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Autonomic cardiac nerves: literature review

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The aim of this paper was to summarise the knowledge about the autonomic cardiac innervation. It is generally known, that the cardiac nervous system consists of nerve plexoganglionic structures located mostly around the strategic regions of the heart. They consist of two main types of components: parasympathetic neurons, which exert an inhibitory effect, and sympathetic postganglionic nerve fibres, which stimulate the cardiac conduction system, and myocardial cells. However, many authors describe that cardiac ganglia contain various populations of neurons. The largest group are classical cholinergic neurons. The second group of cardiac neurons are cells of dual, cholinergic-adrenergic character. There is also subpopulation of small intensely fluoroscent cells of typically adrenergic phenotype. Moreover, many authors indicated the presence of various neurotransmitters in various combinations. In this way, the neurons in cardiac ganglia are a neurochemical complex beyond the classical vision of parasympathetic ganglia. (Folia Morphol 2015; 74, 1: 1–8)

Key words: heart innervations, cardiac neurons, neurochemical characteristic

INTRODUCTION

Scientists have been interested in the issue of cardiac nerves for over 200 years. The first paper on the topic appeared already in 1794 [Scarpa, in: 33]. Since then, the problem has been studied and described for many amphibian, reptile, bird, and mammal species. Such strong interest in cardiac nerve structures is due to their important function: they regulate the function of the cardiac conduction system, the heart rhythm, and coronary circulation [56, 65].

It is known that, initially, the heart develops independently from its nerve system. In humans, it starts contracting already in the 21st–22nd day of development. It is only during the 5th week of development, however, that neural crest cells begin migrating to the heart [22]. The nerve system of the heart includes three types of nerves: sympathetic, parasympathetic, and sensory. Sensory cardiac nerves originate in the sensory ganglionic cells of the vagus nerve (nodose ganglion), which originate in the ectodermal plate (vagus nerve placodes) [31]. Sympathetic nerve fibres originate from sympathetic trunk cells in the thoracic segment, while neurons of the trunk come from the neural crest in the thoracic segment [31]. Parasympathetic nerves originate in the 'cardiac component' of the cranial neural crest. Cardiac ganglia that constitute second order parasympathetic neurons migrate directly from the neural crest of the heart. A little later, preganglionic neurons (the parasympathetic nucleus of the vagus nerve) gain access to the heart (through contact with cardiac ganglia cells) via the vagus nerve [22, 31]. According to the results of studies by Hasan [22] and Woźniak et al. [66], several important phases can be differentiated in the development of the autonomic heart nerves: (a) neural crest cells migration to the dorsal aorta, (b) differentiation of neural crest cells into neurons, (c) aggregation/migration of neurons to form either the paravertebral sympathetic chains or the parasympathetic cardiac ganglia, (d) extension of axonal projections into cardiac tissue and terminal differentiation.

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The migration of cells from neural crests is conditioned by factors of the trophic glial line, mostly by the glial cell line-derived neurotrophic factor, released in the area of the dorsal aorta and along large blood vessels [22]. Other trophic factors of the bone morphogenetic proteins are also known; they are produced in the area of the dorsal aorta and influence the phenotype of neural crest cells [66]. It has been demonstrated that the parasympathetic cardiac innervation in the embryonic development of rats and birds slightly precedes sympathetic innervation, and neuroeffector transmission for the parasympathetic system is established at stage E21 (transmission significantly greater in the atria than in the ventricles) [22]. The results of studies by Navaratnam [44] demonstrate that true cholinesterase first appears in the cytoplasm of the cardiac nerve cells in man between the 4th and 7th month of gestation. In the rat it appears at about the 4th day of postnatal life, in the rabbit between the 24th and 27th day of gestation and in the guinea pig about the 30th day. On the other hand, Marvin et al. [40] found that the activity of acetylcholine synthesis enzymes in rats appears on the 19th day of development.

Today, it is generally known that the autonomous nervous system, a system of plexoganglia, is crucial for the cardiac nervous system. It consists of two types of components: sympathetic, which stimulate the cardiac conduction system and myocardial cells, and parasympathetic, which exert an inhibitory effect [7, 45, 64]. Sympathetic cardiac nerves come mainly from both stellate ganglia as well as from the superior cervical ganglion, middle cervical ganglion, and 10 thoracic ganglia [24, 45, 64]. Sympathetic neurons primarily utilise norepinephrine as their principal neurotransmitter, although other neuropeptides, such as neuropeptide Y (NPY) and galanin (GAL) are also co-released from sympathetic terminals. Among other functions, NPY and GAL decrease acetylcholine release from adjacent parasympathetic terminals [22].

Studies using retrograde tracing carried out on various animal species lead to the determination of neurons projecting axons to the heart (the conduction system, cardiomyocytes, and coronary vessels) and thus the identification of their location [28, 35, 42, 52, 64]. In cats, most cardiac neurons were located in the stellate ganglion (2679 cells, on average) and in the ipsilateral ganglions of segments C8–T9 (213 cells, on average) [35]. Hasan [22] presents similar data: as much as 92% of neurons determined retrospectively were found in the stellate ganglion. Hopkins and Armour [28] on the other hand, observed a large number of marked neurons in central cervical ganglia on both sides of the sympathetic trunk following injection into the heart, aorta, and cardiac sac in dogs. Cranial parts of bilateral stellate ganglia were the secondary location of neurons. They also appeared sporadically in upper cervical ganglia and in small ganglia along cardiopulmonary nerves. Wallis et al. [64] demonstrated that the stellate ganglion is the main source of cardiac innervation in dogs. Nerves also originated from the area of T1 and T2 segments. These authors also described the morphology of putative cardiac neurons. They are oval or polygonal in shape with a surface of about 463 μm^2. Their axons are not divided, and the dendritic tree is diversified, usually consisting from 7 dendrites. The results of studies by Mo et al. [42] were similar. Moreover, authors found that the neurons include both phasic and tonic ones, and they were depolarised due to the effect of muscarine antagonists: angiotensin and substance P (SP). Richardson et al. [52] observed that 'cardiac neurons' in the stellate ganglion of rats were located along the medial edges of those ganglia. They also demonstrated that ganglia formed 4 neurochemical populations of neurons demonstrating immunoreactivity (IR) to: (a) both calbindine (CALB) and NPY; (b) only CALB; (c) only NPY; (d) no reactivity to CALB or NPY. These results may indicate the existence of various functional groups of sympathetic cardiac nerves.

The results of studies by Hopkins et al. [28] with the injection of horseradish peroxidase to specific areas of the heart in dogs suggest that postganglionic sympathetic neurons which project efferent axons to a specific cardiac region are not located in a specific region of a sympathetic ganglion or a specific sympathetic ganglion. Rather, neurons in one region of a sympathetic ganglion project axons to widespread areas of the myocardium.

Parasympathetic preganglionic fibres come from both vagus nerves [24, 37, 41, 45, 64]. Ai et al. [1] arrived at interesting results in a study of the cardiac nerves of rats using anterograde transport; they showed that vagal efferent nerve fibres forming baskets around cardiac neurons came from the *nucleus ambiguous*.

It turns out various neurotrophic factors influence the distribution of cardiac nerves. Sympathetic fibres are influenced by the nerve growth factor, while parasympathetic nerves are influenced by neurturin,

a factor known for the development of cholinergic nerves during organogenesis. However, Mabe et al. [37] found that it is also present in the hearts of adult rats. Moreover, studies have shown that various regions of the myocardium have a nerve system of varying degree and density. They found the greatest number of cholinergic fibres in the immediate vicinity of sino-atrial and atrioventricular nodes. Fibres were also found in the area of the ventricular conduction system, in the atrial septum, and in the lamina muscularis of the right atrium. The smallest number of parasympathetic fibres were found in the lamina muscularis of the right ventricle. Similar results were also obtained by Hoover et al. [26], who studied the heart of the guinea pig, and Yasuhara et al. [67], who studied the rat.

Cardiac plexuses contain clusters of neurons forming a polymorphic system of cardiac ganglia, connected with bundles of nervous fibres. The shape, size, and location of cardiac ganglia vary significantly across species. Usually, they form several nerve clusters located in the epicardium, in several regions of the heart: the base of the heart as well as in the region of the interatrial, coronary, and interventricular grooves. Studies have shown that the human heart contains an average of 836 ganglia [49]; this translates into about 14,000 neurons [5], The heart of a pig has 82 ganglia formed by over 1,600 neurons [6], while the heart of a dog has about 440 small ganglia containing an average of 2,800 neurons, about 20 medium-sized ganglia containing an average of 6,400 cells, and 1–3 large ganglia containing over 44,000 neurons [50]. On the other hand, Yuan et al. [68] showed the presence of only about 260 cardiac ganglia in dogs. Rodents usually have 20–30 ganglia; for example midday gerbils, Egyptian spiny mice, and chinchillas have about 30 ganglia [34]. Rysevaite et al. [56] found 20 ganglia (formed by about 1,100 neuronal somata) in mice, while Ai et al. [1] found only 18 ganglia. Leger et al. [36] found over 1,500 neurons creating ganglia in the heart of the guinea pig, of which 85% were formed by 20 neurons. There are few studies on the subject regarding birds. The issue has been described only for the Japanese quail — about 90 ganglia [32] — and for the pigeon — about 40 ganglia [34]. Some authors suggest that the number of cardiac ganglia can be correlated with body size and the activity of the heart [34], but there is no simple and direct correlation. Moreover, the studies of Akamatsu et al. [2] showed that the number of neurons in cardiac ganglia changes with age. In young rats (aged 3 months) authors observed from 50 to 100 neurons in ganglia, which totalled to an average of 1,086 neurons, while in rats aged 20 months an average of 20 neurons per ganglion were found. The number of ganglia also decreased to 245, which is only 21% in comparison with younger rats. On the other hand, the average area of neurons increased with age from 702 μm^2 to 1065 μm^2. The similar results were found by Batulevicius et al. [9]. Given the above results as well as the significant interspecies and individual variation, one should approach the comparative analysis and possible correlations very carefully.

TOPOGRAPHY OF CARDIAC PLEXUSES

A precise analysis of cardiac ganglia topography shows that they are located in the epicardium, mostly around the strategic regions of the heart. In most species, they are located around the sino-atrial node, the opening of the venae cavae and pulmonary veins as well as around the atrioventricular node. The location of cardiac ganglia varies significantly across species. For example, there are three plexoganglia in mice and in rats: (a) around the sino-atrial node, (b) around the opening of the left pulmonary vein to the left atrium and the opening of the inferior vena cava to the right atrium, i.e. in the vicinity of the atrioventricular node, and (c) around the opening of the inferior pulmonary vein to the left atrium [1]. Kuder and Tekieli [33], on the other hand, described two plexoganglia in the epicardium of the atria and one plexoganglium in the area of the left coronary groove. The largest ganglion is located in the cupping between the right atrial auricle and the aorta. Two other ganglia are located in the area of the right vena cava openings. Several ganglia were also observed on the central surface of the right atrium. A similar location of cardiac ganglia was described by Maifrino et al. [38]. In a study on mice, Rysevaite et al. [56] also found the presence of ganglia spread in the immediate vicinity of the vena cava and the pulmonary veins. However, they found no ganglia around the coronary groove. Cardiac ganglia were located similarly in other species: midday gerbil, Egyptian spiny mouse, chinchilla [34], rat, and guinea pig [48] and rabbit [57]. In the Egyptian mouse, a plexoganglium on the dorsal side of the left ventricle, in the immediate vicinity of the interventricular groove, was found apart from atrial plexoganglia. Horackova et al. [30] found cardiac

ganglia in the guinea pig mostly in the atrial septum and around the openings of venae cavae to the right atrium; they contained 85–90% of neurons, while the rest were located individually. The results of studies by Singh et al. [60] performed in the hearts of adult humans during autopsy or during transplantation procedures have shown that the largest populations of cardiac ganglia are near the sinoatrial and atrioventricular nodes. Smaller collections of ganglia exist on the superior left atrial surface, the interatrial septum, and the atrial appendage–atrial junctions. Ganglia also exist at the base of the great vessels and the base of the ventricles. The right atrial free wall, atrial appendages, trunk of the great vessels, and most of the ventricular myocardium are devoid of cardiac ganglia.

A literature review shows that cardiac ganglia were found more rarely in the epicardium of the ventricles. This is the case in the Egyptian mouse, the pigeon, the Japanese quail [34], and in humans [49]. On the other hand, Caralesu and Luis [12] found no ganglia in the coronary area in cats. The location of cardiac ganglia, mostly in the basal region of the atria and in the anterior or posterior interventricular groove and the coronary groove, coincides partly with the location of the cardiac conduction system. It seems that cardiac ganglia in the ventricles are more common in birds than in mammals. This may be related to the different activity and lifestyle of these classes.

MORPHOLOGY OF CARDIAC GANGLIA

There is significant polymorphism, both between species and individuals, regarding the shape of cardiac ganglia. A review of literature shows that they can be oval, spherical, fusiform, or elongated. Pauza et al. [48] studied cardiac ganglia in several mammalian species (rat, guinea pig, dog, and human) and, despite this polymorphism, they discerned two main morphological types of ganglia: spherical and straight (flat). In spherical ganglia, nerve cells were densely packed and contained 100–200 neurons. In flat ganglia, several neurons were set linearly one next to another. Apart from significant variation across species, significant individual variation of cardiac ganglia is also observed. Pauza et al. [49] describe a range of individual variation in the number of ganglia in some regions of the human heart, between 0 and 70. They consist of several, several dozen, or even several hundred neurons, e.g. about 400 in humans [49]. In most cases, however, 7–20 cells were observed in transverse sections. They take up from a quarter to a half of the section area. Neuronal somata have a diameter of 17–36 μm, like in most autonomous ganglia. Many authors distinguish several morphological types of neurons in cardiac ganglia. Pauza et al. [51], for example, distinguished two neuron types in the cardiac ganglia of rats and guinea pigs: monopolar, constituting 61.2% of the total, and multipolar, constituting 38.8%. There were no essential morphometric differences between these cell types. The authors suggest various functional properties of the two neuron types. This is also confirmed by the results of a study by Hardwick et al. [21], who found two types of neurons in the cardiac ganglia of guinea pigs: phasic, constituting about 95%, and tonic (the remaining 5%). Horackova et al. [30] also found two morphological types of neurons: about 80% of large neurons, 15–40 μm in diameter, and about 20% of the so-called small neurons. Moreover, the authors demonstrated the presence of three cell subpopulations varying in neurochemical properties. Edwards et al. [15], on the other hand, differentiated three neuron types in the cardiac ganglia of guinea pigs, varying in electrophysiological properties. The first type are the so-called S-cells, in which action potential is caused by short hyperpolarisation, while in types 2 and 3, the potential is created due to prolonged hyperpolarisation. The membrane potential of type 2 cells (P-cells) was generated very close to resting potential, and type 3 cells (SAH cells) differed only in that hyperpolarisation was longer than in the case of S-cells. The individual cell types differed morphologically: S-cells were monopolar, most P-cells were bipolar or pseudomonopolar, while SAH-cells were multipolar. The authors suggest that the three cell types can have various functions in the heart. Baptista and Kirby [8] also described the presence of cardiac neurons in mammals that varied in size and shape. The cells can be multipolar as well as bi- or monopolar. Small intensely fluorescent (SIF) cells were also present. The presence of the latter is described by other authors as well, e.g. Shvalev and Sosunov [59], who list three functions of those cells: endocrine, chemoreceptive, and interneuronal. Cheng et al. [13] presented interesting study results. The authors used methods of reverse transport to demonstrate that some monopolar or pseudomonopolar cells in the cardiac ganglia of rats originate in the inferior ganglion of the vagus nerve, hence they might be sensory cells.

NEUROCHEMICAL CHARACTERISTIC OF CARDIAC GANGLIA

In accordance to the organisation of the autonomic nervous system (ANS), only parasympathetic postganglionic neurons should be found in cardiac ganglia. Such was the opinion for many years, and it was also confirmed by studies [7, 20, 21]. However, numerous studies in recent years have shown that the nervous system in the heart of mammals contains populations of immunohistochemically varied neurons. There are reports of minimal numbers of adrenergic neurons in those ganglia [4, 17, 68]. Many authors suggest that ganglionic cardiac neurons are heterogeneous and include two main transmitter types: cholinergic and adrenergic [23, 30, 61, 65].

As is known, there are functional groups of neurons in the nervous system, in the ANS in particular, defined by the particular combination of neurotransmission expression. Such correlation of chemical phenotype and neuron function is called chemical coding [19]. Many papers have been written on this topic. Richardson et al. [53] showed that all main neurons in the cardiac ganglia of rats contain acetyltransferase and NPY. Some neurons also contain nitric oxide synthase (NOS) or calcium-binding proteins, or they are surrounded by endings containing CALB. The authors suggest that this chemical variety of cardiac neurons can represent their various functional groups.

Parsons et al. [47] studied the nervous tissue in the cardiac septum of the mudpuppy and they found that some neurons included dopamine and serotonin (5-HT) as well as SP fibres, i.e. immunoreactive fibres creating a web around parasympathetic ganglionic somata. This was also found by Hardwick et al. [21]. Hoard et al. [24] showed that about 30% of cholinergic bodies in the cardiac ganglionic neurons of mice contain tyrosine hydroxylase (TH), dopamine beta-hydroxylase (DBH), and norepinephrine transporters, indicating the possibility of catecholamine synthesis and metabolism. However, there was no vesicular monoamine transporter type 2 in the bodies of cholinergic neurons, so there is no possibility of storing and releasing follicular noradrenaline. The authors believe that noradrenaline can be released from those neurons in pathophysiological circumstances. Weihe et al. [65] demonstrated neuron subpopulations (about 40–50%) with the coexistence of TH or vesicular monoamine transporter type 2 and vesicular acetyl choline transporter in the cardiac ganglia of rhesus monkeys and humans. Forsgren et al. [16] showed that some ganglionic cells in the subepicardial ganglia in rats revealed IR to DBH and NPY, while they were negative to TH, vasoactive intestinal polypeptide (VIP), calcitonin gene-related peptide (CGRP), SP, and enkephalin. On the other hand, TH-IR was found in the so-called SIF cells. Those cells also showed SP-IR, and some of them were immunoreactive to DBH and CGRP. Hoard et al. [25] studied cultured cardiac neurons from adult mice and found that all of them were cholinergic in nature and 21% of them were immunopositive to TH.

Moravec et al. [43] found that ganglionic cells in epicardial ganglia and around the terminal groove (*sulcus terminalis*) are negative to VIP and TH and immunopositive to NPY and DBH. On the other hand, the bodies of intramural ganglia located between the right and the left branch of the His bundle are highly immunoreactive to TH and DBH.

Studies by Steele et al. [62] on the cardiac ganglia of guinea pigs showed various neuron subpopulations. Many neurons included somatostatin (SOM) with numerous combinations of IR: dynorphin B (DYNB), SP, NPY and NOS. There were also numerous small neuron populations containing a combination of VIP, NPY, DYNB, SP, and NOS. Authors also demonstrated that pericellular baskets of sensory and sympathetic nerve endings were present around ganglionic neurons. They believe it is highly probable that parasympathetic preganglionic transmission from the vagus nerve is modified by sympathetic and sensory neurons as well as by ganglionic interneurons, so that cardiac ganglia are a complex integrating neuronal activity rather than transmitting individual signals. High SOM concentrations are found in various cardiac regions of rats: right atrium, right ventricle, and atrioventricular node [14]. Mawe et al. [39] also demonstrated NOS expression in 5% of cardiac neurons of guinea pigs. This cell subpopulation was immunopositive to choline acetyl transferase (ChAT) at the same time.

The immunohistochemical characteristic of cardiac nerve ganglia in mice showed that 83% of neuronal bodies (of 1,100) were ChAT-positive, while only 4% were TH-positive [55]. At the same time, 14% of ganglia cells were biophenotypic for ChAT and TH. Moreover, both transmitters were present inside nerves reaching the heart. Most ChAT-IR axons reached large cardiac ganglia at the base of the heart and the opening of the vena cava to the right atrium. In turn, most TH-IR fibres reached the dorsal and

abdominal part of the left atrium. SP-IR and CGRP-IR fibres were also present in the epicardium and inside ganglia around the hilum of the heart. SP and CGRP peptides are used to define sensory nerves in the heart [24]. It turns out that sensory receptors for the SP neuropeptide are present not only in neurons, but also on the surface of cardiomyocytes and endothelial cells. Moreover, studies showed the effect of the SP peptide on the depolarisation of cardiac neurons, triggering action potential, and contributing to dilated cardiomyopathy as well as to encephalomyocarditis and viral myocarditis. SP and CGRP proteins can also have a significant counter-regulatory effect in arterial hypertension and blood circulation in coronary vessels [24].

Immunohistochemical studies of Horackova et al. [30] on the intracardiac ganglia of guinea pigs showed that ganglionic neurons can be divided into three subpopulations differentiated by size and the expression of various transmitters. About 80% of neurons located in ganglia had large diameters of up to 40 μm, and about 15% had small diameters below 15 μm. Both groups were immunoreactive to protein gene product 9.5 (PGP 9.5) as well as ChAT-IR, TH-IR, VIP-IR, and SP-IR. The remaining small neurons (about 5%) were immunoreactive to TR, but not to microtubule-associated protein 2 (MAP-2), PGP 9.5, ChAT, and NPY. Moreover, about 10–15% of neurocytes that were loosely distributed instead of clustered in ganglia showed positive IR to TH, ChAT, VIP, NPY, and SP, but not to MAP-2 and PGP 9.5.

In a study on mice, Maifrino et al. [38] showed that about 10% of over 5,000 cardiac neurons, grouped in three regions, were immunopositive to nicotinamide adenine dinucleotide phosphatise (NADPH). In this subpopulation of neurons, most were monopolar (79%), but there were also some bi- and multipolar neurons. The authors suggest they have various functions.

Parsons et al. [46] showed VIP-immunoreactive fibres in 70% cardiac ganglia (intrinsic) in guinea pigs. VIP occurred together with NOS, while it was deficient in ChAT, TH, and SP-positive fibres. On the other hand, about 3% of cardiac neurons showed the coexistence of ChAT and VIP and well as VIP and NOS. The authors also suggest that those fibres come from sensory vagal ganglia.

Many studies describing the presence of cocaine- and amphetamine-related transcript peptide (CART) proteins in cardiac ganglia have been conducted recently [10, 18, 19, 53, 54, 58]. The CART neuropeptide is also known as an anorexigen from other areas in the nervous system, mostly the hypothalamus, although there is an increasing number of reports of yet another function as a neurotransmitter in the ANS. It was found in nerve endings in the gastro-intestinal tract and in parasympathetic ganglia. Most authors describe the presence of the CART neuropeptide in cardiac nerve fibres; however, there are also reports of it being found in cardiac neurons. In guinea pigs, CART was found in vagus nerve endings in the heart and when it was injected in the solitary tract nucleus, baroreflex was impaired and bradycardia ensued [58]. Calupa et al. [10] demonstrated that most (but not all) cardiac ganglia in guinea pigs were innervated by nerve fibres immunoreactive to CART. Only few neurocytes in cardiac ganglia were positive to CART. They also contained ChAT or NOS. Gonsalves et al. [19] demonstrated that immunoreactive neurons are found in the stellate ganglion and the superior cervical ganglion and they project to the heart, mostly to vasoconstrictor neurons. Hasan and Smith [23] found that the sympathetic regulation of the parasympathetic neurochemical phenotype and the synthesis of neurotrophins can play a role in cardiac dysregulation and in other pathophysiological circumstances. Similar conclusions were reached in the study by Armour [3]. The data correspond with the results of the study by Hopkins et al. [29]. Studying the hearts of patients with ischaemia, the authors found that 35% of 473 studied cardiac ganglions had pathological lesions identifiable in light and electron miscroscopy. Ricardson et al. [54] observed IR to CART in 46% of cardiac neuron bodies in rats, some of which also contained NOS or calbindin. Around ca. 10% of CART-positive neurons, nerve endings containing SOM, coexisting with ChAT, were found. Somatostatin is a neurotransmitter participating in cardiac regulation. It was also identified as a possible neurotransmitter, which acts together with acetylcholine in decelerating the heart action in frogs [11]. The inhibitory function of SOM was demonstrated in relation to the contraction of the myocardium in humans [62]. Day et al. [14] demonstrated that various regions of the heart in rats (right atrium, right ventricle, atrioventricular node) contain a high concentration of SOM. Its presence was also found in a small number of vagus nerve endings in guinea pigs [58] and in numerous cardiac neuron subpopulations [62].

In turn, another endogenous neuropeptide, pituitary adenylate cyclase-activated peptide (PACAP), can increase heart excitability. This was demonstrated by Tompkins et al. [63] in *in vitro* studies regarding intrinsic cardiac neurons. The exogenous administration of this neuropeptide increased the excitability of cardiac neurons. The authors suggest that, *in vivo*, PACAP can be released from postganglionic endings of the vagus nerve and act to regulate heart excitability. VIP, another neuropeptide from the same group as PACAP, has a similar effect. The exogenous administration of VIP to isolated heart leads to tachycardia, like in the case of PACAP [27].

SUMMARY

The above report indicates that cardiac ganglia contain various populations of neurons. They are reached by preganglionic parasympathetic fibres from both vagus nerves and postganglionic sympathetic fibres from the sympathetic trunk, mostly from both stellate ganglia, as well as from superior cervical ganglion, middle cervical ganglion, and 10 thoracic ganglia [24, 45, 64]. Moreover, the cardiac plexus also includes sensory fibres in the vagus nerve, originating in the inferior ganglion (*ganglion nodose*) and in parasympathetic fibres, originating in spinal ganglia [3]. Some authors also describe the presence of sensory neurons in cardiac ganglia [3, 13] as well as interneurons [41] and sensory fibres coming from the *nucleus ambiguous* [1]. Neurons forming cardiac ganglia can be divided into several subpopulations varying morphologically, neurochemically, and functionally. The largest group are cholinergic neurons, releasing acetylcholine as the main neurotransmitter. Neurostimulation from those neurons causes the hyperpolarisation of cardiomyocytes and the preganglionic inhibition of sympathetic neurotransmitters, further leading to the deceleration of heart rate. The second group of cardiac neurons are cells of dual, cholinergic-adrenergic, character, with acetylcholine as a neurotransmitter, but also with the expression of enzymes necessary for noradrenaline synthesis, but with no ability to store it. These likely play an important role in pathophysiological processes. There are also small aggregations of SIF cells, of typically adrenergic character. The situation is also complicated by colocalisation and cotransmission, that is the presence of various neurotransmitters in various combinations and representing various functional groups (acetylcholine and TH, DBH, ChAT and NPY, ChAT and CART, CART and NOS, CART and CALB, as well as PACAP and NADPH). It is also possible that other substances participate in ganglionic transmission and neuromodulation. Therefore, neurons in cardiac ganglia are not phenotypically and functionally homogenous. They are a neurochemical complex beyond the classical vision of parasympathetic cardiac ganglia. This is confirmed by various studies, for example by the study of Hoard et al. [24], who demonstrated that cholinergic nerve fibres in the atria are often superimposed on noradrenergic fibres, so mutual 'listening to impulse transmission' is possible. Therefore, the control and regulation of heart rate by the nervous system is a complex process, converting and modulating both exogenous and endogenous interneuronal information that finally reaches cardiomyocytes.

REFERENCES

1. Ai J, Epstein PN, Gozal D, Yang B, Wurster R, Cheng J (2007) Morphology and topography of nucleus ambiguous projections to cardiac ganglia in rats and mice. Neuroscience, 149: 845–860.
2. Akamatsu FE, De-Souza RR, Liberti EA (1999) Fall in number of intracardiac neurons in aging rats. Mech Ageing Dev, 109: 153–164.
3. Armour JA (1999) Myocardial ischaemia and the cardiac nervous system. Cardiocasc Res, 41: 41–54.
4. Armour JA, Hopkins DA (1990) Activity of in situ canine left atrial ganglion neurons. Am J Physiol, 259: 1207–1215.
5. Armour JA, Murphy DA, Yuan BX, Macdonald S, Hopkins DA (1997) Gross and microscopic anatomy of the human cardiac nervous system. Anat Rec, 247: 289–298.
6. Arora RC, Waldmann M, Hopkins DA, Armour JA (2003) Porcine intrinsic cardiac ganglia. Anat Rec A Discov Mol Cell Evol Biol. 27: 249–258.
7. Baluk P, Gabella G (1990) Some parasympathetic neurons in the guinea-pig heart express aspects of the catecholaminergic phenotype in vivo. Cell Tissue Res, 261: 275–285.
8. Baptista CA, Kirby ML (1997) The cardiac ganglia: cellular and molecular aspects. Kaohsiung J Med Sci, 13: 42–54.
9. Batulevicius D, Pauziene N, Pauza DH (2003) Topographic morphology and age-related analysis of the neuronal number of the rat intracardiac nerve plexus. Ann Anat, 185: 449–459.
10. Calupa MA, Locknar SA, Zhang L, Harrison TA, Hoover DB, Parson RL (2001) Distribution of cocaine- and amphetamine regulated transcript peptide in the guinea pig intrinsinc cardiac nervous system and colocalization with neuropeptides or transmitter synthetic enzymes. J Comp Neurol, 439: 73–86.
11. Campbel G, Jackson F (1985) Independent co-release of acetylcholine and somatostatin from cardiac vagal neurones in toad. Neurosci Lett, 60: 47–50.
12. Caralesu FR, Luis AJ (1967) Topography of numerical distribution of intracardiac ganglion cells in the cat. J Comp Neurol, 131: 55–66.
13. Cheng Z, Powley TL, Schwaber JS, Doyle FJ (1997) Vagal afferent innervations of the atria of the rat heart reconstructed with confocal microscopy. J Comp Neurol, 381: 1–17.
14. Day SM, Gu SL, Polak JM, Blum SR (1985) Somatostatin in the human heart and comparison with guinea pig and rat heart. Br Heart J, 53: 153–157.
15. Edwards FR, Hirst GHS, Klemm MF, Steele PA (1995) Different types of ganglion cell in the cardiac plexus of guinea pigs. J Physiol, 486: 453–471.
16. Forsgren S, Moravec M, Moravec J (1990) Catecholamine synthesizing enzymes and neuropeptides in rat heart epicardial ganglia an immunohistochemical study. Histochem J, 22: 667–676.
17. Gagliardi M, Randal WC, Bieger D, Wurster RD, Hopkins DA, Armour JA (1988) Activity In neurones In the In situ panine heart. Am J Physiol, 255: 789–800.
18. Girard BM, Yound BA, Buttolph TR, Locknar SA, White SL, Parson RL (2006) Trophic factor modulation of cocaine- and amphetamine regulated transcript peptide expression in explants cultured guinea-pig cardiac neurons. Neuroscience, 139: 1329–1341.

19. Gonsalves DG, Kerman IA, McAllen RM, Anderson CR (2010) Chemical coding for cardiovascular sympathetic preganglionic neurons in rat. J Neurosci, 30: 1781–1791.
20. Hancock JC, Hoover DB, Hougland MW (1987) Distribution of muscarine receptors and acetylocholinesterase in the rat heart. J Auton Nerv Syst, 19: 59–66.
21. Hardwick JC, Mawe GM, Parsons RL (1995) Evidence for afferent fiber innervations of parasympathetic neurons of the guinea-pig cardiac ganglion. J Auton Nerv Syst, 53: 166–174.
22. Hasan W (2013) Autonomic cardiac innervations. Development and adult plasticity. Organogenesis, 9: 176–193.
23. Hasan W, Smith PG (2009) Modulation of rat parasympathetic cardiac ganglion phenotype and NGF synthesis by adrenergic nerves. Autonomic Neuroscience, 145: 17–26.
24. Hoard JL, Hoover DB, Mabe AM, Blakely RD, Feng N, Paolocci N (2008) Cholinergic neurons of mouse intrinsic cardiac ganglia contain noradrenergic enzymes, norepinephrine transporters, and the neurotrophin receptors TrkA and p75. Neuroscience, 156:129–142.
25. Hoard JL, Hoover DB, Wondergem R (2007) Phenotypic properties of adult mouse intrinsic cardiac neurons maintained in culture. Am J Physiol Cell Physiol, 293: C1875–1883.
26. Hoover DB, Ganote CE, Ferguson SM, Blakely RD, Parsons RL (2004) Localization of cholinergic innervation in guinea pig heart by immunohistochemistry for high-affinity choline transporters. Cardiovasc Res, 62: 112–121.
27. Hoover DB, Tompkins JD, Parsons LR (2009) Differential activation of guinea pig intrinsic cardiac neurons by the PAC1 agonists maxadilan and pituitary adenylate cyclase-activating polypeptide 27 (PACAP 27). J Pharmacol Exp Ther, 331: 97–203.
28. Hopkins DA, Armour JA (1984) Localization of sympathetic postganglionic and parasympathetic preganglionic neurons which innervate different regions of the dog heart. J Comp Neurol, 229: 186–198.
29. Hopkins DA, MacDonalds SE, Murphy DA, Armour JA (2000) Patology of intrinsic cardiac neurons from ischemic human hearts. Anat Rec, 259: 424–236.
30. Horackova M, Armour JA, Byczko Z (1999) Distribution of intrinsinc cardiac neurons in whole-mount guinea-pig atria identified by multiple neurochemical coding. Confocale microscopic study. Cell Tissue Res, 297: 409–421.
31. Kimura K, Ieda M, Fukuda D (2012) Development, maturation and transdifferentiation of cardiac sympathetic nerves. Circ Res, 110: 325–336.
32. Kuder T, Tekieli A (1999) Cardiac ganglia of Japanese quail: distribution and morphology. Ann Anat, 181: 467–473.
33. Kuder T, Tekieli A (2000) Gross and light microscopic studies on the mouse cardiac parasympathetic nervous system. Zool Pol, 45: 157–167.
34. Kuder T, Nowak E, Szczurkowski A, Kuchinka J (2003) A comparative study on cardiac ganglia in midday gerbil, Egyptian spiny mouse, chinchilla laniger and pigeon. Anat Histol Embryol, 32: 134–140.
35. Kuo D, Oravitz JJ, DeGroat WC (1984) Tracing of afferent and efferent pathways in the left inferior cardiac nerve of the cat using retrograde and transganglionic transport of horseradish peroxidase. Brain Res, 321: 111–118.
36. Leger J, Croll RP, Smith FM (1999) Regional distribution and extrinsic innervation of intrinsic cardiac neurons in the guinea pig. J Comp Neurol, 407: 303–17.
37. Mabe AM, Hoard JL, Duffourfc MM, Hoover DB (2006) Localization of cholinergic innervations and neutrin receptors in adult mouse heart and expression of the neutrin gene. Cell Tissue Res, 326: 57–67.
38. Maifrino LBM, Liberti EA, Castelucci P, De Souza RR (2006) NADPH-Diaphorase positive cardiac neurons in the atria of mice. A morphoquantitative study. BMC Neuroscience, 2: 7–10.
39. Mawe GM, Talmage EK, Lee KP, Parsons RL (1996) Expression of choline acetyltransferase immunoreactivity in guinea pig cardiac ganglia. Cell Tissue Res, 285: 281–286.
40. Marvin WJ, Hermsmeyer JK, McDonald RI, Roskoski LM, Roskoski R (1980) Ontogenesis of cholinergic innervations in the rat heart. Circ Res, 46: 690–695.
41. McAllen RM, Salo LM, Paton JFR, Pickering AE (2011) Processing of control and relllex vagal drives by rat cardiac ganglion neurons: an intracellular analysis. J Physiol, 589: 5801–5818.
42. Mo N, Wallis DI, Watson A (1994) Properties of putative cardiac and non-cardiac neurons in the rat stellate ganglion. J Auton Nerv Syst, 47: 7–22.
43. Moravec M, Moravec J, Forsgren S (1990) Catecholaminergic and peptidergic nerve components of intramural ganglia in the rat heart. Cell Tiss Res, 262: 315–327.
44. Navaratnam V (1965) The ontogenesis of cholinesterase activity within the heart and cardiac ganglia in man, rat, rabbit and guinea pig. J Anat, 99: 459–467.
45. Pardini BJ, Lund DD, Schmid PG (1998) Organisation of the sympathetic postganglionic innervations of the rat heart. J Auton Nerv Syst, 28: 193–201.
46. Parsons RL, Locknar SA, Young BA, Hoard JL, Hoover DB (2006) Presence and co-localization of vasoactive intestinal polypeptide with neuronal nitric oxide synthase in cells and nerve fibres within guinea pig intrinsic cardiac ganglia and cardiac tissue. Cell Tissue Res, 323: 197–209.
47. Parsons RL, Neel DS, McKeon TW, Carraway RE (1987) Organization of a vertebrate ganglion: a correlated biochemical and histochemical study. J Neurosci, 7: 837–846.
48. Pauza DH, Pauziene N, Pakeltyte G, Stropus R (2002) Comparative quantitative study of the intrinsic cardiac ganglia and neurons in the rat, guinea pig, dog and human as revealed by histochemical staining for acetylcholinesterase. Ann Anat, 184: 125–136.
49. Pauza DH, Skripka V, Pauziene N, Stropus R (2000) Morphology, distribution, and variability of the epicardiac neural ganglionated subplexuses in the human heart. Anat Rec, 259: 353–382.
50. Pauza D.H. Scripka V, Pauziene N, Stropus R (1999) Anatomical study of the neuronal ganglionated plexus in the canine right atrium: implication for selective denervation and electrophysiology of the sinoatrial node in dog. Anat Rec, 225: 271–294.
51. Pauza DH, Skripkiene G, Scripka V, Pauziene N, Stropus R (1997) Morphological study of neurons in the nerve plexus on the heart base of rats and guinea pigs. J Auton Nerve Syst, 62: 1–12.
52. Richardson RJ, Grkovic I, Allen AM, Anderson CR (2006a) Separate neurochemical classes of sympathetic postganglionic neurons project to the left ventricle of the rat heart. Cell Tissue Res, 324: 9–16.
53. Richardson RJ, Grkovic I, Anderson CR (2003) Immunohistochemical analysis of intracardiac ganglia of the rat heart. Cell Tissue Res, 314: 337–350.
54. Richardson RJ, Grkovic I, Anderson CR (2006b) Cocaine- and amphetamine-related transcript peptide and somatostatin in rat intracardiac ganglia. Cell Tissue Res, 324: 17–24.
55. Rysevaite K, Saburkina I, Pauziene N, Vaitkevicius R, Noujaim S, Jalife J, Pauza DH (2011a) Immunohistochemical characterization of the intrinsic cardiac neuronal plexus in whole-mount mouse heart preparations. Heart Rhytm, 8: 731–738.
56. Rysevaite K, Saburkina I, Pauziene N, Noujaim S, Jalife J, Pauza DH (2011b) Morphologic pattern of the intrinsic ganglionated nerve plexus in the mouse heart. Heart Rhytm, 8: 448–454.
57. Saburkina J, Gukauskiene L, Rysevaite K, Brack KE, Pauza AG, Pauziene N, Pauza DH (2014) Morphological patern of intrinsic nerve plexus distributed on the rabbit heart and interatrial septum. J Anat, 224: 583–593.
58. Scruggs P, Dun SL, Dun NJ (2003) Cocaine- and amphetamine-related transcript peptide attenuates phenylephrine-induced bradycardia in anesthetized rats. Am J Physiol Regul Integr Comp Physiol, 285: 1496–1503.
59. Shvalev VN, Sosunov AA (1985) A light and electron microscopic study of cardiac ganglia in mammals. Z Mikrosk-Anat Forsch, 99: 676–694.
60. Singh S, Johnson PI, Lee RE, Ortei E, Lonchyna VA, Sullivan HJ, Montoya A, Tran H, Wehrmacher Wh, Wurster RD (1996) Topography of cardiac ganglia in the adult heart. J Thorac Cardiovasc Surg, 112: 943–953.
61. Slaviková J, Kuncová J, Reischig J, Dvořaková M (2003) Catecholaminergic neurons in the rat intrinsic cardiac nervous system. Neurochem Res, 28: 593–598.
62. Steele PA, Gibbins IL, Morris JL, Mayer B (1994) Multiple populations of neuropeptide containing intrinsic neurons in the guinea-pig heart. Neuroscience, 62: 241–250.
63. Tompkins JD, Ardell JL, Hoover DB, Parson RL (2007) Neurally released pituitary adenylate cyclase-activiting polypeptide enhances guinea pig intrinsic cardiac neurone excitability. J Physiol, 582: 87–93.
64. Wallis D, Watson AH, Mo N (1996) Cardiac neurones of autonomic ganglia. Microsc Res Tech, 35: 69–79.
65. Weihe E, Schutz B, Hartschuh W, Anlauf M, Schafer MK, Eiden LE (2005) Coexpression of cholinergic and noradrenergic phenotypes in human and nonhuman autonomic nervous system. J Comp Neurol, 492: 370–378.
66. Woźniak W, Grzymisławska M, Łupicka J (2009) The first appearance of sympathetic ganglia in human embryos at stage 13. Folia Morphol, 68: 215–217.
67. Yasuhara O, Matsuo A, Bellier JP, Aimi Y (2007) Demonstration of choline acetyltransferase of a peripheral type in the rat heart. J Histochem Cytochem, 55: 287–299.
68. Yuan BX, Ardell JL, Hopkins DA, Losier AM, Armour JA (1994) Gross and microscopic anatomy of the canine intrinsic cardiac nervous system. Anat Rec, 239: 75–87.