dysfunction, arrhythmias and sudden death [21]. Of note, is that visualization of the ICNS and the presence of interneuron connections is particularly challenging [22–25]; however, several immune histochemical techniques which target specific neurotransmitters within parasympathetic and sympathetic neurons have been particularly successful [26–30]. Neuroimaging techniques, cardioneural optical mapping and optogenetics are also being used to define the complex anatomy of the cardiac nervous system in animals and living humans to evaluate the role of the ANS in normal cardiac function as well as pathogenesis of cardiac disease [4, 31–33].

http://dx.doi.org/10.5772/intechopen.77087

3. Vasoregulation

Arteries normally respond to humoral, metabolic, mechanical and neural stimulation; local metabolic control occurs secondarily to myocardial metabolic change [34, 35]. In the heart, blood flow across the ventricular wall is precisely coordinated to metabolic requirements via adjustments in vessel tone; flow is therefore independent of external physical factors since metabolism is the ultimate determinant of regional perfusion over the operative range of autoregulation [36–38]. The ANS contributes to regulation of myocardial blood flow; sympathetic nerve stimulation produces a biphasic response, which trends to coronary dilatation resulting from increases in myocardial oxygen demand as well as perfusion pressure [39, 40]. Neuropeptide chemicals elevate local catecholamine levels that modulate cardiac dynamics and indirectly increase blood flow across the left ventricular wall [40–42]. In dogs, with an intact cardiac nervous system, we documented significant increases in myocardial blood flow following application of nicotine or bradykinin to selected ganglionated plexi on the heart [40]; stimulation of nicotine sensitive neurons increases cardiac metabolic demand (i.e. higher heart rate and LV systolic pressure) but stimulation with bradykinin produces a similar result without affecting LV pressure. On the other hand, Vergroesen et al. documented that intact cardiac nerves were not essential for regulation of coronary blood flow [43]; however, they suggested that cardiac nerves essentially alter the speed of response of the coronary vascular bed to changes in heart rate and perfusion pressure. The cardiac nervous reflexes thought to be responsible for these effects has not been established but diverse cardiac afferent fibers and receptor subtypes (i.e. ventricular, coronary artery) have been studied.

Stimulation of ventricular mechanoreceptors causes an increase in arterial perfusion pressure, which results in greater blood volume and reflex coronary vasodilatation [44, 45]; higher perfusion pressures promote vasoconstriction. However, stimulation of coronary artery baroreceptors also promote reflex vasodilatation [46]. These reflex responses following mechanoreceptor stimulation may confer protection against arterial injury and thereby slow progression of coronary artery disease.

Local release of prostaglandins, nitric oxide (NO) and endothelium-derived relaxation factors stimulate activation of select populations of cardiac neurons that contribute to vasoregulation. NO contributes to neuronal mediated vasoregulation; NO induced vasodilatation involves adrenergic, myogenic and hormonal influences [47, 48]. NO in concert with other vasoactive mediators effectively counteracts vasoconstrictor mechanisms [49–51]; these effects may be gender dependent. Three nitric oxide synthase (NOS) isoforms that synthesize NO from L-arginine have been documented; of these, two are constitutively expressed Ca^{2+}-dependent isoforms—eNOS (endothelial) is localized in cardiocytes as well as vascular and endocardial endothelium while nNOS (neuronal) is found in cardiac neurons [52–54]. The ubiquitous nature of NO implies a role in regulation of central nervous system, myocardium and vascular function [55]; nNOS and cardiac inhibitory G protein are believed to work in parallel in order to reduce sinus node rate and thereby modulate heart rate variability [56]. NO directly affects intrinsic cardiac neurons; almost 40% of these neurons are NOS positive [57]. Altered neuronal effects of NO may be important in pathogenesis of hypertension, septic shock, diabetes mellitus, etc.

Studies from our laboratory, in dogs subject to acute cardiac decentralization, indicated that intrinsic cardiac neurons function independently of central neuronal inputs. In decentralized and innervated hearts coronary autoregulation was similar (**Figure 1**) despite substantial reductions in myocardial oxygen demand (in decentralized hearts) [58]. In addition, perfusion across the ventricular wall (in decentralized hearts; **Figure 2**) was preserved thus confirming

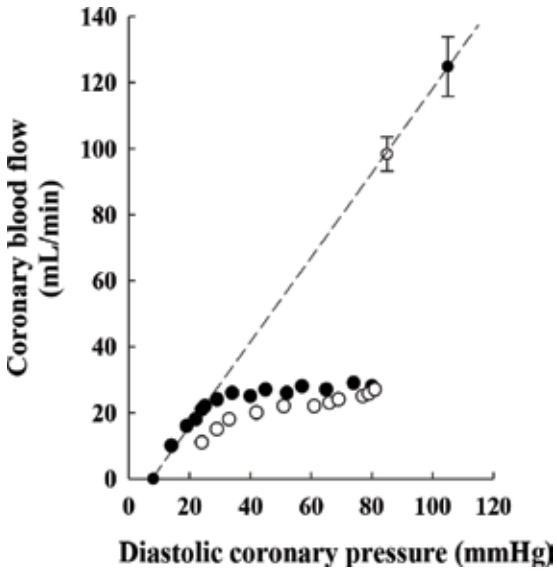

Figure 1. Coronary blood flow versus diastolic coronary artery pressure during autoregulation. Pressure-flow relations in dogs with intact cardiac nerves (closed circles) and after extracardiac nerve ablation (open circles) are shown. Note the similarity between the two curves; reactive hyperemia blood flow was lower in decentralized dogs as shown.

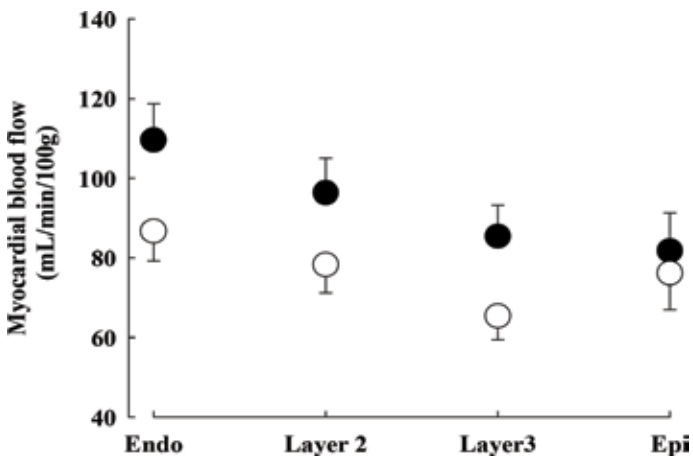

Figure 2. Myocardial blood flow distribution across the ventricular wall (measured with microspheres) in hearts from dogs with intact cardiac nerves (closed symbols) and after acute cardiac decentralization (open symbols). Data are means ± SEM.

earlier findings of Rimoldi and coworkers [59] who reported no change in transmural distribution of myocardial blood flow after regional sympathetic denervation. Stability of perfusion across the ventricular wall was suggested to be due to several factors including neuronal modulation, autoregulation and variations in coronary resistance at the microvessel level (<100 μm). Interestingly, in neuropathy patients the innervation/ventricular perfusion ratio during reactive hyperemia is lower in innervated compared to denervated regions within the ventricular wall [60]. These findings are considerably different from those reported in human subjects after suppression of adenosine-mediated sympathetic activation [61].

4. Ischemic injury

4.1. Nervous system

Ischemia significantly modulates function of intrinsic cardiac neurons because of local accumulations of metabolic by-products (i.e. reactive oxygen species, purinergic compounds, etc.) [62–64]. A limited number of animal studies have investigated the overall effects of ischemia on activity of nerves that course over, or through infarcted myocardium [65]; findings indicate that blood supply to these cardiac nerves is not a determining factor for conduction of action potentials [66]. The question of whether, or not, cardiac nerves are more, or less, sensitive to ischemia is less adequately studied; consequently, the injury threshold of cardiac nerves and neurons remains unknown. However, it is possible that activity of cardiac neurons post-ischemia is preserved consequent to stimulation of ventricular sensory neurites that transduce mechanical and chemical milieu in the myocardium [67]. During acute myocardial ischemia, norepinephrine is released from sympathetic nerves; this triggers sympathetic nerve regeneration (i.e. sprouting, budding) and nerve remodeling to promote sympathetic hyperinnervation, which ultimately plays a role in arrhythmogenesis [68–72]. Function of cardiac sympathetic neurons post-ischemia can also be triggered by the elevated production of intra-neuronal galanin (i.e. promotes regeneration of sympathetic axons) [73]; galanin modifies synaptic transmission and contributes to arrhythmogenesis and sudden cardiac death. Additionally, multiple autacoids (adenosine, bradykinin, NO, reactive oxygen species, etc.) produced during ischemia stimulate the central nervous system, cardiac autonomic ganglia and sympathetic efferent postganglionic axons in coronary vessels [74, 75]. Neuropeptide chemicals released from sensory neurites also modulate intrinsic cardiac neuronal activity [41, 76]. It is interesting to speculate that common survival pathways of cardiac neurons may be shared with cardiocytes but this has not been established.

4.2. Heart

Infarction causes major changes between peripheral and central aspects of the cardiac nervous system; structural and functional alterations at the cardiomyocyte level include; (1) changes in collagen matrix [77], (2) induction of electromechanical dyssynchrony [78], (3) ventricular contractile dysfunction [79], apoptosis [80], etc. In the heart, ischemia affects the ICNS which is the convergence point for cardiac neural control. As such, afferent inputs are modulated

along with descending neural inputs [78] (i.e. including reflex-induced sympathoexcitation and reduced central drive from parasympathetic nerves [81, 82]). Heightened sympathetic tone partly mediated by neurotransmission through the stellate ganglia has been linked to cardiac pathogenesis as well as risk of cardiac arrhythmias and sudden death [83, 84].

Acute coronary artery occlusion produces distinct alterations in cardiomyocyte pathology that ultimately contribute to cell death; for cardiac myocytes a transmural gradient of cell death occurs in relation to duration and degree of ischemia [85, 86]. Transmural necrosis is mostly manifest by 6 h after acute coronary occlusion; the potential for tissue salvage after this time is limited (i.e. species dependent). Physiopathology of ischemic injury is generally well-documented [87–90]; numerous cytoprotective strategies to limit ischemic injury (i.e. pharmacologic, endogenous, etc.) have been tested but none has achieved widespread clinical use [90–92]. Post-ischemic remodeling of sympathetic neurons in stellate ganglia is not well established; however, a potential relation exists between ganglion inflammation and oxidative stress [93]. A recent study in rodents documented greater oxidative stress (i.e. lipofuscin accumulation, mitochondrial degeneration, etc.), metabolic activity (higher rate of lipid peroxidation) and inflammation in stellate glial cells [94]. These physiopathological mechanisms are believed to contribute to local inflammation (i.e. leukocyte infiltration) within stellate ganglia; this stimulates neuronal activity and oxidative stress, which increases cardiac afferent neurotransmission [95]. Other contributing factors include circulating neurohormonal compounds (i.e. angiotensin II, etc.) and brainstem-mediated increases in efferent sympathetic outflow [96–98]. Equally, cardiac inflammation and oxidative stress produced by repeated defibrillation are involved in ganglion pathology [99].

The importance of cardiac nerves for the pathogenesis of post-ischemic infarct development and cardiac dysfunction has been investigated in experimental models of ischemia-reperfusion injury. In cardiac decentralized pigs, significant ventricular dysfunction accompanied by patchy subendocardial necrosis occurs after acute coronary occlusion [100]; myocardial perfusion-function relations in these animals were not affected by nerve status. In addition, we reported coronary vascular reserve to be comparable after nerve ablation albeit in a different experimental model [101], which is consistent with most published findings [102–105]. We also confirmed a trend towards smaller infarcts in dogs subject to extracardiac nerve ablation or pharmacologic decentralization using the autonomic ganglionic blocker hexamethonium bromide (**Figure 3**) [106]; these findings are also in agreement with earlier studies documenting increased tolerance to ischemic injury and a reduction in ventricular fibrillation threshold post-decentralization [102, 107, 108]. Reduced oxygen demand and improved perfusion of affected tissues could be responsible for increased ischemic tolerance of myocytes [43, 104, 105, 109] in the absence of intact cardiac nerves. Of note, extracardiac surgical ablation of sympathetic and parasympathetic efferent neuronal inputs produces a decentralized (not denervated) heart without complete elimination of parasympathetic involvement [110, 111]; on the other hand, pharmacologic ganglionic blockade with hexamethonium bromide blocks transmission within peripheral autonomic ganglia and vagal cardio-acceleration [112]. Continued research into the identification of endogenous compounds that modulate or activate intrinsic neuronal populations to induce cellular protection remains a priority.

http://dx.doi.org/10.5772/intechopen.77087

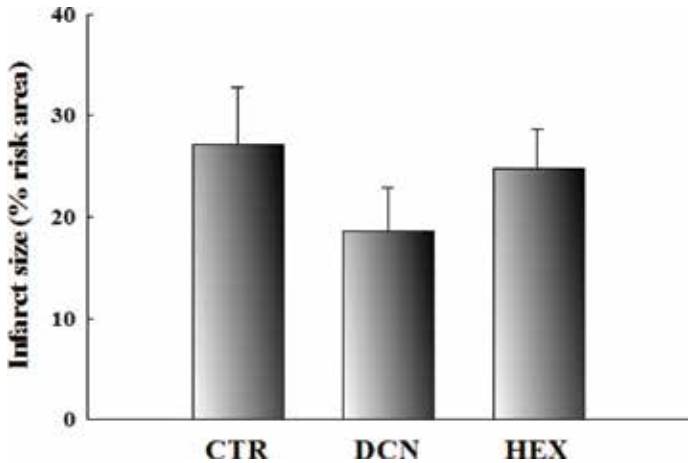

Figure 3. Histogram of myocardial infarct size (as percent of anatomic area at risk) in dogs undergoing ischemia–reperfusion injury. Three distinct groups are shown: (1) control (CTR); (2) acute cardiac decentralized (DCN) and (3) hexamethonium bromide (HEX). Data are mean ± 1SD; n = 8/group.

4.3. Cardiac arrhythmogenesis

Sudden cardiac death due to ventricular arrhythmias is highly relevant to cardiovascular disease related mortality [113]; autonomic neuronal dysfunction is a major contributor to induction of atrial and ventricular dysrhythmias [114–116]. Pathologically induced disturbances in neural processing within the cardiac neural hierarchy affect efferent neuronal outputs throughout the myocardium [19, 117] (i.e. intrinsic and extrinsic cardiac ganglia, central reflexes [95, 118–121]). Cardiac neurons are generally classified as afferent, efferent or convergent on the basis of responses to various cardiovascular stimuli [31, 120]. A study from Ardell's laboratory examined functional remodeling of neuronal elements within the context of myocardial infarction [120]; they showed that: (1) morphological and phenotypic remodeling of intracardiac ganglia is dependent on the site of injury, (2) attenuation of afferent neural signals to intrinsic cardiac neurons (i.e. within ischemic zone) but preservation of these signals in remote and border regions (i.e. neural sensory border zone), (3) autonomic efferent inputs to intrinsic cardiac neurons are maintained, (4) transduction capacity increases in convergent intrinsic local circuit neurons (of the heart) and (5) connectivity of intrinsic cardiac neurons is reduced. Current findings suggest that neuronal remodeling can occur independently of direct injury to specific neuron subsets; as such, neuronal plasticity within the cardiac neuroaxis is crucial post-infarction and during progression of cardiovascular disease [122, 123]. Indeed, healed myocardium provides a particular challenge to electrical propagation and regulation of cardiac function [124, 125]; abnormal cardiac efferent signaling results in continuous discord between central and peripheral aspects within the neural hierarchy that produces fatal arrhythmias due to excessive sympathoexcitation [122]. The peri-infarct region (i.e. interface between dense scar and surviving myocardium) also has an increased potential for ectopic beats [71, 126]. Ajijola and coworkers recently determined that (1) despite scarring, myocyte loss and ion channel remodeling significant regulation of electrical activation

occurs via cardiac sympathetic nerves within the peri-infarct region, and (2) there is significant remodeling of sympathetic innervation within the anteroapical region [127]; additionally, they emphasized the critical role of adrenergic activation in modulating propagation patterns.

Premature ventricular contractions (PVC; contraction of the ventricles caused by abnormal electrical activity) often lead to cardiovascular events, left ventricle dysfunction and sudden cardiac death [128]; multiple mechanisms have been proposed including mechanical dyssynchrony, abnormalities in calcium handling and oxygen consumption and autonomic imbalance [122, 129, 130]. Hamon and coworkers documented (using *in vivo* cardioneural mapping) that PVCs are a powerful stressor for the ICNS and that PVC-induced neural and electrophysiological changes are critical for arrhythmogenesis and remodeling. PVCs with variable coupling intervals have a more complex impact on cardiac neurons than those with fixed short or long coupling intervals [128]. The unpredictability of coupling intervals could trigger a sympathovagal imbalance that influences cardiomyocyte function and leads to electric instability. Greater neuronal responses (particularly within convergent neurons that are responsible for reflex processing) to variable compared to constant stimulus (i.e. neural adaptation) have been described in sensory neurons of the visual, auditory and olfactory systems [131, 132]. In the heart sympathetic nerve activity is greater during irregular cardiac pacing and is independent of hemodynamic changes [133]. As such, increased variability of PVC coupling interval could play a role in reflex activation of the ANS. Greater understanding of underlying mechanoelectric mechanisms of PVC-induced arrhythmogenesis should help to improve risk stratification in cardiac patients that would allow use of more aggressive pharmacologic and non-pharmacologic therapeutics (i.e. specifically targeting the ANS) in prophylactic management (cf. **Table 1**).

	Pertinent studies
Pharmacological interventions	
• Neuregulin-1	[134–138]
• Ghrelin	[139]
• Vasopressin	[140]
• Anesthetic preconditioning	[141]
Non-pharmacological interventions	
• Transcutaneous electrical nerve stimulation (TENS)	[142–144]
• Bioelectronic block	[145–148]
• Spinal cord stimulation (SCS)	[118, 149–152]
• Vagal nerve stimulation (VNS)	[78, 153, 154]
• Renal nerve denervation	[155–157]
• Cardiac decentralization and carotid body ablation	[158–162]
• Cardiac conditioning (ischemia, exercise)	[21, 163, 164]

Table 1. Management strategies that target the autonomic nervous system.

http://dx.doi.org/10.5772/intechopen.77087

5. Therapeutic interventions

5.1. Pharmacological

Pharmacologic interventions can play an important role in post-ischemic nerve repair; though most medications reduce the incidence of arrhythmias some can be proarrhythmic [165]. Significant improvement in acute and chronic ischemic cardiomyopathy, myocarditis and vagus nerve remodeling have recently been reported in clinical and experimental studies with different pharmacological approaches such as epidermal growth factor neuregulin-1 (NRG1) [134, 138, 166–168]. NRG1 is a key factor for cardiac development [136, 169]; NRG1 activates tyrosine kinase causing a host of cardiovascular effects: (1) regulation of structure and function in cardiomyocytes (i.e. apoptosis, cell proliferation), (2) promotion of angiogenesis and (3) downregulation of sympathetic nerve mRNA and protein expression levels to inhibit nerve remodeling [134, 135, 137, 170].

5.2. Non-pharmacological

Cardiovascular disease is often accompanied by increased activity of carotid body chemoreceptors, which induces an autonomic imbalance [161]; ablation of carotid bodies has been documented to markedly improve post-ischemic cardiovascular end-points in clinical and animal studies [159, 160, 171]. Catheter ablation techniques have been used effectively in patients with ventricular tachyarrhythmias [147]; in addition, bilateral cardiac stellate decentralization (removes excessive sympathetic input to cardiomyocytes) is used in subjects that do not respond to conventional treatments [158]. A drawback of the latter intervention is that it is permanent and generally accompanied by secondary effects [172]. Of note is that the ICNS preserves the ability to coordinate neural activity and electrical stability even after disconnection of inputs from higher central command (i.e. brain) [173].

Specific neuron subpopulations can be targeted for neuromodulation therapy [174–176]; spinal cord stimulation (SCS), vagus nerve stimulation (VNS) and bioelectronic therapy (i.e. charge-balanced direct current, axonal modulation, kilohertz (kHz) frequency alternating current, etc.) are used in ischemic heart disease patients to abate reflex activation of peripheral ganglia [118, 147, 148, 174, 177, 178]. Application of electric current by stimulation/suppression of action potential propagation along nerves modulates neuron and organ function [145, 179]. Blockade of action potential propagation is produced by either kHz frequency alternating current or direct current; these protocols are used repeatedly and safely in patients [145, 147, 180].

SCS stimulates sympathetic afferents to transduce signals, which initiate from the ischemic myocardium, to spinal cord dorsal horn neurons [121, 181]. In the majority of patients receiving this treatment beneficial effects (i.e. improved exercise capacity, quality of life, etc.) last for more than a year [182, 183]. Additionally, SCS augments resistance to stress in myocytes by modifying myocyte energetics [177, 184]; in our laboratory, we documented that concurrent SCS did not influence post-ischemic ventricular perfusion [150].

VNS, on the other hand, protects myocardium [185–187] through anti-adrenergic interactions (i.e. higher parasympathetic activity stimulates muscarinic receptor activation that

limits excess adrenergic receptor activation [188]) within intrinsic cardiac ganglia [189, 190] combined with reduced release of norepinephrine from presynaptic mechanisms in ischemic myocardium [191]. VNS also influences myocyte energetics due to its regulatory effects on glycogen metabolism [78, 185]; all of these factors can change sensory transduction within the cardiac milieu in the event of disparities between oxygen and nutrient supply and demand.

Salavation and co-workers have examined potential differences between SCS and VNS with regard to their ability to alter cardiac sensory neurons in nodose ganglia to transduce the ischemic myocardium; they reported that these interventions differentially obtund nociceptive-related nodose afferent neuronal inputs to the medulla but do not affect mechanosensitive transduction capabilities [192]. These nerve stimulation techniques are presently being tested in a number of clinical trials in heart failure patients (i.e. NECTAR-HF, ANTHEM-HF, INOVATE-HF) with promising results [153, 193, 194].

Intact neural pathways may be unimportant for protection of ischemic myocardium; this is most apparent in the transplanted heart where autonomic ganglia are disconnected from central neurons [121, 195]. Endogenous compounds released into the bloodstream or locally near nerves, neurons and cardiomyocytes, etc. during ischemia could stimulate intracellular pathways that transduce cytoprotective mechanisms. For instance, cardiac conditioning, which significantly delays development of post-ischemic tissue injury [91, 196–198], might involve activation of the ICNS (cf. recent review [90]). A variety of conditioning stratagems (both pharmacologic and non-pharmacologic) that trigger cellular transduction pathways (guanylate cyclase, kinases, etc.) mediate cellular protection through end-effectors; significant cross-tolerance exists with regard to the mechanisms involved [106, 199, 200].

6. Conclusions

Neurocardiology involves dynamic exchange between neurohumoral control systems and the cardiac milieu; bi-directional interactions between sympathetic and parasympathetic efferent pathways regulate inter-organ communications at different levels of the neuraxis. This is evident in the cardiac conditioning paradigm (i.e. pre-, per-, post- and remote) where endogenous ligands and catecholamines trigger intracellular transduction pathways to mediate cytoprotective end-effectors that promote cell survival [201, 202]. Strategies that protect against non-lethal ischemic injury could depend on nervous system status the question of how cytoprotective signals are transmitted between organs remains crucial. New findings support the concept that disorders within the ANS contribute to pathogenesis of organ injury, co-morbidities [203, 204] and even survival. Improved comprehension of modifications within the cardiac-neuro axis at the molecular, cellular, organ and whole body levels are critical for development of therapeutic strategies.

Acknowledgements

The authors would like to thank Professor Chantale Simard, Faculty of Pharmacy at Laval University for helpful suggestions during the preparation of this review.

Conflict of interest

The authors have no conflicts of interest to declare.

Author details

John G. Kingma[1,2]*, Denys Simard[2] and Jacques R. Rouleau[1,2]

*Address all correspondence to: john.kingma@fmed.ulaval.ca

1 Department of Medicine, Faculty of Medicine, Laval University, Québec, QC, Canada

2 Centre de Recherche de l'institut de Cardiologie et de Pneumologie du Québec, Université Laval, Québec, QC, Canada

References

[1] Hopkins DA, Armour JA. Localization of sympathetic postganglionic and parasympathetic preganglionic neurons which innervate different regions of the dog heart. The Journal of Comparative Neurology. 1984;**229**(2):186-198. DOI: 10.1002/cne.902290205

[2] Janes RD, Brandys JC, Hopkins DA, Johnstone DE, Murphy DA, Armour JA. Anatomy of human extrinsic cardiac nerves and ganglia. The American Journal of Cardiology. 1986;**57**(4):299-309. PubMed PMID: 3946219

[3] Kawashima T. The autonomic nervous system of the human heart with special reference to its origin, course, and peripheral distribution. Anatomy and Embryology. 2005;**209**(6):425-438. DOI: 10.1007/s00429-005-0462-1

[4] Hanna P, Rajendran PS, Ajijola OA, Vaseghi M, Andrew Armour J, Ardell JL, et al. Cardiac neuroanatomy—Imaging nerves to define functional control. Autonomic Neuroscience. 2017;**207**:48-58. DOI: 10.1016/j.autneu.2017.07.008

[5] Pardini BJ, Lund DD, Schmid PG. Organization of the sympathetic postganglionic innervation of the rat heart. Journal of the Autonomic Nervous System. 1989;**28**(3):193-201

[6] Wallis D, Watson AH, Mo N. Cardiac neurones of autonomic ganglia. Microscopy Research and Technique. 1996;**35**(1):69-79. DOI: 10.1002/(SICI)1097-0029(19960901)35:1<69::AID-JEMT6>3.0.CO;2-N

[7] Lymperopoulos A, Rengo G, Koch WJ. Adrenergic nervous system in heart failure: Pathophysiology and therapy. Circulation Research. 2013;**113**(6):739-753. DOI: 10.1161/CIRCRESAHA.113.300308. PubMed PMID: 23989716

[8] Kuder T, Nowak E. Autonomic cardiac nerves: Literature review. Folia Morphologica. 2015;**74**(1):1-8. DOI: VM/OJS/J/38007 [pii];10.5603/FM.2015.0003

[9] Hopkins DA, MacDonald SE, Murphy DA, Armour JA. Pathology of intrinsic cardiac neurons from ischemic human hearts. The Anatomical Record. 2000;**259**:424-436

[10] Hasan W. Autonomic cardiac innervation: Development and adult plasticity. Organogenesis. 2013;**9**(3):176-193. DOI: 24892 [pii];10.4161/org.24892

[11] Kimura K, Ieda M, Fukuda K. Development, maturation, and transdifferentiation of cardiac sympathetic nerves. Circulation Research. 2012;**110**(2):325-336. DOI: 110/2/325 [pii];10.1161/CIRCRESAHA.111.257253

[12] Ardell JL, Randall WC. Selective vagal innervation of sinoatrial and atrioventricular nodes in canine heart. American Journal of Physiology-Heart and Circulatory Physiology. 1986;**251**:H764-HH73

[13] Randall WC, Milosavljevic M, Wurster RD, Geis GS, Ardell JL. Selective vagal innervation of the heart. Annals of Clinical and Laboratory Science. 1986;**16**(3):198-208

[14] Singh S, Johnson PI, Lee RE, Orfei E, Lonchyna VA, Sullivan HJ, et al. Topography of cardiac ganglia in the adult human heart. The Journal of Thoracic and Cardiovascular Surgery. 1996;**112**(4):943-953. DOI: S0022-5223(96)70094-6

[15] Horackova M, Armour JA, Byczko Z. Distribution of intrinsic cardiac neurons in whole-Mount Guinea pig atria identified by multiple neurochemical coding. A confocal microscope study. Cell and Tissue Research. 1999;**297**(3):409-421. DOI: 92970409.441

[16] Slavikova J, Kuncova J, Reischig J, Dvorakova M. Catecholaminergic neurons in the rat intrinsic cardiac nervous system. Neurochemical Research. 2003;**28**(3-4):593-598

[17] Weihe E, Schutz B, Hartschuh W, Anlauf M, Schafer MK, Eiden LE. Coexpression of cholinergic and noradrenergic phenotypes in human and nonhuman autonomic nervous system. The Journal of Comparative Neurology. 2005;**492**(3):370-379. DOI: 10.1002/cne.20745

[18] Ai J, Epstein PN, Gozal D, Yang B, Wurster R, Cheng ZJ. Morphology and topography of nucleus ambiguus projections to cardiac ganglia in rats and mice. Neuroscience. 2007;**149**(4):845-860. DOI: S0306-4522(07)00953-0

[19] Armour JA. Myocardial ischaemia and the cardiac nervous system. Cardiovascular Research. 1999;**41**:41-54

[20] McAllen RM, Salo LM, Paton JF, Pickering AE. Processing of central and reflex vagal drives by rat cardiac ganglion neurones: An intracellular analysis. The Journal of Physiology. 2011;**589**(Pt 23):5801-5818. DOI: jphysiol.2011.214320

[21] Ardell JL, Andresen MC, Armour JA, Billman GE, Chen PS, Foreman RD, et al. Translational neurocardiology: Preclinical models and cardioneural integrative aspects. The Journal of Physiology. 2016;**594**(14):3877-3909. DOI: 10.1113/JP271869

[22] Bentivoglio M, Kuypers HG, Catsman-Berrevoets CE, Loewe H, Dann O. Two new fluorescent retrograde neuronal tracers which are transported over long distances. Neuroscience Letters. 1980;**18**(1):25-30. PubMed PMID: 6189013

[23] Grkovic I, Fernandez K, McAllen RM, Anderson CR. Misidentification of cardiac vagal pre-ganglionic neurons after injections of retrograde tracer into the pericardial space in the rat. Cell and Tissue Research. 2005;**321**(3):335-340. DOI: 10.1007/s00441-005-1145-1

[24] Kuypers HG, Bentivoglio M, van der Kooy D, Catsman-Berrevoets CE. Retrograde transport of bisbenzimide and propidium iodide through axons to their parent cell bodies. Neuroscience Letters. 1979;**12**(1):1-7. PubMed PMID: 88694

[25] Tomney PA, Hopkins DA, Armour JA. Axonal branching of canine sympathetic postganglionic cardiopulmonary neurons. A retrograde fluorescent labeling study. Brain Research Bulletin. 1985;**14**(5):443-452. PubMed PMID: 2411359

[26] Batulevicius D, Pauziene N, Pauza DH. Architecture and age-related analysis of the neuronal number of the Guinea pig intrinsic cardiac nerve plexus. Annals of Anatomy. 2005;**187**(3):225-243. DOI: 10.1016/j.aanat.2005.01.004

[27] Karnovsky MJ, Roots L. A "direct-coloring" thiocholine method for cholinesterases. The Journal of Histochemistry and Cytochemistry. 1964;**12**:219-221. DOI: 10.1177/12.3.219

[28] Kawano H, Okada R, Yano K. Histological study on the distribution of autonomic nerves in the human heart. Heart and Vessels. 2003;**18**(1):32-39. DOI: 10.1007/s003800300005

[29] Pauza DH, Rysevaite-Kyguoliene K, Vismantaite J, Brack KE, Inokaitis H, Pauza AG, et al. A combined acetylcholinesterase and immunohistochemical method for precise anatomical analysis of intrinsic cardiac neural structures. Annals of Anatomy. 2014;**196**(6):430-440. DOI: 10.1016/j.aanat.2014.08.004

[30] Wang X, Pinol RA, Byrne P, Mendelowitz D. Optogenetic stimulation of locus ceruleus neurons augments inhibitory transmission to parasympathetic cardiac vagal neurons via activation of brainstem alpha1 and beta1 receptors. The Journal of Neuroscience. 2014;**34**(18):6182-6189. DOI: 10.1523/JNEUROSCI.5093-13.2014

[31] Beaumont E, Salavatian S, Southerland EM, Vinet A, Jacquemet V, Armour JA, et al. Network interactions within the canine intrinsic cardiac nervous system: Implications for reflex control of regional cardiac function. The Journal of Physiology. 2013;**591**(18): 4515-4533. DOI: 10.1113/jphysiol.2013.259382

[32] Bravo PE, Lautamaki R, Carter D, Holt DP, Nekolla SG, Dannals RF, et al. Mechanistic insights into sympathetic neuronal regeneration: Multitracer molecular imaging of catecholamine handling after cardiac transplantation. Circulation. Cardiovascular Imaging. 2015;**8**(8):e003507. DOI: 10.1161/CIRCIMAGING.115.003507

[33] Calkins H, Lehmann MH, Allman K, Wieland D, Schwaiger M. Scintigraphic pattern of regional cardiac sympathetic innervation in patients with familial long QT syndrome using positron emission tomography. Circulation. 1993;**87**(5):1616-1621. PubMed PMID: 8491017

[34] Feigl EO, Van Winkle DM, Miyashiro JK. Cholinergic vasodilatation of coronary resistance vessels in dogs, baboons and goats. Blood Vessels. 1990;**27**:94-105

[35] Feigl EO. Coronary physiology. Physiological Reviews. 1983;**63**:1-205

[36] Downey JM. Myocardial contractile force as a function of coronary blood flow. The American Journal of Physiology. 1976;**230**:1-6

[37] Boatwright RB, Downey HF, Bashour FA, Crystal GJ. Transmural variation in autoregulation of coronary blood flow in hyperperfused canine myocardium. Circulation Research. 1980;**47**(4):599-609

[38] Rouleau JR, Boerboom LE, Surjadhana A, Hoffman JIE. The role of autoregulation and tissue diastolic pressures in the transmural distribution of left ventricular blood flow in anesthetized dogs. Circulation Research. 1979;**45**(6):804-815

[39] Mohrman DE, Feigl EO. Competition between sympathetic vasoconstriction and metabolic vasodilation in the canine coronary circulation. Circulation Research. 1978;**42**:79-86

[40] Kingma JG Jr, Armour JA, Rouleau JR. Chemical modulation of in situ intrinsic cardiac neurones influences myocardial blood flow in the anaesthetised dog. Cardiovascular Research. 1994;**28**(9):1403-1406. PubMed PMID: 7954653

[41] Armour JA, Huang MH, Smith FM. Peptidergic modulation of in situ canine intrinsic cardiac neurons. Peptides. 1993;**14**(2):191-202

[42] Butler CK, Smith FM, Cardinal R, Murphy DA, Hopkins DA. Cardiac responses to electrical stimulation of discrete loci in atrial and ventricular ganglionated plexi. American Journal of Physiology-Heart and Circulatory Physiology. 1990;**259**:H1365-H1H73

[43] Vergroesen I, Merkus D, Van Teeffelen JWGE, Dankelman J, Spaan JAE, Van Wezel HB, et al. Chronic cardiac denervation affects the speed of coronary vascular regulation. Cardiovascular Research. 1999;**44**:615-622

[44] Dankelman J, Vergroesen I, Han Y, Spaan JAE. Dynamic response of coronary regulation to heart rate and perfusion changes in dogs. American Journal of Physiology-Heart and Circulatory Physiology. 1992;**263**:H447-HH52

[45] Vergroesen I, Noble MI, Spaan JA. Intramyocardial blood volume change in first moments of cardiac arrest in anesthetized goats. The American Journal of Physiology. 1987;**253**(2 Pt 2):H307-H316. DOI: 10.1152/ajpheart.1987.253.2.H307

[46] McMahon NC, Drinkhill MJ, Hainsworth R. Reflex vascular responses from aortic arch, carotid sinus and coronary baroreceptors in the anaesthetized dog. Experimental Physiology. 1996;**81**(3):397-408. PubMed PMID: 8737074

[47] Zanzinger J. Role of nitric oxide in the neural control of cardiovascular function. Cardiovascular Research. 1999;**43**:639-649

[48] Paton JF, Kasparov S, Paterson DJ. Nitric oxide and autonomic control of heart rate: A question of specificity. Trends in Neurosciences. 2002;**25**(12):626-631. PubMed PMID: 12446130

[49] Toda N, Kitamura Y, Okamura T. Neural mechanism of hypertension by nitric oxide synthase inhibitor in dogs. Hypertension. 1993;**21**:3-8

[50] Thorin E, Huang PL, Fishman MC, Bevan JA. Nitric oxide inhibits a2-adrenoceptor-mediated endothelium-dependent vasodilation. Circulation Research. 1998;**82**:1323-1329

[51] Thorin E, Atkinson J. Modulation by the endothelium of sympathetic vasoconstriction in an in vitro preparation of the rat tail artery. British Journal of Pharmacology. 1994;**111**(1):351-357

[52] Amezcua JL, Palmer RMJ, Souza BM, Moncada S. Nitric oxide synthesized from L-arginine regulates vascular tone in the coronary circulation of the rabbit. British Journal of Pharmacology. 1989;**97**:1119-1124

[53] Schulz R, Smith JA, Lewis MJ, Moncada S. Nitric oxide synthase in cultured endocardial cells of the pig. British Journal of Pharmacology. 1991;**104**:21-24

[54] Sosunov AA, Hassall CJS, Loesch A, Turamine M, Burnstock G. Ultrastructural investigation of nitric oxide synthase immunoreactive nerves associated with coronary blood vessels of rat and Guinea-pig. Cell and Tissue Research. 1995;**280**:575-582

[55] Christopherson KS, Bredt DS. Nitric oxide in excitable tissues: Physiological roles and disease. The Journal of Clinical Investigation. 1997;**100**:2424-2429

[56] Brown AM. Regulation of heartbeat by G protein-coupled ion channels. The American Journal of Physiology. 1990;**259**:H1621-H16H8

[57] Armour JA, Smith FM, Losier AM, Ellenberger HH, Hopkins DA. Modulation of intrinsic cardiac neuronal activity by nitric oxide donors induces cardiodynamic changes. The American Journal of Physiology. 1995;**268**(2 Pt 2):R403-RR13

[58] Rouleau JR, Simard D, Rodrigue N, Blouin A, Kingma JG Jr. Myocardial blood flow after chronic cardiac decentralization in anesthetized dogs: Effects of ACE-inhibition. Autonomic Neuroscience. 2002;**97**(1):12-18. DOI: S1566-0702(02)00002-4

[59] Rimoldi OE, Drake-Holland AJ, Noble MI, Camici PG. Basal and hyperaemic myocardial blood flow in regionally denervated canine hearts: An in vivo study with positron emission tomography. European Journal of Nuclear Medicine and Molecular Imaging. 2007;**34**(2):197-205. DOI: 10.1007/s00259-006-0233-0

[60] Stevens MJ, Dayanikli F, Raffel DM, Allman KC, Sandford T, Feldman EL, et al. Scintigraphic assessment of regionalized defects in myocardial sympathetic innervation and blood flow regulation in diabetic patients with autonomic neuropathy. Journal of the American College of Cardiology. 1998;**31**(7):1575-1584. DOI: S0735-1097(98)00128-4

[61] Kaufmann PA, Rimoldi O, Gnecchi-Ruscone T, Bonser RS, Luscher TF, Camici PG. Systemic inhibition of nitric oxide synthase unmasks neural constraint of maximal myocardial blood flow in humans. Circulation. 2004;**110**(11):1431-1436. DOI: 10.1161/01.CIR.0000141294.25130.54

[62] Huang H-S, Pan H-L, Stahl GL, Longhurst JC. Ischemia- and reperfusion-sensitive cardiac sympathetic afferents: Influence of H_2O_2 and hydroxyl radicals. American Journal of Physiology-Heart and Circulatory Physiology. 1995;**269**:H888-H901

[63] Huang MH, Sylven C, Horackova M, Armour JA. Ventricular sensory neurons in canine dorsal root ganglia: Effects of adenosine and substance P. The American Journal of Physiology. 1995;**269**(2 Pt 2):R318-RR24

[64] Thompson GW, Horackova M, Armour JA. Sensitivity of canine intrinsic cardiac neurons to H_2O_2 and hydroxyl radical. The American Journal of Physiology 1998;275(4 Pt 2):H1434-H1H40

[65] Barber MJ, Mueller TM, Henry DP, Felten SY, Zipes DP. Transmural myocardial infarction in the dog produces sympathectomy in noninfarcted myocardium. Circulation. 1983;**67**:787-796

[66] Janes RD, Johnstone DE, Armour JA. Functional integrity of intrinsic cardiac nerves located over an acute transmural myocardial infarction. Canadian Journal of Physiology and Pharmacology. 1987;**65**:64-69

[67] Arora RC, Armour JA. Adenosine A1 receptor activation reduces myocardial reperfusion effects on intrinsic cardiac nervous system. American Journal of Physiology. Regulatory, Integrative and Comparative Physiology. 2003;**284**(5):R1314-R1R21

[68] Holmes JW, Laksman Z, Gepstein L. Making better scar: Emerging approaches for modifying mechanical and electrical properties following infarction and ablation. Progress in Biophysics and Molecular Biology. 2016;**120**(1-3):134-148. DOI: 10.1016/j.pbiomolbio.2015.11.002

[69] Prabhu SD, Frangogiannis NG. The biological basis for cardiac repair after myocardial infarction: From inflammation to fibrosis. Circulation Research. 2016;**119**(1):91-112. DOI: 10.1161/CIRCRESAHA.116.303577

[70] Wang Y, Suo F, Liu J, Hu H, Xue M, Cheng W, et al. Myocardial infarction induces sympathetic hyperinnervation via a nuclear factor-kappaB-dependent pathway in rabbit hearts. Neuroscience Letters. 2013;**535**:128-133. DOI: 10.1016/j.neulet.2012.12.059

[71] Cao JM, Fishbein MC, Han JB, Lai WW, Lai AC, Wu TJ, et al. Relationship between regional cardiac hyperinnervation and ventricular arrhythmia. Circulation. 2000;**101**(16):1960-1969. PubMed PMID: 10779463

[72] Yu FS, Zhang Y, Feng Y, Zhang L, Ma YH, Song W, et al. Nerve remodeling in a canine model of atrial fibrillation induced by 48 hours right atrial pacing. Zhonghua Xin Xue Guan Bing Za Zhi. 2010;**38**(7):644-647. PubMed PMID: 21055291

[73] Habecker BA, Gritman KR, Willison BD, Van Winkle DM. Myocardial infarction stimulates galanin expression in cardiac sympathetic neurons. Neuropeptides. 2005;**39**(2):89-95. DOI: S0143-4179(04)00151-9 [pii];10.1016/j.npep.2004.11.003

[74] Abe T, Morgan DA, Gutterman DD. Role of adenosine receptor subtypes in neural stunning of sympathetic coronary innervation. American Journal of Physiology-Heart and Circulatory Physiology. 1997;**272**(41):H25-H34

[75] Allen TGJ, Burnstock G. The actions of adenosine 5′-triphosphate on Guinea-pig intracardiac neurones in culture. British Journal of Pharmacology. 1990;**100**:269-276

[76] Croom JE, Foreman RD, Chandler MJ, Barron KW. Cutaneous vasodilation during dorsal column stimulation is mediated by dorsal roots and CGRP. The American Journal of Physiology. 1997;**272**:H950-H9H7

[77] Dobaczewski M, de Haan JJ, Frangogiannis NG. The extracellular matrix modulates fibroblast phenotype and function in the infarcted myocardium. Journal of Cardiovascular Translational Research. 2012;**5**(6):837-847. DOI: 10.1007/s12265-012-9406-3

[78] Beaumont E, Southerland EM, Hardwick JC, Wright GL, Ryan S, Li Y, et al. Vagus nerve stimulation mitigates intrinsic cardiac neuronal and adverse myocyte remodeling postmyocardial infarction. American Journal of Physiology. Heart and Circulatory Physiology. 2015;**309**(7):H1198-H1206. DOI: 10.1152/ajpheart.00393.2015

[79] Mollema SA, Liem SS, Suffoletto MS, Bleeker GB, van der Hoeven BL, van de Veire NR, et al. Left ventricular dyssynchrony acutely after myocardial infarction predicts left ventricular remodeling. Journal of the American College of Cardiology. 2007;**50**(16):1532-1540. DOI: 10.1016/j.jacc.2007.07.025

[80] Crow MT, Mani K, Nam YJ, Kitsis RN. The mitochondrial death pathway and cardiac myocyte apoptosis. Circulation Research. 2004;**95**(10):957-970. DOI: 10.1161/01.RES.0000148632.35500.d9

[81] Armour JA. Potential clinical relevance of the 'little brain' on the mammalian heart. Experimental Physiology. 2008;**93**(2):165-176

[82] Billman GE. A comprehensive review and analysis of 25 years of data from an in vivo canine model of sudden cardiac death: Implications for future anti-arrhythmic drug development. Pharmacology & Therapeutics. 2006;**111**(3):808-835. DOI: 10.1016/j.pharmthera.2006.01.002

[83] Shivkumar K, Ardell JL. Cardiac autonomic control in health and disease. The Journal of Physiology. 2016;**594**(14):3851-3852. DOI: 10.1113/JP272580

[84] Shivkumar K, Ajijola OA, Anand I, Armour JA, Chen PS, Esler M, et al. Clinical neurocardiology defining the value of neuroscience-based cardiovascular therapeutics. The Journal of Physiology. 2016;**594**(14):3911-3954. DOI: 10.1113/JP271870. PubMed PMID: 27114333

[85] Reimer KA, Lowe JE, Rasmussen MM, Jennings RB. The wavefront phenomenon of ischemic cell death: I. Myocardial infarct size vs. duration of coronary occlusion in dogs. Circ. 1977;**56**:786-794

[86] Jennings RB. Historical perspective on the pathology of myocardial ischemia/reperfusion injury. Circulation Research. 2013;**113**(4):428-438. DOI: 10.1161/CIRCRESAHA.113.300987

[87] Kloner RA, Jennings RB. Consequences of brief ischemia: Stunning, preconditioning, and their clinical implications: Part 1. Circulation. 2001;**104**(24):2981-2989

[88] Kloner RA, Jennings RB. Consequences of brief ischemia: Stunning, preconditioning, and their clinical implications: Part 2. Circulation. 2001;**104**(25):3158-3167

[89] Yellon DM, Hausenloy DJ. Myocardial reperfusion injury. The New England Journal of Medicine. 2007;**357**(11):1121-1135. DOI: 357/11/1121

[90] Kingma JG, Simard D, Rouleau JR. Influence of cardiac nerve status on cardiovascular regulation and cardioprotection. World Journal of Cardiology. 2017;**9**(6):508-520. Epub June 26, 2017. DOI: 10.4330/wjc.v9.i6.508

[91] Hausenloy DJ, Barrabes JA, Botker HE, Davidson SM, Di Lisa F, Downey J, et al. Ischaemic conditioning and targeting reperfusion injury: A 30 year voyage of discovery. Basic Research in Cardiology. 2016;**111**(6):70. DOI: 10.1007/s00395-016-0588-8

[92] Bulluck H, Yellon DM, Hausenloy DJ. Reducing myocardial infarct size: Challenges and future opportunities. Heart. 2016;**102**(5):341-348. DOI: 10.1136/heartjnl-2015-307855. PubMed PMID: 26674987

[93] James TN, Zipes DP, Finegan RE, Eisele JW, Carter JE. Cardiac ganglionitis associated with sudden unexpected death. Annals of Internal Medicine. 1979;**91**(5):727-730. PubMed PMID: 496105

[94] Ajijola OA, Hoover DB, Simerly TM, Brown TC, Yanagawa J, Biniwale RM, et al. Inflammation, oxidative stress, and glial cell activation characterize stellate ganglia from humans with electrical storm. JCI Insight. 2017;**2**(18). DOI: 10.1172/jci.insight.94715

[95] Wang HJ, Wang W, Cornish KG, Rozanski GJ, Zucker IH. Cardiac sympathetic afferent denervation attenuates cardiac remodeling and improves cardiovascular dysfunction in rats with heart failure. Hypertension. 2014;**64**(4):745-755. DOI: 10.1161/HYPERTENSIONAHA.114.03699

[96] Lambert C, Massillon Y, Meloche S. Upregulation of cardiac angiotensin II AT1 receptors in congenital cardiomyopathic hamsters. Circulation Research. 1995;**77**(5):1001-1007

[97] Zucker IH, Gao L. The regulation of sympathetic nerve activity by angiotensin II involves reactive oxygen species and MAPK. Circulation Research. 2005;**97**(8):737-739. DOI: 10.1161/01.RES.0000188261.94569.1f

[98] Lambert GW, Kaye DM, Lefkovits J, Jennings GL, Turner AG, Cox HS, et al. Increased central nervous system monoamine neurotransmitter turnover and its association with sympathetic nervous activity in treated heart failure patients. Circulation. 1995;**92**(7):1813-1818. PubMed PMID: 7545554

[99] Moss AJ, Ryan DH, Yeaney GA. Ganglionitis and genetic cardiac arrhythmias: More questions than answers. Circulation. Arrhythmia and Electrophysiology. 2014;**7**(2):190-191. DOI: 10.1161/CIRCEP.114.001589

[100] Huang CH, Vatner SF, Peppas AP, Yang G, Kudej RK. Cardiac nerves affect myocardial stunning through reactive oxygen and nitric oxide mechanisms. Circulation Research. 2003;**93**(9):866-873. DOI: 10.1161/01.RES.0000097762.64561.D2 [doi];01.RES.0000097762.64561.D2

[101] Kingma JG, Simard D, Voisine P, Rouleau JR. Influence of cardiac decentralization on cardioprotection. PLoS One. 2013;**8**(11):e79190. DOI: 10.1371/journal.pone.0079190 [doi];PONE-D-13-17924 [pii]

[102] Jones CE, Devous MD Sr, Thomas JX Jr, Dupont E. The effect of chronic cardiac denervation on infarct size following acute coronary occlusion. American Heart Journal. 1978;**95**(6):738-746

[103] Jones CE, Beck LY, Dupont E, Barnes GE. Effects of coronary ligation of the chronically sympathectomized dog ventricle. The American Journal of Physiology. 1978;**235**(4):H429-HH34

[104] Dupont E, Jones CE, Luedecke RA, Smith EE. Chronic ventricular sympathectomy: Effect on myocardial perfusion after ligation of the circumflex coronary artery in dogs. Circulatory Shock. 1979;**6**(4):323-331

[105] Lavallee M, Amano J, Vatner SF, Manders WT, Randall WC, Thomas JXJ. Adverse effects of chronic cardiac denervation in conscious dogs with myocardial ischemia. Circulation Research. 1985;**57**(3):383-392

[106] Kingma JG Jr, Simard D, Voisine P, Rouleau JR. Role of the autonomic nervous system in cardioprotection by remote preconditioning in isoflurane-anaesthetized dogs. Cardiovascular Research. 2011;**89**(2):384-391. DOI: 10.1093/cvr/cvq306

[107] Shen YT, Knight DR, Vatner SF, Randall WC, Thomas JX Jr. Responses to coronary artery occlusion in conscious dogs with selective cardiac denervation. The American Journal of Physiology. 1988;**255**(3 Pt 2):H525-H533. DOI: 10.1152/ajpheart.1988.255.3.H525

[108] Kliks BR, Burgess MJ, Abildskov JA. Influence of sympathetic tone on ventricular fibrillation threshold during experimental coronary occlusion. The American Journal of Cardiology. 1975;**36**(1):45-49. PubMed PMID: 1146697

[109] Acad B-A, Joselevitz-Goldman J, Scholz PM, Weiss HR. Improved distribution of regional oxygenation in denervated ischemic dog myocardium. Circulation Research. 1988;**62**(5):1041-1048

[110] Brunsting JR, Schuil HA, Zijlstra WG. Intrinsic heart rate in the dog determined by pharmacologic denervation. The American Journal of Physiology. 1983;**245**(4):H592-H5H7

[111] Barber MJ, Mueller TM, Davies BG, Zipes DP. Phenol topically applied to canine left ventricular epicardium interrupts sympathetic but not vagal afferents. Circulation Research. 1984;**55**(4):532-544

[112] Sonoyama K, Tajima K, Fujiwara R, Kasai T. Intravenous infusion of hexamethonium and atropine but not propranolol diminishes apolipoprotein A-IV gene expression in rat ileum. The Journal of Nutrition. 2000;**130**(3):637-641

[113] Chugh SS, Reinier K, Teodorescu C, Evanado A, Kehr E, Al Samara M, et al. Epidemiology of sudden cardiac death: Clinical and research implications. Progress in Cardiovascular Diseases. 2008;**51**(3):213-228. DOI: 10.1016/j.pcad.2008.06.003

[114] Armour JA, Hageman GR, Randall WC. Arrhythmias induced by local cardiac nerve stimulation. The American Journal of Physiology. 1972;**223**(5):1068-1075. DOI: 10.1152/ajplegacy.1972.223.5.1068

[115] Gelband H, Rosen MR, Myerburg RJ, Bush HL, Bassett AL, Hoffman BF. Restorative effect of epinephrine on the electrophysiologic properties of depressed human atrial tissue. Journal of Electrocardiology. 1977;**10**(4):313-320. PubMed PMID: 915399

[116] Zipes DP. Antiarrhythmic therapy in 2014: Contemporary approaches to treating arrhythmias. Nature Reviews. Cardiology. 2015;**12**(2):68-69. DOI: 10.1038/nrcardio.2014.211

[117] Kember G, Armour JA, Zamir M. Neural control hierarchy of the heart has not evolved to deal with myocardial ischemia. Physiological Genomics. 2013;**45**(15):638-644. DOI: physiolgenomics.00027.2013 [pii];10.1152/physiolgenomics.00027.2013

[118] Foreman RD, Linderoth B, Ardell JL, Barron KW, Chandler MJ, Hull SSJ, et al. Modulation of intrinsic cardiac neurons by spinal cord stimulation: Implications for its therapeutic use in angina pectoris. Cardiovascular Research. 2000;**47**:367-375

[119] Ajijola OA, Yagishita D, Reddy NK, Yamakawa K, Vaseghi M, Downs AM, et al. Remodeling of stellate ganglion neurons after spatially targeted myocardial infarction: Neuropeptide and morphologic changes. Heart Rhythm. 2015;**12**(5):1027-1035. DOI: 10.1016/j.hrthm.2015.01.045

[120] Rajendran PS, Nakamura K, Ajijola OA, Vaseghi M, Armour JA, Ardell JL, et al. Myocardial infarction induces structural and functional remodelling of the intrinsic cardiac nervous system. The Journal of Physiology. 2016;**594**(2):321-341. DOI: 10.1113/JP271165

[121] Ardell JL, Cardinal R, Vermeulen M, Armour JA. Dorsal spinal cord stimulation obtunds the capacity of intrathoracic extracardiac neurons to transduce myocardial ischemia. American Journal of Physiology. Regulatory, Integrative and Comparative Physiology. 2009;**297**(2):R470-R477. DOI: 10.1152/ajpregu.90821.2008

[122] Fukuda K, Kanazawa H, Aizawa Y, Ardell JL, Shivkumar K. Cardiac innervation and sudden cardiac death. Circulation Research. 2015;**116**(12):2005-2019. DOI: CIRCRESAHA.116.304679 [pii];10.1161/CIRCRESAHA.116.304679

[123] Hardwick JC, Ryan SE, Beaumont E, Ardell JL, Southerland EM. Dynamic remodeling of the Guinea pig intrinsic cardiac plexus induced by chronic myocardial infarction. Autonomic Neuroscience. 2014;**181**:4-12. DOI: 10.1016/j.autneu.2013.10.008

[124] Rubart M, Zipes DP. Mechanisms of sudden cardiac death. The Journal of Clinical Investigation. 2005;**115**(9):2305-2315. DOI: 10.1172/JCI26381

[125] Vaseghi M, Shivkumar K. The role of the autonomic nervous system in sudden cardiac death. Progress in Cardiovascular Diseases. 2008;**50**(6):404-419. DOI: S0033-0620(08)00004-2 [pii];10.1016/j.pcad.2008.01.003

[126] Vracko R, Thorning D, Frederickson RG. Nerve fibers in human myocardial scars. Human Pathology. 1991;**22**(2):138-146. PubMed PMID: 1705914

[127] Ajijola OA, Lux RL, Khahera A, Kwon O, Aliotta E, Ennis DB, et al. Sympathetic modulation of electrical activation in normal and infarcted myocardium: Implications for arrhythmogenesis. American Journal of Physiology. Heart and Circulatory Physiology. 2017;**312**(3):H608-H621. DOI: 10.1152/ajpheart.00575.2016

[128] Hamon D, Rajendran PS, Chui RW, Ajijola OA, Irie T, Talebi R, et al. Premature ventricular contraction coupling interval variability destabilizes cardiac neuronal and electrophysiological control: Insights from simultaneous cardioneural mapping. Circulation. Arrhythmia and Electrophysiology. 2017;**10**(4):e004937. DOI: 10.1161/CIRCEP.116.004937

[129] Hamon D, Blaye-Felice MS, Bradfield JS, Chaachoui N, Tung R, Elayi CS, et al. A new combined parameter to predict premature ventricular complexes induced cardiomyopathy: Impact and recognition of epicardial origin. Journal of Cardiovascular Electrophysiology. 2016;**27**(6):709-717. DOI: 10.1111/jce.12967

[130] Wang Y, Eltit JM, Kaszala K, Tan A, Jiang M, Zhang M, et al. Cellular mechanism of premature ventricular contraction-induced cardiomyopathy. Heart Rhythm. 2014;**11**(11):2064-2072. DOI: 10.1016/j.hrthm.2014.07.022

[131] De Palo G, Facchetti G, Mazzolini M, Menini A, Torre V, Altafini C. Common dynamical features of sensory adaptation in photoreceptors and olfactory sensory neurons. Scientific Reports. 2013;**3**:1251. DOI: 10.1038/srep01251

[132] Grill-Spector K, Henson R, Martin A. Repetition and the brain: Neural models of stimulus-specific effects. Trends in Cognitive Sciences. 2006;**10**(1):14-23. DOI: 10.1016/j.tics.2005.11.006

[133] Segerson NM, Sharma N, Smith ML, Wasmund SL, Kowal RC, Abedin M, et al. The effects of rate and irregularity on sympathetic nerve activity in human subjects. Heart Rhythm. 2007;**4**(1):20-26. DOI: 10.1016/j.hrthm.2006.09.017

[134] Lai X, Zhong L, Fu HX, Dang S, Wang X, Zhang N, et al. Effects of neuregulin-1 on autonomic nervous system remodeling post-myocardial infarction in a rat model. Neural Regeneration Research. 2017;**12**(11):1905-1910. DOI: 10.4103/1673-5374.219054

[135] Odiete O, Hill MF, Sawyer DB. Neuregulin in cardiovascular development and disease. Circulation Research. 2012;**111**(10):1376-1385. DOI: 10.1161/CIRCRESAHA.112.267286

[136] Mei L, Xiong WC. Neuregulin 1 in neural development, synaptic plasticity and schizophrenia. Nature Reviews. Neuroscience. 2008;**9**(6):437-452. DOI: 10.1038/nrn2392

[137] Mendes-Ferreira P, De Keulenaer GW, Leite-Moreira AF, Bras-Silva C. Therapeutic potential of neuregulin-1 in cardiovascular disease. Drug Discovery Today. 2013;**18**(17-18):836-842. DOI: 10.1016/j.drudis.2013.01.010

[138] Jabbour A, Hayward CS, Keogh AM, Kotlyar E, McCrohon JA, England JF, et al. Parenteral administration of recombinant human neuregulin-1 to patients with stable chronic heart failure produces favourable acute and chronic haemodynamic responses. European Journal of Heart Failure. 2011;**13**(1):83-92. DOI: 10.1093/eurjhf/hfq152

[139] Camargo-Silva G, Turones LC, da Cruz KR, Gomes KP, Mendonca MM, Nunes A, et al. Ghrelin potentiates cardiac reactivity to stress by modulating sympathetic control and beta-adrenergic response. Life Sciences. 2018;**196**:84-92. DOI: 10.1016/j.lfs.2018.01.019

[140] Lozic M, Sarenac O, Murphy D, Japundzic-Zigon N. Vasopressin, central autonomic control and blood pressure regulation. Current Hypertension Reports. 2018;**20**(2):11. DOI: 10.1007/s11906-018-0811-0

[141] Pasqualin RC, Mostarda CT, Souza LE, Vane MF, Sirvente R, Otsuki DA, et al. Sevoflurane preconditioning during myocardial ischemia-reperfusion reduces infarct size and preserves autonomic control of circulation in rats. Acta Cirúrgica Brasileira. 2016;**31**(5):338-345. DOI: 10.1590/S0102-865020160050000008

[142] Jessurun GA, Tio RA, De Jongste MJ, Hautvast RW, Den Heijer P, Crijns HJ. Coronary blood flow dynamics during transcutaneous electrical nerve stimulation for stable angina pectoris associated with severe narrowing of one major coronary artery. The American Journal of Cardiology. 1998;**82**(8):921-926. PubMed PMID: 9794345

[143] Wang Z, Yu L, Wang S, Huang B, Liao K, Saren G, et al. Chronic intermittent low-level transcutaneous electrical stimulation of auricular branch of vagus nerve improves left

ventricular remodeling in conscious dogs with healed myocardial infarction. Circulation. Heart Failure. 2014;**7**(6):1014-1021. DOI: 10.1161/CIRCHEARTFAILURE.114.001564

[144] Stavrakis S, Humphrey MB, Scherlag BJ, Hu Y, Jackman WM, Nakagawa H, et al. Low-level transcutaneous electrical vagus nerve stimulation suppresses atrial fibrillation. Journal of the American College of Cardiology. 2015;**65**(9):867-875. DOI: 10.1016/j.jacc.2014.12.026

[145] Vrabec T, Bhadra N, Wainright J, Bhadra N, Franke M, Kilgore K. Characterization of high capacitance electrodes for the application of direct current electrical nerve block. Medical & Biological Engineering & Computing. 2016;**54**(1):191-203. DOI: 10.1007/s11517-015-1385-5

[146] Vrabec T, Bhadra N, Van Acker G, Bhadra N, Kilgore K. Continuous direct current nerve block using multi contact high capacitance electrodes. IEEE Transactions on Neural Systems and Rehabilitation Engineering. 2017;**25**(6):517-529. DOI: 10.1109/TNSRE.2016.2589541

[147] Chui RW, Buckley U, Rajendran PS, Vrabec T, Shivkumar K, Ardell JL. Bioelectronic block of paravertebral sympathetic nerves mitigates post-myocardial infarction ventricular arrhythmias. Heart Rhythm. 2017;**14**(11):1665-1672. DOI: 10.1016/j.hrthm.2017.06.025

[148] Buckley U, Chui RW, Rajendran PS, Vrabec T, Shivkumar K, Ardell JL. Bioelectronic neuromodulation of the paravertebral cardiac efferent sympathetic outflow and its effect on ventricular electrical indices. Heart Rhythm. 2017;**14**(7):1063-1070. DOI: 10.1016/j.hrthm.2017.02.020

[149] de Vries J, De Jongste MJ, Spincemaille G, Staal MJ. Spinal cord stimulation for ischemic heart disease and peripheral vascular disease. Advances and Technical Standards in Neurosurgery. 2007;**32**:63-89. PubMed PMID: 17907475

[150] Kingma JG Jr, Linderoth B, Ardell JL, Armour JA, DeJongste MJ, Foreman RD. Neuromodulation therapy does not influence blood flow distribution or left-ventricular dynamics during acute myocardial ischemia. Autonomic Neuroscience. 2001;**91**(1-2):47-54. DOI: 10.1016/S1566-0702(01)00285-5

[151] de Vries J, Svilaas T, De Jongste MJ, Nieuwland W, Hoekstra-Mars EW, Zijlstra F. Impact of electrical neurostimulation on persistent ST elevation after successful reperfusion by primary percutaneous coronary intervention. Journal of Electrocardiology. 2007;**40**(6):522-526. DOI: 10.1016/j.jelectrocard.2007.05.014

[152] de Vries J, De Jongste MJ, Durenkamp A, Zijlstra F, Staal MJ. The sustained benefits of long-term neurostimulation in patients with refractory chest pain and normal coronary arteries. European Journal of Pain. 2007;**11**(3):360-365. DOI: 10.1016/j.ejpain.2006.04.002

[153] Zannad F, De Ferrari GM, Tuinenburg AE, Wright D, Brugada J, Butter C, et al. Chronic vagal stimulation for the treatment of low ejection fraction heart failure: Results of the NEural cardiac TherApy foR heart failure (NECTAR-HF) randomized controlled trial. European Heart Journal. 2015;**36**(7):425-433. DOI: 10.1093/eurheartj/ehu345

[154] Janig W. Autonomic nervous system and inflammation. Autonomic Neuroscience. 2014; **182**:1-3. DOI: S1566-0702(14)00032-0 [pii];10.1016/j.autneu.2014.02.002

[155] Bradfield JS, Vaseghi M, Shivkumar K. Renal denervation for refractory ventricular arrhythmias. Trends in Cardiovascular Medicine. 2014;**24**(5):206-213. DOI: 10.1016/j.tcm.2014.05.006

[156] Chen C, Upadhyay A. Renal denervation for uncontrolled hypertension: Critical review of the evidence. Current Opinion in Nephrology and Hypertension. 2017;**26**(2):114-122. DOI: 10.1097/MNH.0000000000000300

[157] Nammas W, Airaksinen JK, Paana T, Karjalainen PP. Renal sympathetic denervation for treatment of patients with atrial fibrillation: Reappraisal of the available evidence. Heart Rhythm. 2016;**13**(12):2388-2394. DOI: 10.1016/j.hrthm.2016.08.043

[158] Vaseghi M, Gima J, Kanaan C, Ajijola OA, Marmureanu A, Mahajan A, et al. Cardiac sympathetic denervation in patients with refractory ventricular arrhythmias or electrical storm: Intermediate and long-term follow-up. Heart Rhythm. 2014;**11**(3):360-366. DOI: 10.1016/j.hrthm.2013.11.028. PubMed PMID: 24291775

[159] Del Rio R, Marcus NJ, Schultz HD. Carotid chemoreceptor ablation improves survival in heart failure: Rescuing autonomic control of cardiorespiratory function. Journal of the American College of Cardiology. 2013;**62**(25):2422-2430. DOI: 10.1016/j.jacc.2013.07.079. PubMed PMID: 24013056

[160] Marcus NJ, Del Rio R, Schultz EP, Xia XH, Schultz HD. Carotid body denervation improves autonomic and cardiac function and attenuates disordered breathing in congestive heart failure. The Journal of Physiology. 2014;**592**(2):391-408. DOI: 10.1113/jphysiol.2013.266221

[161] McBryde FD, Abdala AP, Hendy EB, Pijacka W, Marvar P, Moraes DJ, et al. The carotid body as a putative therapeutic target for the treatment of neurogenic hypertension. Nature Communications. 2013;**4**:2395. DOI: 10.1038/ncomms3395

[162] Paton JF, Sobotka PA, Fudim M, Engelman ZJ, Hart EC, McBryde FD, et al. The carotid body as a therapeutic target for the treatment of sympathetically mediated diseases. Hypertension. 2013;**61**(1):5-13. DOI: 10.1161/HYPERTENSIONAHA.111.00064

[163] Kudej RK, Shen YT, Peppas AP, Huang CH, Chen W, Yan L, et al. Obligatory role of cardiac nerves and alpha1-adrenergic receptors for the second window of ischemic preconditioning in conscious pigs. Circulation Research. 2006;**99**(11):1270-1276

[164] Basalay M, Barsukevich V, Mastitskaya S, Mrochek A, Pernow J, Sjoquist PO, et al. Remote ischaemic pre- and delayed postconditioning—Similar degree of cardioprotection but distinct mechanisms. Experimental Physiology. 2012;**97**(8):908-917. DOI: expphysiol.2012.064923 [pii];10.1113/expphysiol.2012.064923

[165] Priori SG, Blomstrom-Lundqvist C, Mazzanti A, Blom N, Borggrefe M, Camm J, et al. 2015 ESC guidelines for the management of patients with ventricular arrhythmias

and the prevention of sudden cardiac death: The task force for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death of the European Society of Cardiology (ESC)endorsed by: Association for European Paediatric and Congenital Cardiology (AEPC). Europace. 2015;**17**(11):1601-1687. DOI: 10.1093/europace/euv319

[166] Murphy S, Krainock R, Tham M. Neuregulin signaling via erbB receptor assemblies in the nervous system. Molecular Neurobiology. 2002;**25**(1):67-77. DOI: 10.1385/MN:25:1:067

[167] Yamada S, Marutsuka M, Inoue M, Zhang J, Abe S, Ishibashi K, et al. The interaction of the ErbB4 intracellular domain p80 with alpha-enolase in the nuclei is associated with the inhibition of the neuregulin1-dependent cell proliferation. International Journal of Biochemistry and Molecular Biology. 2014;**5**(1):21-29

[168] Gao R, Zhang J, Cheng L, Wu X, Dong W, Yang X, et al. A phase II, randomized, double-blind, multicenter, based on standard therapy, placebo-controlled study of the efficacy and safety of recombinant human neuregulin-1 in patients with chronic heart failure. Journal of the American College of Cardiology. 2010;**55**(18):1907-1914. DOI: 10.1016/j.jacc.2009.12.044

[169] Pascal D, Giovannelli A, Gnavi S, Hoyng SA, de Winter F, Morano M, et al. Characterization of glial cell models and in vitro manipulation of the neuregulin1/ErbB system. BioMed Research International. 2014;**2014**:310215. DOI: 10.1155/2014/310215

[170] Rohrbach S, Yan X, Weinberg EO, Hasan F, Bartunek J, Marchionni MA, et al. Neuregulin in cardiac hypertrophy in rats with aortic stenosis. Differential expression of erbB2 and erbB4 receptors. Circulation. 1999;**100**(4):407-412. PubMed PMID: 10421602

[171] Abdala AP, McBryde FD, Marina N, Hendy EB, Engelman ZJ, Fudim M, et al. Hypertension is critically dependent on the carotid body input in the spontaneously hypertensive rat. The Journal of Physiology. 2012;**590**(17):4269-4277. DOI: 10.1113/jphysiol.2012.237800

[172] Gossot D, Kabiri H, Caliandro R, Debrosse D, Girard P, Grunenwald D. Early complications of thoracic endoscopic sympathectomy: A prospective study of 940 procedures. The Annals of Thoracic Surgery. 2001;**71**(4):1116-1119. PubMed PMID: 11308146

[173] Vaseghi M, Lellouche N, Ritter H, Fonarow GC, Patel JK, Moriguchi J, et al. Mode and mechanisms of death after orthotopic heart transplantation. Heart Rhythm. 2009;**6**(4):503-509. DOI: S1547-5271(09)00013-7

[174] Salavatian S, Beaumont E, Longpre JP, Armour JA, Vinet A, Jacquemet V, et al. Vagal stimulation targets select populations of intrinsic cardiac neurons to control neurally induced atrial fibrillation. American Journal of Physiology. Heart and Circulatory Physiology. 2016;**311**(5):H1311-H1H20. DOI: 10.1152/ajpheart.00443.2016

[175] Ardell JL, Cardinal R, Beaumont E, Vermeulen M, Smith FM, Andrew Armour J. Chronic spinal cord stimulation modifies intrinsic cardiac synaptic efficacy in the suppression of atrial fibrillation. Autonomic Neuroscience. 2014;**186**:38-44. DOI: 10.1016/j.autneu.2014.09.017

[176] Stavrakis S, Nakagawa H, Po SS, Scherlag BJ, Lazzara R, Jackman WM. The role of the autonomic ganglia in atrial fibrillation. JACC: Clinical Electrophysiology. 2015;**1**(1-2): 1-13. DOI: 10.1016/j.jacep.2015.01.005

[177] Southerland EM, Milhorn DM, Foreman RD, Linderoth B, DeJongste MJ, Armour JA, et al. Preemptive, but not reactive, spinal cord stimulation mitigates transient ischemia-induced myocardial infarction via cardiac adrenergic neurons. American Journal of Physiology. Heart and Circulatory Physiology. 2007;**292**(1):H311-H3H7

[178] Lopshire JC, Zhou X, Dusa C, Ueyama T, Rosenberger J, Courtney N, et al. Spinal cord stimulation improves ventricular function and reduces ventricular arrhythmias in a canine postinfarction heart failure model. Circulation. 2009;**120**(4):286-294. DOI: 10.1161/CIRCULATIONAHA.108.812412

[179] McCreery DB, Agnew WF, Yuen TG, Bullara LA. Comparison of neural damage induced by electrical stimulation with faradaic and capacitor electrodes. Annals of Biomedical Engineering. 1988;**16**(5):463-481. PubMed PMID: 3189974

[180] Kilgore KL, Bhadra N. Reversible nerve conduction block using kilohertz frequency alternating current. Neuromodulation. 2014;**17**(3):242-254; discussion 54-5. DOI: 10.1111/ner.12100

[181] Ding X, Ardell JL, Hua F, McAuley RJ, Sutherly K, Daniel JJ, et al. Modulation of cardiac ischemia-sensitive afferent neuron signaling by preemptive C2 spinal cord stimulation: Effect on substance P release from rat spinal cord. American Journal of Physiology. Regulatory, Integrative and Comparative Physiology. 2008;**294**(1):R93-R101. DOI: 10.1152/ajpregu.00544.2007

[182] Bagger JP, Jensen BS, Johannsen G. Long-term outcome of spinal cord electrical stimulation in patients with refractory chest pain. Clinical Cardiology. 1998;**21**(4):286-288. PubMed PMID: 9562939

[183] de Jongste MJ, Haaksma J, Hautvast RW, Hillege HL, Meyler PW, Staal MJ, et al. Effects of spinal cord stimulation on myocardial ischaemia during daily life in patients with severe coronary artery disease. A prospective ambulatory electrocardiographic study. British Heart Journal. 1994;**71**(5):413-418. PubMed PMID: 8011403

[184] Ardell JL. Heart failure: Mechanisms of spinal cord neuromodulation for heart disease. Nature Reviews. Cardiology. 2016;**13**(3):127-128. DOI: 10.1038/nrcardio.2016.8

[185] Beaumont E, Wright GL, Southerland EM, Li Y, Chui R, KenKnight BH, et al. Vagus nerve stimulation mitigates intrinsic cardiac neuronal remodeling and cardiac hypertrophy induced by chronic pressure overload in Guinea pig. American Journal of Physiology. Heart and Circulatory Physiology. 2016;**310**(10):H1349-H1359. DOI: 10.1152/ajpheart.00939.2015

[186] Katare RG, Ando M, Kakinuma Y, Arikawa M, Handa T, Yamasaki F, et al. Vagal nerve stimulation prevents reperfusion injury through inhibition of opening of mitochondrial permeability transition pore independent of the bradycardiac effect. The Journal of Thoracic and Cardiovascular Surgery. 2009;**137**(1):223-231. DOI: S0022-5223(08)01404-9

[187] Shinlapawittayatorn K, Chinda K, Palee S, Surinkaew S, Kumfu S, Kumphune S, et al. Vagus nerve stimulation initiated late during ischemia, but not reperfusion, exerts cardioprotection via amelioration of cardiac mitochondrial dysfunction. Heart Rhythm. 2014;**11**(12):2278-2287. DOI: 10.1016/j.hrthm.2014.08.001

[188] Olshansky B, Sabbah HN, Hauptman PJ, Colucci WS. Parasympathetic nervous system and heart failure: Pathophysiology and potential implications for therapy. Circulation. 2008;**118**(8):863-871. DOI: 10.1161/CIRCULATIONAHA.107.760405

[189] Furukawa Y, Hoyano Y, Chiba S. Parasympathetic inhibition of sympathetic effects on sinus rate in anesthetized dogs. American Journal of Physiology-Heart and Circulatory Physiology. 1996;**271**(40):H44-H50

[190] McGuirt AS, Schmacht DC, Ardell JL. Autonomic interactions for control of atrial rate are maintained after SA nodal parasympathectomy. American Journal of Physiology-Heart and Circulatory Physiology. 1997;**272**(41):H2525-H2H33

[191] Kawada T, Yamazaki T, Akiyama T, Li M, Ariumi H, Mori H, et al. Vagal stimulation suppresses ischemia-induced myocardial interstitial norepinephrine release. Life Sciences. 2006;**78**(8):882-887. DOI: 10.1016/j.lfs.2005.05.087

[192] Salavatian S, Beaumont E, Gibbons D, Hammer M, Hoover DB, Armour JA, et al. Thoracic spinal cord and cervical vagosympathetic neuromodulation obtund nodose sensory transduction of myocardial ischemia. Autonomic Neuroscience. 2017;**208**:57-65. DOI: 10.1016/j.autneu.2017.08.005

[193] Premchand RK, Sharma K, Mittal S, Monteiro R, Dixit S, Libbus I, et al. Extended follow-up of patients with heart failure receiving autonomic regulation therapy in the ANTHEM-HF study. Journal of Cardiac Failure. 2016;**22**(8):639-642. DOI: 10.1016/j.cardfail.2015.11.002

[194] DiCarlo LA, Libbus I, Kumar HU, Mittal S, Premchand RK, Amurthur B, et al. Autonomic regulation therapy to enhance myocardial function in heart failure patients: The ANTHEM-HFpEF study. ESC Heart Failure. 2018;**5**(1):95-100. DOI: 10.1002/ehf2.12241

[195] Ardell JL, Butler CK, Smith FM, Hopkins DA, Armour JA. Activity of in vivo atrial and ventricular neurons in chronically decentralized canine hearts. American Journal of Physiology-Heart and Circulatory Physiology. 1991;**260**(29):H713-HH21

[196] Hausenloy DJ, Yellon DM. The therapeutic potential of ischemic conditioning: An update. Nature Reviews. Cardiology. 2011;**8**(11):619-629. DOI: 10.1038/nrcardio.2011.85

[197] Cabrera-Fuentes HA, Alba-Alba C, Aragones J, Bernhagen J, Boisvert WA, Botker HE, et al. Meeting report from the 2nd international symposium on new frontiers in cardiovascular research. Protecting the cardiovascular system from ischemia: Between bench and bedside. Basic Research in Cardiology. 2016;**111**(1):7. DOI: 10.1007/s00395-015-0527-0

[198] Kloner RA, Rezkalla SH. Preconditioning, postconditioning and their application to clinical cardiology. Cardiovascular Research. 2006;**70**(2):297-307. DOI: 10.1016/j.cardiores.2006.01.012

[199] Lynch C III. Anesthetic preconditioning: Not just for the heart? Anesthesiology. 1999;**91**(3):606-608

[200] Minguet G, Joris J, Lamy M. Preconditioning and protection against ischaemia-reperfusion in non-cardiac organs: A place for volatile anaesthetics? European Journal of Anaesthesiology. 2007;**24**(9):733-745. DOI: S0265021507000531 [pii];10.1017/S0265021507000531

[201] Hausenloy DJ, Yellon DM. Preconditioning and postconditioning: New strategies for cardioprotection. Diabetes, Obesity & Metabolism. 2008;**10**(6):451-459. DOI: DOM762 [pii];10.1111/j.1463-1326.2007.00762.x

[202] Hausenloy DJ, Yellon DM. Preconditioning and postconditioning: Underlying mechanisms and clinical application. Atherosclerosis. 2009;**204**(2):334-341. DOI: S0021-9150(08)00748-X

[203] Gerritsen J, Dekker JM, TenVoorde BJ, Kostense PJ, Heine RJ, Bouter LM, et al. Impaired autonomic function is associated with increased mortality, especially in subjects with diabetes, hypertension, or a history of cardiovascular disease: The Hoorn study. Diabetes Care. 2001;**24**(10):1793-1798

[204] Pop-Busui R. Cardiac autonomic neuropathy in diabetes: A clinical perspective. Diabetes Care. 2010;**33**(2):434-441. DOI: 33/2/434 [pii];10.2337/dc09-1294

Chapter 4

Inflammation and Autonomic Function

Ângela Leal, Mafalda Carvalho, Isabel Rocha and Helder Mota-Filipe

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.79280

Abstract

Inflammation is generally a temporary and limited condition but may lead to a chronic one if immune and physiological homeostasis are disrupted. The autonomic nervous system has an important role in the short- and, also, long-term regulation of homeostasis and, thus, on inflammation. Autonomic modulation in acute and chronic inflammation has been implicated with a sympathetic interference in the earlier stages of the inflammatory process and the activation of the vagal inflammatory reflex to regulate innate immune responses and cytokine functional effects in longer processes. The present review focuses on the autonomic mechanisms controlling proinflammatory responses, and we will discuss novel therapeutic options linked to autonomic modulation for diseases associated with a chronic inflammatory condition such as sepsis.

Keywords: inflammation, autonomic nervous system, heart rate variability, anti-inflammatory pathway, inflammatory reflex, sepsis

1. Introduction

Inflammation is the physiological response to invading pathogens and tissue damage, such as exposure to extreme heat or cold, ischemia, and trauma [1, 2]. The inflammatory response can be divided into acute or chronic inflammation. An acute inflammatory response is a controlled process, with a short time window of minutes up to a few hours and it is characterized by the abundant presence of a specific type of immune competent cells (neutrophils), responsible for clearing invading pathogens and promote tissue repair, thus restoring homeostasis. However, uncontrolled inflammation, which extends from days up to years, may cause more severe complications. In the latter, if an accumulation of lymphocytes in the inflamed tissue predominates,

both immune and physiological homeostasis are disrupted, thereby the inflammation can progress to a chronic condition [1, 2]. In its most severe form, it can lead to permanent tissue damage, organ dysfunction, and, ultimately, death [3]. The immune cells residing in tissues, i.e., macrophages, fibroblasts, mast cells, and dendritic cells, as well as circulating leukocytes, including monocytes and neutrophils, recognize pathogen invasion and/or cell damage with intracellular or surface-expressed pattern recognition receptors (PRRs). These receptors detect, either directly or indirectly, pathogen-associated molecular patterns (PAMPs), such as, microbial nucleic acids, lipoproteins, and carbohydrates, often essential for microbe survival, or damage-associated molecular patterns (DAMPs), endogenous molecules normally found in cells, that are released during necrosis contributing to sterile inflammation. Activated PRRs in response to PAMPs and DAMPs, oligomerize and assemble large multisubunit factors, such as, nuclear factor kappa B (NF-kB), activator protein 1 (AP1), cellular transcription factor (CREB), CCAAT-enhancer-binding proteins (c/EBP), and interferon regulatory factors (IRF) transcription factors, which will in turn initiate complex downstream signaling cascades, resulting in the increased expression of key pro- and anti-inflammatory genes [2, 3]. For instance, protease caspase-1, activated by a subset of PRRs, causes maturation of cytokines interleukins IL-1b and IL-18.

Expression of genes encoding enzymes, chemokines, cytokines, adhesion molecules, and regulators of the extracellular matrix promotes the further recruitment and activation of leukocytes to the region, which are crucial for eliminating foreign particles and host debris [2, 3]. Cell adhesion molecules and chemokines facilitate leukocyte extravasation from the circulation to the affected site, the chemokines stimulating G-protein-coupled receptors (GPCRs) [3]. Thus, the immune system plays a crucial and defining role in the overall inflammatory response processes through recruitment of various immune cell types, in addition to the release of pro-inflammatory cytokines into the bloodstream, including interleukin 1 (IL-1), interleukin 6 (IL-6), and tumor necrosis factor alpha (TNFα), which are inhibited by mediators of inflammation resolution, such as anti-inflammatory cytokines, that restore cellular homeostasis and defend the organism from external injuries [2].

The interaction between nervous and immune systems, or the "supersystems" as described by Tada [4], is, in fact, critical in the maintenance and regulation of homeostasis, not only under a daily routine, but also, in adverse environmental conditions caused by injury, infections, and exposure to toxins [5–7]. Indeed, the autonomic nervous system controls the inflammatory processes and immune responses, by finding a balance between pro-inflammatory and anti-inflammatory responses, ensuring an adequate host defense with minimal collateral damage due to overly aggressive responses of the innate immune system [6]. Both parasympathetic and sympathetic efferent nerves have been suggested to affect immune cells and inflammatory responses. The latter interfere in the earlier stages of the inflammatory process, while the parasympathetic nervous system is important to regulate innate immune responses and cytokine functional effects in chronic processes [6, 8, 9].

2. Autonomic nervous system and inflammation

Over the last few years, association between inflammation and common human diseases (e.g., sepsis, obesity, diabetes, rheumatoid arthritis) remains an unsolved mystery of current biology and medicine [10–13]. Inflammation as a response to infection interacts with different

parts of the nervous system. Indeed, recent studies indicate that systemic inflammation can be attenuated by the autonomic nerve fibers [14].

The autonomic nervous system (ANS) includes the sympathetic and parasympathetic nervous system (SNS and PNS, respectively) as its motor systems, and regulates and integrates many human physiological systems and functions, such as the cardiovascular system, the endocrine and exocrine systems, and the digestive system [14, 15]. As reviewed by Kenney and Ganta, to balance the functions of autonomic effector organs, the SNS and PNS work antagonistically, synergistically, or independently [14]. Previous studies have shown that both the SNS and PNS can sense inflammation and influence development and severity of inflammatory processes in animal models [12, 16].

There are many chronic autoimmune diseases in which there is an imbalance of the ANS, for example, rheumatoid arthritis (RA), caused by synovial inflammation, leading to bone erosions, cartilage damage, and ultimately joint deformities and disability [8, 12]. Patients with RA have autonomic modifications, with lower parasympathetic activity and less frequently, alterations in sympathetic function [17]. These alterations are correlated with higher levels of inflammatory markers, such as C-reactive protein (CRP) concentrations and erythrocyte sedimentation rate [17, 18]. Another example is obesity, which consists in the accumulation of abnormal and excessive fat that may interfere with the maintenance of an optimal state of health and is accompanied by an increased morbidity and mortality [19]. This condition is generally associated with other clinical comorbidities including, cardiovascular impairment, atherosclerosis, insulin resistance, and diabetes mellitus [19, 20]. The excess of macronutrients in the adipose tissues stimulates them to release inflammatory mediators (TNFα, Il-6) and reduces production of adiponectin, predisposing to a pro-inflammatory state and oxidative stress [20]. The ANS has a significant role in the integrated short-term regulation of weight, modulating the satiety signal and energy expenditure. The afferent vagal pathways are probably the most important link between the gut and the brain and interact in a complex way with gut hormones. SNS has the physiological function of increasing lipolysis and energy expenditure, through sympathetic innervation in white and brown adipose tissues. However, in obesity, SNS activity is compromised and might trigger alterations in sympathetic regulation of cardiovascular function, thus favoring the development of cardiovascular complications, such as hypertension [21, 22] and organ dysfunction [23, 24].

Another two examples of immune/inflammatory diseases are sepsis and severe burn injury, both characterized by severe global changes to the entire immune system [25]. The immunopathological response to the intense disruptions to the body's homeostatic balance can contribute to the development of systemic inflammatory response syndrome (SIRS), serious metabolic disturbances, and subsequent multiple organ failure and death [25, 26]. During the acute phase, following burn injury, there is an increase in sympathetic activity, which is important for the modulation of energy substrate mobilization, cardiovascular, and hemodynamic compensation and wound repair [27]. Nevertheless, prolonged or excessive sympathetic activity due to the activation of positive feedback mechanism can also be deleterious [27]. Furthermore, the increased susceptibility to infection and other systemic disorders are also accompanied by excessive inflammatory responses that underline the observed cardiac dysfunction, acute respiratory distress syndrome, acute renal failure, increased intestinal permeability resulting in bacterial translocation, hypermetabolism, hypercatabolism, and ultimately, sepsis [28, 29]. Sepsis can, therefore, be

an associated comorbidity of burn injuries, but in its essence is a highly common heterogeneous syndrome in the general population and will be further reviewed in Section 3 [25].

2.1. The role of sympathetic nervous system in the inflammatory processes

The sympathetic nervous system (SNS) is responsible for the "fight-or-flight" response to threatening situations and consists of neural hardwiring emanating from the spinal cord to innervate target organs, including primary and secondary lymphoid organs. About 25% of sympathetic nerve fibers arise from cranial nerves III, VII, and IX and from the second and third sacral spinal nerves [30]. Diverse stimuli (stressors, cytokines, and infection) trigger the SNS and consequent catecholamine release, inducing functional alterations in immune system susceptibility to respond to an invasive infection and other pathologies. As previously mentioned, the SNS interacts in several different manners with the immune system to maintain immune homeostasis under basal conditions, by enhancing host defenses to eliminate pathogens, promoting healing after tissue injury, and restoring homeostasis after pathogen elimination and/or tissue repair. This communication with all immune competent cells occurs directly by stimulated release of its major neurotransmitter, i.e., norepinephrine—NE, and subsequent intercellular signaling via postsynaptic adrenergic receptors (ARs) expressed in closely apposed immunocytes, i.e., T and B lymphocytes, antigen-presenting cells, stromal cells, granulocytes, macrophages, and mast cells [15]. The SNS is highly adaptive, and to appropriately regulate the immune system, it acts through the:

1. Constant up- and down-regulation of diverse target cell functions across time (i.e., expansion, differentiation, apoptosis, and cytokine secretion) and
2. Detection and interaction with the diverse signaling pathways that mediate the above cellular functions [14].

In the initial stages of the inflammatory processes, the body assumes an "inflammatory configuration" with increased systemic SNS and hypothalamic-pituitary-adrenal (HPA) axis activity through the chemoreceptor reflex, the ultimate protective, which can be interpreted as an "energy appeal reaction," resulting in the provision of enough energy-rich fuels, like glucose and free fatty acids, to fulfill the needs of an activated immune system together with the maintenance of appropriate oxygen blood levels. If inflammation evolves to a more severe state, the system changes into a "chronic inflammatory condition," that, according to Pongratz and Straub, is characterized by an increased systemic activity of the SNS, an increased activity of the HPA axis but without immunosuppression (glucocorticoid receptor desensitization and inadequacy), and a local repulsion of SNS fibers from inflamed tissue, including lymphoid organs, to create zones of permitted inflammation [8]. The immune response is more or less uncoupled from central regulation to avoid the anti-inflammatory influence of the brain. All mechanisms ensure an optimal fight against an invading antigen. Nevertheless, if a prolonged or inappropriate activation of either the SNS or immune system persists, the effects are detrimental and can result in the collapse of these two systems, ultimately failing in re-establishing immune system homeostasis within normal physiological ranges [8, 15]. Under such conditions, the immune system and/or SNS can promote pathological and lethal effects, including chronic inflammation, toxic shock, tissue damage, immune deficiency, autoimmunity, and cancer [15], as well as, cachexia, high blood pressure, insulin resistance, leading to increased levels of cardiovascular mortality [8].

Several studies have demonstrated the role of the SNS in inflammation. Martelli et al. showed that in a rat model of intravenous endotoxin, a bilateral section of splenic sympathetic nerves deeply increases inflammatory cytokine release; however, bilateral vagotomy was ineffective, which suggests a splanchnic sympathetic efferent reflex arc of the anti-inflammatory neural pathway [31]. Another clinical phenomenon is immunosuppression after stroke [32]. Indeed, the 6-hydroxydopamine, which blocks a nonselective α-adrenoreceptor and causes pharmacological ablation of the SNS, may also attenuate stroke-induced immunological abnormalities, prevent infections, and improve the survival, and thus SNS activation, instead of the PNS, has a significant role in the immunosuppression response [6, 32–34]. Additionally, in hypertensive patients, the central inhibition of the SNS decreased peripheral TNF serum levels [35]. Moreover, del Rey and colleagues have also found in an animal model of arthritis that during protracted inflammation, there might be a disruption of this communication between the brain and the immune system [36]. There are also several studies indicating that the sympathetic nervous system might be influencing different forms of cancer [37]. In fact, epidemiological studies showed that breast cancer and melanoma improve with the use of beta-blockers, while other studies imply that psychological stress might modulate SNS activity, with a significant impact on inflammation and consequently on the pathogenesis of some cancers [37, 38].

Finally, recent studies show that the SNS plays a significant role in several immune-mediated or immune-related diseases, including sepsis [39], colitis [40], allergic asthma [41], chronic eye inflammation [42], arthritis [8, 36], among others.

2.2. The role of parasympathetic nervous system in the inflammatory processes

The parasympathetic nervous system (PSNS) innervates multiple organ systems, including cardiovascular, respiratory, immune, and endocrine systems [43] and plays a critical role in a diverse array of physiological processes, such as inflammation, immune response, heart rate, gastrointestinal peristalsis, and digestion [13]. About 75% of parasympathetic innervation comes from the tenth cranial nerve, the vagus nerve (VN), that extends throughout the body, and is the largest nerve and main parasympathetic division of the autonomic nervous system [13, 30, 44]. Vagus nerve comprises both sensory afferent neurons, crucial for conducting peripheral immune signals to the brain, which integrate the visceral sensory information and coordinates the autonomic function and visceral activity [13, 33, 34], and motor efferent neurons, which integrate the information that was delivered to the central nervous system and control the peripheral effectors [30, 45]. The vagus nerve not only regulates gut physiology but also mediates cholinergic anti-inflammatory pathway, the inflammatory reflex that controls immune function, and pro-inflammatory responses during infection and injury [35, 45, 46]. The sensory afferent vagus nerve fibers detect peripheral inflammatory mediators, such as cytokines, released by activated macrophages and other immune cells, revealing its pro-inflammatory properties; however, a potent anti-inflammatory effect is exhibited by the efferent branch [46]. The animal models of acute inflammation reveal that the activation of the efferent vagus nerve, probably due to binding of acetylcholine on the alpha-7 subunit-containing nicotinic receptors (α7nAChR), essential for the vagal anti-inflammatory action [47, 48] resulted in reduced systemic production of pro-inflammatory cytokines [46, 49]. This suggests that in the initial phase of inflammation processes, the neuroimmune path eliminates the infectious agent, and in the posterior phase re-establishes the homeostasis [13, 33, 34, 50].

In human studies, the parasympathetic neurotransmitter acetylcholine attenuated proinflammatory cytokine release (e.g., TNFα) in lipopolysaccharide-stimulated macrophage cultures [49], so nicotine was more effective than muscarine in inhibiting TNF release. Human macrophages express α7nAChR subunit, and its knockdown makes macrophages less responsive to nicotine-mediated TNF inhibition [49].

Activating efferent vagus nerve can significantly suppress systemic pro-inflammatory cytokine levels in endotoxemia animal models, as the acetylcholine (Ach) released from the nerve terminals binds to the α7n-AChR expressed on macrophages, to modulate the immune system response [51]. α7nAChR, expressed in the nervous and immune systems, is important for mediating anti-inflammatory signaling by inhibiting NF-κB nuclear translocation and activating the JAK2/STAT3 pathway [48, 49, 51], being also a crucial neural component connecting the parasympathetic vagus nerve with the sympathetic splenic nerve at the mesenteric ganglion [52]. The endotoxemia animal model reveals that peripheral vagal afferents can be activated by responding directly to bacterial lipopolysaccharide (LPS) and cytokines, such as TNFα, interleukin-1 (IL-1), interleukin-6 (IL-6), and interferon-gamma (IFN-γ). IL-1 receptors, expressed on vagal afferents, can be activated by inflammatory stimulation to regulate the immune responses [23, 48]. It has been shown that an excess of proinflammatory cytokine was released in α7nAChR knockout mice, and macrophages from these animals fail to respond to cholinergic agonists [6, 48]. Further, electrical vagus nerve stimulation reduced systemic TNFα concentrations and prevented septic shock in rats [49], and in mice, the vagus nerve stimulation (VNS), as well as splenic nerve electrical stimulation, inhibits lipopolysaccharide (LPS)-induced TNFα release [49, 50, 52, 53]; however, those results were quite surprising because the spleen does not have vagal innervation [53].

Several experiments demonstrated that the sympathetic splenic nerve connects the vagus nerve to the spleen [47, 52]. It is possible that the α7nAchR regulates both the neuronal connection and the macrophage activation. Moreover, the connection between the vagus and splenic nerves has been a matter of constant debate [43]. For example, anatomical and physiological studies have demonstrated no connection between the vagus and splenic nerves [54]. Additionally, denervation of the arterial splenic nerve in mice led to the inhibition of the cholinergic anti-inflammatory pathway [52]. To resolve this inhibition is essential to find a non-neural link in the anti-inflammatory pathway from vagus to spleen. Some authors have proposed an unconventional and theoretical model, where vagus nerve stimulation activates multiple cell types, the choline acetyltransferase positive (CHAT+), epithelial cells, endothelial cells, muscle fibers, and immune cells (such as lymphocytes and macrophages) that are not resident in the spleen, migrating in direction of this organ and subsequently releasing acetylcholine [12, 46, 55]. This electrical nerve stimulation therapy could be applied concomitantly with a pharmacological treatment for a better response. The human studies reveal that the great advantage of this model is the stimulation of ACh/norepinephrine release, reducing interventions with higher doses of anti-inflammatory drugs or even halting their administration [56].

In addition, it has been shown that other organs display a cholinergic control of inflammation, such as gut, kidney, and liver. Despite lung exhibiting vagal innervation, activation of the cholinergic anti-inflammatory pathway is not sufficient to regulate inflammation; however, it is necessary to maintain the homeostasis. In this sense, vagus and/or splenic nerve stimulation appeared as an efficient procedure to minimize inflammation [57]. Furthermore,

in experimental glomerulonephritis, a genetic α7nAChR deletion exacerbates inflammation and fibrosis [6, 54]. Recently, Cedillo et al. showed that increased α7nAChR expression on peripheral blood mononuclear cells was associated with better control of inflammation, disease severity, and clinical outcome in septic patients and prognosis [58].

Concluding, both animal and human studies have suggested that the vagus nerve stimulation has a potential protective regulating systemic inflammation in various pathologies, such as ischemia/reperfusion, sepsis, epilepsy, hemorrhagic shock, migraine, and others [13, 51, 59–61]. However, additional studies are needed to determine the interplay between the vagus and the splenic nerves, and their respective roles in modulating inflammation [6, 12]. According to Martelli et al., several treatments are currently undergoing development, based on the cholinergic anti-inflammatory pathway [46].

3. Autonomic function and sepsis

Sepsis is an important cause of admission in intensive care units (ICU) and remains a major clinical and scientific challenge in modern medicine [53]; this is a huge and expensive medical problem throughout the world, with a mortality rate ranging between 30 and 50% [10, 62]. It is defined as life-threatening acute organ dysfunction, secondary to infection [63], characterized by abnormal body temperature, mental confusion, hypotension, diminished urine output, or thrombocytopenia [53, 63]. Over the past two and a half decades, there has been a tremendous effort to develop standardized diagnostic definitions of sepsis, as described in (**Table 1**). The most prevalent sites of infection, responsible to trigger sepsis in humans, are the lungs, abdominal cavity, urinary tract, and primary infections of the blood stream. After the unsuccessful treatment of sepsis, the patient may develop circulatory, cellular, and metabolic abnormalities, such as, respiratory or renal failure, changes in coagulation, and profound and unresponsive hypotension [53], as well as modifications in cardiovascular, autonomic, neurological, hormonal, metabolic and clotting systems [72, 73]. These marked alterations are characterized by septic shock, the leading causes of death in sepsis [63].

The pathophysiology of sepsis is characterized as a host reaction to infection that involves a balanced inflammatory response, critical to fight the infection, and an unregulated pro- and anti-inflammatory response to induce organ damage in the host [73]. Thereby, the immune response in sepsis results in the increased levels of cytokines, designated hyperinflammatory phase and subsequently evolves to hypoinflammatory phase (immune-suppressive function) [39], the latter being more destructive and aggressive than the initial infection [73]. This imbalance is determined by several factors, such as pathogen virulence, bacterial (i.e., lipoteichoic acid and bacterial lipopolysaccharide—LPS) [74] and patient-related factors (i.e., genetic background, age, and comorbidities) [53], leading the immune system to detect PAMPs (including components of bacterial, fungal, and viral pathogens) and DAMPS (endogenous molecules released from damaged host cells, including ATP, mitochondrial DNA, and high mobility group box 1 or HMGB1) [75]. For transcription of type I interferons and proinflammatory cytokines (i.e., TNF-8, interleukin (IL)-1, and IL-6) initiation, both DAMPs and PAMPs activate innate immune and some epithelial cells through pattern recognition receptors on the

Sepsis definitions	
Previous definitions [64–68]	
Diagnosis	Signs and symptoms
Systemic inflammatory response syndrome (SIRS)	**Two of the following symptoms**: • Body temperature > 38 or < 36°C • Heart rate > 90 beats/min • Respiratory rate > 20 breaths/min or arterial CO_2 < 32 mmHg • White blood cell count >12.000/mm^3, <4000/mm^3 or > 10% immature forms
Sepsis	SIRS and proven or suspected infection
Severe sepsis	Sepsis in combination with multiple organ dysfunction (MODS).
Septic shock	Sepsis and persistent hypotension (mean arterial pressure [MAP] <65 mmHg) after fluid resuscitation and/or lactate >4 mmol (36 mg/dL)
Revised definitions [63, 69, 70]	
Diagnosis	Signs
Sepsis	• Life-threatening organ dysfunction caused by a dysregulated host response to infection • Suspected or documented infection and an acute increase of >2 sequential (sepsis related) organ failure assessment (SOFA) points (SOFA score [71] is a proxy for organ dysfunction)
Septic shock	• Septic shock is a subset of sepsis in which underlying circulatory and cellular/metabolic abnormalities are profound enough to substantially increase mortality • Sepsis and vasopressor therapy needed to increase MAP ≥65 mmHg and lactate >2 mmol/L (18 mg/dL) despite adequate fluid resuscitation

Table 1. Sepsis: previous and revised definition.

cell surface (toll-like receptors and C-type lectin receptors) or in the cytosol (NOD-like receptors, RIG-I-like receptors) [76, 77]. In the case of bacterial infection, when a microbiological diagnosis is made, about half of the cases show that 60% are caused by Gram-negative and Gram-positive bacterium in the remaining cases [53, 62, 78]. Lipopolysaccharide (LPS) from Gram-negative bacteria (an example of a PAMP) reacts with toll-like receptor 4 (TLR4), causing phagocytic cells to robustly generate a variety of proinflammatory cytokines signaling, leading to systemic inflammatory response syndrome (SIRS) [10].

In sepsis, not only the response of immune cells is highly context dependent to stimuli, but also, the nervous system itself depends on the inflammatory context [10]. Several evidences demonstrate that immune and inflammatory responses are regulated by the autonomic nervous system through PNS and SNS activities [8, 14]. Essentially, to inhibit the inflammatory cytokine production by innate immune cells in the spleen, gut, and other organ, the carotid body chemoreceptors, afferent sensory vagal fibers, and brain areas with a permeable blood barrier respond to local and systemic cytokines, signaling to brainstem nuclei, which in turn send vagal, cholinergic efferents to the periphery [10, 79]. After bacterial infection, one of the

first and local responses is the release of vasoactive peptides by spinal afferent C-fibers and the ensuing neurogenic inflammation. Vagal afferent C-fibers can exert neurogenic inflammatory reflex actions, like those underlying some forms of diarrhea; however, in the proposed vagal anti-inflammatory reflex, the exact role of efferent parasympathetic vagal fibers remains to be elucidated, as these fibers do not seem to directly innervate the major immune organs [79, 80]. The work developed by Tracey and colleagues have shown that, in animal models of sepsis and in another inflammatory conditions (e.g., colitis, hemorrhagic shock, and ischemia-reperfusion injury), neural reflex involving the vagus nerve causes T cells to release acetylcholine and, therefore, interacts with the α7nAch receptor on macrophages to dampen the release of powerful proinflammatory mediators such as TNF-α and HMGB-1 [49, 50, 81]. In an animal model of cecal ligation puncture (CLP), improvements in survival and suppression of the SIRS response of sepsis were described after stimulation of the vagal nerve [81], as does the use of a selective or a universal synthetic agonist for α7nAchR on macrophages [49]. Another recent work indicates that vagal stimulation also reduces symptoms and inflammation in patients suffering from rheumatoid arthritis and Crohn's disease [80].

Contrary to the lack of information on vagal innervation of immune organs and cells, there is longstanding evidence in favor of sympathetic nervous system innervation of primary and secondary immune organs (including, thymus, spleen, bone marrow, and lymph nodes) [82]. Depending on the kind of bacterial infection, there are different effects of SNS on bacterial dissemination, innate immune cell responses, and inflammatory mediators [83]. During septic systemic inflammation, noradrenaline increases in immune organs where it can act on α and β receptors present on macrophages, and adrenaline release into the blood also increases, implying that almost any tissue macrophage could be exposed to adrenaline, which has been shown to modulate pro-inflammatory cytokine secretion by cultured blood cells [81]. Noradrenaline, which is both released and often administered during sepsis [84], may, along with adrenaline, exert pro-inflammatory actions through stimulation of β1 adrenergic receptors, as antagonists of this receptor have been shown to exert anti-inflammatory effects in experimental sepsis [85].

These findings show a specific interest, since, in clinical severe sepsis and septic shock, the selective β1 receptor blocker, esmolol, has shown beneficial effects on microcirculation and myocardial oxygen [84, 85]. Interestingly, in a rat model of CLP, esmolol has similar beneficial effects on vascular and cardiac function [86], and at the same time, it increases anti-inflammatory and reduces pro-inflammatory cytokine production, reduces bacterial component, and improves gut barrier function, ultimately increasing animal survival rates [87]. Although the SNS can influence infection-induced immune responses, depending on the type of bacteria and the timing of treatment, this kind of adrenergic drugs may have beneficial or detrimental effects on the active molecules. Notwithstanding, the promising anti-inflammatory effects of the β1 antagonist esmolol need to be confirmed in clinical trials on septic patients [80].

4. Autonomic modulation and therapeutics in sepsis

Several advancements have been made over time to understand the neuroimmune mechanisms for maintaining and restoring homeostasis during normal and pathophysiologic conditions.

Some studies have already reported that, in adverse conditions, basic reflex mechanisms respond through efferent vagal and sympathetic circuits and that neurotransmitters influence leukocytes with important clinical implications [8, 43, 88]. In this heading, we will review the most relevant therapies associated with autonomic modulation, developed and tested over the last 3 years.

Regarding the importance of sympathetic downstream signaling in anti-inflammation processes, one promising pharmacological approach is the inhibition of phosphodiesterase 4 (PDE4), an enzyme that degrades cAMP [89]. It is reported that, by inhibiting this enzyme, the cAMP increases, and, consequently, shows promising results in several diseases, as psoriatic arthritis, rheumatoid arthritis, Behçet's syndrome [90], and sepsis [91]. Focusing on sepsis studies, inhibitors of PDE4 reduce systemic vascular resistance and improve cardiac contractility and renal function [91]. PDE4 inhibitors also have a potent anti-inflammatory activity effect, by reducing microvascular leakage, all of which could be beneficial in infants with severe sepsis [92]. **Table 2** summarizes the main treatments developed in the last 3 years based on pharmacologic PDE inhibition in sepsis.

It is known that studies in humans have their limitations and confounding variables, such as, differences between groups in age and sex, body mass index, disease severity, smoking, frailty, and physical activity [97]. However, interestingly, clinical responses could be attained through autonomic nervous system modulation, as well as pro- and anti-inflammatory interventions [6, 98]. Several approaches, such as lifestyle interventions, medications, and devices, could be repurposed or further expanded to target inflammation. For example, to lessen systemic inflammation, in metabolic and cardiovascular diseases, it is necessary to develop measures to attenuate sympathetic activity [6, 98, 99]. Similarly, observations in animal models showed that sympathetic inhibition could improve the immunosuppression associated with strokes, and thereby, prevent infectious complications and deaths [6]. At the moment, there are some clinical trials in progress, to evaluate the effects of the cholinergic anti-inflammatory pathway by vagus nerve stimulation in patients with sepsis, severe sepsis, and shock septic, but there are also, at least, five clinical trials that are evaluating the oxytocin in endotoxemia model (https://clinicaltrials.gov/ct2/results?cond=Sepsis&term=esmolol&cntry=&state=&city=&dist).

Taking into account the cholinergic anti-inflammatory pathway, the major discoveries have been associated with: vagus nerve stimulation (VNS) and transvenous vagus nerve stimulation (tVNS) in anti-inflammatory responses, the identification of α7nAChRs in different cell types (macrophages, dendritic cells, and microglial cells) as targets for suppression of inflammation, and the integration of cholinergic T cells into the efferent neuroimmune pathway within the spleen, and also, some pharmacological approaches [98]. Although the vagal neuroimmune pathway is still controversial in specific situations (e.g., sterile or pathogen-induced inflammation), the effect of vagal stimulation could be beneficial to the host by inhibiting exacerbated cytokine production and inappropriate neutrophil entrapment into vital organs [12, 16]. By contrast, the cholinergic anti-inflammatory pathway can inhibit specific innate immune responses that are crucial to eliminate the bacteria (e.g., initial neutrophil migration) and subsequently increase the mortality in sepsis [100, 101]. Nevertheless, while VNS will unlikely replace the standard intensive care therapy, it is quite possible that, in the future, autonomic modulation through VNS would become an adjunct to benefit septic

Pharmacological approach						
	Target	**Study**	**Method**	**Conclusions**	**Perspectives**	**Year**
Dibutyryl-cAMP Rolipram Roflumilast	Inhibition of phosphodiesterase 4B **(PDE4)**	***In vitro:*** macrophage cell line (mouse) Animal (mice)	Cell: LPS (10 ng/ml) Mice: LPS (10 mg/kg)	Upregulates anti-inflammatory cytokine IL-1Ra production in vitro and in vivo	PDE4B-selective inhibitors may retain the anti-inflammatory effects of nonselective PDE4 inhibitors	2017 [93]
Roflumilast	Effects on a CLP-model-induced sepsis	Animal (mice)	CLP	Reduce of bacterial load Inhibition of the IL-6 and TNFα expression Alleviation of liver injury	May be an appropriate treatment of sepsis Needed more studies.	2017 [94]
Rolipram Azithromycin Ethyl pyruvate Metformin	Investigate potential antiendotoxic effects	*In vitro* (cells of horses)	LPS (1 μg/ml)	Rolipram: the most potent inhibitor of cytokine production	Further work is required to investigate the potential use in vivo.	2015 [95]
Rolipram	Impact of the PDE4 inhibition on hepatic integrity	Animal (rat)	LPS (2.5 mg/kg, i.v.)	Improvement of hepatic microcirculation and integrity Protective effect on hepatoma cell line viability	Further studies needed to determine clinical applicability of PD-4-I	2015 [96]

Table 2. Pharmacologic phosphodiesterase (PDEs) inhibition in sepsis disease.

http://dx.doi.org/10.5772/intechopen.79280

	Target	Study	Method	Conclusions	Perspectives	Year
Autonomic modulation						
Chemoreflex stimulation and pharmacological intervention	Chemoreflex activation Pharmacological blockade (methylatropine or propranolol)	Animal (rat)	LPS (1.5 mg/kg, i.v.)	Attenuates the use of TNFα, IL-1β, and IL-6 plasma levels Increases the IL-10 plasma levels	Methods to stimulate CSN, which is a promising therapeutic strategy	2017 [102]
Vagal nerve stimulation B(VNS)	Assessed changes in IL-6 and TNFα	Animal (rat)	LPS (0.5 mg/kg, intratracheally)	Attenuates the upregulation of IL-6 and TNFα Viable alternative to antibiotics	Provides a suggestive link between VNS and potential clinical application to treat sepsis in preterm infants	2016 [103]
	Effects on sepsis-associated encephalopathy	Animal (rat)	LPS (i.v.)	Activates anti-inflammatory effect through cholinergic pathway Improves the cerebral function Reduces systemic and cerebral inflammatory reaction	Requires more research	2015 [104]
	Potential role of prostaglandin in cholinergic neuro-regulation	Animal (mice)	LPS (2 mg/kg, i.p.)	VNS decreases the release of pro-inflammatory cytokines both in serum and spleen	Further development of therapeutic directed of inflammatory reflex modulation, and immunosuppression in chronic inflammatory diseases	2015 [105]
Transvenous vagus nerve stimulation (tVNS)	Determine the feasibility and safety, investigated its putative anti-inflammatory effects	Randomized double-blind study	LPS (2 ng/kg, i.v).	tVNS is feasible and safe but does not influence the systemic inflammatory response in vivo	Short-term tVNS does not modulate the innate immune response in humans Require more studies	2015 [106]
	α7 gene expression level in peripheral blood mononuclear cells (PBMC) as marker for CAP	Pilot study	Septic patients within the first 24 hours of diagnosing sepsis	PBMC α7 gene expression level is a clinically relevant marker for CAP activity in sepsis: the higher the α7 expression, the better the inflammation control and the prognosis	The CAP activation in high-risk septic patients has therapeutic potential: this activation could be used as an adjunctive therapy	2015[58]

	Target	Study	Method	Conclusions	Perspectives	Year
Pharmacological intervention						
Esmolol	Effects on reducing apoptosis and inflammation	Animal (rat)	CLP	Reduces apoptosis and inflammatory reaction and protect key organs	Needed clinical trials to confirm the use of esmolol in sepsis treatment	2017 [87]
	Effect on tissue perfusion and the clinical prognosis of patients with severe sepsis	Prospective cohort clinical trial	Continuous infusion of esmolol via central venous catheter	Controlled heart rate reduced the duration of mechanical ventilation No significant effects on circulatory or tissue perfusion	Requires further research.	2016 [84]
	Effects on myocardial and vascular function	Animal (rat)	CLP	Enhances intrinsic cardiac contractility and improves vascular responsiveness to catecholamines	Further investigation needed to determine the effects of β1-blockade on other organ functions and inflammatory patterns in septic shock.	2015 [86]
Oxytocin	Effects on the cardiorespiratory activity	Animal (rat)	LPS (0.1 mg/kg; intraperitoneally)	Potential cardioprotective peptide Diminished tachypnea and restore the cardiorespiratory interactions Provokes a less anticorrelated pattern in HRV Decreased mean heart rate Reduce lethargy and moderated the hyperthermia	These findings confirm the suitability of the electrocardiogram-derived respiration technique to obtain the estimated respiration signal in rodent models	2017 [107] 2016 [108]
Xanomeline	Effect on brain muscarinic acetylcholine receptor (mAChR)-mediated cholinergic signaling	Animal (mice, rat)	LPS (6 mg/kg, i.p) CLP	Suppresses serum and splenic TNF levels Alleviates sickness behavior, and increased survival	Further studies centrally-acting M1 mAChR agonists as experimental therapeutic agents in a broader spectrum of inflammatory conditions	2015 [109]
Other						
ANS indices	Investigate how changes in cardiovascular indices can be a sign of progression of organ failure	Prospective observational cohort study	Follow-up during the first 3 days of ICU stay after development of septic shock	Variability of heart rate significantly increases in septic shock patients presenting improvement of organ function from ICU day 1 to day 3.	Further investigations are required to find specific associations with drug dosage, namely, drugs acting on the sympathetic system, and clinically relevant outcomes	2017 [110]

Table 3. Sepsis: autonomic modulation, therapeutics, and treatments based on the PNS therapy.

patients [100, 101]. The most relevant treatments and therapies based on parasympathetic nervous system developed over the last 3 years, in animal models and human studies, are shown in **Table 3**.

5. Conclusion

The nervous and immune systems are not fully independent. When the body is inflamed, both systems produce neurotransmitters and cytokines and express receptors that are involved in important physiological functions and in the maintenance of homeostasis. The reactions of the immune competent cells to neurotransmitters are variable, depending on the context of receptor engagement, such as, activation state of the cell, expression pattern of neurotransmitter receptors, microenvironment, cytokine, and distance from the catecholamine source (concentration). It is already well described that autonomic modulation in acute and chronic inflammation has been implicated with a sympathetic interference in the earlier stages of the inflammatory process and the activation of the vagal inflammatory reflex to regulate innate immune responses and cytokine functional effects in longer more chronic processes. The present chapter reviewed the overall autonomic mechanisms controlling inflammatory responses in several conditions such as, burn processes, arthritis rheumatoid, obesity, with a special focus on the inflammatory processes associated with sepsis. Furthermore, the most relevant therapeutic options for the latter, through autonomic modulation, were also reviewed and summarized.

In summary, it is quite clear that sepsis remains a worldwide clinical challenge and therapies' outcomes depend largely on host factors. Henceforth, continuous searching for new and more effective therapies during the initial phases of sepsis is utterly important, in order to reduce the mortality associated with this syndrome (condition).

Conflict of interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author details

Ângela Leal[2]*, Mafalda Carvalho[2], Isabel Rocha[1,2] and Helder Mota-Filipe[2,3]

*Address all correspondence to: araqueleal@hotmail.com

1 Department of Physiology, Faculty of Medicine, Universidade de Lisboa, Portugal

2 CCUL, Universidade de Lisboa, Portugal

3 Department of Social Pharmacy, Faculty of Pharmacy, Universidade de Lisboa, Portugal

References

[1] Báez-Pagán CA, Delgado-Vélez M, Lasalde-Dominicci JA. Activation of the macrophage $\alpha 7$ nicotinic acetylcholine receptor and control of inflammation. Journal of Neuroimmune Pharmacology. 2015;**10**(3):468-476

[2] Maldonado-Ruiz R, Fuentes-Mera L, Camacho A. Central modulation of neuroinflammation by neuropeptides and energy-sensing hormones during obesity. BioMed Research International. 2017;**2017**:7949582

[3] Newton K, Dixit VM. Signaling in innate immunity and inflammation. Cold Spring Harbor Perspectives in Biology. 2012;**4**(3)

[4] Tada T. The immune system as a supersystem. Annual Review of Immunology. 1997; **15**:1-13

[5] Hoover DB. Cholinergic modulation of the immune system presents new approaches for treating inflammation. Pharmacology & Therapeutics. 2017;**179**:1-16

[6] Chobanyan-Jürgens K, Jordan J. Autonomic nervous system activity and inflammation: Good ideas, good treatments, or both? American Journal of Physiology. Heart and Circulatory Physiology. 2015;**309**(12):H1999-H2001

[7] Elenkov IJ, Wilder RL, Chrousos GP, Vizi ES. The sympathetic nerve–an integrative interface between two supersystems: The brain and the immune system. Pharmacological Reviews. 2000;**52**(4):595-638

[8] Pongratz G, Straub RH. The sympathetic nervous response in inflammation. Arthritis Research & Therapy. 2014;**16**(6):504

[9] Jänig W. Sympathetic nervous system and inflammation: A conceptual view. Autonomic Neuroscience. 2014;**182**:4-14

[10] Bosmann M, Ward PA. The inflammatory response in sepsis. Trends in Immunology. 2013;**34**(3):129-136

[11] Han C, Rice MW, Cai D. Neuroinflammatory and autonomic mechanisms in diabetes and hypertension. American Journal of Physiology. Endocrinology and Metabolism. 2016; **311**(1):E32-E41

[12] Koopman FA, van Maanen MA, Vervoordeldonk MJ, Tak PP. Balancing the autonomic nervous system to reduce inflammation in rheumatoid arthritis. Journal of Internal Medicine. 2017;**282**(1):64-75

[13] Sheng Y, Zhu L. The crosstalk between autonomic nervous system and blood vessels. International Journal of Physiology, Pathophysiology and Pharmacology. 2018; **10**(1):17-28

[14] Kenney MJ, Ganta CK. Autonomic nervous system and immune system interactions. Comprehensive Physiology. 2014;**4**(3):1177-1200

[15] Furness JB. The organisation of the autonomic nervous system: Peripheral connections. Autonomic Neuroscience. 2006;**130**(1-2):1-5

[16] Koopman FA, Stoof SP, Straub RH, Van Maanen MA, Vervoordeldonk MJ, Tak PP. Restoring the balance of the autonomic nervous system as an innovative approach to the treatment of rheumatoid arthritis. Molecular Medicine. 2011;**17**(9-10):937-948

[17] Adlan AM, Lip GY, Paton JF, Kitas GD, Fisher JP. Autonomic function and rheumatoid arthritis: A systematic review. Seminars in Arthritis and Rheumatism. 2014;**44**(3):283-304

[18] Lazzerini PE, Acampa M, Capecchi PL, Hammoud M, Maffei S, Bisogno S, et al. Association between high sensitivity C-reactive protein, heart rate variability and corrected QT interval in patients with chronic inflammatory arthritis. European Journal of Internal Medicine. 2013;**24**(4):368-374

[19] Guarino D, Nannipieri M, Iervasi G, Taddei S, Bruno RM. The role of the autonomic nervous system in the pathophysiology of obesity. Frontiers in Physiology. 2017;**8**:665

[20] Ellulu MS, Patimah I, Khaza'ai H, Rahmat A, Abed Y. Obesity and inflammation: The linking mechanism and the complications. Archives of Medical Science. 2017;**13**(4): 851-863

[21] Geraldes V, Goncalves-Rosa N, Tavares C, Paton JFR, Rocha I. Reversing gene expression in cardiovascular target organs following chronic depression of the paraventricular nucleus of hypothalamus and rostral ventrolateral medulla in spontaneous hypertensive rats. Brain Research. 2016;**1646**:109-115

[22] Geraldes V, Gonçalves-Rosa N, Liu B, Paton JF, Rocha I. Chronic depression of hypothalamic paraventricular neuronal activity produces sustained hypotension in hypertensive rats. Experimental Physiology. 2014;**99**(1):89-100

[23] Pavlov VA, Tracey KJ. The vagus nerve and the inflammatory reflex–linking immunity and metabolism. Nature Reviews. Endocrinology. 2012;**8**(12):743-754

[24] Laranjo S, Geraldes V, Oliveira M, Rocha I. Insights into the background of autonomic medicine. Revista Portuguesa de Cardiologia. 2017;**36**(10):757-771

[25] Greenhalgh DG. Sepsis in the burn patient: A different problem than sepsis in the general population. Burns Trauma. 2017;**5**:23

[26] Rocha J, Eduardo-Figueira M, Barateiro A, Fernandes A, Brites D, Pinto R, et al. Erythropoietin reduces acute lung injury and multiple organ failure/dysfunction associated to a scald-burn inflammatory injury in the rat. Inflammation. 2015;**38**(1):312-326

[27] O'Halloran E, Shah A, Dembo L, Hool L, Viola H, Grey C, et al. The impact of non-severe burn injury on cardiac function and long-term cardiovascular pathology. Scientific Reports. 2016;**6**:34650

[28] Liu L, Li X, Yang J, Chai J, Yu Y, Duan H, et al. Comparison of systemic inflammation response and vital organ damage induced by severe burns in different area. International Journal of Clinical and Experimental Pathology. 2015;**8**(6):6367-6376

[29] Farina JA, Rosique MJ, Rosique RG. Curbing inflammation in burn patients. International Journal of Inflammation. 2013;**2013**:715645

[30] van Westerloo I D, Giebelen T AJ, van der Poll T. The Central and Autonomic Nervous Systems: Essential Regulators of the Immune Response. Yearbook of Intensive Care and Emergency Medicine; 2005. p. 421-33

[31] Martelli D, Yao ST, McKinley MJ, McAllen RM. Reflex control of inflammation by sympathetic nerves, not the vagus. The Journal of Physiology. 2014;**592**(7):1677-1686

[32] Prass K, Meisel C, Höflich C, Braun J, Halle E, Wolf T, et al. Stroke-induced immunodeficiency promotes spontaneous bacterial infections and is mediated by sympathetic activation reversal by poststroke T helper cell type 1-like immunostimulation. The Journal of Experimental Medicine. 2003;**198**(5):725-736

[33] Goehler LE, Gaykema RP, Hansen MK, Anderson K, Maier SF, Watkins LR. Vagal immune-to-brain communication: A visceral chemosensory pathway. Autonomic Neuroscience. 2000;**85**(1-3):49-59

[34] Marquette C, Linard C, Galonnier M, Van Uye A, Mathieu J, Gourmelon P, et al. IL-1beta, TNFalpha and IL-6 induction in the rat brain after partial-body irradiation: Role of vagal afferents. International Journal of Radiation Biology. 2003;**79**(10):777-785

[35] Pöyhönen-Alho MK, Manhem K, Katzman P, Kibarskis A, Antikainen RL, Erkkola RU, et al. Central sympatholytic therapy has anti-inflammatory properties in hypertensive postmenopausal women. Journal of Hypertension. 2008;**26**(12):2445-2449

[36] del Rey A, Wolff C, Wildmann J, Randolf A, Hahnel A, Besedovsky HO, et al. Disrupted brain-immune system-joint communication during experimental arthritis. Arthritis and Rheumatism. 2008;**58**(10):3090-3099

[37] Fitzgerald PJ. Beta blockers, norepinephrine, and cancer: An epidemiological viewpoint. Clinical Epidemiology. 2012;**4**:151-156

[38] Straub RH, Kalden JR. Stress of different types increases the proinflammatory load in rheumatoid arthritis. Arthritis Research & Therapy. 2009;**11**(3):114

[39] Boomer JS, Green JM, Hotchkiss RS. The changing immune system in sepsis: Is individualized immuno-modulatory therapy the answer? Virulence. 2014;**5**(1):45-56

[40] Straub RH, Grum F, Strauch U, Capellino S, Bataille F, Bleich A, et al. Anti-inflammatory role of sympathetic nerves in chronic intestinal inflammation. Gut. 2008;**57**(7):911-921

[41] Frieri M, Kumar K, Boutin A. Review: Immunology of sinusitis, trauma, asthma, and sepsis. Allergy & Rhinology (Providence). 2015;**6**(3):205-214

[42] Steinle JJ. Sympathetic neurotransmission modulates expression of inflammatory markers in the rat retina. Experimental Eye Research. 2007;**84**(1):118-125

[43] Browning KN, Verheijden S, Boeckxstaens GE. The vagus nerve in appetite regulation, mood, and intestinal inflammation. Gastroenterology. 2017;**152**(4):730-744

[44] Fisher JT, Brundage KL, Waldron MA, Connelly BJ. Vagal cholinergic innervation of the airways in newborn cat and dog. The Journal of Applied Physiology (1985). 1990; **69**(4):1525-1531

[45] Ukena C, Mahfoud F, Ewen S, Cremers B, Laufs U, Böhm M. Renal denervation in the treatment of hypertension. Current Hypertension Reports. 2013;**15**(4):363-369

[46] Martelli D, McKinley MJ, McAllen RM. The cholinergic anti-inflammatory pathway: A critical review. Autonomic Neuroscience. 2014;**182**:65-69

[47] Parrish WR, Rosas-Ballina M, Gallowitsch-Puerta M, Ochani M, Ochani K, Yang LH, et al. Modulation of TNF release by choline requires alpha7 subunit nicotinic acetylcholine receptor-mediated signaling. Molecular Medicine. 2008;**14**(9-10):567-574

[48] Wang H, Yu M, Ochani M, Amella CA, Tanovic M, Susarla S, et al. Nicotinic acetylcholine receptor alpha7 subunit is an essential regulator of inflammation. Nature. 2003; **421**(6921):384-388

[49] Borovikova LV, Ivanova S, Zhang M, Yang H, Botchkina GI, Watkins LR, et al. Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. Nature. 2000;**405**(6785):458-462

[50] Tracey KJ. The inflammatory reflex. Nature. 2002;**420**(6917):853-859

[51] Pavlov VA, Wang H, Czura CJ, Friedman SG, Tracey KJ. The cholinergic anti-inflammatory pathway: A missing link in neuroimmunomodulation. Molecular Medicine. 2003; **9**(5-8):125-134

[52] Vida G, Peña G, Deitch EA, Ulloa L. α7-cholinergic receptor mediates vagal induction of splenic norepinephrine. Journal of Immunology. 2011;**186**(7):4340-4346

[53] Kanashiro A, Sônego F, Ferreira RG, Castanheira FV, Leite CA, Borges VF, et al. Therapeutic potential and limitations of cholinergic anti-inflammatory pathway in sepsis. Pharmacological Research. 2017;**117**:1-8

[54] Truong LD, Trostel J, Garcia GE. Absence of nicotinic acetylcholine receptor α7 subunit amplifies inflammation and accelerates onset of fibrosis: An inflammatory kidney model. The FASEB Journal. 2015;**29**(8):3558-3570

[55] Wessler I, Kilbinger H, Bittinger F, Unger R, Kirkpatrick CJ. The non-neuronal cholinergic system in humans: Expression, function and pathophysiology. Life Sciences. 2003; **72**(18-19):2055-2061

[56] Kwan H, Garzoni L, Liu HL, Cao M, Desrochers A, Fecteau G, et al. Vagus nerve stimulation for treatment of inflammation: Systematic review of animal models and clinical studies. Bioelectronic Medicine. 2016;**3**:1-6

[57] Pereira MR, Leite PE. The involvement of parasympathetic and sympathetic nerve in the inflammatory reflex. Journal of Cellular Physiology. 2016;**231**(9):1862-1869

[58] Cedillo JL, Arnalich F, Martín-Sánchez C, Quesada A, Rios JJ, Maldifassi MC, et al. Usefulness of α7 nicotinic receptor messenger RNA levels in peripheral blood mono-nuclear

cells as a marker for cholinergic antiinflammatory pathway activity in septic patients: Results of a pilot study. The Journal of Infectious Diseases. 2015;**211**(1):146-155

[59] Jiang Y, Li L, Liu B, Zhang Y, Chen Q, Li C. Vagus nerve stimulation attenuates cerebral ischemia and reperfusion injury via endogenous cholinergic pathway in rat. PLoS One. 2014;**9**(7):e102342

[60] Fernandez R, Nardocci G, Navarro C, Reyes EP, Acuña-Castillo C, Cortes PP. Neural reflex regulation of systemic inflammation: Potential new targets for sepsis therapy. Frontiers in Physiology. 2014;**5**:489

[61] Kox M, Pickkers P. Modulation of the innate immune response through the Vagus nerve. Nephron. 2015;**131**(2):79-84

[62] Alberti C, Brun-Buisson C, Burchardi H, Martin C, Goodman S, Artigas A, et al. Epidemiology of sepsis and infection in ICU patients from an international multicentre cohort study. Intensive Care Medicine. 2002;**28**(2):108-121

[63] Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, et al. The third international consensus definitions for sepsis and septic shock (Sepsis-3). Journal of the American Medical Association. 2016;**315**(8):801-810

[64] American College of Chest Physicians/Society of Critical Care Medicine consensus conference: Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. Critical Care Medicine. 1992;**20**(6):864-874

[65] Funk DJ, Parrillo JE, Kumar A. Sepsis and septic shock: A history. Critical Care Clinics. 2009;**25**(1):83-101 viii

[66] Marshall JC. Why have clinical trials in sepsis failed? Trends in Molecular Medicine. 2014;**20**(4):195-203

[67] Cohen J, Vincent JL, Adhikari NK, Machado FR, Angus DC, Calandra T, et al. Sepsis: A roadmap for future research. The Lancet Infectious Diseases. 2015;**15**(5):581-614

[68] Bone RC, Balk RA, Cerra FB, Dellinger RP, Fein AM, Knaus WA, et al. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM consensus conference committee. American College of Chest Physicians/Society of Critical Care Medicine. Chest. 1992;**101**(6):1644-1655

[69] Seymour CW, Liu VX, Iwashyna TJ, Brunkhorst FM, Rea TD, Scherag A, et al. Assessment of clinical criteria for sepsis: For the third international consensus definitions for sepsis and septic shock (Sepsis-3). Journal of the American Medical Association. 2016; **315**(8):762-774

[70] Shankar-Hari M, Phillips GS, Levy ML, Seymour CW, Liu VX, Deutschman CS, et al. Developing a new definition and assessing new clinical criteria for septic shock: For the third international consensus definitions for sepsis and septic shock (Sepsis-3). Journal of the American Medical Association. 2016;**315**(8):775-787

[71] Vincent JL, Moreno R, Takala J, Willatts S, De Mendonça A, Bruining H, et al. The SOFA (Sepsis-related organ failure assessment) score to describe organ dysfunction/failure. On behalf of the working group on Sepsis-related problems of the European Society of Intensive Care Medicine. Intensive Care Medicine. 1996;**22**(7):707-710

[72] Rello J, Valenzuela-Sánchez F, Ruiz-Rodriguez M, Moyano S. Sepsis: A review of ad-vances in management. Advances in Therapy. 2017;**34**(11):2393-2411

[73] Medzhitov R. Septic shock: On the importance of being tolerant. Immunity. 2013;**39**(5): 799-800

[74] Erridge C. Endogenous ligands of TLR2 and TLR4: Agonists or assistants? Journal of Leukocyte Biology. 2010;**87**(6):989-999

[75] Gotts JE, Matthay MA. Sepsis: Pathophysiology and clinical management. BMJ. 2016; **353**:i1585

[76] Takeuchi O, Akira S. Pattern recognition receptors and inflammation. Cell. 2010;**140**(6): 805-820

[77] Schulte W, Bernhagen J, Bucala R. Cytokines in sepsis: Potent immunoregulators and potential therapeutic targets–an updated view. Mediators of Inflammation. 2013;**2013**: 165974

[78] Angus DC. The search for effective therapy for sepsis: Back to the drawing board? Journal of the American Medical Association. 2011;**306**(23):2614-2615

[79] Bonaz B, Sinniger V, Pellissier S. Anti-inflammatory properties of the vagus nerve: Potential therapeutic implications of vagus nerve stimulation. The Journal of Physiology. 2016;**594**(20):5781-5790

[80] Griton M, Konsman JP. Neural pathways involved in infection-induced inflammation: Recent insights and clinical implications. Clinical Autonomic Research. 2018;**28**(3):289-299

[81] Andersson U, Tracey KJ. Reflex principles of immunological homeostasis. Annual Re-view of Immunology. 2012;**30**:313-335

[82] Nance DM, Sanders VM. Autonomic innervation and regulation of the immune system (1987-2007). Brain, Behavior, and Immunity. 2007;**21**(6):736-745

[83] Madden KS. Sympathetic neural-immune interactions regulate hematopoiesis, thermo-regulation and inflammation in mammals. Developmental and Comparative Immunology. 2017;**66**:92-97

[84] Shang X, Wang K, Xu J, Gong S, Ye Y, Chen K, et al. The effect of esmolol on tissue perfusion and clinical prognosis of patients with severe sepsis: A prospective cohort study. BioMed Research International. 2016;**2016**:1038034

[85] Morelli A, Donati A, Ertmer C, Rehberg S, Kampmeier T, Orecchioni A, et al. Microvascular effects of heart rate control with esmolol in patients with septic shock: A pilot study. Critical Care Medicine. 2013;**41**(9):2162-2168

[86] Kimmoun A, Louis H, Al Kattani N, Delemazure J, Dessales N, Wei C, et al. β1-adrenergic inhibition improves cardiac and vascular function in experimental septic shock. Critical Care Medicine. 2015;**43**(9):e332-e340

[87] Lu Y, Yang Y, He X, Dong S, Wang W, Wang D, et al. Esmolol reduces apoptosis and inflammation in early sepsis rats with abdominal infection. The American Journal of Emergency Medicine. 2017;**35**(10):1480-1484

[88] Murch O, Collin M, Sepodes B, Foster SJ, Mota-Filipe H, Thiemermann C. Lysophosphatidylcholine reduces the organ injury and dysfunction in rodent models of gram-negative and gram-positive shock. British Journal of Pharmacology. 2006;**148**(6):769-777

[89] Van Wagoner DR, Lindsay BD. Phosphodiesterase-4 activity: A critical modulator of atrial contractility and arrhythmogenesis. Journal of the American College of Cardiology. 2012;**59**(24):2191-2192

[90] Poole RM, Ballantyne AD. Apremilast: First global approval. Drugs. 2014;**74**(7):825-837

[91] Carcillo JA, Herzer WA, Mi Z, Thomas NJ, Jackson EK. Treatment with the type IV phosphodiesterase inhibitor Ro 20-1724 protects renal and mesenteric blood flow in endotoxemic rats treated with norepinephrine. The Journal of Pharmacology and Experimental Therapeutics. 1996;**279**(3):1197-1204

[92] Sims CR, Singh SP, Mu S, Gokden N, Zakaria D, Nguyen TC, et al. Rolipram improves outcome in a rat model of infant sepsis-induced cardiorenal syndrome. Frontiers in Pharmacology. 2017;**8**:237

[93] Yang JX, Hsieh KC, Chen YL, Lee CK, Conti M, Chuang TH, et al. Phosphodiesterase 4B negatively regulates endotoxin-activated interleukin-1 receptor antagonist responses in macrophages. Scientific Reports. 2017;**7**:46165

[94] Feng H, Chen J, Wang H, Cheng Y, Zou Z, Zhong Q, et al. Roflumilast reverses polymicrobial sepsis-induced liver damage by inhibiting inflammation in mice. Laboratory Investigation. 2017;**97**(9):1008-1019

[95] Bauquier JR, Tudor E, Bailey SR. Anti-inflammatory effects of four potential anti-endotoxaemic drugs assessed in vitro using equine whole blood assays. Journal of Veterinary Pharmacology and Therapeutics. 2015;**38**(3):290-296

[96] Wollborn J, Wunder C, Stix J, Neuhaus W, Bruno RR, Baar W, et al. Phosphodiesterase-4 inhibition with rolipram attenuates hepatocellular injury in hyperinflammation in vivo and in vitro without influencing inflammation and HO-1 expression. Journal of Pharmacology and Pharmacotherapeutics. 2015;**6**(1):13-23

[97] Nørgaard M, Ehrenstein V, Vandenbroucke JP. Confounding in observational studies based on large health care databases: Problems and potential solutions - a primer for the clinician. Clinical Epidemiology. 2017;**9**:185-193

[98] Murray K, Reardon C. The cholinergic anti-inflammatory pathway revisited. Neurogastroenterology and Motility. 2018;**30**(3)

[99] He B, Lu Z, He W, Huang B, Jiang H. Autonomic modulation by electrical stimulation of the parasympathetic nervous system: An emerging intervention for cardiovascular diseases. Cardiovascular Therapeutics. 2016;**34**(3):167-171

[100] van der Poll T, van de Veerdonk FL, Scicluna BP, Netea MG. The immunopathology of sepsis and potential therapeutic targets. Nature Reviews. Immunology. 2017; **17**(7):407-420

[101] Lelubre C, Vincent JL. Mechanisms and treatment of organ failure in sepsis. Nature Reviews. Nephrology. 2018

[102] Santos-Almeida FM, Domingos-Souza G, Meschiari CA, Fávaro LC, Becari C, Castania JA, et al. Carotid sinus nerve electrical stimulation in conscious rats attenuates systemic inflammation via chemoreceptor activation. Scientific Reports. 2017;**7**(1):6265

[103] Johnson RL, Murray ST, Camacho DK, Wilson CG. Vagal nerve stimulation attenuates IL-6 and TNFα expression in respiratory regions of the developing rat brainstem. Respiratory Physiology & Neurobiology. 2016;**229**:1-4

[104] Li N, Li Z, Xiang H, Wang X, Zhang X, Li J. Protective effects of vagus nerve stimulation on rats with sepsis-associated encephalopathy. Zhonghua Wei Zhong Bing Ji Jiu Yi Xue. 2015;**27**(6):509-513

[105] Le Maître E, Revathikumar P, Idborg H, Raouf J, Korotkova M, Jakobsson PJ, et al. Impaired vagus-mediated immunosuppression in microsomal prostaglandin E synthase-1 deficient mice. Prostaglandins & Other Lipid Mediators. 2015;**121**(Pt B):155-162

[106] Kox M, van Eijk LT, Verhaak T, Frenzel T, Kiers HD, Gerretsen J, et al. Transvenous vagus nerve stimulation does not modulate the innate immune response during experimental human endotoxemia: A randomized controlled study. Arthritis Research & Therapy. 2015;**17**:150

[107] Elorza-Ávila AR, Reyes-Lagos JJ, Hadamitzky M, Peña-Castillo M, Echeverría JC, Ortiz-Pedroza MD, et al. Oxytocin's role on the cardiorespiratory activity of endotoxemic rats. Respiratory Physiology & Neurobiology. 2017;**236**:19-22

[108] Reyes-Lagos JJ, Hadamitzky M, Peña-Castillo M, Echeverría JC, Bösche K, Lückemann L, et al. Exogenous oxytocin reduces signs of sickness behavior and modifies heart rate fluctuations of endotoxemic rats. Physiology & Behavior. 2016;**165**:223-230

[109] Rosas-Ballina M, Valdés-Ferrer SI, Dancho ME, Ochani M, Katz D, Cheng KF, et al. Xanomeline suppresses excessive pro-inflammatory cytokine responses through neural signal-mediated pathways and improves survival in lethal inflammation. Brain, Behavior, and Immunity. 2015;**44**:19-27

[110] Carrara M, Bollen B, Bendjelid K, Baselli G, Ferrario M. Autonomic nervous system indices in the progression of organ failure in early septic shock patients. Journal of Critical Care. 2017;**38**:1-372

Section 4

Autonomic Nervous System and its Organization

Chapter 5

Regulation of Dendritogenesis in Sympathetic Neurons

Vidya Chandrasekaran and Pamela J. Lein

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.80480

Abstract

In postganglionic sympathetic neurons, the size of the dendritic arbor determines presynaptic convergence, which correlates with tonic activity, and aberrant dendritic morphology is associated with disease. There is, therefore, great interest in understanding how dendritic morphology is regulated in these neurons. Early studies established a role for target-derived nerve growth factor (NGF) in regulating the size of the dendritic arbor of sympathetic neurons *in vivo*. However, *in vitro* studies revealed that even in the presence of optimal concentrations of NGF, rat sympathetic neurons cultured in the absence of serum or non-neuronal cells survive and elaborate extensive axonal arbors, but fail to form dendrites. Subsequently, it was discovered that bone morphogenetic proteins (BMPs) trigger cultured sympathetic neurons to extend a dendritic arbor comparable to that of their *in vivo* counterparts. The goals of this chapter are to: (i) summarize these early experiments; (ii) discuss evidence substantiating a role for BMPs in glial-induced dendritic growth *in vitro* and regulation of dendritic growth *in vivo*; (iii) review what is known about the molecular mechanisms by which NGF, BMPs and other factors influence dendritic arborization of sympathetic neurons; and (iv) identify key data gaps in understanding of how dendrites are regulated in sympathetic neurons.

Keywords: afferent input, BMPs, dendrites, neuronal polarity, NGF, p75, reactive oxygen species (ROS), Rit, Smad, STAT, sympathetic neurons, target-derived factors

1. Introduction

Differences in dendritic morphology between neurons are a striking feature of the vertebrate nervous system with important functional implications. The shape of dendrites influences the propagation and integration of postsynaptic potentials [1], and determines presynaptic

convergence [2, 3]. These observations coupled with evidence that aberrant dendritic structure is strongly associated with neurologic disease [4, 5] have generated significant interest in understanding how dendrites are regulated.

Postganglionic sympathetic neurons are a well-characterized model for studying dendrite development and plasticity [6]. The dendritic arbor of these neurons is relatively complex with an average of two to six primary dendrites, depending on the animal species, and multiple orders of branching [7]. In postganglionic sympathetic neurons, the size of the dendritic arbor correlates with not only the number and pattern of synaptic inputs [3, 8], but also tonic activity [8, 9]. As is true of central neurons, aberrant morphology of sympathetic neuron dendrites is associated with disease. For example, dendritic hypertropy of sympathetic neurons in stellate and superior cervical ganglia (SCG) is observed in the spontaneously hypertensive rat [10, 11], and is thought to contribute to the pathogenesis of hypertension in this model [11]. Therapeutic intervention with statins not only decreases sympathetic activity and normalizes blood pressure in the spontaneously hypertensive rat [12], but also decreases dendritic arborization of both stellate and SCG neurons [13].

In this chapter, we will review what is known about the molecular and cellular mechanisms that regulate dendrites in postganglionic sympathetic neurons, and identify key data gaps.

2. Early studies of dendritic growth in sympathetic neurons

The majority of dendritic growth in postganglionic sympathetic neurons occurs during the postnatal period; however, dendrites continue to grow into adulthood [14–16], and *in situ* imaging of mature sympathetic neurons has demonstrated that their dendritic arbors continually grow and retract throughout life [17, 18]. In the rat, neonatal deafferentation has negligible effect on dendritic growth in SCG neurons throughout the first month of life [19–21], indicating that dendritic growth in sympathetic neurons does not require afferent input. In contrast, target tissues strongly influence dendritic growth in these neurons. Experimentally reducing target size causes dendritic arbors of SCG neurons to be smaller than normal; conversely, increasing the target size significantly increases the size of the dendritic arbor [16]. The influence of target is further illustrated by observations that sympathetic neurons within the same ganglion that project to different targets exhibit varying dendritic morphologies [18, 19].

The effect of target tissues on dendritic growth in sympathetic neurons is mediated, at least in part, by nerve growth factor (NGF) [22–25]. Separation of neurons from target tissues by axonal ligation causes dendritic atrophy in the few neurons that survive, and this effect is attenuated by systemic administration of NGF [26, 27]. However, exogenous NGF reverses axotomy-induced dendritic retraction by <50%, even though cell survival is completely rescued [27], indicating that additional target-derived factors are needed to fully account for the effects of target on dendritic growth. Consistent with this conclusion, the dendritic complexity of axotomized sympathetic neurons recovers to control levels upon ganglion cell reinnervation of the periphery [28]. *In vitro* studies further suggest that factors in addition to NGF are

required for dendritic growth in sympathetic neurons. When grown in low-density cultures in the absence of serum or non-neuronal cells, but in the presence of optimal concentrations of NGF, sympathetic neurons form an axon but no dendrites [29, 30].

In vitro studies have further revealed that the initiation and maintenance of dendritic growth in sympathetic neurons is regulated by trophic interactions. Addition of serum to NGF-containing medium stimulates sympathetic neurons to form dendrites, although under these culture conditions, the dendritic arbor is significantly less complex than is observed *in vivo* [31]. In contrast, co-culture with ganglionic glial cells causes these neurons to form a dendritic arbor comparable to that of their *in vivo* counterparts [32]. Subsequently, it was discovered that the addition of bone morphogenetic proteins (BMPs) to the culture medium similarly triggers sympathetic neurons to form a complex dendritic arbor [29, 33]. Multiple BMP family members have been shown to stimulate dendritic growth in cultured sympathetic neurons, including BMPs 2, 4, 5, 6, 7 and 60A; however, this activity appears to be restricted to the dpp and 60A BMP subfamilies since BMP-3 and other members of the TGFβ superfamily, including TGFβ1, TGFβ2, TGFβ3, activin A, inhibin, and GDNF, have no effect on dendritic growth in cultured sympathetic neurons [29, 33, 34].

The trophic actions of BMPs are specific to dendritic growth in that BMPs do not support cell survival, nor do they enhance axonal growth in cultured sympathetic neurons [29]. Consistent with observations of dendritic growth in sympathetic neurons *in vivo*, the dendrite-promoting activity of BMP-7 is independent of synaptic or electrical activity [35], but is modulated by NGF [29, 36]. Importantly, the dendritic arbor induced by BMPs in cultured sympathetic neurons is comparable to that of their *in vivo* counterparts with respect to not only size and complexity, but also accumulation and post-translational modification of dendrite-specific cytoskeletal and membrane proteins, exclusion of axonal proteins, transport of select mRNA, and formation of synaptic contacts of the appropriate polarity [29, 34, 35]. These observations indicate that BMPs selectively induce the execution of a developmental program in sympathetic neurons that controls both quantitative and qualitative aspects of dendritic growth.

These observations suggest that BMPs mediate the effects of ganglionic glia and target tissues on dendritic growth in sympathetic neurons. Immunocytochemical and *in situ* hybridization studies indicate that the spatiotemporal expression of BMPs 5, −6, and −7 in rat SCG is consistent with a role in the initial stages of dendritogenesis [33, 37]. *In vitro*, both SCG glia and neurons express BMP mRNA and protein when grown in the absence or presence of each other [37]. However, co-culture of sympathetic neurons with ganglionic glia markedly increases BMP protein coincident with a significant decrease in levels of the soluble BMP antagonists, follistatin and noggin [37]. Functional assays indicate that glial-induced dendritic growth is significantly reduced by BMP-7 antibodies and completely blocked by exogenous noggin and follistatin [37]. Collectively, these data suggest a model in which glia influence the rapid perinatal expansion of the dendritic arbor in sympathetic neurons by increasing BMP activity via modulation of the balance between BMPs and their antagonists. Whether this model holds true *in vivo* has yet to be tested.

The question of whether BMPs also contribute to target effects on dendritic growth has yet to be addressed experimentally. Sympathetic targets, including the eye, heart, lung, kidney, and

blood vessels, express significant levels of BMPs during embryonic development, throughout the postnatal period, and into adulthood [38–40]. Thus, target tissues may be a source of BMPs to sympathetic neurons not only during initial expansion of the dendritic arbor, but also in the maintenance and remodeling of dendritic arbors that continues throughout the life of the animal.

3. Signaling pathways that regulate dendritic growth in sympathetic neurons

Research over the past few decades has provided insights into the signaling pathways and molecular mechanisms that control dendritic growth in sympathetic neurons. As discussed in the preceding section, BMPs and NGF play predominant roles in the initiation and maintenance of dendrites in these autonomic neurons. While the importance of these growth factors as regulators of dendritic growth in sympathetic neurons is well established, the downstream effectors that link BMP and NGF to increased dendritic growth are not fully understood. In this section, we will discuss the signaling pathways activated by these growth factors, the evidence implicating downstream effectors of BMPs and NGF in dendritic regulation, and the identification of factors that interact with these signaling pathways to alter their influence on the dendritic arborization of sympathetic neurons.

3.1. BMP signaling

BMPs mediate their cellular effects by binding to a heteromeric receptor complex of transmembrane serine/threonine kinas receptor subunits comprised of a type I receptor [BMP type I receptor A (BMPR1A), which is also known as activin receptor-like kinase-3 (ALK-3) or BMP type I receptor B (BMPRIB, also known as ALK-6)], and a type II receptor [BMP type II receptor (BMPRII), activin type II receptor (ActRII), or activin type IIB receptor (ActRIIB)] [41, 42]. Ligand binding causes type II receptors to phosphorylate type I receptors, which then phosphorylate Smads 1, 5, and/or 8, also known as receptor Smads (R-Smads). Phosphorylated R-Smads complex with Smad 4, triggering translocation of the Smad complex to the nucleus to regulate gene transcription [42, 43]. Studies in *Smad* knockout animals and *Smad* deficient cells suggest that BMPs can also signal through Smad-independent pathways via activation of mitogen-activated protein kinase (MAPK), c-jun amino terminal kinase (JNK), p38-MAPK, phosphoinositol-3-kinase (PI3K), LIM kinase 1 (LIMK1) or small GTPases [43–46].

BMP signaling pathways are active in sympathetic ganglia during developmental periods corresponding to the initiation, extension and maintenance of dendrites. Quantitative PCR, *in situ* hybridization, and immunocytochemistry studies have confirmed the presence of mRNA and protein for BMPRIA, BMPRIB, ActRII and BMPRII in neurons of mouse SCG starting as early as embryonic day 14, and persisting into postnatal development and even adulthood [47, 48]. A role for BMPRIA in regulating dendritic growth in sympathetic neurons *in vivo* was confirmed in a BMPRIA conditional knockout mouse generated by crossing a *Dbhi-Cre* mouse line to a *BmprIa* floxed line [49]. *Dbhi-Cre* is expressed in sympathetic ganglia around E10.5, thus, *BmprIa* expression is knocked out after neuronal specification, but before initiation of dendritic growth. The loss of BMPRIA expression in sympathetic ganglia significantly

diminished but did not completely block dendritic growth *in vivo* [49]. At postnatal day 3, the number of primary dendrites and the size of the dendritic arbor were not significantly different in sympathetic neurons of SCG from *BmprIa* conditional knockout mice *vs*. SCG neurons from congenic wildtype controls. At later developmental times, however, dendritic arborization was significantly decreased in SCG neurons of *BmprIa* knockout vs. wildtype mice. These data suggest that, *in vivo*, BMP signaling is required for the maintenance of dendrites, but not for the initiation and early growth of dendrites in sympathetic neurons.

Several caveats of the *in vivo* study suggest that the lack of effect of *BmprIa* knockout on early stages of dendritic growth *in vivo* may not be discrepant with *in vitro* data showing that BMPs are necessary and sufficient for induction of dendritic growth in sympathetic neurons [29, 49]. First, while the authors confirmed that *BmprIa* mRNA was not generated in the SCG after embryonic day 11.5, they did not examine BMPRIA protein levels in the SCG of knockout animals. Thus, the possibility that BMPRIA protein was still present during early stages of dendritic growth cannot be ruled out. Moreover, recent biochemical studies have shown that BMPRs cycle between various cellular compartments, and the association of the receptors with endocytosis machinery is required for Smad-mediated signal transduction [50]. Thus, additional studies are needed to characterize BMPR turnover and changes in receptor localization in wildtype *vs*. *BMPR* knockouts. Another, perhaps more likely, explanation is that sympathetic neurons in the *BmprIa* knockout mice express other type I receptors, such as the activin receptors, that may be activated by BMPs to trigger dendritic growth at early developmental stages. Further studies targeting other BMPRs are warranted to fully elucidate the role of BMP signaling in dendritogenesis *in vivo*.

3.1.1. Smad-dependent transcriptional regulation of dendritic growth

Canonical BMP signaling involves the Smad family of transcription factors. Immunocytochemical analyses of primary rat SCG neurons have demonstrated that Smad 1/5/8 translocates to the nucleus within 20 minutes of exposure to BMP-7, with maximal nuclear translocation observed within 2 hours of adding BMP-7 to the culture medium. Transfection with a Smad1 dominant negative mutant, Smad1 (3SA), blocked BMP-7-induced dendritic growth in primary sympathetic neurons [51], indicating that Smad 1 activation is necessary for induction of dendritic growth by BMP-7. In contrast to the *BmprIa* conditional knockouts, conditional knockdown of *Smad4* in sympathetic neurons, generated by crossing *Dbhi-Cre* mice to *Smad4* floxed mice, increased dendritic length and the size of the dendritic arbor in SCG neurons [49], suggesting that Smad 4 may play a role in limiting dendritic growth *in vivo*.

A comprehensive analyses of Smad-dependent dendritic growth in sympathetic neurons provided evidence for early transcriptional regulation of dendritic growth downstream of BMP-7 [52]. In neuronal cell cultures from embryonic rat SCG, BMP-7-induced dendritic growth could be blocked by pharmacologic inhibition of transcription with actinomycin-D when the inhibitor was added within the first 24 hours of BMP-7 exposure but not when it was added after 48 hours of BMP-7 exposure. Microarray analyses identified over 250 genes that were differentially regulated by BMP-7 within the first 24 hours after adding BMP-7 to the culture medium. Of these, 56 mRNAs were altered within the first 6 hours and 185 mRNAs were differentially regulated at 24 hours after BMP exposure [52]. Many of the differentially regulated genes were linked to signaling pathways previously implicated in dendritogenesis in other

neuronal cell types or neuronal morphogenesis and/or axonal guidance, such as BMP, Notch, integrin, Wnt, and NGF signaling molecules. However, the functional relevance of most of these genes to dendritic growth in sympathetic neurons has yet to be determined. Moreover, recent reports of limited correlation between transcriptome and proteome analysis in yeast, plants and mice [53, 54], suggest that in order to generate a more complete understanding of the molecular pathways that link BMPs to dendritic growth in sympathetic neurons, detailed proteome analyses are needed to complement the existing transcriptomic dataset.

One gene identified as being strongly upregulated by BMP-7 in primary sympathetic neurons, the gene encoding the p75 neurotrophin receptor ($p75^{NTR}$) [52], has been evaluated for a role in BMP-induced dendritic growth. A member of the tumor necrosis factor (TNF) receptor family, $p75^{NTR}$ regulates diverse neurobiological processes, including axonal growth, synaptic plasticity, dendritic growth in central neurons, and neuronal cell death [55–61]. $p75^{NTR}$ binds diverse ligands to mediate its effects, including NGF, other neurotrophins, myelin-derived polypeptides, such as myelin-associated glycoprotein (MAG) or Nogo, and β-amyloid peptide [60, 62, 63]. In cultured embryonic rat SCG neurons, $p75^{NTR}$ mRNA and protein expression are significantly upregulated within 24 hours of exposure to BMP-7 [52, 64], and pharmacologic inhibition of signaling via BMPRI prevents induction of $p75^{NTR}$ protein expression in primary sympathetic neurons exposed to BMP-7 [64]. Functional studies revealed that BMP7 does not trigger dendritic growth in primary sympathetic neurons derived from SCG of $p75^{NTR}$ knockout mice; conversely, ligand-independent activation of $p75^{NTR}$ via overexpression of a $p75^{NTR}$ cDNA construct in $p75^{NTR-/-}$ neurons [65], phenocopies the dendrite-promoting effects of BMP-7 [64]. Morphometric analyses of SCG from wildtype *vs*. $p75^{NTR}$ knockout mice at 3, 6 and 12–16 weeks of age indicated that genetic deletion of $p75^{NTR}$ does not prevent dendritic growth, but does significantly stunt dendritic maturation in sympathetic neurons. These data support the hypotheses that $p75^{NTR}$ is involved in downstream signaling events that mediate BMP7-induced dendritic growth in sympathetic neurons, and suggest that $p75^{NTR}$ signaling positively modulates dendritic complexity in sympathetic neurons *in vivo*.

An outstanding question regarding $p75^{NTR}$ effects on dendritic growth in sympathetic neurons is the identity of ligand(s) and co-receptor(s) that $p75^{NTR}$ interacts with to mediate BMP-induced dendritic growth. Several lines of evidence argue against a direct interaction between $p75^{NTR}$ and the BMP receptor complex: (*i*) inhibiting transcription blocks both BMP-induced dendritic growth and upregulation of $p75^{NTR}$ mRNA [52]; (*ii*) pharmacologic blockade of BMPRI signaling blocks $p75^{NTR}$ induction [64]; and (*iii*) overexpressing $p75^{NTR}$ in cultured $p75^{NTR-/-}$ neurons induces dendritic growth even in the absence of BMP-7 [64]. NGF is a potential activating ligand, and NGF is required for BMP-induced dendritic growth [29, 36]. However, the observation that $p75^{NTR}$ interactions with the primary receptor for NGF, TrkA, serve to limit synapse formation in sympathetic neurons [66], argues against $p75^{NTR}$-TrkA interactions enhancing dendritic development. Likewise, pro-neurotrophin activation of the $p75^{NTR}$-sortilin complex serves to promote sympathetic axon degeneration and cell death [67], suggesting that this interaction is also unlikely to stimulate dendritic growth. The identity of the $p75^{NTR}$ ligand that mediates BMP-7-induced dendritic growth remains an outstanding question.

Similarly, the downstream effector molecule(s) that link $p75^{NTR}$ to increased dendritic arborization remain to be determined. Key candidates include the Rho GTPases. Rho GTPases function as central regulators of dendritic morphology, linking extracellular signals to changes

in the dendritic actin cytoskeleton [68, 69]. $p75^{NTR}$ has been shown to interact with RhoA in the yeast two-hybrid system [70]. In cultured rat sympathetic neurons, exposure to BMP-7 increases the levels of the GTP-bound form of RhoA, but not GTP-Rac1 or GTP-Cdc42, as determined by a GTP pull down assay, and triggers RhoA translocation from the cytoplasm to the membrane [13]. The observation that BMP-7-induced dendritic growth in primary sympathetic neurons requires RhoA activation [13], suggests a model in which BMP-7 sequentially activates BMPRIA, Smad 1/5/8, $p75^{NTR}$, and then RhoA to induce dendritic growth in sympathetic neurons.

3.2. Signaling pathways that interact with Smad signaling to modulate BMP-induced dendritic growth

The shape of the dendritic arbor of developing sympathetic neurons is determined by interactions between positive and negative regulators of dendritic growth. A number of signaling pathways have been shown to interact with BMP signaling to modulate the number of dendrites, total dendritic length and dendritic branching in sympathetic neurons. In this section, we will review known positive and negative regulators of Smad signaling that impact BMP-induced dendritogenesis in sympathetic neurons.

3.2.1. Positive regulators of Smad signaling that enhance dendritic growth

Proteasome-mediated signaling. The ubiquitin-proteasome pathway is unique in that it is one of the few signaling pathways identified thus far whose interactions with BMP signaling enhance dendritic arborization in sympathetic neurons. In the nervous system, proteasome-mediated protein degradation pathways are important for self-renewal of neurons, axonal growth, vesicle release, receptor turnover, signal transduction and synaptic plasticity [71–73]. The ubiquitin-proteasome pathway has been implicated in regulating dendritic growth in the mammalian central nervous system, with multiple E3 ubiquitin ligases, including Anaphase promoting complex (APC), cullin RING-type E3 ubiquitin ligases (CUL) and neuronal precursor cell expressed and developmentally downregulated protein (Nedd4), determined to be necessary for the extension and maintenance of the dendritic arbor of central neurons [71, 74–76].

Biochemical studies have shown that R-Smads interact with components of the proteasome complex, as well as enzymes and proteins involved in the ubiquitination-deubiquitination of proteins, in many systems, and the ability of Smads to regulate transcription is dependent on association with the proteasome complex in various cell types [77, 78]. Interactions between BMP signaling molecules and the proteasome pathway have been reported in perinatal rat sympathetic neuronal cultures prior to dendritic growth induction by BMP-7 [51]. In this study, interactions between Smad1 and multiple proteasome components were confirmed using a yeast two-hybrid assay, and pharmacologic inhibition of proteasome activity by lactacystin and ALLN (N-acetyl-Leu-Leu-norleucinal) selectively blocked BMP-7-induced dendritic growth in primary sympathetic neurons in the absence of any effect on axonal growth [51]. These proteasome inhibitors also suppressed Smad-mediated transcriptional regulation in a biochemical assay using a Tlx -luciferase construct transfected into P19 cells [51]. One caveat of this study is that although there was clearly a functional interaction between BMP signaling and the proteasome pathway in the context of dendritic growth, a biochemical

interaction between Smads and proteasomes were not demonstrated in primary sympathetic neurons. Further studies are necessary to fully understand the genetic and biochemical interactions between Smads and ubiquitin-proteasome pathway during dendritogenesis in sympathetic neurons.

Signaling mediated by reactive oxygen species (ROS). Under physiological conditions, ROS are generated by the mitochondrial electron transport chain or as a consequence of the activity of NADPH oxidase (NOX) [79–81]. While the deleterious effects of ROS at high concentrations are well documented, over the past decade, there is growing appreciation of the beneficial role of ROS at physiological concentrations [80–84], and of the importance of the NOX family of enzymes in regulating many aspects of neuronal development including neurogenesis, neurite outgrowth and synaptic plasticity [80, 83, 85]. NOX2 is expressed in neonatal rat SCG neuronal cell cultures [86], and exposure of cultured embryonic SCG neurons to BMP-7 increases the expression of NOX2 [87]. Pharmacologic inhibition or siRNA knockdown of NOX2 significantly decreases BMP-7-induced dendritic growth in primary sympathetic neurons, an effect that is also observed in cultures co-exposed to BMP-7 and any of three mechanistically and structurally distinct antioxidants that block ROS generation [87]. Antioxidants block BMP-induced dendritic growth downstream of Smad signaling since BMP-induced nuclear translocation of Smads is unaffected by antioxidant treatment [87].

Collectively, these data support the hypothesis that ROS are involved in the downstream signaling events that mediate BMP7-induced dendritic growth, and suggest that ROS-mediated signaling positively modulates dendritic complexity in sympathetic neurons. One caveat of this study, however, is that while BMP-7 was observed to increase NOX2 levels and oxygen consumption in sympathetic neurons, increased ROS levels were not detected in sympathetic neurons exposed to BMP-7. Likely, this reflects the fact that physiologic BMP signaling generates levels of ROS that are below the detection threshold for standard ROS detection assays. Further work is needed to determine whether these *in vitro* observations translate to a role for ROS in regulating dendritic growth in sympathetic ganglia *in vivo*.

3.2.2. Negative regulators of SMAD signaling that influence dendritic growth

STAT signaling. The interferons (IFN) and the neuropoietic cytokines, which include interleukins (IL), leukemia inhibitory factor (LIF) and ciliary neurotrophic factor (CNTF), signal through the activation and nuclear translocation of signal transducers and activators of transcription (STAT) proteins, which then regulate transcription of target genes [88]. Previous studies have shown that cytokines that signal through STATs *vs*. members of the TGFβ superfamily of signaling molecules tend to exert opposing effects on cellular function [89–91]. Similar antagonistic interactions between STAT-dependent cytokine signaling pathways and the BMP signaling pathways have been documented during dendritogenesis in perinatal sympathetic neurons. In primary perinatal sympathetic neurons, addition of LIF, CNTF or gamma-IFN (IFNγ) significantly decreased the number of primary dendrites and total dendritic length in cultures exposed to BMP-7, but had no effect on axonal growth or cell viability [92–94]. In addition, all three cytokines triggered retraction of preexisting dendrites [92, 94], suggesting a role for them in the dendritic retraction observed following neuronal injury. Addition of LIF, CNTF or IFNγ to primary sympathetic neurons resulted in nuclear translocation of STAT, and transfection of a dominant negative STAT1 construct blocked

IFNγ-mediated dendritic retraction [92, 94]. These data provide evidence that neuropoietic cytokines negatively regulate dendritic growth in perinatal sympathetic neurons via activation of STAT proteins. However, many questions remain, including the mechanism by which nuclear translocation of STAT proteins limits the activity of SMAD proteins, and the downstream targets of STAT and SMAD signaling during dendritogenesis.

Mitogen-activated protein kinase (MAPK) signaling. MAPK family members, including extracellular signal-regulated kinase (ERK), p38, and c-Jun NH_2-terminal kinase (JNK), are serine–threonine kinases that function as downstream effectors of many extracellular signaling molecules, including NGF, epidermal growth factor (EGF), and fibroblast growth factor (FGF) [95, 96]. The activation of MAPK regulates gene expression to alter various cellular activities such as cell division, growth, survival and cell death [95, 96]. Previous studies in *Xenopus* embryos and cells such as radial glia, astrocytes, hippocampal neurons, and PC12 cells, have demonstrated interplay between Smad and MAPK signaling pathways, and shown that phosphorylation of the linker on the Smad protein by MAPK is necessary for coordinating the cellular effects of BMPs and other growth factors [43, 97–99]. Interplay between BMP and MAPK signaling pathways is also observed during dendritogenesis in rat and mouse sympathetic neurons. Pharmacologic inhibition of MAPK signaling with PD98059 or transfection of a dominant negative MEK1 or ERK2 mutant increases the number of dendrites and total dendritic arbor induced following BMP-7 exposure, whereas overexpression of MEK1 decreases BMP-7 induced dendritic growth [100]. Inhibition of MAPK signaling in primary sympathetic neurons also increases Smad nuclear accumulation following BMP-7 exposure, and FGF was identified as one of the ligands responsible for activating the ERK signaling pathway in these neurons [100]. These findings suggest that, in perinatal sympathetic neurons, FGF activates the MAPK pathway, which inhibits the nuclear accumulation of SMADs, thereby limiting BMP-induced dendritic growth.

In addition to modulating BMP effects on dendritic arborization via activation of MAPK signaling, FGFs regulate neuronal differentiation via the integrative nuclear FGFR1 signaling (INFS) pathway [101]. FGFR1 is expressed in the nucleus of adult rat SCG neurons following axotomy [102]. Nuclear localization of FGFR1 is also increased in perinatal rat sympathetic neurons following exposure to BMP-7, and transfection of a mutant FGFR1 receptor inhibits FGFR1 nuclear localization and decreases the dendritic response to BMP-7 [103]. These data suggest that the INFS-mediated FGF signaling pathway functions downstream of BMP signaling to limit BMP-induced dendritogenesis in sympathetic neurons.

Rit GTPase, a member of the small GTPase family, has also been shown to activate ERK1/2 in primary sympathetic neurons, and the transfection of dominant negative (dn) Rit or constitutively active (ca) Rit were observed to increase or decrease BMP-induced dendritic growth, respectively [36]. Rit GTPase also negatively modulates dendritic growth as a downstream target of IFNγ signaling as demonstrated by inhibition of IFNγ-mediated dendritic retraction in primary sympathetic neurons transfected with dnRit constructs [104]. Addition of IFNγ to cultures of pheochromocytoma cells, which are often used as a model for sympathetic neurons, increased levels of GTP-Rit, and transfection of dnRit inhibited IFNγ-induced activation of p38 MAPK [104]. These observations suggest that a novel Rit-p38 MAP kinase signaling pathway functions in parallel with the canonical JAK–STAT signaling pathway to mediate IFNγ-induced dendritic retraction. Collectively, these studies provide evidence for crosstalk

between BMP signaling and MAPK signaling during dendritogenesis in sympathetic neurons, and suggest that in contrast to its effects in central neurons, MAPK signaling functions as a negative regulator of BMP-induced dendritic growth in sympathetic neurons.

Retinoic acid signaling. In the developing nervous system, BMPs and retinoic acid work synergistically to regulate neural tube patterning and specification of neuronal identity [105–108]. Similarly, interplay between BMP and retinoic acid signaling have been documented during dendritogenesis in sympathetic neurons; however, their interaction in this latter context is antagonistic [106]. Genes required for synthesis of retinoic acid are expressed in cultured perinatal rat SCG neurons, and retinoids synthesized by SCG explants were able to activate transcriptional reporters in retinoic acid-responsive F9 cells [106]. The addition of retinoic acid, or specific retinoic acid receptor and retinoid X receptor agonists, to the medium of cultured sympathetic neurons inhibited BMP-induced dendritic growth without altering axonal growth or cell viability [106]. What is not known, however, is the point of convergence between these two pathways in the context of dendritic growth. Further studies are also needed to determine whether retinoic acid regulates the dendritic arborization of sympathetic neurons *in vivo*.

Signaling by pituitary adenylate cyclase (PACAP) and vasoactive intestinal peptide (VIP). PACAP is a member of the secretin/VIP family of peptides that regulates the development of cells within the sympathoadrenal lineage [109]. In sympathetic neurons, PACAP regulates cell survival, proliferation, and catecholamine secretion, and release of PACAP and VIP from preganglionic neurons stimulates depolarization of sympathetic neurons [109]. In cultured perinatal rat sympathetic neurons, PACAP38 and VIP decrease BMP-induced dendritic growth, as evidenced by a decrease in the percentage of cells with dendrites, number of dendrites per neuron, and size of the dendritic arbor [110]. Using receptor specific antagonists and antibodies against phosphorylated cyclic AMP response element binding (CREB) protein, the PACAP response was shown to be mediated by PAC_1 receptor activation, which results in the nuclear accumulation of phospho-CREB. Inhibition of adenylate cyclase activity by SQ22526 overcomes the inhibitory effects of PACAP on dendritic growth, and agents that increase cAMP levels, such as forskolin, inhibit BMP-induced dendritic growth [110]. These data suggest that peptides released by preganglionic nerves modulate dendritic growth in postganglionic sympathetic neurons by a cAMP-dependent mechanism.

In summary, signaling by cytokines, growth factors, small molecules, and peptides, such as retinoic acid, PACAP and VIP, antagonize BMP signaling during dendritogenesis in sympathetic neurons. Most of the relevant data were collected from studies of primary perinatal sympathetic neurons cultured from rodent SCG. While the findings from this model have provided a glimpse into the complexity of the interactions that influence dendritic arborization of these neurons, further studies are required to understand the mechanisms by which these factors interact to regulate dendritic growth, how these pathways are spatially and temporally coordinated to influence dendritic arborization of sympathetic neurons *in vivo*, and to determine their relevance to the human condition.

3.3. Other pathways that regulate dendritic growth in sympathetic neurons

As described earlier, NGF is an important regulator of dendritic growth in sympathetic neurons. However, the molecular mechanisms by which NGF regulates dendritic growth are

not well characterized. Early growth response-3 (Egr3), a transcriptional regulator known to be induced by NGF via MAPK signaling, has been identified as a potential downstream regulator of NGF-induced dendritic growth [111]. Sympathetic neurons from a conditional *Egr3* knockout generated using *Dbhi-Cre* have fewer primary dendrites and shorter dendritic arbors compared to sympathetic neurons from congenic wildtype animals. In addition, *Egr3* knockout animals exhibit defects in axonal guidance, and in innervation of autonomic targets [111]. Although, Egr3 had previously been shown to not be required for NGF effects on neuronal survival [112], some neuronal cell death was observed in germline mutants for Egr3. It is, therefore, possible that the dendritic effects of knocking out *Egr3* are secondary to adverse effects on neuronal cell viability or changes in target innervation.

Neuronal depolarization induced by electric field stimulation or the addition of potassium chloride to primary postnatal sympathetic neurons cultured was shown to trigger the formation of dendrites in the presence of NGF that retracted in the absence of neuronal activity [113]. Neuronal depolarization enhanced stability of microtubules and activated calcium calmodulin dependent kinase II (CaMKII) in dendrites. The latter was shown to be causally related to the effects of neuronal depolarization on dendritic growth: pharmacologic inhibition of CaMKII activity using KN62 or mAIP completely blocks activity-dependent dendritic growth in cultured sympathetic neurons [113].

Signaling by integrin-linked kinase (ILK) and glycogen synthase kinase-3β (GSK-3β) have also been shown to be downstream effectors of activity-dependent dendritic growth in postnatal sympathetic neurons [114]. ILK and GSK-3β are serine threonine kinases that are downstream effectors of integrin and neurotrophin signaling [115]. ILK has been shown to phosphorylate and inactivate GSK-3β to regulate NGF-mediated axonal growth [116]. Increased phosphorylation of GSK-3β protein was observed in cultured postnatal rat SCG neurons in response to increased neuronal activity, and inhibition of ILK activity by QLT0254, as well as transfection of dominant negative ILK or siRNA for ILK, blocked activity-dependent dendritic growth in these neurons. Similarly, inhibition of GSK-3β activity using kenpaullone or genetic knockdown of GSK-3β expression increased the number of primary dendrites formed in response to potassium chloride, suggesting that GSK-3β inhibition is necessary for early stages of activity-dependent dendritic growth in sympathetic neurons.

Interestingly, unlike BMP-induced dendritic growth, inhibition of ERK activity inhibited activity-dependent dendritic growth in postnatal sympathetic neurons *in vitro* [113, 114]. Pharmacologic inhibition of ERK by PD98059 blocked activity-dependent dendritic growth, inhibition of GSK-3β increased depolarization-dependent ERK activation, and inhibition of ERK reversed the enhanced dendritic growth observed with GSK-3β inhibition [113, 114]. The reasons for the opposing roles of ERK in BMP-induced vs. activity-dependent dendritic growth remain unexplored.

4. The path forward

The experimental evidence clearly implicate NGF, BMP and neuronal activity as positive regulators of dendritic growth in perinatal sympathetic neurons *in vitro*. However, the interaction between these three pathways and their relative contributions to the induction and

maintenance of dendritic growth in sympathetic neurons *in vivo* are not fully understood. Multiple mechanistically and structural diverse signaling molecules have been implicated as negative regulators of dendritic growth *in vitro*. The spatial and temporal regulation of these pathways, how they interact with each other and with positive modulators of dendritic growth to shape dendritic arbors, and their functional significance *in vivo* remain outstanding questions. In addition, further studies are needed to understand the genes and proteins that are regulated downstream of each of these signaling pathways and to identify those that serve as hubs for interactions between multiple signaling pathways to regulate the final dendritic arbor of sympathetic neurons. Finally, a key data gap is the relevance of these observations in experimental models to the human condition.

Acknowledgements

The authors acknowledge Dennis Higgins, PhD (University of Buffalo) who dedicated his research career to understanding the regulation of dendritic growth in sympathetic neurons, and whose enthusiasm for this topic, and scientific research in general, was the inspiration and motivation for much of the authors' own work in this field. This work was supported by the National Institutes of Health (grants R01 NS097808, R01 ES014901 and R21 NS45037).

Conflict of interest

The authors declare no conflict of interest.

Author details

Vidya Chandrasekaran[1] and Pamela J. Lein[2]*

*Address all correspondence to: pjlein@ucdavis.edu

1 Department of Biology, Saint Mary's College of California, Moraga, CA, USA

2 Department of Molecular Biosciences, University of California, Davis, CA, USA

References

[1] Miller JP, Jacobs GA. Relationships between neuronal structure and function. The Journal of Experimental Biology. 1984;**112**:129-145

[2] Purpura DP. Comparative physiology of dendrites. In: Quarton GC, Melnechuk T, Schmitt FO, editors. The Neurosciences: A Study Program. New York: Rockefeller University Press; 1967. pp. 372-393

[3] Purves D. Body and Brain: A Trophic Theory of Neural Connections. Cambridge, MA: Harvard University Press; 1988

[4] Kweon JH, Kim S, Lee SB. The cellular basis of dendrite pathology in neurodegenerative diseases. BMB Reports. 2017;**50**(1):5-11

[5] Copf T. Impairments in dendrite morphogenesis as etiology for neurodevelopmental disorders and implications for therapeutic treatments. Neuroscience and Biobehavioral Reviews. 2016;**68**:946-978. DOI: 10.1016/j.neubiorev.2016.04.008

[6] Lein PJ, Fryer AD, Higgins D. Cell culture: Autonomic and enteric neurons. In: Squire LR, editor. Encyclopedia of Neuroscience. Oxford, UK: Academic Press; 2009. pp. 625-632

[7] Purves D, Lichtman JW. Geometrical differences among homologous neurons in mammals. Science. 1985;**228**(4697):298-302

[8] Ivanov A, Purves D. Ongoing electrical activity of superior cervical ganglion cells in mammals of different size. The Journal of Comparative Neurology. 1989;**284**(3):398-404

[9] De Castro F, Sanchez-Vives MV, Munoz-Martinez EJ, Gallego R. Effects of postganglionic nerve section on synaptic transmission in the superior cervical ganglion of the guinea-pig. Neuroscience. 1995;**67**(3):689-695

[10] Peruzzi D, Hendley ED, Forehand CJ. Hypertrophy of stellate ganglion cells in hypertensive, but not hyperactive, rats. The American Journal of Physiology. 1991;**261**(4 Pt 2): R979-R984

[11] Kondo M, Terada M, Shimizu D, Fujiwara T, Tabei R. Morphometric study of the superior cervical and stellate ganglia of spontaneously hypertensive rats during the prehypertensive stage. Virchows Archiv. B, Cell Pathology Including Molecular Pathology. 1990;**58**(5):371-376

[12] Kishi T, Hirooka Y, Mukai Y, Shimokawa H, Takeshita A. Atorvastatin causes depressor and sympatho-inhibitory effects with upregulation of nitric oxide synthases in stroke-prone spontaneously hypertensive rats. Journal of Hypertension. 2003;**21**(2):379-386

[13] Kim WY, Gonsiorek EA, Barnhart C, Davare MA, Engebose AJ, Lauridsen H, Bruun D, Lesiak A, Wayman G, Bucelli R, Higgins D, Lein PJ. Statins decrease dendritic arborization in rat sympathetic neurons by blocking RhoA activation. Journal of Neurochemistry. 2009;**108**(4):1057-1071

[14] Andrews TJ, Li D, Halliwell J, Cowen T. The effect of age on dendrites in the rat superior cervical ganglion. Journal of Anatomy. 1994;**184**(Pt 1):111-117

[15] Snider WD. Rostrocaudal differences in dendritic growth and synaptogenesis in rat sympathetic chain ganglia. The Journal of Comparative Neurology. 1986;**244**(2):245-253. DOI: 10.1002/cne.902440210

[16] Voyvodic JT. Peripheral target regulation of dendritic geometry in the rat superior cervical ganglion. The Journal of Neuroscience. 1989;**9**(6):1997-2010

[17] Purves D, Hadley RD, Voyvodic JT. Dynamic changes in the dendritic geometry of individual neurons visualized over periods of up to three months in the superior cervical ganglion of living mice. The Journal of Neuroscience. 1986;**6**(4):1051-1060

[18] Andrews TJ, Thrasivoulou C, Nesbit W, Cowen T. Target-specific differences in the dendritic morphology and neuropeptide content of neurons in the rat SCG during development and aging. The Journal of Comparative Neurology. 1996;**368**(1):33-44

[19] Luebke JI, Wright LL. Characterization of superior cervical ganglion neurons that project to the submandibular glands, the eyes, and the pineal gland in rats. Brain Research. 1992;**589**(1):1-14

[20] Smolen AJ, Beaston-Wimmer P. Dendritic development in the rat superior cervical ganglion. Brain Research. 1986;**394**(2):245-252

[21] Voyvodic JT. Development and regulation of dendrites in the rat superior cervical ganglion. The Journal of Neuroscience. 1987;**7**(3):904-912

[22] Goedert M, Otten U, Thoenen H. Biochemical effects of antibodies against nerve growth factor on developing and differentiated sympathetic ganglia. Brain Research. 1978;**148**(1):264-268

[23] Purves D, Snider WD, Voyvodic JT. Trophic regulation of nerve cell morphology and innervation in the autonomic nervous system. Nature. 1988;**336**(6195):123-128

[24] Ruit KG, Osborne PA, Schmidt RE, Johnson EM Jr, Snider WD. Nerve growth factor regulates sympathetic ganglion cell morphology and survival in the adult mouse. The Journal of Neuroscience. 1990;**10**(7):2412-2419

[25] Ruit KG, Snider WD. Administration or deprivation of nerve growth factor during development permanently alters neuronal geometry. The Journal of Comparative Neurology. 1991;**314**(1):106-113

[26] Purves D, Nja A. Effect of nerve growth factor on synaptic depression after axotomy. Nature. 1976;**260**(5551):535-536

[27] Nja A, Purves D. The effects of nerve growth factor and its antiserum on synapses in the superior cervical ganglion of the guinea-pig. The Journal of Physiology. 1978;**277**:55-75

[28] Yawo H. Changes in the dendritic geometry of mouse superior cervical ganglion cells following postganglionic axotomy. The Journal of Neuroscience. 1987;**7**(11):3703-3711

[29] Lein P, Johnson M, Guo X, Rueger D, Higgins D. Osteogenic protein-1 induces dendritic growth in rat sympathetic neurons. Neuron. 1995;**15**(3):597-605

[30] Bruckenstein DA, Higgins D. Morphological differentiation of embryonic rat sympathetic neurons in tissue culture. I. Conditions under which neurons form axons but not dendrites. Developmental Biology. 1988;**128**(2):324-336

[31] Bruckenstein DA, Higgins D. Morphological differentiation of embryonic rat sympathetic neurons in tissue culture. II. Serum promotes dendritic growth. Developmental Biology. 1988;**128**(2):337-348. DOI: 10.1016/0012-1606(88)90296-5

[32] Tropea M, Johnson MI, Higgins D. Glial cells promote dendritic development in rat sympathetic neurons in vitro. Glia. 1988;**1**(6):380-392

[33] Beck HN, Drahushuk K, Jacoby DB, Higgins D, Lein PJ. Bone morphogenetic protein-5 (bmp-5) promotes dendritic growth in cultured sympathetic neurons. BMC Neuroscience. 2001;**2**:12

[34] Guo X, Rueger D, Higgins D. Osteogenic protein-1 and related bone morphogenetic proteins regulate dendritic growth and the expression of microtubule-associated protein-2 in rat sympathetic neurons. Neuroscience Letters. 1998;**245**(3):131-134

[35] Lein P, Guo X, Hedges AM, Rueger D, Johnson M, Higgins D. The effects of extracellular matrix and osteogenic protein-1 on the morphological differentiation of rat sympathetic neurons. International Journal of Developmental Neuroscience. 1996;**14**(3):203-215

[36] Lein PJ, Guo X, Shi GX, Moholt-Siebert M, Bruun D, Andres DA. The novel GTPase Rit differentially regulates axonal and dendritic growth. The Journal of Neuroscience. 2007;**27**(17):4725-4736. DOI: 10.1523/JNEUROSCI.5633-06.2007

[37] Lein PJ, Beck HN, Chandrasekaran V, Gallagher PJ, Chen HL, Lin Y, Guo X, Kaplan PL, Tiedge H, Higgins D. Glia induce dendritic growth in cultured sympathetic neurons by modulating the balance between bone morphogenetic proteins (BMPs) and BMP antagonists. The Journal of Neuroscience. 2002;**22**(23):10377-10387

[38] Lyons KM, Hogan BL, Robertson EJ. Colocalization of BMP 7 and BMP 2 RNAs suggests that these factors cooperatively mediate tissue interactions during murine development. Mechanisms of Development. 1995;**50**(1):71-83

[39] Vukicevic S, Latin V, Chen P, Batorsky R, Reddi AH, Sampath TK. Localization of osteogenic protein-1 (bone morphogenetic protein-7) during human embryonic development: High affinity binding to basement membranes. Biochemical and Biophysical Research Communications. 1994;**198**(2):693-700

[40] Wall NA, Blessing M, Wright CV, Hogan BL. Biosynthesis and in vivo localization of the decapentaplegic-Vg-related protein, DVR-6 (bone morphogenetic protein-6). The Journal of Cell Biology. 1993;**120**(2):493-502

[41] Katagiri T, Watabe T. Bone morphogenetic proteins. Cold Spring Harbor Perspectives in Biology. 2016;**8**(6). DOI: 10.1101/cshperspect.a021899

[42] Massague J, Wotton D. Transcriptional control by the TGF-beta/Smad signaling system. The EMBO Journal. 2000;**19**(8):1745-1754. DOI: 10.1093/emboj/19.8.1745

[43] Derynck R, Zhang YE. Smad-dependent and Smad-independent pathways in TGF-beta family signalling. Nature. 2003;**425**(6958):577-584

[44] Lee-Hoeflich ST, Causing CG, Podkowa M, Zhao X, Wrana JL, Attisano L. Activation of LIMK1 by binding to the BMP receptor, BMPRII, regulates BMP-dependent dendritogenesis. The EMBO Journal. 2004;**23**(24):4792-4801

[45] Xu X, Han J, Ito Y, Bringas P Jr, Deng C, Chai Y. Ectodermal Smad4 and p38 MAPK are functionally redundant in mediating TGF-beta/BMP signaling during tooth and palate development. Developmental Cell. 2008;**15**(2):322-329. DOI: 10.1016/j.devcel.2008.06.004

[46] Yu L, Hebert MC, Zhang YE. TGF-beta receptor-activated p38 MAP kinase mediates Smad-independent TGT-beta responses. The EMBO Journal. 2002;**21**(14):3749-3759. DOI: 10.1093/emboj/cdf366

[47] O'Keeffe GW, Gutierrez H, Howard L, Laurie CW, Osorio C, Gavalda N, Wyatt SL, Davies AM. Region-specific role of growth differentiation factor-5 in the establishment of sympathetic innervation. Neural Development. 2016;**11**:4. DOI: 10.1186/s13064-016-0060-3

[48] Zhang D, Mehler MF, Song Q, Kessler JA. Development of bone morphogenetic protein receptors in the nervous system and possible roles in regulating trkC expression. The Journal of Neuroscience. 1998;**18**(9):3314-3326

[49] Majdazari A, Stubbusch J, Muller CM, Hennchen M, Weber M, Deng CX, Mishina Y, Schutz G, Deller T, Rohrer H. Dendrite complexity of sympathetic neurons is controlled during postnatal development by BMP signaling. The Journal of Neuroscience. 2013;**33**(38):15132-15144. DOI: 10.1523/JNEUROSCI.4748-12.2013

[50] Ehrlich M. Endocytosis and trafficking of BMP receptors: Regulatory mechanisms for fine-tuning the signaling response in different cellular contexts. Cytokine & Growth Factor Reviews. 2016;**27**:35-42. DOI: 10.1016/j.cytogfr.2015.12.008

[51] Guo X, Lin Y, Horbinski C, Drahushuk KM, Kim IJ, Kaplan PL, Lein P, Wang T, Higgins D. Dendritic growth induced by BMP-7 requires Smad1 and proteasome activity. Journal of Neurobiology. 2001;**48**(2):120-130

[52] Garred MM, Wang MM, Guo X, Harrington CA, Lein PJ. Transcriptional responses of cultured rat sympathetic neurons during BMP-7-induced dendritic growth. PLoS One. 2011;**6**(7):e21754. DOI: 10.1371/journal.pone.0021754

[53] Ghazalpour A, Bennett B, Petyuk VA, Orozco L, Hagopian R, Mungrue IN, Farber CR, Sinsheimer J, Kang HM, Furlotte N, Park CC, Wen PZ, Brewer H, Weitz K, Camp DG 2nd, Pan C, Yordanova R, Neuhaus I, Tilford C, Siemers N, Gargalovic P, Eskin E, Kirchgessner T, Smith DJ, Smith RD, Lusis AJ. Comparative analysis of proteome and transcriptome variation in mouse. PLoS Genetics. 2011;**7**(6):e1001393. DOI: 10.1371/journal.pgen.1001393

[54] Nie L, Wu G, Culley DE, Scholten JC, Zhang W. Integrative analysis of transcriptomic and proteomic data: Challenges, solutions and applications. Critical Reviews in Biotechnology. 2007;**27**(2):63-75. DOI: 10.1080/07388550701334212

[55] Dechant G, Barde YA. The neurotrophin receptor p75(NTR): Novel functions and implications for diseases of the nervous system. Nature Neuroscience. 2002;**5**(11):1131-1136. DOI: 10.1038/nn1102-1131

[56] Horton A, Laramee G, Wyatt S, Shih A, Winslow J, Davies AM. NGF binding to p75 enhances the sensitivity of sensory and sympathetic neurons to NGF at different stages of development. Molecular and Cellular Neurosciences. 1997;**10**(3-4):162-172. DOI: 10.1006/mcne.1997.0650

[57] Kenchappa RS, Zampieri N, Chao MV, Barker PA, Teng HK, Hempstead BL, Carter BD. Ligand-dependent cleavage of the P75 neurotrophin receptor is necessary for NRIF nuclear translocation and apoptosis in sympathetic neurons. Neuron. 2006;**50**(2):219-232. DOI: 10.1016/j.neuron.2006.03.011

[58] Roux PP, Barker PA. Neurotrophin signaling through the p75 neurotrophin receptor. Progress in Neurobiology. 2002;**67**(3):203-233

[59] Salama-Cohen P, Arevalo MA, Meier J, Grantyn R, Rodriguez-Tebar A. NGF controls dendrite development in hippocampal neurons by binding to p75NTR and modulating the cellular targets of Notch. Molecular Biology of the Cell. 2005;**16**(1):339-347. DOI: 10.1091/mbc.E04-05-0438

[60] Shu YH, Lu XM, Wei JX, Xiao L, Wang YT. Update on the role of $p75^{NTR}$ in neurological disorders: A novel therapeutic target. Biomedicine & Pharmacotherapy. 2015;**76**:17-23. DOI: 10.1016/j.biopha.2015.10.010

[61] Underwood CK, Coulson EJ. The p75 neurotrophin receptor. The International Journal of Biochemistry & Cell Biology. 2008;**40**(9):1664-1668. DOI: 10.1016/j.biocel.2007.06.010

[62] Bengoechea TG, Chen Z, O'Leary DA, Masliah E, Lee KF. P75 reduces beta-amyloid-induced sympathetic innervation deficits in an Alzheimer's disease mouse model. Proceedings of the National Academy of Sciences of the United States of America. 2009;**106**(19):7870-7875. DOI: 10.1073/pnas.0901533106

[63] Wang KC, Kim JA, Sivasankaran R, Segal R, He Z. P75 interacts with the Nogo receptor as a co-receptor for Nogo, MAG and OMgp. Nature. 2002;**420**(6911):74-78. DOI: 10.1038/nature01176

[64] Courter LA, Shaffo FC, Ghogha A, Parrish DJ, Lorentz CU, Habecker BA, Lein PJ. BMP7-induced dendritic growth in sympathetic neurons requires p75(NTR) signaling. Developmental Neurobiology. 2016;**76**(9):1003-1013. DOI: 10.1002/dneu.22371

[65] Roux PP, Bhakar AL, Kennedy TE, Barker PA. The p75 neurotrophin receptor activates Akt (protein kinase B) through a phosphatidylinositol 3-kinase-dependent pathway. The Journal of Biological Chemistry. 2001;**276**(25):23097-23104. DOI: 10.1074/jbc.M011520200

[66] Sharma N, Deppmann CD, Harrington AW, St Hillaire C, Chen ZY, Lee FS, Ginty DD. Long-distance control of synapse assembly by target-derived NGF. Neuron. 2010;**67**(3):422-434. DOI: 10.1016/j.neuron.2010.07.018

[67] 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**(6977):843-848. DOI: 10.1038/nature02319

[68] Redmond L, Ghosh A. The role of Notch and Rho GTPase signaling in the control of dendritic development. Current Opinion in Neurobiology. 2001;**11**(1):111-117

[69] Van Aelst L, Cline HT. Rho GTPases and activity-dependent dendrite development. Current Opinion in Neurobiology. 2004;**14**(3):297-304

[70] Yamashita T, Tucker KL, Barde YA. Neurotrophin binding to the p75 receptor modulates rho activity and axonal outgrowth. Neuron. 1999;**24**(3):585-593

[71] Hamilton AM, Zito K. Breaking it down: The ubiquitin proteasome system in neuronal morphogenesis. Neural Plasticity. 2013;**2013**:196848. DOI: 10.1155/2013/196848

[72] Oddo S. The ubiquitin-proteasome system in Alzheimer's disease. Journal of Cellular and Molecular Medicine. 2008;**12**(2):363-373. DOI: 10.1111/j.1582-4934.2008.00276.x

[73] Sun L, Trausch-Azar JS, Ciechanover A, Schwartz AL. Ubiquitin-proteasome-mediated degradation, intracellular localization, and protein synthesis of Myod and Id1 during muscle differentiation. The Journal of Biological Chemistry. 2005;**280**(28):26448-26456. DOI: 10.1074/jbc.M500373200

[74] Kawabe H, Neeb A, Dimova K, Young SM Jr, Takeda M, Katsurabayashi S, Mitkovski M, Malakhova OA, Zhang DE, Umikawa M, Kariya K, Goebbels S, Nave KA, Rosenmund C, Jahn O, Rhee J, Brose N. Regulation of Rap2A by the ubiquitin ligase Nedd4-1 controls neurite development. Neuron. 2010;**65**(3):358-372. DOI: 10.1016/j.neuron.2010.01.007

[75] Kim AH, Puram SV, Bilimoria PM, Ikeuchi Y, Keough S, Wong M, Rowitch D, Bonni A. A centrosomal Cdc20-APC pathway controls dendrite morphogenesis in postmitotic neurons. Cell. 2009;**136**(2):322-336. DOI: 10.1016/j.cell.2008.11.050

[76] Litterman N, Ikeuchi Y, Gallardo G, O'Connell BC, Sowa ME, Gygi SP, Harper JW, Bonni A. An OBSL1-Cul7Fbxw8 ubiquitin ligase signaling mechanism regulates Golgi morphology and dendrite patterning. PLoS Biology. 2011;**9**(5):e1001060. DOI: 10.1371/journal.pbio.1001060

[77] Izzi L, Attisano L. Regulation of the TGFbeta signalling pathway by ubiquitin-mediated degradation. Oncogene. 2004;**23**(11):2071-2078. DOI: 10.1038/sj.onc.1207412

[78] Zhu H, Kavsak P, Abdollah S, Wrana JL, Thomsen GH. A smad ubiquitin ligase targets the BMP pathway and affects embryonic pattern formation. Nature. 1999;**400**(6745):687-693. DOI: 10.1038/23293

[79] Brennan AM, Suh SW, Won SJ, Narasimhan P, Kauppinen TM, Lee H, Edling Y, Chan PH, Swanson RA. NADPH oxidase is the primary source of superoxide induced by NMDA receptor activation. Nature Neuroscience. 2009;**12**(7):857-863. DOI: 10.1038/nn.2334

[80] Kennedy KA, Sandiford SD, Skerjanc IS, Li SS. Reactive oxygen species and the neuronal fate. Cellular and Molecular Life Sciences. 2012;**69**(2):215-221. DOI: 10.1007/s00018-011-0807-2

[81] Kowaltowski AJ, de Souza-Pinto NC, Castilho RF, Vercesi AE. Mitochondria and reactive oxygen species. Free Radical Biology & Medicine. 2009;**47**(4):333-343. DOI: 10.1016/j.freeradbiomed.2009.05.004

[82] Colavitti R, Pani G, Bedogni B, Anzevino R, Borrello S, Waltenberger J, Galeotti T. Reactive oxygen species as downstream mediators of angiogenic signaling by vascular endothelial growth factor receptor-2/KDR. The Journal of Biological Chemistry. 2002;**277**(5):3101-3108. DOI: 10.1074/jbc.M107711200

[83] Munnamalai V, Suter DM. Reactive oxygen species regulate F-actin dynamics in neuronal growth cones and neurite outgrowth. Journal of Neurochemistry. 2009;**108**(3):644-661. DOI: 10.1111/j.1471-4159.2008.05787.x

[84] Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. The International Journal of Biochemistry & Cell Biology. 2007;**39**(1):44-84. DOI: 10.1016/j.biocel.2006.07.001

[85] Massaad CA, Klann E. Reactive oxygen species in the regulation of synaptic plasticity and memory. Antioxidants & Redox Signaling. 2011;**14**(10):2013-2054. DOI: 10.1089/ars.2010.3208

[86] Cao X, Demel SL, Quinn MT, Galligan JJ, Kreulen D. Localization of NADPH oxidase in sympathetic and sensory ganglion neurons and perivascular nerve fibers. Autonomic Neuroscience. 2009;**151**(2):90-97. DOI: 10.1016/j.autneu.2009.07.010

[87] Chandrasekaran V, Lea C, Sosa JC, Higgins D, Lein PJ. Reactive oxygen species are involved in bmp-induced dendritic growth in cultured rat sympathetic neurons. Molecular and Cellular Neurosciences. 2015;**67**:116-125. DOI: 10.1016/j.mcn.2015.06.007

[88] O'Shea JJ, Gadina M, Kanno Y. Cytokine signaling: Birth of a pathway. Journal of Immunology. 2011;**187**(11):5475-5478. DOI: 10.4049/jimmunol.1102913

[89] Iwamoto T, Oshima K, Seng T, Feng X, Oo ML, Hamaguchi M, Matsuda S. STAT and SMAD signaling in cancer. Histology and Histopathology. 2002;**17**(3):887-895. DOI: 10.14670/HH-17.887

[90] Tang LY, Heller M, Meng Z, Yu LR, Tang Y, Zhou M, Zhang YE. Transforming growth factor-beta (TGF-beta) directly activates the JAK1-STAT3 axis to induce hepatic fibrosis in coordination with the SMAD pathway. The Journal of Biological Chemistry. 2017;**292**(10):4302-4312. DOI: 10.1074/jbc.M116.773085

[91] Ulloa L, Doody J, Massague J. Inhibition of transforming growth factor-beta/SMAD signalling by the interferon-gamma/STAT pathway. Nature. 1999;**397**(6721):710-713. DOI: 10.1038/17826

[92] Guo X, Chandrasekaran V, Lein P, Kaplan PL, Higgins D. Leukemia inhibitory factor and ciliary neurotrophic factor cause dendritic retraction in cultured rat sympathetic neurons. The Journal of Neuroscience. 1999;**19**(6):2113-2121

[93] Guo X, Metzler-Northrup J, Lein P, Rueger D, Higgins D. Leukemia inhibitory factor and ciliary neurotrophic factor regulate dendritic growth in cultures of rat sympathetic neurons. Brain Research. Developmental Brain Research. 1997;**104**(1-2):101-110

[94] Kim IJ, Beck HN, Lein PJ, Higgins D. Interferon gamma induces retrograde dendritic retraction and inhibits synapse formation. The Journal of Neuroscience. 2002;**22**(11):4530-4539

[95] Kim EK, Choi EJ. Pathological roles of MAPK signaling pathways in human diseases. Biochimica et Biophysica Acta. 2010;**1802**(4):396-405. DOI: 10.1016/j.bbadis.2009.12.009

[96] Plotnikov A, Zehorai E, Procaccia S, Seger R. The MAPK cascades: Signaling components, nuclear roles and mechanisms of nuclear translocation. Biochimica et Biophysica Acta. 2011;**1813**(9):1619-1633. DOI: 10.1016/j.bbamcr.2010.12.012

[97] Guo X, Wang XF. Signaling cross-talk between TGF-beta/BMP and other pathways. Cell Research. 2009;**19**(1):71-88. DOI: 10.1038/cr.2008.302

[98] Massague J. Integration of Smad and MAPK pathways: A link and a linker revisited. Genes & Development. 2003;**17**(24):2993-2997

[99] Pera EM, Ikeda A, Eivers E, De Robertis EM. Integration of IGF, FGF, and anti-BMP signals via Smad1 phosphorylation in neural induction. Genes & Development. 2003;**17**(24):3023-3028. DOI: 10.1101/gad.1153603

[100] Kim IJ, Drahushuk K, Kim W, Lein PJ, Andres DA, Higgins D. Extracellular signal-regulated kinases regulate dendritic growth in rat sympathetic neurons. The Journal of Neuroscience. 2004;**20**(8):2013-2037

[101] Stachowiak MK, Fang X, Myers JM, Dunham SM, Berezney R, Maher PA, Stachowiak EK. Integrative nuclear FGFR1 signaling (INFS) as a part of a universal "feed-forward-and-gate" signaling module that controls cell growth and differentiation. Journal of Cellular Biochemistry. 2003;**90**(4):662-691. DOI: 10.1002/jcb.10606

[102] Klimaschewski L, Meisinger C, Grothe C. Localization and regulation of basic fibroblast growth factor (FGF-2) and FGF receptor-1 in rat superior cervical ganglion after axotomy. Journal of Neurobiology. 1999;**38**(4):499-506

[103] Horbinski C, Stachowiak EK, Chandrasekaran V, Miuzukoshi E, Higgins D, Stachowiak MK. Bone morphogenetic protein-7 stimulates initial dendritic growth in sympathetic neurons through an intracellular fibroblast growth factor signaling pathway. Journal of Neurochemistry. 2002;**80**(1):54-63

[104] Andres DA, Shi GX, Bruun D, Barnhart C, Lein PJ. Rit signaling contributes to interferon-gamma-induced dendritic retraction via p38 mitogen-activated protein kinase activation. Journal of Neurochemistry. 2008;**107**(5):1436-1447. DOI: 10.1111/j.1471-4159.2008.05708.x

[105] Altmann CR, Brivanlou AH. Neural patterning in the vertebrate embryo. International Review of Cytology. 2001;**203**:447-482

[106] Chandrasekaran V, Zhai Y, Wagner M, Kaplan PL, Napoli JL, Higgins D. Retinoic acid regulates the morphological development of sympathetic neurons. Journal of Neurobiology. 2000;**42**(4):383-393

[107] Melton KR, Iulianella A, Trainor PA. Gene expression and regulation of hindbrain and spinal cord development. Frontiers in Bioscience. 2004;**9**:117-138

[108] Wilson L, Maden M. The mechanisms of dorsoventral patterning in the vertebrate neural tube. Developmental Biology. 2005;**282**(1):1-13. DOI: 10.1016/j.ydbio.2005.02.027

[109] Ghzili H, Grumolato L, Thouennon E, Tanguy Y, Turquier V, Vaudry H, Anouar Y. Role of PACAP in the physiology and pathology of the sympathoadrenal system. Frontiers in Neuroendocrinology. 2008;**29**(1):128-141. DOI: 10.1016/j.yfrne.2007.10.001

[110] Drahushuk K, Connell TD, Higgins D. Pituitary adenylate cyclase-activating polypeptide and vasoactive intestinal peptide inhibit dendritic growth in cultured sympathetic neurons. The Journal of Neuroscience. 2002;**22**(15):6560-6569

[111] Quach DH, Oliveira-Fernandes M, Gruner KA, Tourtellotte WG. A sympathetic neuron autonomous role for Egr3-mediated gene regulation in dendrite morphogenesis and target tissue innervation. The Journal of Neuroscience. 2013;**33**(10):4570-4583. DOI: 10.1523/JNEUROSCI.5481-12.2013

[112] Eldredge LC, Gao XM, Quach DH, Li L, Han X, Lomasney J, Tourtellotte WG. Abnormal sympathetic nervous system development and physiological dysautonomia in Egr3-deficient mice. Development. 2008;**135**(17):2949-2957. DOI: 10.1242/dev.023960

[113] Vaillant AR, Zanassi P, Walsh GS, Aumont A, Alonso A, Miller FD. Signaling mechanisms underlying reversible, activity-dependent dendrite formation. Neuron. 2002;**34**(6):985-998

[114] Naska S, Park KJ, Hannigan GE, Dedhar S, Miller FD, Kaplan DR. An essential role for the integrin-linked kinase-glycogen synthase kinase-3 beta pathway during dendrite initiation and growth. The Journal of Neuroscience. 2006;**26**(51):13344-13356. DOI: 10.1523/JNEUROSCI.4462-06.2006

[115] Dedhar S, Williams B, Hannigan G. Integrin-linked kinase (ILK): A regulator of integrin and growth-factor signalling. Trends in Cell Biology. 1999;**9**(8):319-323

[116] Arevalo JC, Chao MV. Axonal growth: Where neurotrophins meet Wnts. Current Opinion in Cell Biology. 2005;**17**(2):112-115. DOI: 10.1016/j.ceb.2005.01.004

Chapter 6

A5 and A6 Noradrenergic Cell Groups: Implications for Cardiorespiratory Control

Manuel Víctor López-González,
Marta González-García and
Marc Stefan Dawid-Milner

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.79389

Abstract

Central pontine A5 and A6 noradrenergic cell groups are two of the main sources of noradrenaline release at the spinal cord, at the level of the superficial dorsal horn, the motoneuron pools of the ventral horn, lamina X and the thoracic and sacral intermediolateral cell columns. Noradrenergic ascending or descending pathways originating in the A5 or A6 noradrenergic cell groups are highly sensitive to stress and to other high-arousal states. These noradrenergic groups present extensive projections that play a key role in the modulation of all antinociceptive and autonomic responses elicited by painful or threatening situations. Depending on the locations of these projections, different possible roles for each noradrenergic cell groups are suggested. The A6 noradrenergic cell group might have the greatest effect on somatosensory transmission and the A5 group on sympathetic function. Consistent with this, stimulation of central noradrenergic pathways evokes an array of stresslike and antinociceptive effects, including changes in blood pressure, heart rate and respiratory rate. In addition, it also produces an increase in excitability, which leads to a high degree of arousal and a potentiation of cortical and subcortical mechanism generating the necessary cognitive, behavioral and autonomic responses to confront these physical or psychological situations.

Keywords: pontine noradrenergic cell groups, A5 region, locus coeruleus, cardiovascular control, analgesia

1. Introduction

Noradrenergic (NA) central pathways located at the level of the brainstem were initially described by Dahlström and Fuxe in 1964 and contain several clusters or groups of neurons

classified from A1 to A7. These clusters extend rostrocaudally from the lateral pons to the caudal ventrolateral medulla. Afferent and efferent connections are sent and come from very different locations along the central nervous system (CNS) and are implicated in physiological and behavioral functions associated with a wide cascade of processes, such as homeostasis, arousal, memory, learning, autonomic and behavioral responses to stress and pain, among others [1, 2].

NA neurons are characterized by the presence, within the synaptic terminal, of the cytoplasmatic enzymatic machinery, which is necessary to biosynthesize noradrenaline from the amino acid tyrosine through a precise and sequential enzymatic reaction. Tyrosine hydroxylase (TH) is the limiting enzyme. It transforms tyrosine into dihydroxyphenylalanine (L-DOPA), which is converted into dopamine by L-DOPA decarboxylase. Finally, dopamine is used as a substrate by dopamine-β-hydroxylase (DBH), which transforms dopamine into noradrenaline [3]. DBH immunodetection is specific for NA neurons and NA central demand [4]. Although once the noradrenaline is a precursor to adrenaline synthesis, the immunodetection of DBH is not restricted to noradrenergic neurons except in the cases where the referred group is isolated from adrenergic neurons (as A6 and A5). After its release into the synaptic cleft, noradrenaline can bind to the pre- or post-synaptic adrenergic receptors and activates intracellular signaling cascades depending on the specific function of the subtype of the adrenergic receptor activated (facilitatory or inhibitory receptors).

Briefly, in terms of the precise location of the different NA cell groups: the A1 NA cell group is found in the ventrolateral medulla; the A2, located close to the dorsal vagal complex, has an intimate relationship (as part of) with caudal NTS complex, starting in very caudal level of medulla until the open of fourth ventricle; A3 neurons are included within the medullary reticular formation, and neurons of the A4 cell group are situated in the surroundings of the fourth ventricle. The precise location of the most studied NA cell groups, the A5, A6 and A7, is the following: the A5 NA cell group is located in the ventrolateral pons; A6, which represents the locus coeruleus, is located in the lateral floor of the fourth ventricle and, finally, A7 is found in the lateral part of the pons. These last three groups of NA neurons represent the most important NA clusters with projections to the spinal cord [5, 6].

Early studies using retrograde transport of horseradish peroxidase combined with immunostaining for DBH or retrograde transport of anti-DBH antibodies demonstrated that the NA endings of the spinal cord arise from the A5, A6 and A7 cell groups in the pons [7]. The projections from the neurons located in the A5, A6 and A7 cell groups are found throughout the spinal cord, but the highest density of synaptic contacts is established at the level of the superficial dorsal horn, the motoneuron pools of the ventral horn, lamina X and the thoracic and sacral intermediolateral cell columns (IML) [5].

In this chapter, the main focus is centered on the main pontine NA cell groups, which project to the spinal cord (A5 and A6), and their implications for cardiorespiratory control.

2. Spinal projections

The A5 and A6 pontine NA clusters of neurons project widely across the spinal cord [5]. These projections reach the dorsal and ventral horns (laminae I-VII) and the IML of the spinal cord

at thoracic levels. These descending projections of the NA cell groups are crucial in explaining their functional implications in central cardiorespiratory control and in other important autonomic functions involved in behavioral responses to stress or pain.

2.1. Spinal projections from the A6 (locus coeruleus)

The projections from A6 cells use two main pathways: through the spinal cord in the ventral funiculi and through the dorsal surface of the dorsal horn. The A6 NA cell group supplies the highest concentration of synaptic endings at all levels. It includes all regions of the spinal gray matter, but it is especially dense at the level of the dorsal horn, although it has a small number of axons to the ventral horn and IML [5]. Extensive literature for this exists, not only at an anatomical level [6–12] but also with electrophysiological evidence [13, 14]. Intra and extracellular neuronal recording studies provide the assignment to caudal A6 NA neurons with a role in regulating the excitability of the cell bodies of somatic alpha motoneurons located within the ventral horn of the spinal cord.

2.2. Spinal projections from the A5

It is well established that the spinally projecting axons of the A5 NA group mainly travel through the spinal cord within the lateral funiculi to end at the level of the IML cell column

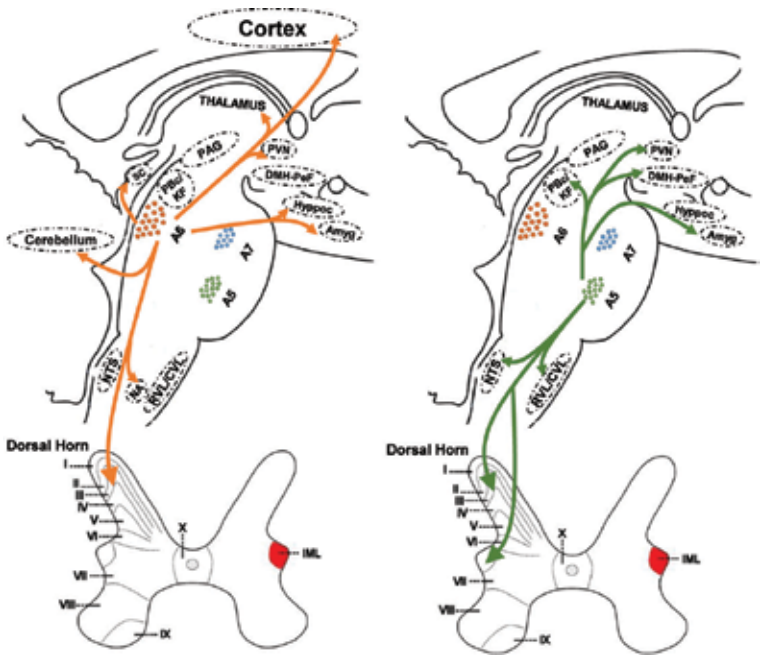

Figure 1. Schematic diagram of a sagittal section of human brain in which the main pontine noradrenergic nuclei (A5 and A6) and their main efferent connections are positioned. (A5) A5 noradrenergic cell group. (A6) A6 noradrenergic cell group, Locus Coeruleus. (Amyg) Amygdala. (CVL) Caudal ventrolateral medulla. (DMH-PeF) Dorsomedial Hypothalamic nucleus and perifornical area. (Hyppoc) Hyppocampus. (IML) Intermedio lateral cell collum of the spinal cord. (KF) Kölliker-Fuse nucleus. (LH) Lateral Hypothalamus. (NTS) Nucleus Tractus Solitarii. (PAG) Periaqueductal gray. (PBc) Parabrachial complex. (PVN) Paraventricular nucleus. (RVL) Rostral ventrolateral medulla.

of the thoracic spinal cord segments [5, 15–17]. There are also projections to the dorsal horn of the spinal cord (laminae IV–VII) [5, 16], where a high density of nociceptive neurons can be observed [18]. The A5 NA cell group contributes only with sparse projections to the dorsal and ventral horns at cervical and lumbosacral levels, but it supplies the thoracic IML with the densest projections, particularly to sympathetic preganglionic neurons [5].

In summary, the projections of A5 and A6 NA cell groups to the spinal cord are distributed in a complementary and topographic way. This suggests a different possible role for each of these cell groups, which depend on the precise location of their projections. Therefore, the A6 NA cell group might have its main effect on somatosensory transmission, and the A5 group on sympathetic autonomic function (**Figure 1**).

3. Functional pathways related to central and spinal projections

Although the previously described spinal projections are enough to explain the roles of each NA cell group, the efferent connections that these nuclei send to other areas of the CNS involved in autonomic control are what reinforce their role in autonomic control and homeostasis.

3.1. A6

The pontine A6 NA cell group, also called "locus coeruleus," is the most exhaustively studied NA nucleus in the brain. This NA region, which projects mainly to the dorsal horn of the spinal cord, has been linked with antinociception or modulation of pain together with the A7 NA cell group in Harlan Sprague-Dawley and Wistar rats [2, 19–23].

Neurons of the A6 region, as other catecholaminergic nuclei, are known to be immunoreactive for TH and DBH, the two enzymes critically involved in noradrenaline biosynthesis. A6 NA neurons also express a wide selection of neuropeptides including neuropeptide Y, somatostatin and cholecystokinin [24]. Most of the A6 NA neurons have different neurochemical characteristics and morphologies, presenting predominantly a medium size with fusiform and polar morphology, and three or four long thin dendrites [25].

A6 NA neurons send axons with extensive bifurcations, which travel long distances and establish connections even with cortical domains [26]. In addition, neurons located in the rostral part of the A6 NA region have widely branched axons that innervate forebrain areas, providing the main source of noradrenalin to the neocortex, hippocampus, amygdala, thalamus and cerebellum [27, 28]. Specifically, at the level of the hypothalamus, the A6 region makes contact with the paraventricular and supraoptic nuclei [29]. Other projections from the A6 NA neurons target the superior colliculus [30]. An activation of all these superior structures enhances arousal, vigilance and attention to sensory stimuli [31]. It has been reported that electrical stimulation of the A6 region also elicits a pressor response [32]. Furthermore, pharmacological inhibitions or activations of the activity of the A6 NA neurons also evoke changes in blood pressure [33].

With regard to these multiple ascending pathways, it is known that the A6 NA region has a critical role in stress responses, autonomic function, emotional memory, attention and the control modulation of motor and sensory functions. Furthermore, it has been shown that noradrenalin exerts potent neuromodulatory actions, reducing neuronal baseline activity and increasing the responsiveness of target cells to novel synaptic stimuli. Within the neocortex, hippocampus, amygdala and cerebellum, noradrenaline also facilitates synaptic plasticity, including long-term potentiation [34–36].

Tracing and immunocytochemical studies clearly describe all the descending projections from the A6 NA neurons to the brainstem and spinal cord [37]. These studies show the differences between the projections that originate from the subcoeruleus and coeruleus regions. However, the A6 NA neurons primarily project to the parasympathetic neurons of the dorsal motor nucleus of the vagus, nucleus ambiguus and sacral spinal cord, and subcoeruleus neurons send their projections to sympathetic preganglionic neurons and somatic cranial nerve nuclei. Both pathways have widespread projections to the brainstem reticular formation and dorsal horn of the spinal cord [38], and to the region surrounding the central canal and the ventral horn [37, 38].

Finally, A6 NA neurons also play a major role in behavioral and autonomic responses to stress [39]. A6 NA cells orexin 1 receptors are activated by stress-related orexin axons projecting from neuronal cell bodies located in the perifornical hypothalamus [40]. Furthermore, A6 noradrenergic neurons also modulate the interaction between the amygdala and hippocampus, thus promoting emotional memory [41], which involves an activation of β receptors within the basolateral amygdala [39]. In a recent report [42], it has been shown that A6 noradrenergic neurons participate in the tachycardia evoked during autonomic responses to stress and also are recognized as central chemoreceptors [43, 44].

3.2. A5

Multiple reports demonstrate that A5 neurons provide the major component of NA input to sympathetic preganglionic neurons of the IML of the spinal cord. Once there, they branch and establish buttons along the cell bodies and proximal dendrites of cholinergic preganglionic neurons, thus sustaining the earlier anatomical [5, 17] and physiological studies [45–50], which indicate a role for the A5 region in regulating sympathetic function.

The A5 region contains NA and non-NA neurons. The non-NA cells are mainly located at the level of the most caudal part of the A5 region [51]. These neurons seem to have similar properties to respiratory chemoreceptors cells previously identified in the rostral medulla oblongata [52]. By employing immunocytochemical and in situ hybridization techniques, neurons of the A5 region are shown to express ionotropic and metabotropic glutamate receptors. Ionotropic NMDA receptors show NR1-NR2D subunits [53], while the non-NMDA types are both AMPA and kainate [54]. The A5 metabotropic receptors observed within the A5 region are mGluR I, II and III [55].

Focusing on the descending connections from the A5 region, there is a dense connectivity with several medullary nuclei. These include the nucleus tractus solitarius (NTS), caudal ventrolateral medulla (CVLM), rostral ventrolateral medulla (RVLM), the caudal pressor area and the retrotrapezoid nucleus. There is also significant ascending connectivity, showing reciprocal

projections with the Kölliker-Fuse, medial and lateral parabrachial nuclei in the pons, the perifornical area and the paraventricular nucleus in the hypothalamus and with the amygdala [15, 56–60]. The location and connectivity of A5 region cells, the so-called ventrolateral pons, with an entire network of ascending and descending connections with other regions of the CNS involved in cardiorespiratory regulation, supports the idea that these neurons are the perfect candidates to drive and modulate the control of both sympathetic activity and cardiorespiratory function (**Figure 1**) [15, 45, 56, 58, 59, 61–63].

We have studied the functional relations between this sympathetic NA region and other hypothalamic, pontine and medullary regions involved in cardiorespiratory control. We first demonstrated that the stimulation of A5 NA cell bodies with glutamate mainly produces an increase in both blood pressure and heart rate [47] (**Figure 2**). It is known that the simultaneous increase of sympathetic vasomotor activity, arterial blood pressure and heart rate implies a reset of the baroreceptor reflex but without attenuation in the sensitivity of the reflex [64]. Furthermore, A5 neurons are activated during baroreceptor unloading [45] and carotid chemoreceptors stimulation [65]. Thus, it has been proposed that A5 neurons may play an important role in the carotid sympathetic chemoreflex triggered by hypoxia [66–68].

However, not only do A5 NA neurons have a cardiovascular role, but they also play an important role in respiratory control, modulating the activity of respiratory neurons [69]. A5 neurons are also synaptically connected to phrenic motoneurons [70] and contribute to the

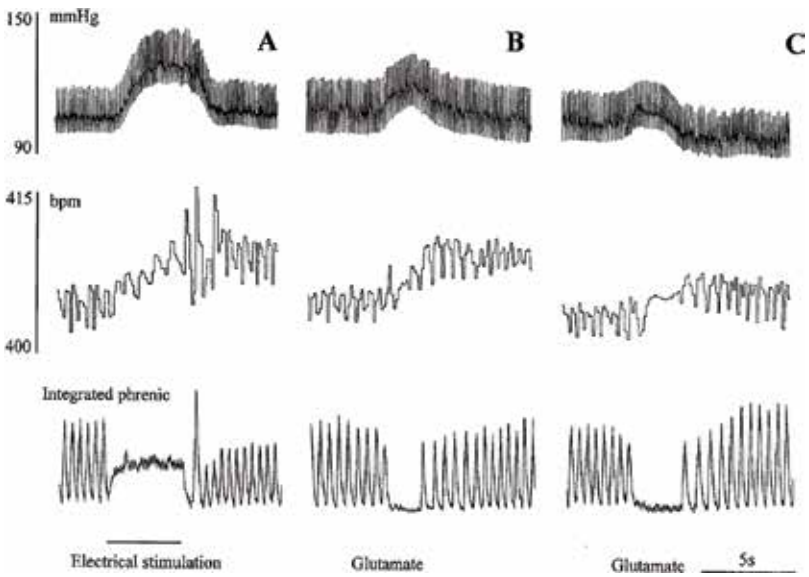

Figure 2. (A), (B), (C). Cardiorespiratory responses to A5 region stimulation in spontaneously breathing animals. Blood pressure (upper traces), heart rate (middle traces) and integrated phrenic activity (lower traces) during (A) electrical stimulation (10 μA, 0.4 ms, 50 Hz for 5 s) and (B) glutamate injection (1.5 nmol, 15 nl, over 5 s) in the same animal showing a decrease in respiratory rate with an increase in blood pressure and heart rate. (C) The response of another animal to glutamate injection (2.5 nmol, 25 nl, over 5 s), in which the respiratory response is similar to (B), but the cardiovascular response is bi-phasic and the increase in heart rate smaller.

http://dx.doi.org/10.5772/intechopen.79389

respiratory responses evoked by hypoxia and hypercapnia [66, 68, 71]. We have also demonstrated that the A5 region and medial Parabrachial and Kölliker Fuse nuclei have a role in modifying the activity of laryngeal motoneurons localized in the nucleus ambiguus, producing laryngeal constriction and increasing subglottic pressure (**Figure 3**) [50]. Finally, A5 NA neurons also participate in the cardiorespiratory response elicited by the activation of the parabrachial complex (**Figure 4**) [46], which is a critical component of the brainstem respiratory network required for eupnoea [72].

Similarly to A6 NA neurons, the A5 region is also involved in the control of stress-related responses. The terms "defense region" or "defense response" have been classically used in the

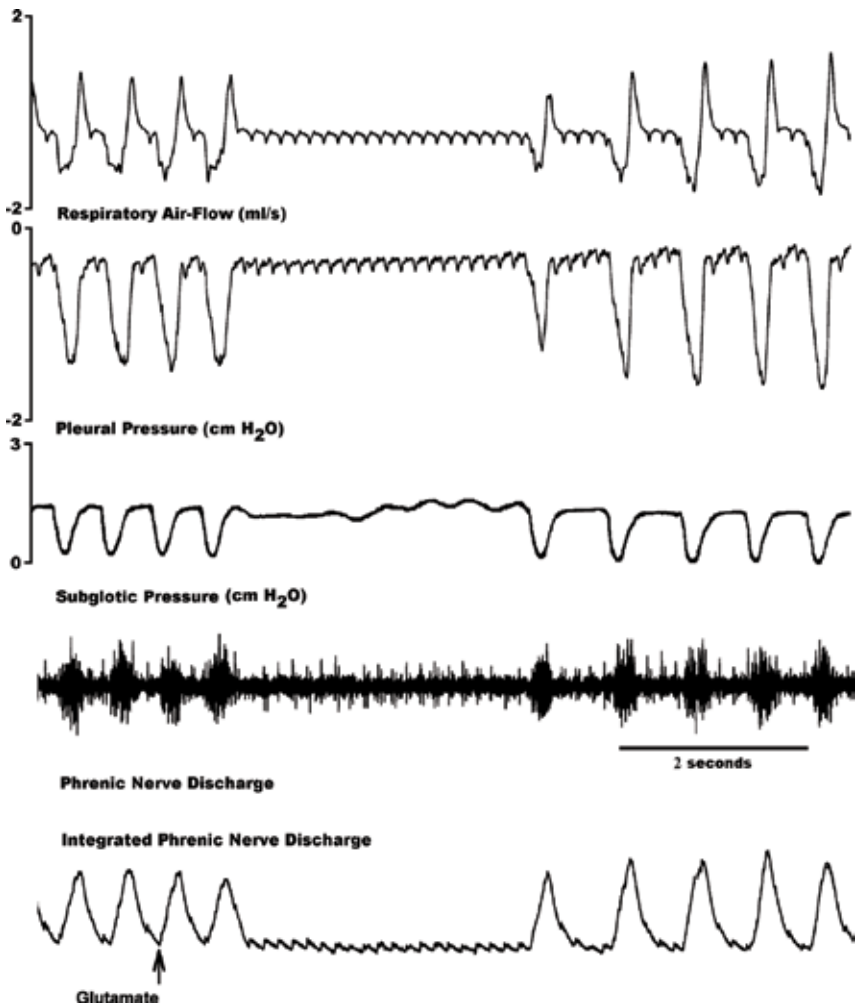

Figure 3. Laryngeal and respiratory responses to glutamate microinjection in the A5 region. Respiratory airflow, pleural pressure, subglottic pressure, phrenic nerve discharge and integrated phrenic nerve discharge, showing a expiratory facilitatory response with increase of subglottic pressure during a glutamate injection (10 nl over 5 s) in the A5 region. The arrows shows the onset of injection.

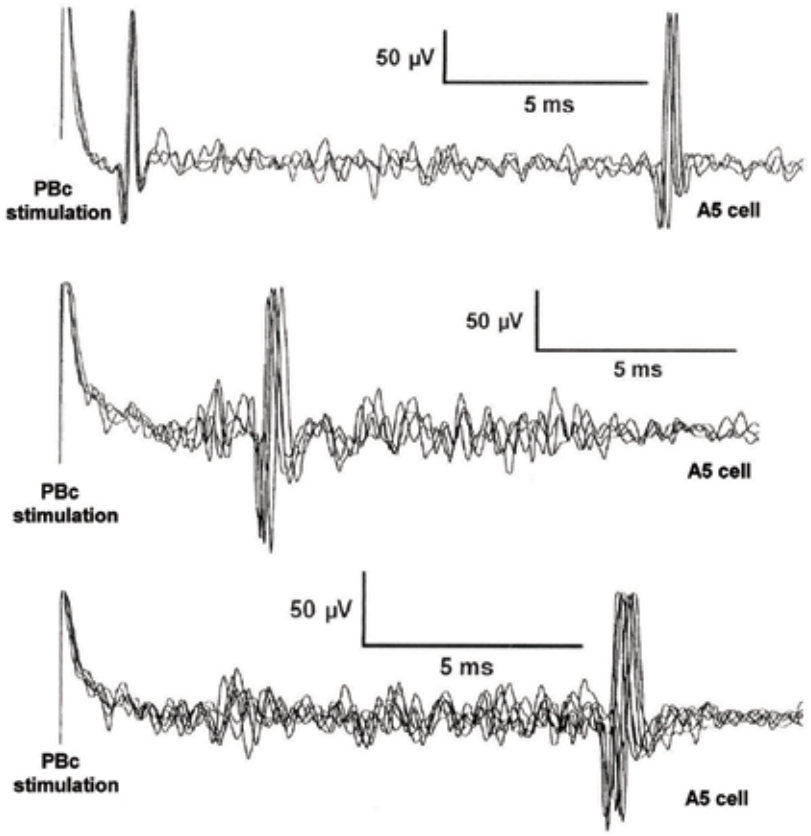

Figure 4. Extracellular recordings of three cells (superimposed sweeps) from the A5 region showing electrophysiological relations between the Parabrachial complex and the A5 region.

literature to describe the areas of the CNS from which we can evoke a pattern of autonomic and behavioral changes that are typically observed when an animal is confronted with threatening stimuli from different types of stressors [73–75]. The complexity of defensive behavior requests different interconnected regions, which plays specific roles according to the origin of the stressor agent or source of fear. It has been reported that there are two important regions from which this "defense response" can be elicited: the dorsomedial hypothalamic and perifornical area (DMH-PeF) in the hypothalamus, and the dorsolateral periaqueductal gray (dlPAG) in the midbrain [76]. The DMH-PeF and the dlPAG are part of an extensive network that coordinates defensive behavior.

The defense response is characterized by hypertension, tachycardia and tachypnea. As previously described, the simultaneous increase of arterial blood pressure, heart rate and sympathetic vasomotor activity implies that the baroreceptor reflex is reset to higher levels of arterial pressure, but without attenuation in the sensitivity of the reflex. A potentiation of the chemoreceptor reflex is known to be involved in this effect [77], as well as an activation of GABAergic mechanisms at the level of the NTS [78, 79].

With electrophysiological and neuropharmacological techniques, we have demonstrated the functional and anatomical interrelations between the Parabrachial complex and the A5 NA region in modulating the cardiorespiratory response evoked from DMH-PeF [80, 81]

http://dx.doi.org/10.5772/intechopen.79389

(**Figures 5** and **6**) and that glutamate is a possible neurotransmitter candidate involved in these interactions [81, 82]. In unpublished observations, we have obtained similar results with the interactions between the dlPAG and the A5 region [83].

We have also shown that the tachycardia evoked from these defense regions is decreased when the A5 region is pharmacologically blocked with the GABA agonist muscimol. For this reason, we propose the existence of two different pathways that subserve the tachycardia and the pressor response elicited from the stimulation of these defense regions [81, 84]. The tachycardia and the hypertension evoked during defense stimulation involve a direct activation of the neurons of the RVLM. These neurons send direct projections to preganglionic neurons of the IML that are ultimately responsible for the abrupt increase in blood pressure [85]. In addition, a direct activation of the adrenal medulla contributes to a secondary increase in blood pressure due to the liberation of adrenaline. Furthermore, in a parallel pathway to the activation of the RVLM and the preganglionic neurons in the IML, the stimulation of defense regions increases the intensity of the chemoreceptor reflex by means of an excitation or facilitation of chemoreceptor neurons in the NTS [77]. In a parallel circuit, an inhibition of the response to baroreceptor inputs is produced by disfacilitation or inhibition of baroreceptor neurons at the level of the NTS [78, 86]. This inhibition seems to be mediated by GABAergic interneurons in the NTS [78].

Other groups have also suggested the existence of these separates pathways [76]. It has been hypothesized that cardiorespiratory sympathoexcitatory changes evoked during defense stimulation are produced via indirect polysynaptic projections from the dlPAG to the medulla through connections with the DMH-PeF, Parabrachial complex and cuneiform nucleus. Our results suggest that the A5 region is one of the best candidates to mediate in these cardiorespiratory descending pathways because of its excitatory direct connections with the IML and the inhibitory direct projections with the CVLM, which are a source of inhibition to the RVLM [59]. Therefore, the stimulation of both defense regions, DMH-PeF and dlPAG, results in an activation of the A5 region. Thus, this activation will reinforce the pressor response, supporting the hypothesis that neurons within the A5 region are involved in the decrease of the sensitivity of the baroreceptor reflex at the level of the NTS, after the activation of the so-called defense regions, DMH-PeF and dlPAG.

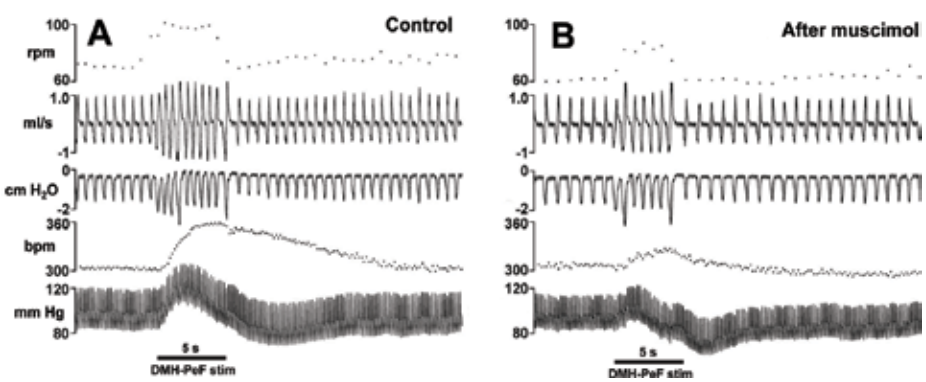

Figure 5. Instantaneous respiratory rate (upper trace), respiratory flow, pleural pressure, instantaneous heart rate and blood pressure in a spontaneously breathing rat, showing the cardiorespiratory response evoked on DMH-PeF stimulation before (A) and after the microinjection of muscimol (50 nl over 5 s) in the A5 region (B) The segment shows the duration (5 s) of the DMH-PeF electrical stimulation.

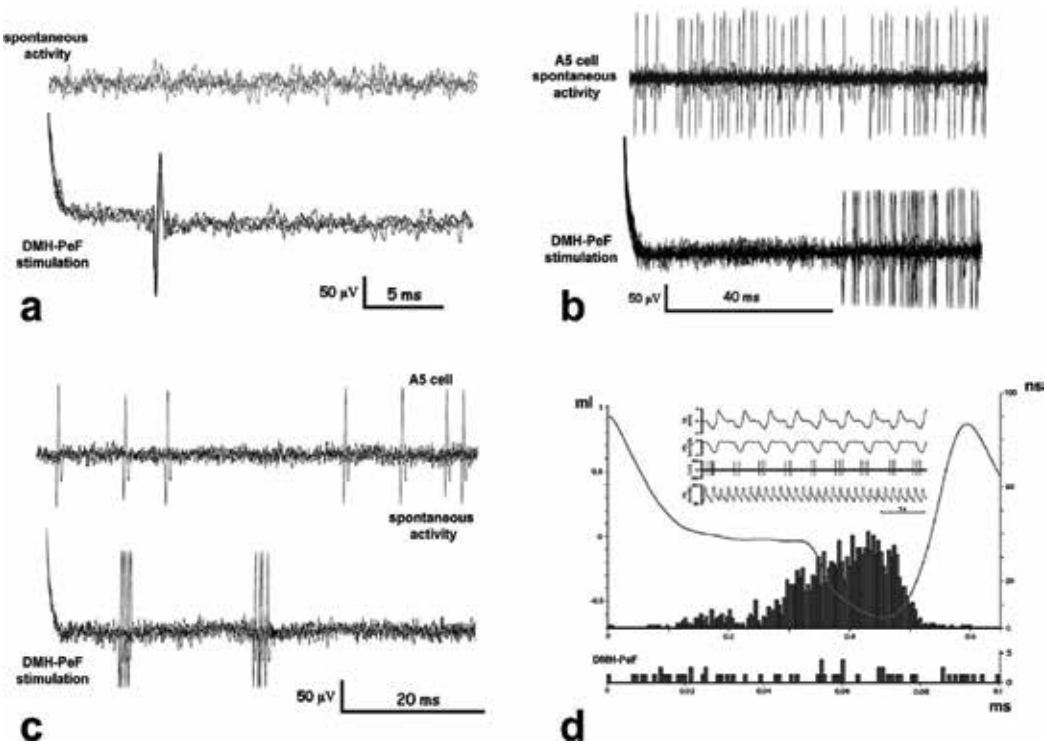

Figure 6. Extracellular recordings (superimposed sweeps) from the A5 region showing electrophysiological relations between the DMH-PeF and the A5 region: (a) Silent axon (upper trace) with constant-latency responses to DMH-PeF stimulation (lower trace). (b) Spontaneously active A5 cell (upper trace) inhibited by DMH-PeF stimulation (lower trace). (c) Spontaneously active A5 cell (upper trace) excited with double short- and long-latency responses to DMH-PeF stimulation (lower trace). (d) Inset shows recording of respiratory flow, pleural pressure, neuronal activity of a putative respiratory-modulated A5 cell and blood pressure. Main graph shows respiratory flow (inspiration downwards), and neuronal activity, while lower trace shows DMH-PeF-triggered histograms. This recordings show the complexity of the neuronal interactions between A5 and DMH-Pef.

4. Clinical implications

The A5 region is also involved in the impairment of sympathetic cardiovascular and respiratory control observed in multiple system atrophy (MSA) [87] and in syndromes such as Sudden Infant Death Syndrome, Rett syndrome, Ondine's syndrome and other genetic failures related to Phox2a, Ret, Mecp2, BDNF and Phox2b mutations [88].

Growing evidence supports the presence of earlier noradrenaline deficiency in neurodegenerative disorders including Parkinson disease (PD). PD dysautonomic symptoms are common, especially in cardiovascular, gastrointestinal and genitourinary systems. Most patients with PD have imaging evidence of cardiac sympathetic denervation. Selective degeneration of the noradrenergic neurons of the A6 NA cell group precedes that of dopaminergic neurons of the substantia nigra pars compacta and has been increasingly recognized as a potential

major contributor to cognitive manifestations in early PD, particularly impaired attention. This makes the A6 NA system a major contributor to the pathophysiology and potential target for therapy of PD [19, 89, 90].

5. Summary and perspectives

This chapter focuses on the different spinal projections and main modulatory actions of the two main NA pontine cell groups derived from this connectivity. Among these NA modulatory actions, a high variety of physiological and behavioral processes can be found that involve multiple cortical and subcortical structures. The diversity of anatomical, morphological, pharmacological and electrophysiological studies carried out in these NA cell groups has demonstrated that A5 and A6 NA pontine cell groups seem to be the best neuronal substrate to articulate the necessary responses to a wide range of psychological and physical stressors. A6 NA neurons present the necessary projections to modulate analgesic responses, while the A5 NA region seems to modulate all of the necessary autonomic responses needed to confront threatening stimuli or situations.

Regarding pain, bidirectional NA modulatory actions of spinal nociceptive processing depends on the type of pain. Moreover, this modulation is not only referred to by the type of nociceptive stimulus but, in addition, is affected by other CNS structures that are involved in emotional, motivational or attentional states. As has been previously explained, A6 and A5 NA cell groups may be the key centers for all modulatory actions exerted from superior structures within the CNS, which inhibit nociceptive transmission at the level of the spinal dorsal horn acting via presynaptic alpha2 receptors.

This chapter has laid the groundwork for further investigations on the topic and numerous unanswered questions remain. For example, how do these noradrenergic nuclei respond when functional or structural diseases caused by genetic or epigenetic factors appear? Are all these centers and their connections equally affected under these different pathological states? Are NA cells of these nuclei affected in the same manner by different external stressors or do they have different functional responses depending on their location within each nucleus or their projections? Does the selective degeneration that occurs in A5 and A6 neurons in diseases, such as MSA and PD, have a relationship with the evolution of the dysautonomia or the cognitive alterations observed in these patients?

Further basic and clinical studies are needed to assess the role of the NA pontine cell groups on physiology and pathophysiology based on these questions.

Acknowledgements

The study was supported by a program grant Junta de Andalucía, Group n° CTS-156, Spain.

Author details

Manuel Víctor López-González[1,2]*, Marta González-García[1,2] and
Marc Stefan Dawid-Milner[1,2]

*Address all correspondence to: msdawid@uma.es

1 Departamento de Fisiología Humana, Histología Humana, Anatomía Patológica y Educación Física y Deportiva, Facultad de Medicina, Universidad de Málaga, Málaga, Spain

2 Unidad de Neurofisiología del Sistema Nervioso Autónomo (CIMES), Universidad de Málaga, Málaga, Spain

References

[1] Dahlström A, Fuxe K. Localization of monoamines in the lower brain stem. Experientia. 1964;**20**:398-399. DOI: 10.1007/BF02147990

[2] Pertovaara A. Noradrenergic pain modulation. Progress in Neurobiology. 2006;**80**:53-83. DOI: 10.1016/j.pneurobio.2006.08.001

[3] Armstrong DM, Ross CA, Pickel VM, et al. Distribution of dopamine; noradrenaline and adrenaline-containing cell bodies in the rat medulla oblongata: Demonstrated by the immunocytochemical localization of catecholamine biosynthetic enzymes. The Journal of Comparative Neurology. 1982;**212**:173-187. DOI: 10.1002/cne.902120207

[4] Bacopoulos NG, Bhatnagar RK. Correlation between tyrosine hydroxylase activity and catecholamine concentration or turnover in brain regions. Journal of Neurochemistry. 1977;**29**:639-643. DOI: 10.1111/j.1471-4159.1977.tb07780.x

[5] Bruinstroop E, Cano G, Vanderhorst VGJM, et al. Spinal projections of the A5, A6 (locus coeruleus), and A7 noradrenergic cell groups in rats. The Journal of Comparative Neurology. 2012;**520**:1985-2001. DOI: 10.1002/cne.23024

[6] Proudfit HK, Clark FM. The projections of locus coeruleus neurons to the spinal cord. Progress in Brain Research. 1991;**88**:123-141

[7] Westlund KN, Bowker RM, Ziegler MG, Coulter JD. Noradrenergic projections to the spinal cord of the rat. Brain Research. 1983;**263**:15-31. DOI: 10.1016/0006-8993(83)91196-4

[8] Clark FM, Yeomans DC, Proudfit HK. The noradrenergic innervation of the spinal cord: Differences between two substrains of Sprague-Dawley rats determined using retrograde tracers combined with immunocytochemistry. Neuroscience Letters. 1991;**125**:155-158. DOI: 10.1016/0304-3940(91)90015-L

[9] Clark FM, Proudfit HK. The projection of locus coeruleus neurons to the spinal cord in the rat determined by anterograde tracing combined with immunocytochemistry. Brain Research. 1991;**538**:231-245. DOI: 10.1016/0006-8993(91)90435-X

[10] Clark FM, Proudfit HK. The projection of noradrenergic neurons in the A7 catecholamine cell group to the spinal cord in the rat demonstrated by anterograde tracing combined with immunocytochemistry. Brain Research. 1991;**547**:279-288. DOI: 10.1016/0006-8993(91)90972-X

[11] Clark FM, Proudfit HK. Anatomical evidence for genetic differences in the innervation of the rat spinal cord by noradrenergic locus coeruleus neurons. Brain Research. 1992;**591**: 44-53

[12] Proudfit HK. The behavioral pharmacology of the noradrenergic descending system. In: JMR B, Guilbaud, editors. Towards the Use of Noradrenergic Agonists. Amsterdam: Elsevier; 1992

[13] Chan JYH, Fung SJ, Chan SHH, Barnes CD. Facilitation of lumbar monosynaptic reflexes by locus coeruleus in the rat. Brain Research. 1986;**369**:103-109. DOI: 10.1016/0006-8993(86)90517-2

[14] Fung SJ, Manzoni D, Chan JY, et al. Locus coeruleus control of spinal motor output. Progress in Brain Research. 1991;**88**:395-409

[15] Byrum CE, Guyenet PG. Afferent and efferent connections of the A5 noradrenergic cell group in the rat. The Journal of Comparative Neurology. 1987;**261**:529-542. DOI: 10.1002/cne.902610406

[16] Clark FM, Proudfit HK. The projections of noradrenergic neurons in the A5 catecholamine cell group to the spinal cord in the rat: Anatomical evidence that A5 neurons modulate nociception. Brain Research. 1993;**616**:200-210

[17] Loewy AD, McKellar S, Saper CB. Direct projections from the A5 catecholamine cell group to the intermediolateral cell column. Brain Research. 1979;**174**:309-314

[18] Light A. The initial processing of pain and its descending control: Spinal and trigeminal systems. In: Pain and Headache. Switzerland: Karger; 1992

[19] Benarroch EE. Locus coeruleus. Cell and Tissue Research. 2017;**373**(1):221-232. DOI: 10.1007/s00441-017-2649-1

[20] Holden JE, Schwartz EJ, Proudfit HK. Microinjection of morphine in the A7 catecholamine cell group produces opposing effects on nociception that are mediated by alpha1- and alpha2-adrenoceptors. Neuroscience. 1999;**91**:979-990

[21] Holden JE, Van Poppel AY, Thomas S. Antinociception from lateral hypothalamic stimulation may be mediated by NK(1) receptors in the A7 catecholamine cell group in rat. Brain Research. 2002;**953**:195-204

[22] Iwamoto ET, Marion L. Adrenergic, serotonergic and cholinergic components of nicotinic antinociception in rats. The Journal of Pharmacology and Experimental Therapeutics. 1993;**265**:777-789

[23] Nuseir K, Proudfit HK. Bidirectional modulation of nociception by GABA neurons in the dorsolateral pontine tegmentum that tonically inhibit spinally projecting noradrenergic A7 neurons. Neuroscience. 2000;**96**:773-783

[24] Miller MA, Kolb PE, Leverenz JB, et al. Preservation of noradrenergic neurons in the locus ceruleus that coexpress galanin mRNA in Alzheimer's disease. Journal of Neurochemistry. 1999;**73**:2028-2036

[25] Chan-Palay V, Asan E. Quantitation of catecholamine neurons in the locus coeruleus in human brains of normal young and older adults and in depression. The Journal of Comparative Neurology. 1989;**287**:357-372. DOI: 10.1002/cne.902870307

[26] Foote SL, Morrison JH. Extrathalamic modulation of cortical function. Annual Review of Neuroscience. 1987;**10**:67-95. DOI: 10.1146/annurev.ne.10.030187.000435

[27] Levitt P, Moore RY. Origin and organization of brainstem catecholamine innervation in the rat. The Journal of Comparative Neurology. 1979;**186**:505-528. DOI: 10.1002/cne.901860402

[28] Mason ST, Fibiger HC. Regional topography within noradrenergic locus coeruleus as revealed by retrograde transport of horseradish peroxidase. The Journal of Comparative Neurology. 1979;**187**:703-724. DOI: 10.1002/cne.901870405

[29] Ginsberg SD, Hof PR, Young WG, Morrison JH. Noradrenergic innervation of the hypothalamus of rhesus monkeys: Distribution of dopamine-beta-hydroxylase immunoreactive fibers and quantitative analysis of varicosities in the paraventricular nucleus. The Journal of Comparative Neurology. 1993;**327**:597-611. DOI: 10.1002/cne.903270410

[30] Morrison JH, Foote SL. Noradrenergic and serotoninergic innervation of cortical, thalamic, and tectal visual structures in old and new world monkeys. The Journal of Comparative Neurology. 1986;**243**:117-138. DOI: 10.1002/cne.902430110

[31] Aston-Jones G, Shipley MT, Chouvet G, et al. Afferent regulation of locus coeruleus neurons: Anatomy, physiology and pharmacology. Progress in Brain Research. 1991;**88**:47-75

[32] Gurtu S, Pant KK, Sinha JN, Bhargava KP. An investigation into the mechanism of cardiovascular responses elicited by electrical stimulation of locus coeruleus and subcoeruleus in the cat. Brain Research. 1984;**301**:59-64

[33] Valentino RJ, Martin DL, Suzuki M. Dissociation of locus coeruleus activity and blood pressure. Effects of clonidine and corticotropin-releasing factor. Neuropharmacology. 1986;**25**:603-610

[34] Hagena H, Hansen N, Manahan-Vaughan D. β-Adrenergic control of hippocampal function: Subserving the choreography of synaptic information storage and memory. Cerebral Cortex. 2016;**26**:1349-1364. DOI: 10.1093/cercor/bhv330

[35] Lim EP, Tan CH, Jay TM, Dawe GS. Locus coeruleus stimulation and noradrenergic modulation of hippocampo-prefrontal cortex long-term potentiation. The International Journal of Neuropsychopharmacology. 2010;**13**:1219-1231. DOI: 10.1017/S1461145709991131

[36] Lippiello P, Hoxha E, Volpicelli F, et al. Noradrenergic modulation of the parallel fiber-Purkinje cell synapse in mouse cerebellum. Neuropharmacology. 2015;**89**:33-42. DOI: 10.1016/j.neuropharm.2014.08.016

[37] Westlund KN, Craig AD. Association of spinal lamina I projections with brainstem catecholamine neurons in the monkey. Experimental Brain Research. 1996;**110**:151-162

[38] Westlund KN, Coulter JD. Descending projections of the locus coeruleus and subcoeruleus/medial parabrachial nuclei in monkey: Axonal transport studies and dopamine-beta-hydroxylase immunocytochemistry. Brain Research. 1980;**2**:235-264

[39] Roozendaal B, McGaugh JL. Memory modulation. Behavioral Neuroscience. 2011;**125**: 797-824. DOI: 10.1037/a0026187

[40] Johnson PL, Federici LM, Fitz SD, et al. Orexin 1 and 2 receptor involvement in CO_2-induced panic-associated behavior and autonomic responses. Depression and Anxiety. 2015;**32**: 671-683. DOI: 10.1002/da.22403

[41] Strange BA, Dolan RJ. Adrenergic modulation of emotional memory-evoked human amygdala and hippocampal responses. Proceedings of the National Academy of Sciences. 2004;**101**:11454-11458. DOI: 10.1073/pnas.0404282101

[42] Wang X, Pinol RA, Byrne P, Mendelowitz D. Optogenetic stimulation of locus ceruleus neurons augments inhibitory transmission to parasympathetic cardiac vagal neurons via activation of brainstem 1 and 1 receptors. The Journal of Neuroscience. 2014;**34**: 6182-6189. DOI: 10.1523/JNEUROSCI.5093-13.2014

[43] de Carvalho D, Patrone LGA, Marques DA, Vicente MC, Szawka RE, Anselmo-Franci JA, Bícego KC, Gargaglioni LH. Participation of locus coeruleus in breathing control in female rats. Respiratory Physiology & Neurobiology. 2017 Nov;**245**:29-36. DOI: 10.1016/j.resp.2017.06.008

[44] Patrone LGA, Biancardi V, Marques DA, Bícego KC, Gargaglioni LH. Brainstem catecholaminergic neurones and breathing control during postnatal development in male and female rats. The Journal of Physiology. 2018. [Epub ahead of print]. DOI: 10.1113/JP275731

[45] Dampney RAL, Polson JW, Potts PD, et al. Functional organization of brain pathways subserving the baroreceptor reflex: Studies in conscious animals using immediate early gene expression. Cellular and Molecular Neurobiology. 2003;**23**:597-616

[46] Dawid-Milner MS, Lara JP, López de Miguel MP, et al. A5 region modulation of the cardiorespiratory responses evoked from parabrachial cell bodies in the anaesthetised rat. Brain Research. 2003;**982**:108-118

[47] Dawid-Milner MS, Lara JP, Gonzaléz-Barón S, Spyer KM. Respiratory effects of stimulation of cell bodies of the A5 region in the anaesthetised rat. Pflügers Archiv. 2001;**441**: 434-443

[48] Guyenet PG. The sympathetic control of blood pressure. Nature Reviews. Neuroscience. 2006;**7**:335-346. DOI: 10.1038/nrn1902

[49] Huangfu DH, Koshiya N, Guyenet PG. A5 noradrenergic unit activity and sympathetic nerve discharge in rats. American Journal of Physiology. Regulatory, Integrative and Comparative Physiology. 1991;**261**:R393-R402. DOI: 10.1152/ajpregu.1991.261.2.R393

[50] Lara JP, Dawid-Milner MS, López MV, et al. Laryngeal effects of stimulation of rostral and ventral pons in the anaesthetized rat. Brain Research. 2002;**934**:97-106

[51] Goodchild AK, Phillips JK, Lipski J, Pilowsky PM. Differential expression of catecholamine synthetic enzymes in the caudal ventral pons. The Journal of Comparative Neurology. 2001;**438**:457-467

[52] Mulkey DK, Stornetta RL, Weston MC, et al. Respiratory control by ventral surface chemoreceptor neurons in rats. Nature Neuroscience. 2004;**7**:1360-1369. DOI: 10.1038/nn1357

[53] Guthmann A, Herbert H. Expression of N-methyl-D-aspartate receptor subunits in the rat parabrachial and Kölliker-Fuse nuclei and in selected pontomedullary brainstem nuclei. The Journal of Comparative Neurology. 1999;**415**:501-517

[54] Wisden W, Seeburg PH, Monyer H. AMPA, kainate and NMDA ionotropic glutamate receptor expression — an in situ hybridization atlas. In: Ottersen OP, Storm-Mathisen J (eds). Elsevier, Amsterdam: Handbook of Chemical Neuroanatomy: Glutamate; 2000. pp. 99-143

[55] Shigemoto R, Mizuno N. Metabotropic glutamate receptors - immunocytochemical and in situ hybridization analysis. In: Ottersen OP, Storm-Mathisen J (eds) Handbook of Chemical Neuroanatomy: metabotropic glutamate receptors: immunocytochemical and in situ hybridization analyses. Elsevier, London. 2000. pp. 63-98

[56] Abbott SB, Kanbar R, Bochorishvili G, et al. C1 neurons excite locus coeruleus and A5 noradrenergic neurons along with sympathetic outflow in rats. The Journal of Physiology. 2012;**590**:2897-2915. DOI: 10.1113/jphysiol.2012.232157

[57] Madden CJ, Ito S, Rinaman L, et al. Lesions of the C1 catecholaminergic neurons of the ventrolateral medulla in rats using anti-DbetaH-saporin. The American Journal of Physiology. 1999;**277**:R1063-R1075

[58] Rosin DL, Chang DA, Guyenet PG. Afferent and efferent connections of the rat retrotrapezoid nucleus. The Journal of Comparative Neurology. 2006;**499**:64-89. DOI: 10.1002/cne.21105

[59] Tavares I, Lima D, Coimbra A. The pontine A5 noradrenergic cells which project to the spinal cord dorsal horn are reciprocally connected with the caudal ventrolateral medulla in the rat. The European Journal of Neuroscience. 1997;**9**:2452-2461

[60] Usunoff KG, Itzev DE, Rolfs A, et al. Brain stem afferent connections of the amygdala in the rat with special references to a projection from the parabigeminal nucleus: A fluorescent retrograde tracing study. Anatomy and Embryology (Berlin). 2006;**211**:475-496. DOI: 10.1007/s00429-006-0099-8

[61] Spyer KM. Annual review prize lecture. Central nervous mechanisms contributing to cardiovascular control. The Journal of Physiology. 1994;**474**:1-19

[62] Taxini CL, Moreira TS, Takakura AC, et al. Role of A5 noradrenergic neurons in the chemoreflex control of respiratory and sympathetic activities in unanesthetized conditions. Neuroscience. 2017;**354**:146-157. DOI: 10.1016/j.neuroscience.2017.04.033

[63] Taxini CL, Takakura AC, Gargaglioni LH, Moreira TS. Control of the central chemoreflex by A5 noradrenergic neurons in rats. Neuroscience. 2011;**199**:177-186. DOI: 10.1016/j.neuroscience.2011.09.068

[64] McDowall LM, Horiuchi J, Killinger S, Dampney RAL. Modulation of the baroreceptor reflex by the dorsomedial hypothalamic nucleus and perifornical area. American Journal of Physiology. Regulatory, Integrative and Comparative Physiology. 2006;**290**:R1020-R1026. DOI: 10.1152/ajpregu.00541.2005

[65] Guyenet PG, Koshiya N, Huangfu D, et al. Central respiratory control of A5 and A6 pontine noradrenergic neurons. The American Journal of Physiology. 1993;**264**:R1035-R1044. DOI: 10.1152/ajpregu.1993.264.6.R1035

[66] Kanbar R, Depuy SD, West GH, et al. Regulation of visceral sympathetic tone by A5 noradrenergic neurons in rodents. The Journal of Physiology. 2011;**589**:903-917. DOI: 10.1113/jphysiol.2010.198374

[67] Koshiya N, Guyenet PG. A5 noradrenergic neurons and the carotid sympathetic chemoreflex. American Journal of Physiology. Regulatory, Integrative and Comparative Physiology. 1994;**267**:R519-R526. DOI: 10.1152/ajpregu.1994.267.2.R519

[68] Song G, Xu H, Wang H, et al. Hypoxia-excited neurons in NTS send axonal projections to Kölliker-Fuse/parabrachial complex in dorsolateral pons. Neuroscience. 2011;**175**: 145-153. DOI: 10.1016/j.neuroscience.2010.11.065

[69] Hilaire G, Viemari J-C, Coulon P, et al. Modulation of the respiratory rhythm generator by the pontine noradrenergic A5 and A6 groups in rodents. Respiratory Physiology & Neurobiology. 2004;**143**:187-197. DOI: 10.1016/j.resp.2004.04.016

[70] Dobbins EG, Feldman JL. Brainstem network controlling descending drive to phrenic motoneurons in rat. The Journal of Comparative Neurology. 1994;**347**:64-86. DOI: 10.1002/cne.903470106

[71] Schlenker EH, Prestbo A. Elimination of the post-hypoxic frequency decline in conscious rats lesioned in pontine A5 region. Respiratory Physiology & Neurobiology. 2003;**138**: 179-191

[72] WM S-J, Paton JFR. Role of pontile mechanisms in the neurogenesis of eupnea. Respiratory Physiology & Neurobiology. 2004;**143**:321-332. DOI: 10.1016/j.resp.2004.05.010

[73] Carrive P. The periaqueductal gray and defensive behavior: Functional representation and neuronal organization. Behavioural Brain Research. 1993;**58**:27-47

[74] DiMicco JA, Samuels BC, Zaretskaia MV, Zaretsky DV. The dorsomedial hypothalamus and the response to stress: Part renaissance, part revolution. Pharmacology Biochemistry and Behavior. 2002;**71**:469-480

[75] Keay KA, Bandler R. Parallel circuits mediating distinct emotional coping reactions to different types of stress. Neuroscience and Biobehavioral Reviews. 2001;**25**:669-678

[76] Dampney RAL. Central mechanisms regulating coordinated cardiovascular and respiratory function during stress and arousal. American Journal of Physiology. Regulatory, Integrative and Comparative Physiology. 2015;**309**:R429-R443. DOI: 10.1152/ajpregu.00051.2015

[77] Silva-Carvalho L, Dawid-Milner MS, Goldsmith GE, Spyer KM. Hypothalamic modulation of the arterial chemoreceptor reflex in the anaesthetized cat: Role of the nucleus tractus solitarii. The Journal of Physiology. 1995;**487**(Pt 3):751-760

[78] Jordan D, Mifflin SW, Spyer KM. Hypothalamic inhibition of neurones in the nucleus tractus solitarius of the cat is GABA mediated. The Journal of Physiology. 1988;**399**: 389-404

[79] Silva-Carvalho L, Dawid-Milner MS, Spyer KM. The pattern of excitatory inputs to the nucleus tractus solitarii evoked on stimulation in the hypothalamic defence area in the cat. The Journal of Physiology. 1995;**487**(Pt 3):727-737

[80] Díaz-Casares A, López-González MV, Peinado-Aragonés CA, et al. Role of the parabrachial complex in the cardiorespiratory response evoked from hypothalamic defense area stimulation in the anesthetized rat. Brain Research. 2009;**1279**:58-70. DOI: 10.1016/j.brainres.2009.02.085

[81] López-González MV, Díaz-Casares A, González-García M, et al. Glutamate receptors of the A5 region modulate cardiovascular responses evoked from the dorsomedial hypothalamic nucleus and perifornical area. Journal of Physiology and Biochemistry. 2018;**74**(2):325-334. DOI: 10.1007/s13105-018-0612-6

[82] Díaz-Casares A, López-González MV, Peinado-Aragonés CA, et al. Parabrachial complex glutamate receptors modulate the cardiorespiratory response evoked from hypothalamic defense area. Autonomic Neuroscience. 2012;**169**:124-134. DOI: 10.1016/j.autneu.2012.06.001

[83] Peinado-Aragonés CA. Interrelaciones de la sustancia gris periacueductal dorsolateral y la región protuberancial A5 en el control central cardiorrespiratorio. [thesis]. Universidad de Málaga, España; 2016. URI: http://hdl.handle.net/10630/12109

[84] López-González MV, Díaz-Casares A, Peinado-Aragonés CA, et al. Neurons of the A5 region are required for the tachycardia evoked by electrical stimulation of the hypothalamic defence area in anaesthetized rats. Experimental Physiology. 2013;**98**:1279-1294. DOI: 10.1113/expphysiol.2013.072538

[85] Loewy AD. Forebrain nuclei involved in autonomic control. Progress in Brain Research. 1991;**87**:253-268

[86] Mifflin SW, Spyer KM, Withington-Wray DJ. Baroreceptor inputs to the nucleus tractus solitarius in the cat: Modulation by the hypothalamus. The Journal of Physiology. 1988; **399**:369-387

[87] Benarroch EE, Schmeichel AM, Low PA, et al. Loss of A5 noradrenergic neurons in multiple system atrophy. Acta Neuropathologica. 2008;**115**:629-634. DOI: 10.1007/s00401-008-0351-9

[88] Hilaire G. Endogenous noradrenaline affects the maturation and function of the respiratory network: Possible implication for SIDS. Autonomic Neuroscience. 2006;**126-127**: 320-331. DOI: 10.1016/j.autneu.2006.01.021

[89] Espay AJ, LeWitt PA, Kaufmann H. Norepinephrine deficiency in Parkinson's disease: The case for noradrenergic enhancement. Movement Disorders. 2014;**29**:1710-1719. DOI: 10.1002/mds.26048

[90] Kaufmann H, Goldstein DS. Autonomic dysfunction in Parkinson disease. Handbook of Clinical Neurology. 2013;**117**:259-278. DOI: 10.1016/B978-0-444-53491-0.00021-3

Edited by Pavol Svorc

The autonomic nervous system is one of the most important involuntary control mechanisms that primarily controls and modulates the functions of the visceral organs. The book discusses some of the specificities of the autonomic nervous system in terms of dendritic development in the sympathetic compartment, as well as a detailed description of noradrenergic groups and their key role in the modulation of all antinociceptive and autonomic responses elicited by painful or threatening situations. In the book, only those cases are mentioned that are closely related to disorders or changes of function of the autonomic nervous system. This book can evoke interest in many researchers who want to use the information for the advancement of their research towards a better understanding of the autonomic regulatory mechanisms.

Published in London, UK

IntechOpen

ISBN 978-1-83881-720-6