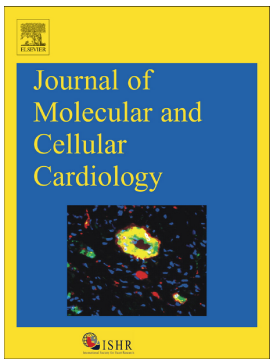

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A brain within the heart: a review on the intracardiac nervous system

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ABSTRACT

Cardiac function is under the control of the autonomic nervous system, composed by the parasympathetic and sympathetic divisions, which are finely tuned at different hierarchical levels. While a complex regulation occurs in the central nervous system involving the insular cortex, the amygdala and the hypothalamus, a local cardiac regulation also takes place within the heart, driven by an intracardiac nervous system. This complex system consists of a network of ganglionic plexuses and interconnecting ganglions and axons. Each ganglionic plexus contains numerous intracardiac ganglia that operate as local integration centres, modulating the intricate autonomic interactions between the extrinsic and intracardiac nervous systems. Herein, we summarize the current understanding on the intracardiac nervous system, and acknowledge its role in the pathophysiology of cardiovascular diseases.

Keywords: Autonomic nervous system, intracardiac nervous system, intracardiac ganglia, intracardiac neurons, cardiovascular diseases.

1. Introduction

Cardiac innervation has been studied for over 200 years and the first paper on the area is dated to 1794 by Scarpa. Such enthusiasm on the study of the intracardiac nervous system (ICNS) is due to its important role in several domains of cardiac physiology such as heart rhythm, conduction system and even coronary circulation [1,2]. In recent decades, strong evidence was collected suggesting that intracardiac neurons significantly modulate several domains of heart function.

Briefly, intracardiac neurons can be divided into several subpopulations according to their morphology, neurochemistry and function [3-5]. This diversity results in the complex array of neurons forming the intracardiac ganglia (ICG). Each ICG is composed by 200 to 1000 intracardiac neurons in humans, strongly suggesting that each acts as a major local integration center of the ICNS [4,6]. Groups of ICG are typically associated with interconnecting nerves forming ganglionated plexi (GP). These are gathered in specific regions of the heart including the atria, the sinoatrial (SA) and atrioventricular (AV) nodes, the interventricular septum and both ventricles. Each group of ICG contains efferent, sensory and local circuit neurons that control multiple cardiac properties [7]. The interconnections between individual ganglia, alongside the interactions between neurons within ICG, provide an anatomical and functional basis of the complex nervous network of the heart, also called its "little brain" [8,9].

Despite the current understanding about the complexity of ICNS, little is known about how this intrinsic system modifies cardiac performance, and how it can underpin cardiovascular disease. In this paper, we will summarize the recent advances on the ICNS, to better understand its complexity, and to grasp concepts fundamental for the development of new therapeutic strategies in the treatment of debilitating cardiac diseases.

2. The development of cardiac autonomic innervation

During human embryogenesis, the development of cardiovascular and nervous systems is simultaneous. While the nervous system originates from neuroectoderm, the cardiovascular system is mainly derived from the mesoderm [10]. The development of the autonomic heart innervation can be divided into four distinct phases: (a) migration of neural crest cells (NCC) to the dorsal aorta, (b) differentiation of NCC into neurons, (c) aggregation/migration of neurons to form the paravertebral sympathetic chains and the parasympathetic cardiac ganglia, and (d) extension of axonal projections into cardiac tissue and terminal differentiation [11-13] (figure 1).

Although the primitive human heart starts to contract in the 21st–22nd day of development, heart development continues until the 50th day and it is during the fifth week, that NCC begins migrating to the heart [12]. NCCs are originated from the dorsal part of the closing neural tube under the ectoderm, creating the parasympathetic and sympathetic nervous systems (SNS) [3,12]. Although autonomic cardiac neurons have a common origin at the neural crest, they undergo a distinct

developmental differentiation as they mature toward their adult phenotype. The migration of NCCs is conditioned by distinct factors: while cardiac glial cell line–derived neurotrophic factor, neurturin and artemin play a role in the survival of parasympathetic neurons, the nerve growth factor (NGF), brain-derived neurotrophic factor, neurotrophin-3 and neurotrophin-4/5 are essential for the survival and outgrowth of sympathetic axons [3,12,14-18]. The sympathetic nerve fibers originate in the thoracic segment, from the sympathetic trunk cells, whereas neurons of the parasympatethic trunk come from the neural crest to the thoracic segment [16]. Contrary to sympathetic ganglia, the NCCs destined to create the parasympathetic cardiac ganglia do not migrate only in the rostral part of the somites, being also able to run through the somites or laterally to them. These NCCs enter the heart along pathways traced by the vagus nerve and do not aggregate in the dorsal aorta [19]. Chronologically, in mammalian embryos, the development of parasympathetic cardiac innervation through NCCs precedes sympathetic innervation, and it becomes functional first. Navaratnam and colleagues [20] showed that true cholinesterase first appears in the cytoplasm of cardiac nerve cells in humans between the 4th and 7th month of gestation and in the rat on the 4th day of postnatal life. In parallel, Marvin and colleagues [21] found that the activity of acetylcholine synthesis enzymes in rats appears on the 19th day of development.

A short note to highlight that in addition to the sympathetic and parasympathetic nerves, which can be regarded as the motor components of the ANS, the sensory nerves originate in the sensory ganglionic cells of the vagus nerve (ganglion nodose), which in turn originate in the ectodermal plaque (vagus nerve placodes) [16]. Several growth factors released from the cardiac tissue are essential for the appropriate development of the sensory peripheral nervous system that innervates the heart.

3. The intracardiac nervous system: organization, morphology and function

The ICNS is a neural network located in the heart that acts as an integration site for the regulation of the autonomic efferent cardiac output [3,7]. This intrinsic system is believed to be a short cardio-cardiac feedback control loop within the cardiac regulation hierarchy, adapting an appropriate response to vascular impedance and body metabolic demands. This hierarchy includes neural networks located not only in the heart, but also in intrathoracic extracardiac ganglia and in the central nervous system (CNS), such as the IC (figure 2) [22,23].

The structural organization of the ICNS has been described in several animal and human studies [24, 25-27]. Concerning the later, is has been purposed that the whole ICNS may be subdivided into seven epicardic neural ganglionated subplexuses: the dorsal right atrial (DRA), ventral right atrial (VRA), left dorsal (LD), ventral left atrial (VLA), middle dorsal (MD), right coronary (RC) and left coronary (LC) plexuses [24]. The ganglionated subplexuses are epicardiac extensions of mediastinal nerves that penetrate the heart through the heart hilum and separately innervate the various regions of the heart. The extrinsic cardiac nerves can access the heart arterially, around the roots of the pulmonary

artery and the aortic root, or through the venous region of the heart hilum, around the roots of the pulmonary veins and the superior vena cava. The LC and RC subplexuses extend from the arterial region to the effector sites on the left and right ventricles, respectively. The remaining five subplexes originate from the venous region around the pulmonary veins: the DRA subplexus can originate either from the superior vena cava or the right caudal vein, and branch to supply the SA node and the dorsal region of the right atrium. The MD subplexus branches between the pulmonary veins towards the dorsal coronary groove with neuronal connections with the LD subplexus, ending on the dorsal left atrial and ventricular regions. The neurons constituting the ventral subplexuses are ventrally directed to the left pulmonary veins forming ICG on the VLA region, and connect the ventral right atrium to the ventro-medial region around the superior vena cava, constituting the VRA subplexus [6]. Along these lines, the node is supplied by abundant postganglionated nerves derived both from the VRA and DRA subplexuses, while the intrinsic nerves that innervate the region of the AV node originate mainly from the MD subplexus, and from the LD subplexus in part. Thus, the human right atrium is innervated by two subplexuses, the left atrium by three, the right ventricle by one, and the left ventricle by three. It was also established that this topography is consistent from heart to heart in post-mortem studies (figure 3), but the structural organization of ICG and intracardiac neurons within subplexuses varies considerably from heart to heart and is greatly affected by age [24,28]. The different regions of the heart may be supplied by nerves from the distinct subplexuses or a sole subplexus. It was proposed, based on the architecture of the neural subplexuses, that specific denervation of the discrete heart regions and/or total denervation of the heart is possible via the destruction of the selected neural subplexuses [24,26].

The location, shape and size of the ICG vary significantly across species. The number of ICGs is also species dependent, being suggested by some authors that the number of ICGs may be correlated with heart activity and body size, although this correlation is not direct [4,24,29]. In large animals, specifically in human, approximately 700–1500 ICGs were identified in the epicardium of hearts, mostly around strategic regions of the heart: the base of the heart as well as in the region of the interatrial, coronary, and interventricular grooves [4,24]. This location of ICGs coincides partly with the cardiac conduction system. The free wall of the right atrium, atrial appendages, trunk of the great vessels, and most of the ventricular myocardium are believed to be devoid of ICGs [9,29].

The ICG neural network comprises a heterogeneous population of neurons made up of autonomic (parasympathetic and sympathetic) efferent neurons, local circuit neurons, and afferent neurons (figure 4) [30,22]. Within the ICG, the autonomous efferent neurons can be activated by local or chemical stimuli, subsequently transmitting this information to local circuit neurons and/or autonomous efferent neurons in order to convey an appropriate modulatory response. The autonomic efferent neurons can still receive sympathetic and parasympathetic preganglionic information from the higher neuronal hierarchy [23,31]. In addition, circulating catecholamines may also exert direct effects on ICNS affecting the cardiac motor output to the heart [23].

In humans, the cardiac sympathetic preganglionic neurons are located in the upper thoracic spinal cord, projecting to synapse with postganglionic neurons in the stellate ganglia and in the inferior/middle cervical ganglion. The cell bodies of most postganglionic sympathetic neurons are in these ganglia, and their axons form the superior, middle, and inferior cardiac nerves that terminate on the surface of the heart. A few postganglionic neurons have been described in the myocardium [32]. Neurons can communicate with each other by establishing direct gap junction connections, or by releasing neurotransmitters. The sympathetic neurons essentially use norepinephrine (NE) as their main neurotransmitter, although other neuropeptides such as the neuropeptide Y (NPY), galanin (GAL), adenosine triphosphate (ATP), serotonin and histamine are also co-released from the sympathetic terminals [3,12,33,34]. Regarding catecholamines, its anabolic pathway depends on tyrosine hydroxylase (TH), which converts tyrosine to L-3,4- dihydroxyphenylanine (L-DOPA), and dopamine β-hydroxylase, which hydroxylates dopamine to produce NE. Catecholamines released from sympathetic nerve terminal increase chronotropism and inotropism by the activation of β1 adrenergic receptors (β1AR) on SA node cells and cardiac myocytes [35]. These events are due to the increase of the intracellular levels of cyclic adenosine monophosphate (cAMP) when NE binds to cardiac β1AR, which in turn accelerates diastolic depolarization by increasing the "funny" cation current gated by hyperpolarization-activated cyclic nucleotide-gated channels [36]. This results in the shortening of the refractory period, almost linearly with the frequency of sympathetic postganglionic discharge, which promotes ventricular contractility and relaxation rate [37]. Among other functions, NPY and GAL reduce the release of acetylcholine from adjacent parasympathetic terminals [11,38,39]. On the other hand, high cardiac sympathetic drive also increases the inotropic state of the heart, since it promotes myocyte calcium influx [40]. Excessive sympathetic activity may result from numerous mechanisms: activation of descending sympathoexcitatory pathways, disinhibition of sympathoexcitatory reflexes, loss of baroreflex buffering, sympathetic activation by hypoxia or ischemia, or loss of inhibitory GABAergic control at diencephalic, brainstem or spinal levels [41-44].

The parasympathetic preganglionic neurons involved in the control of cardiac function are located in the nucleus ambiguus of the medulla oblongata, and to a much lesser extent in the dorsal motor nucleus of the vagus nerve, and the region between these two nuclei. The vagus nerve further divides into the superior and inferior cardiac nerves, finally merging with the postganglionic sympathetic neurons to form a complex set of epicardial GP, which display functional selectivity for the effects on refractory period, conduction time and contractility, and receive synaptic contacts by local sensory neurons [45,46]. Thus, in the parasympathetic system, preganglionic neurons are generally longer than postganglionic neurons. Acetylcholine (ACh) is the principal neurotransmitter in these cardiac ganglion neurons, however, neuropeptides such as vasoactive intestinal polypeptide (VIP) and nitric oxide may also be co-released from parasympathetic terminals [37,47]. Acetylcholine binds to M2 ACh muscarinic receptors, which decreases cAMP levels in myocardial cells. In addition, these receptors also open specific potassium channels that gate a hyperpolarizing current, particularly

under conditions of marked parasympathetic activity [48]. Parasympathetic attenuation of heart rate is effected primarily through hyperpolarization of nodal tissue, both SA and AV [12].

Finally, the intracardiac local circuit neurons are also part of ICG, receiving inputs from both sympathetic and parasympathetic efferent preganglionic neurons. The ability of these neurons to synapse into adjacent neurons of the same ICG, or to project to neurons in other ICGs, represents an undeniable source of computational ability endowed within the ICNS and the eventual influence of cardiodynamics [23,49,50]. The remodeling of the cardiac neuronal hierarchy can initiate or exacerbate heart disease. After a myocardial infarction, the intracardiac neurons perfused by an obstructed coronary artery can undergo pathological alterations, compromising their function [51]. Thus, in ischemic myocardium, the activity of the afferent nerves are generally reduced, whereas intracardiac local circuit neurons become hyperactive to transduce both afferent and efferent inputs from the remaining healthy myocardium [52], stabilizing the function cardiac efferent neurons in the presence of excessive sensorial inputs [53]. On the other hand, excessive activation of the intracardiac local circuit neurons involved in the transduction of afferent signals into mechanical efferent effects plays a role in the induction of atrial fibrillation [54].

3.1. Morphology of Intracardiac Ganglia and Neurochemical Phenotype of Intracardiac Neurons

Regarding the shape of the ICGs, there is a significant polymorphism, both between species and between individuals. ICGs may be oval, spherical, fusiform, or elongated [3]. Pauza et al. [26] studied cardiac ganglia using immunohistochemistry (acetylcholinesterase) in several mammalian species and although the examined ICG varied in their shape and size, with respect to 3D shape, they divided them in two main morphological types of ganglia: spherical (globular) and straight (plain). In spherical ganglia, neurons were densely packed in layers and contained no more than 100-200 neurons, occupying a small area ranging from 0.01 to 0.17 mm^2. The straight ganglia are composed of several neurons arranged linearly side by side, not involving more than 50 somatic neurons. Neuronal somata have a diameter of 15–30 µm and 20–45 µm in size on the short and long axis, like in most autonomous ganglia [3,26,55]. Intracardiac neurons have their nuclei usually located in their periphery, containing a prominent nucleolus [4]. On the other hand, according to Horackova et al., morphologically the intracardiac neurons display a variety of morphologies: unipolar (afferent), bipolar and multipolar, being most frequently bipolar and only rarely unipolar [56,57]. Bipolar neurons were spherical or straight, with two neurites originating from each pole and usually branching further into a few additional neurites. Unipolar neurons were generally spherical and typically had a single long, prominent neurite that did not branch for a relatively long distance.

As will be discussed below, intracardiac neurons display several morphological alterations during cardiac diseases and aging. The area of ICGs, intracardiac neurons and their nuclei increases with age. On the other hand, there is a decrease in satellite cell frequency, neuronal packing density and the area occupied by nerve cells within the ICG. Thus, it is suggested that aging-related changes influence

cardiac activity, such as reduced chronotropic responses in the elderly caused by a significant reduction in the number of intracardiac neurons [58-59].

Although for many years it was believed that only parasympathetic postganglionic neurons existed in the cardiac ganglia, several studies have shown that the nervous system in the mammalian heart contains populations of different neurons [60-62]. Some authors propose a chemical variety of cardiac neurons may represent their various functional groups [12,58]; ganglionic cardiac neurons are heterogeneous and include two main types of transmitters: cholinergic and adrenergic [2,56,63]. It is believed that intrinsic cardiac neurons are mainly cholinergic, releasing acetylcholine as the main neurotransmitter and playing an inhibitory role in cardiac regulation. This fact was evidenced by Horackova et al. [56], who documented the presence of cholinergic, adrenergic and peptidergic (putative sensory) nerve fibers and neurons using double Immunohistochemical labeling. This study showed that the vast majority (~75-100%) of the intracardiac neurons were choline acetyltransferase-positive and only about 10% were tyrosine hydroxylase-positive [2, 12, 56]. The second group of cardiac neurons most prevalent (14%) is cells of dual, cholinergic-adrenergic character, releasing acetylcholine as neurotransmitter [64]. These neurons also have the ability to express enzymes necessary for noradrenaline synthesis, although they do not have the capacity to store it. This subpopulation of neurons probably plays an important role in pathophysiological processes [12].

3.2. Plasticity of Cardiac Autonomic Neurons

The compensation capacity of the nervous system, denominated homeostatic plasticity, has been the subject of intense study. The first reported demonstration of neural plasticity used the opposing actions elicited on myocytes by NE and ACh to identify a transition from noradrenergic transmission to cholinergic transmission in sympathetic neurons cultured with cardiac myocytes [65]. In fact, sympathetic neurons can acquire properties of parasympathetic neurons through a process of cellular remodeling by dedifferentiation, transdifferentiation, or both [16]. Recently, this ability has been documented for the plasticity of intrinsic excitability, via changing ionic conductances, and for changes in chemical synaptic strength, which is implicated in several states of cardiovascular disease [66].

Sympathetic hyperactivity, accompanied by reduction in parasympathetic activity and heart rate variability, is increasingly recognized as a characteristic in the pathogenesis of a number of cardiovascular pathologies [67,68]. As an example, chronic ischemic ventricular tissues may induce pathological neuronal remodeling as the peripheral network becomes grossly destabilized due to the tonic nature of erratic ventricular afferent inputs [22]. Such data were evidenced by Cao et al. [69], who observed that acute ventricular myocardial infarction (MI) in the porcine model can lead to sympathetic denervation of the infarcted myocardium and hyperinnervation of the border areas.

4. The role of ICNS in the pathophysiology of CVD

The ICNS is extremely important in the pathophysiology of various cardiovascular diseases (ischemic heart disease, arrhythmias, heart failure (HF), hypertension) and also in circumstances where the CNS innervation to the heart is artificially interrupted (e.g. following heart transplantation). Dysregulation of this complex system, usually results in increased sympathetic activity and decreased cardiac parasympathetic responsiveness, contributing to a negative prognosis in terms of morbidity and mortality [70].

Several studies have shown that different cardiac diseases cause pathological alterations in intracardiac neurons. Ischemic heart disease negatively affects the myocardium due to lack of oxygen, with neural remodeling occurring at the intra-thoracic and CNS levels, and also within intracardiac neurons [22,71]. Hopkins et al. [71] showed that under ischemic conditions, one third of intracardiac neurons display inclusions, a severe enlargement (66×54 µm vs 40×34 µm for normal neurons) and degenerative changes in their dendrites and axons. In addition, studies published in recent years have shown that intracardiac neurons derived from animals submitted to myocardia infarction (MI) have shown enhanced neuronal excitability, altered synaptic efficacy, and adaptive reorganization of neurochemical phenotypes and neuromodulation within the ICNS [72,73]. The ICNS seems to undergo a significant reorganization - both anatomical and functional - essentially within the first 7 days of the course of MI. After MI, peri-infarct inflammatory cells, including macrophages and myofibroblasts, play a key role in producing NGF, leading to nerve sprouting and sympathetic hyperinnervation [73]. The continuous activity of the SNS contributes to myocardial damage and ultimately to systolic dysfunction and fatal arrhythmias.

There is evidence that neurohormonal activation (sympathetic system overactivation and renin-angiotensin-aldosterone axis deregulation) plays a central role in the pathophysiology of HF. In patients with HF, studies have shown a positive correlation between mortality and plasma levels of epinephrine. It is interesting to note that epinephrine is not only released from the sympatho-adrenal system [74], as the heart itself contains a population of neurons synthesizing catecholamines, constituting an intrinsic adrenergic system [5]. According to the literature [75], there are no substantial changes in the function of the ICNS during the early stage of HF. However, the progression of HF causes cardiac and neuronal hypertrophy, is paralleled by neurons markedly edematous and significantly less excitable [76]. These changes affect the communication of neurons, detrimentally disturbing ganglionic function and underpinning the attenuation in GP neurotransmission. In accordance, in a model of HF induced by tachycardia the response of intracardiac neurons to activation of their nicotinic receptors were shown to be altered [27]. Of notice, a significant reduction in the number of intracardiac neurons in patients with HF has been reported [77]. Moreover, some studies suggest that HF reduces the cell excitability of the cardiac postganglionic parasympathetic neurons, while enhances cell excitability in cardiac postganglionic sympathetic neurons. These changes seem to be dependent on the modulation of N-type Ca^{2+} channel [78,79].

Following heart transplantation, the influence of extracardiac neuronal control is eliminated and cardiac activity is modulated by the microcircuits of the intracardiac nerve network. For this reason, the term post-transplant decentralization is used instead of denervation of the heart [80,81]. This condition also causes changes in the membrane properties of intracardiac neurons that may lead to increased excitability, while maintaining synaptic neurotransmission within the GP [82]. This adaptation of intracardiac neurons reflects their endeavor to compensate a loss of modulatory influence of the CNS. In addition, cardiac surgical procedures (e.g. valvular procedures, congenital reconstructions, septal defect repairs) may also alter the activity of ICNS. This fact is related to incisions made through areas densely populated with ICGs [29].

There are several other examples of the role of ICNS in cardiovascular pathologies. One is Chagas's disease, where a great loss of intracardiac neurons was observed [83]. Similarly in diabetes, atrophy the loss of Nissl substance in intracardiac neurons was also verified [31].

Altogether, these data suggests that the intracardiac network is highly sensible to direct cardiac insults. Given that most of the population of this network is parasympathetic [84], we hypothesize that direct insults to the heart such as ischemia, increased filling pressures or hypertension, among others, may lead to a preferential damage of the parasympathetic system that may contribute to the autonomic imbalance observed in patients with cardiovascular diseases. This is important because the development of strategies that tackle directly the ICNS may be more effective than other strategies used to reestablish the parasympathetic drive to the heart (p.e. vagal stimulation).

5. How to study ICNS?

The study of ICNS has faced many obstacles that hamper a faster understanding of its organization and function. For example, the continuous motion of the heart is a challenging barrier to the performance of more advanced electrophysiological studies *in vivo* or in full organ preparations. Therefore it is not surprising that most of these studies are performed using *in vitro* preparations of intracardiac neurons, what imposes several limitations to the interpretation of the respective findings. At this level the patch clamp technique has been widely used to understand the plasticity and function of intracardiac neurons [79,85], since it is the gold standard technique to analyze the electrical properties and functional connectivity of neurons. In addition the patch clamp technique offers the advantage to study simultaneously the function, organization and network of the ICNS. For example, cardiac vagal neurons in the brainstem responsible for the generation of the parasympathetic activity to the heart can be identified with a retrograde marker and studied using patch-clamp electrophysiological recordings *in vitro* [85].

The study of morphology of intracardiac neurons uses the traditional tools used to study other organs/tissues such as histochemistry using antibodies to acetylcholinesterase (parasympathetic nervous system) and tyrosine hydroxylase (adrenergic neurons). A more detailed study can be performed using intracellular markers injected ionophoretically into the somata of neurons [86].

Another strategy to study intracardiac neurons is to explore their neurochemical properties. In fact, the development of more accurate in vitro measurements of neurotransmitters have provided a better understanding about the neurochemistry of ICG [87,88]. Currently, it is possible to monitor *in vivo* pharmacodynamics and quantify the absorption and clearance of specific substances in living tissues using radiolabeled versions of these neurotransmitters [27,89].

In humans, the study of cardiac innervation can be performed using single photon emission tomography (SPECT) with ^{123}I-metaiodobenzylguanidine (MIBG) or using positron emission tomography (PET) with ^{11}C-HydroxyepHedrIne [90,91]. As the sympathetic neuronal tissue is more sensitive to damage than the myocardium it may be an early marker of cardiovascular diseases [90]. ^{123}I-MIBG is a norepinephrine analog internalized by sympathetic terminals and used to evaluate sympathetic neuron integrity and function. During the last 25 years, studies have shown that reduced myocardial ^{123}I-MIBG uptake is an independent predictor of adverse long-term outcome in HF, and improvement in ^{123}I-MIBG uptake is observed in response to effective HF therapy [92,93]. Thus, HF patients with moderate to severely reduced myocardial ^{123}I-MIBG uptake as a reflection of myocardial denervation have the worst outcomes in terms of morbidity and mortality from pump failure, as well as from unstable arrhythmias that frequently precede the occurrence of sudden cardiac death [94,95]. The characterization of tissue content and turnover of norepinephrine in vivo has also been extensively studied using ^{3}H-norepinephrine. Like ^{123}I-MIBG SPECT, PET imaging can also predict the occurrence of lethal arrhythmias, sudden cardiac death, and all-cause mortality in patients with HF with reduced ejection fraction. However, PET imaging offers clear benefits over ^{123}I-MIBG SPECT as higher spatial resolution, more established quantitative methods and a larger variety of ANS radiotracers [90]. The most clinically relevant PET tracer of cardiac sympathetic neurons is ^{11}C-metahydroxyphedrine, a metabolically resistant analog of NE [96].

5.1. Potential strategies for therapeutic interventions targeting ICNS

With the increasing understanding of the autonomic nervous system and its role in the pathophysiology of cardiac diseases, autonomic modulation may play an important role in the treatment of varied cardiovascular diseases [90,97].

Given the importance of the ICNS in the regulation of different dimensions of cardiac function, intracardiac neurons may be potential targets in the treatment of cardiovascular diseases. In fact, recent studies showed promising results concerning the ablation of GP in the left atrium and around the pulmonary veins in the treatment of atrial fibrillation [98].

On the other hand, the modulation of extrinsic cardiac ANS also has a place in the treatment of cardiovascular diseases. New non-invasive treatment options for extrinsic cardiac ANS such as renal denervation and spinal cord stimulation and direct vagal stimulation are all emerging as possible treatments for hypertension and heart failure, respectively, and may provide interesting future treatment possibilities as non-pharmacological antiarrhythmic strategies for atrial and ventricular

arrhythmias [97]. Experimental data on vagal nerve and spinal cord stimulation suggest that each technique may reduce ventricular arrhythmias. Furthermore, the modification of the sympathetic tone through ablation of the stellate ganglion reduces ventricular arrhythmias and its being useful in patients with inherited arrhythmia disorders, such as long QT syndrome and catecholaminergic polymorphic VT, with drug resistant sympathetically induced arrhythmias [99].

6. Conclusion

The autonomic regulation of the heart involves different levels of regulation from the brain to the periphery. Cardiac postganglionic parasympathetic and sympathetic neurons are arranged in ganglia and plexus in specific regions of the heart and definitively play a role in the modulation of cardiac function in physiological and pathological conditions. A better understanding of these neurons may contribute to the development of new markers of disease or new therapeutic targets that may contribute to a continuous improvement in the management of cardiovascular diseases.

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Figures:

Fig 1. Autonomic heart innervation development: (a) migration of neural crest cells (NCC) to the dorsal aorta, (b) differentiation of NCC into neurons and (c) aggregation/migration of neurons to form the paravertebral sympathetic chains and the parasympathetic cardiac ganglia (Parts the illustration were adapted from medical Servier under a CC BY 3.0 license).

Fig 2. Schematic summary of the proposed model of the autonomic control of the heart involving central and peripheral structures. At a peripheral level, it is mainly regulated by the intrinsic nervous system (ICNS). In this diagram the neural connections between the heart and the ICNS are shown, with the cardiac sensory information being transduced by afferent neuronal somata in ICG and intrathoracic extracardiac ganglia through intrathoracic local circuit neurons to cardiac motor neurons. This cardiac sensory information is then centrally transduced to generate longer-loop medullary and spinal cord reflexes. Chemosensory and mechanosensory feedback to the CNS is also provided by afferent neurons with somata distributed throughout the nodose and dorsal root ganglia. The box at the bottom right shows that circulating catecholamines may also exert direct effects on the ICNS affecting the cardiac motor output to the heart. Sympathetic cardiac nerves (red) originate from cervical ganglia, stellate (cervicothoracic) ganglia, and thoracic ganglia. Parasympathetic innervation (yellow) comes from the medullary centres via the vagus nerve. The sensory nerves (blue) project to the dorsal horn of the spinal cord via dorsal root ganglia.
Red lines - sympathetic; Yellow lines - parasympathetic; Blue lines - afferent projections.
B: β-adrenergic receptor; M2: muscarinic receptor.

***** THIS FIGURE SHOULD BE PRINTED WITH COLOR**

Fig 3. Proposed scheme of innervation by the seven ganglionated subplexuses. A: Dorsal view. B. Right lateral view C. Superior view D: Ventral view. DRA: right dorsal atrial; VRA: ventral right atrial; LD: left dorsal; VLA: ventral left atrial; MD: middle dorsal; RC: right coronary; LC: left coronary.

Fig 4. Schematic drawing of an ICG, showing the different intracardiac neurons (autonomic efferent neurons, local circuit neurons and afferent neurons) and their projections.

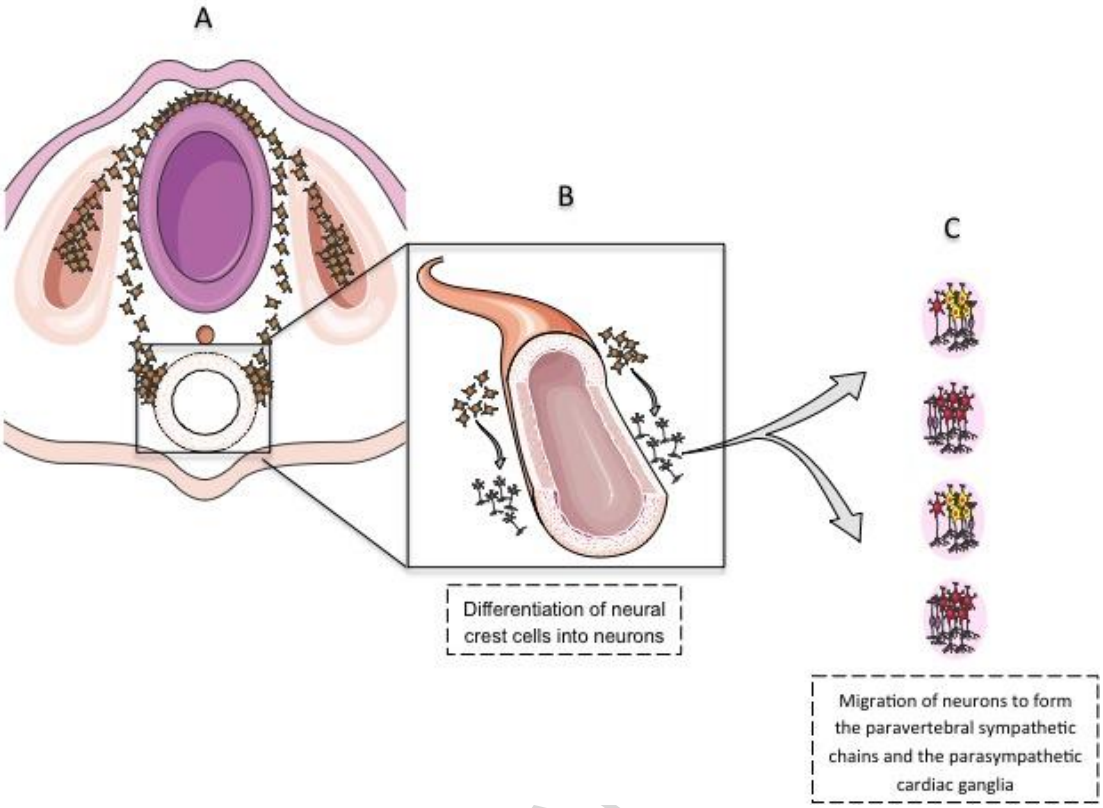

Figure 1

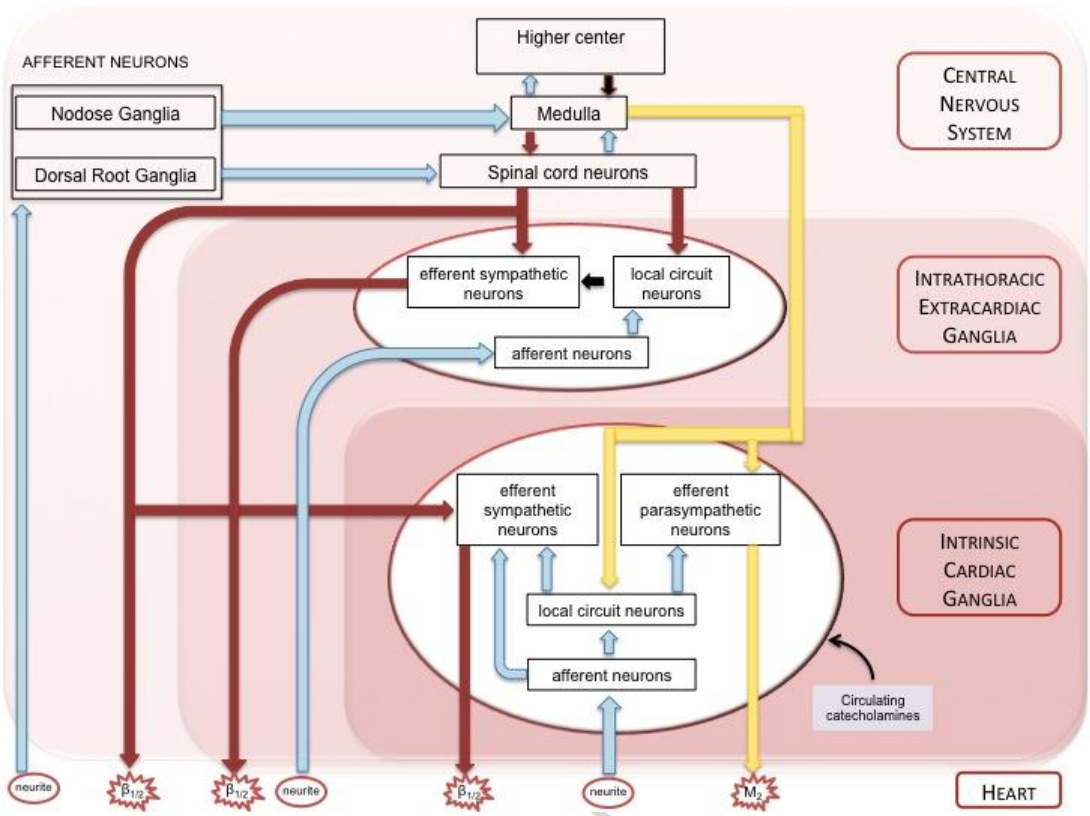

Figure 2

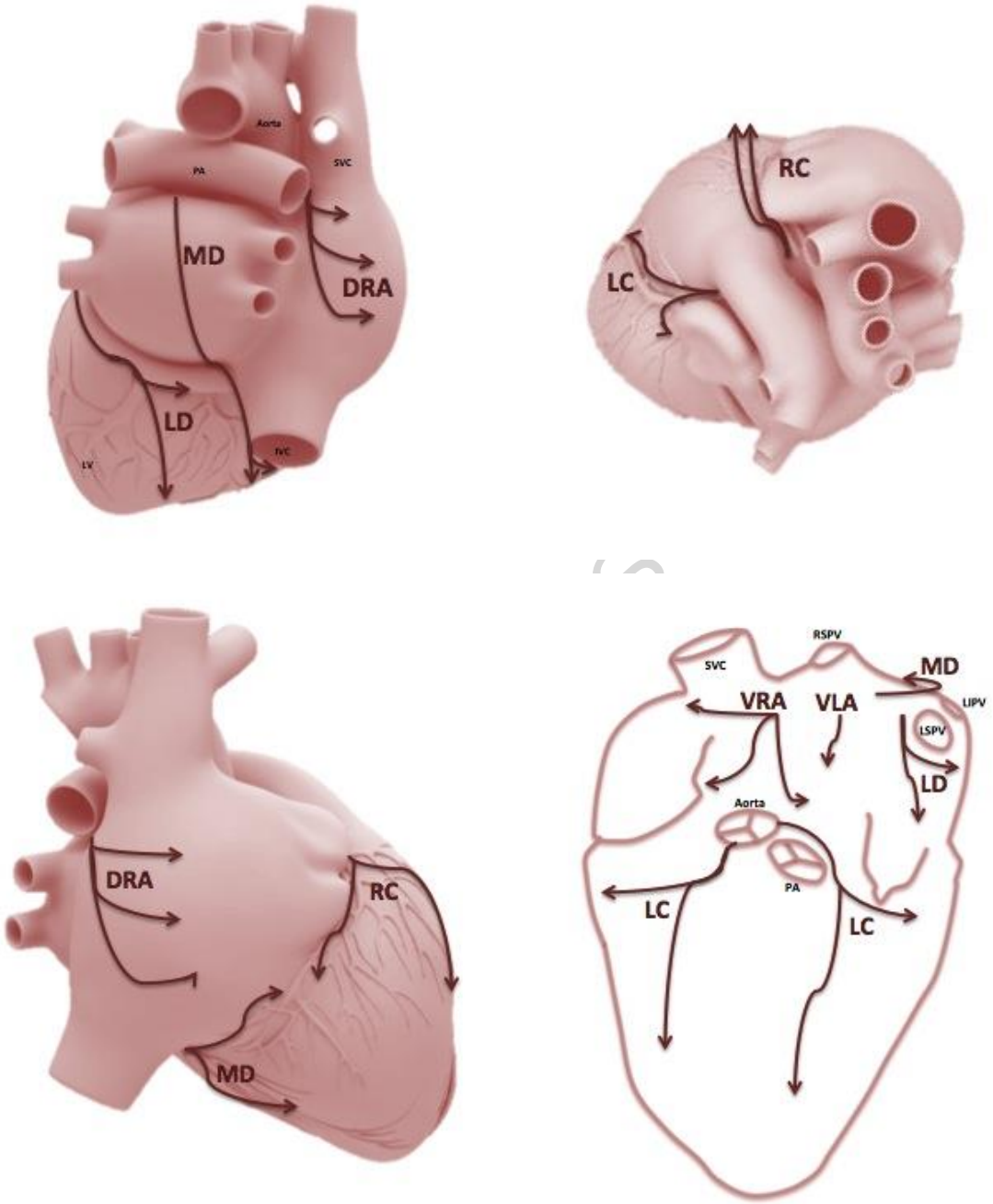

Figure 3

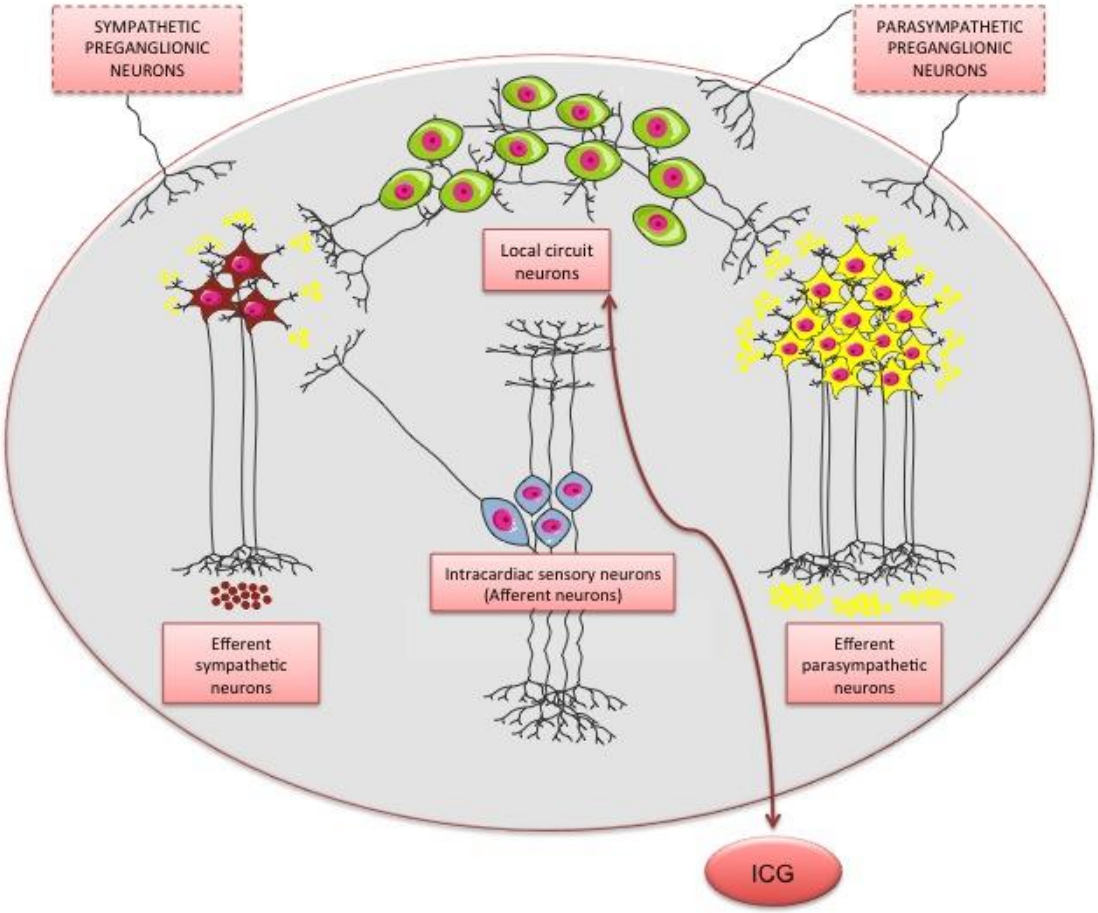

Figure 4

Highlights

- The intracardiac nervous system (ICNS) plays a crucial role in cardiac physiology.
- Cardiovascular diseases (CVD) cause pathological changes in intracardiac neurons.
- More studies are needed in this area in order to know how ICNS can underpin CVD.
- A better understanding of the ICNS may help to develop new treatments for CVD.
- This paper reviews the role and current knowledge of ICNS in CVD.