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Review Article

The Development of the Sympathetic System of the Heart

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Abstract. Development of the sympathetic nervous system begins at about embryonic day 9 in mice with the migration of the neural crest cells to the dorsal aorta and the development of neurons and the sympathetic ganglia. This is followed by the axonal elongation towards the developing cardiac tissue. This process is directed by a series of signal ligands including ephrin-B1, semaphorin 3a (Sema3a), F-Spondin, bone morphogenetic proteins (BMPs), Wnt-1 protein, neurotrophin-3 (NT-3), nerve growth factor (NGF) and artemin (ARTN). Once at the developing heart, the nerve fibres follow the coronary veins in the subepicardium using NGF and the chemorepellent Sema3a as signals. Here they interact with the cardiac conduction system. Although these cardiac neural cells are part of the autonomic system, they are developed later, mainly on the epicardial surface. Bilateral innervation of the heart comes from the middle cervical stellate (MC-S) ganglion. Although the left ventricle and atrium receive noradrenaline from the MC-S on both sides, the right ventricle receives more from the MC-S from the left rather than from the right side. The development of the great vessels also contributes towards the pattern of development of cardiac innervation. The afferent fibres leaving the heart are also described. Their development relates to the sympathetic innervation of the heart and therefore to cardiac sensations. We hypothesise about how this reflects on the patterns of ischaemic cardiac pain.

Keywords: autonomic, sympathetic, visceral afferents, neural, embryology, cardiac development

1 Introduction

The heart is highly vascularised and extensively innervated by autonomic nerves. The autonomic nervous system comprises of both the sympathetic and parasympathetic systems, which work together to maintain homeostasis. Any impairment in the autonomic functions and innervation of the heart syncytium can lead to lethal arrhythmias (Dae et al., 1991; Hildreth, Anderson & Hednderson, 2009). The autonomic system is also vital for the heart during the development itself and the neonatal period, where it promotes the cardiac tissue to regenerate if it is damaged. Unfortunately, this is not the case in adult hearts (White, Gordon, Balkan & Hare, 2015). This highlights the importance of the neurovascular interactions during cardiac development.

The sympathetic nervous supply of the heart regulates chronotropy (contraction rate), inotropy (contraction force), dromotropy (atrioventricular node conduction speed) as well as lusitropy (myocardial relaxation) through the release of norepinephrine and the activation of cardiac β_1-adrenergic receptors (Kimura, Ieda & Fukuda, 2012; Fukuda, Kanazawa, Aizawa, Ardell & Shivkumar, 2015). The heart will suffer dysregulation and even myocardial injury without the sympathetic supply (Y. H. Jiang et al., 2015). The sympathetic innervation density is not uniform within the heart as it is higher in the subepicardium and within the special conduction system. This corresponds to the different areas of influence over cardiac performance (Kimura et al., 2012).

The sympathetic innervation is sculpted throughout development by chemoattractive and chemorepulsive factors such as nerve growth factor (NGF) and semaphorin 3a (Sema3a) respectively (Glebova & Ginty, 2004; Ieda et al., 2007). It is derived from neural crest cells and extends from neurons in the stellate ganglia,

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located on either side of the thoracic vertebra, to the heart. Arterial and venous vascular smooth muscle cells (VSMCs) mediate the proximal axon extension by the secretion of artemin (ARTN), neurotrophin-3 (NT-3) and endothelins. Although the proximal extension from the ganglia is well established, the mechanisms responsible for the distal extension to reach their target cells are not. During distal axon extension, nerves follow a typical pattern and path in target tissues, such as the subepicardium, prior to ultimately innervating the target cells (Nam et al., 2013). Besides the stellate ganglia, which is the main sympathetic supply to the heart, the superior cervical and the thoracic paravertebral ganglia also contribute to some cardiac sympathetic innervation in mice (Armstrong, Ryu, Chieco & Kuruvilla, 2011).

2.1 Formation of the Sympathetic Chain and Neuron Projection Along the Blood Vessels

The development of the autonomic innervation of the heart is composed of four phases: **(1)** the neural crest cells (NCCs) migrate to the dorsal aorta; **(2)** NCCs differentiate into neurons; **(3)** aggregation and migration of the neurons form the paravertebral sympathetic chains (the cardiac ganglia within the heart in the case of the parasympathetic); **(4)** the axon projections elongate towards and into the cardiac tissue until the terminal differentiation into their respective mature sympathetic or parasympathetic neuron (Hasan, 2013). The thoracic NCCs form paravertebral sympathetic ganglia at the dorsal aorta after migrating from the dorsal neural tube through somites during the fifth week of development of the embryo (Moore, Pernaud & Torchia, 2008). Table 1 shows the progress of the sympathetic development of the heart compiled from various animal studies.

The truncal NCCs are split into two groups, which migrate over different pathways at different stages of development. The first group to migrate are responsible for neurons and glial cells, they travel in a ventral direction from the dorsal aspect of the neural tube towards the dorsal aorta. The second group of NCCs give rise to the melanocytes in the skin and they migrate later and laterally from the neural tube (Erickson, Duong & Tosney, 1992; Henion & Weston, 1997). Early studies show that the NCCs responsible for the glial cells and neurons, mainly the dorsal root ganglia (DRG) and sympathetic chain, migrate ventrolaterally and take two pathways. Some migrate from between the somites but the majority migrate into the somite in the region between the sclerotome and the dermomyotome, from where they leave later on, towards the dorsal aorta to develop the sympathetic chain and associate with newly emerging axons from the ventral root (Loring & Erickson, 1987) (Fig. 1). The migration of the NCCs through the somites is split between entering the ventral half of the somatic sclerotome and migration away through the dorsal half of the sclerotome. This is due to the expression of ephrin-B1 ligands at the caudal side of the somite which act as a repulsive cue to the NCCs when binding to their EphB2 tyrosine kinase receptors (Krull et al., 1997). Besides being influenced by the ephrin-B1, the NCCs are also affected by Sema3a and F-spondin within the somites which guide them through the correct somite region. Migration away from the somite sclerotome requires the signalling of neuregulin. At the dorsal aorta clumping of NCCs into columns is regulated by Sema3a (Young, Anderson & Anderson, 2004). Some trunk NCCs, destined to form neurons and glial cells, stop traveling ventrally towards the dorsal aorta and coalesce at the lateral aspect of the neural tube and form the dorsal root ganglia (DRG). Others keep migrating ventrally to reach the dorsal aorta and develop into the sympathetic ganglia (SG) (Loring & Erickson, 1987). According to the study by Kasemeier-Kulesa, Kulesa and Lefcort (2005), the trunk's NCCs travel through the somites towards the dorsal aorta in a chain-like method, stretching from the dorsal to the ventral end of the somite and using active filopodia in the direction of migration. Once the NCCs are outside the ventral border of the somite, they can also travel freely in the rostral and caudal directions to the nearest developing SG and aggregate to it. A subpopulation of these trunk NCCs might still retain their pluripotent and plastic abilities and for some time they can change their location from the DRG to the SG or vice versa as well a from DRG to a nearby DRG until they are eventually inhibited from doing so (Kasemeier-Kulesa et al., 2005). This inhibition can be due to their completed differentiation of sensory and sympathetic cells by factors from the bHLH genes *Ngn2* and *Mash1*, bone morphogenetic proteins (BMPs) and Wnt-1 protein (Parras et al., 2002; Lee et al., 2004; Huber, 2006). Alternatively migration may be inhibited due to an extracellular matrix (ECM) barrier that develops around the cells (Perris & Perissinotto, 2000).

A final migration of the cells occurs at the dorsolateral region of the dorsal aorta. The migration happens either in the rostral or caudal direction in order to form the paravertebral sympathetic chain, which will be then followed by a phase of rapid mitosis (Hasan, 2013). At this region of the dorsal aorta, before the final migration to form the symphatetic chain, the terminal differentiation of the aggregated NCCs occurs, to fulfil their fate to become sympathoadrenal cells. This process is signaled by BMPs released from the aortic smooth muscles (Huber, 2006).

The cellular migration in the rostral direction develops the superior cervical ganglion (SCG). Later on, the extension of axons alongside blood vessels requires ARTN signalling through both GFRalpha3 and Ret re-

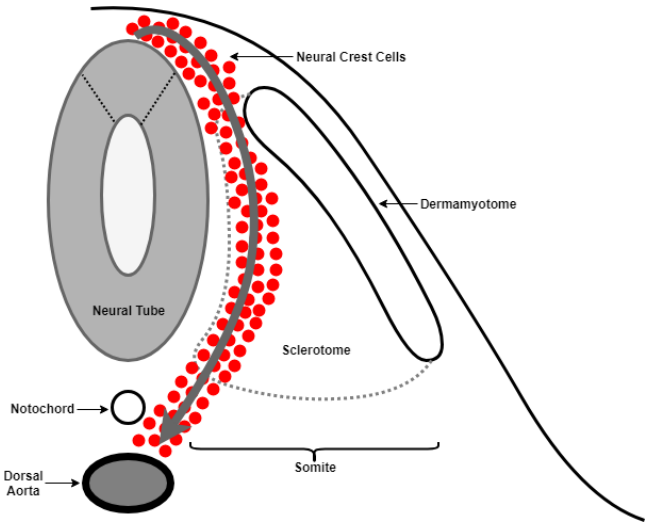

Figure 1: Neural crest cell migration through the somite and towards the dorsal aorta in a cross sectional view.

ceptors (Young et al., 2004). Within the ganglia, the cells adhere together using N-cadherins expressed by the sympathetic progenitor cells (Kasemeier-Kulesa, Bradley, Pasquale, Lefcort & Kulesa, 2006).

Within the superior cervical ganglion of studied foetal rats, axons bud from the side of the cell body, which determines the direction they are about to take. The axon does not branch within the ganglion, only branching slightly in the process of forming the peripheral nerves. The elongation process is rapid and the sympathetic targets are reached within a few days (Rubin, 1985). The sympathetic axons use the signal ligand ARTN, a member of the glial cell line-derived neurotrophic factor (GDNF) ligand family, which is produced and released by the smooth muscles of the developing vessels to find their target tissue by following the vessels. As in NCCs, ARTN acts on the receptor complex made up of both Ret and GFRalpha3 found on the developing sympathetic axon (Honma et al., 2002). The first contact between the differentiating blood vessels and the axons happens just after the formation of the primary sympathetic chain, which starts to develop in the mouse as early as day 9.5 (Enomoto et al., 2001; Wildner, Gierl, Strehle, Pla & Birchmeier, 2008).

2.2 Neurotrophic Factors as Chemoattractants in Final Target Innervation

Besides ARTN, both NT-3 and NGF proteins are required for the growth of the sympathetic axons towards the heart and away from the stellate ganglion. NGF is the prototypic member of the neurotrophin family. It has been under extensive study in the past few years and is essential for terminal sympathetic innervation of the target tissues using tropomyosin receptor kinase A (TrkA) receptors (Kuruvilla et al., 2004)

At the innervation site of the heart, target-derived NGF causes axons to become less sensitive to target-derived NT-3 and depend more on NGF. This is because NGF stimulates p75 neurotrophin receptor expression (Kuruvilla et al., 2004).

The amount of NGF synthesised by a given target tissue (including cardiac tissue) determines its final innervation density. NGF is found in higher levels in the atria than in the ventricles and also causes the expression of β_3-adrenoreceptors in cardiomyocytes especially in the left ventricle (Heumann, Korsching, Scott & Thoenen, 1984; Zhou et al., 2005). Deletion of a single copy of the NGF has been shown to result in apoptosis causing a 50% reduction in sympathetic neurons (Ieda et al., 2004). Studies on transgenic mice demonstrated that the overexpression of NGF leads to hyperinnervation, hyperplasia of ectopic cells in the stellate ganglia as well as cardiac enlargement (Hassankhani et al., 1995).

Besides development, NGF is also important for the maintenence and survival of sympathetic neurons throughout life (Ruit, Osborne, Schmidt, Johnson E. M. & Snider, 1990; Sharma et al., 2010). Mutants without both NGF and Bcl2-associated X protein (*Bax*) have normal extension of sympathetic axons along the extracardiac vasculature. However, sympathetic innervation in the heart is dramatically decreased. The simultaneous knockout of the pro-apoptotic factor *Bax* with NGF, allows neurons to survive in the absence of NGF, validating that NGF has a role in in distal cardiac sympathetic axon growth which is distinct from its impact on survival (Glebova & Ginty, 2004).

Endothelin-1 (ET-1) specifically upregulates the expression of NGF. It has been shown that both NGF expression and cardiac sympathetic innervation were reduced in mouse hearts with deficient ET-1. In such mice, the sympathetic stellate ganglia also demonstrated excessive apoptosis and neuronal loss. Cardiac-specific overexpression of NGF in these mice actually reverses sympathetic nerve retardation. These outcomes show that the ET-1/NGF pathway is critical for the sympathetic innervation of the heart (Ieda et al., 2004; Hasan, Pedchenko, Krizsan-Agbas, Baum & Smith, 2003).

2.3 Sema3a as a Chemorepellent in Cardiac Sympathetic Innervation Patterning

In contrast to neurotrophic factors such as NGF, which act as chemoattractants in cardiac nerve development, the chemorepellents in the heart which repel nerve axons and induce growth-cone collapse are still unidentified. Sema3a, a potent neural chemorepellent, is produced by the trabecular layer during early embryo development and then only by the Purkinje fibres after birth (Ieda et al., 2007; Kawasaki et al., 2002). Sema3a binds to the

Table 1: Cardiac sympathetic nervous system development.

Process	Day	Species	Human equivalent
NCCs migrate through the somites' ventral portion	Day 8.5–9.0 Serbedzija, Fraser and Bronner-Fraser (1990)	Mouse	Week 3 Vegh et al. (2016)
NCCs migrate away from the somite and towards the dorsal aorta where they clump into colums	Day 9.0–9.5 Serbedzija, Fraser and Bronner-Fraser (1990)	Mouse	Week 3-4 Vegh et al. (2016)
Terminal differentiation of NCCs at dorsal aorta before final migration	Day 9.5–10.0 Serbedzija, Fraser and Bronner-Fraser (1990)	Mouse	
Final NCC migration from dorsolateral region of aorta to the caudal or rostral direction forming paravertebral sympathetic chain followed by rapid mitosis	Day 9.5–10.0 Serbedzija, Fraser and Bronner-Fraser (1990)	Mouse	
Sympathetic precursor cells reach the cervical region along the dorsal aorta and start to accumulte in a columnar fashion	Day 12 (*Day 10.5) Rubin (1985)	Rat	Week 4 Witschi (1962)
Precursor cells at the cervical region develop the superior cervical ganglia (C1–C5) and the stellate ganglia further caudally (C8)	Days 12–14 (*Day 10.5–12.5) Rubin (1985)	Rat	
First axons start to bud from cell bodies in the ganglia	Day 12 Rubin (1985)	Rat	
Enhanced and rapid mitosis of the accumulated precurser cells forming the superior cervical and the stellate ganglia	Day 14 (*Day 12.5) Rubin (1985)	Rat	Week 6 Witschi (1962)
At the developing heart, the angiogenic remodeling moves outwards to the subepicardium from the sinus venosum. VSMCs are recruited to the subepicardium and NGF is expressed to stimulate elongation of axons.	Day 13.5 Nam et al. (2013)	Mouse	Week 7 Vegh et al. (2016)
First sympathetic axons start to innervate the subepicardial layer of the developing heart and extend along the developing coronary veins in the layer using NGF secreted by the veins-associated VSMCs	Days 13.5 Nam et al. (2013)	Mouse	
Sympathetic targets reached after a rapid process of axon elongation using ARTN ligand released from vessel smooth muscle to find target (i.e. Axons follow vessels)	Day 15 Rubin (1985)	Mouse	Week 8 Otis and Brent (1954)
Sympathetic axons are present across the whole dorsal aspect of the developing heart while more axons continue to associate with the large coronary veins.	Day 15.5 Nam et al. (2013)	Mouse	
VSMCs in the myocardium during the development of the coronary arteries secretes NGF which directs the sympathetics axons to penetrate the myocardium from the epicardium. *(NGF from the epicardial veins starts to decrease at the dorsal region)* At the same time, in the epicardium, the sympathetic axons extands distally and ventrally from the dorsal aspect along the developing coronary veins in the ventral region of the developing heart.	Day 15.5–16.5 Nam et al. (2013)	Mouse	
All venous secreted NGF has ceased but arterial NGF in the myocardium was still being secreted at continue the attraction of axons into this layer.	Day 17.5 Nam et al. (2013)	Mouse	
Sensory neuron axons reach the subepicardium of the developing heart.	Day 18.5 Nam et al. (2013)	Mouse	

ARTN: artemin, NCC: neural crest cells, NGF: neurve growth factor, VSMC: vascular smooth muscle cell
*Equivalent mouse embryonal dates for comparison purposes.

transmembrane protein receptors known as neurophilin-1 and neurophilin-2, which work to aid axon guidance (Takagi et al., 1991; Kitsukawa et al., 1997).

Sema3a is strongly expressed in the developing heart at mouse embryonic day 12 and it eventually tapers down thereafter (Ieda et al., 2007). It is found in the subendocardium, but not the subepicardium, of the atria and ventricles. This pattern of distribution is the opposite to the epicardial-to-endocardial gradient of sympathetic innervation, which suggests that Sema3a acts as a negative regulator of cardiac innervation. Further evidence is provided by Sema3a knockout mice, which were shown to have a disrupted pattern of sympathetic innervation and malformation of the stellate ganglia, which extend the sympathetic nerves to the heart, along with hypertrophy of the heart muscle and dilatation of the right atrium (Behar, Golden, Mashimo, Schoen & Fishman, 1996). These demonstrate that Sema3a plays an important role by inhibiting sympathetic neural growth in the heart.

Lorentz et al. (2010) demonstrated that the subendocardium innervation of the adult p75 (NGF receptor) knockout mouse ventricles showed similar signs to mice with overexpression of Sema3a, eventually leading to spontaneous ventricular arrhythmias. This shows that as the sympathetic neurons are innervating the heart, NGF/p75 blunt the repulsive effect of Sema3a from the subendocardium. The delicate balance between NGF and Sema3a synthesised in the heart determine the correct epicardial to endocardial transmural symphatetic innervation of the ventricles (Carter, Feng & Paolocci, 2010).

2.4 The Role of Cardiac Neural Crest Cells in Cardivascular Development

Cardiac neural crest cells (CNCs), required for the complete development of the heart, are a subpopulation of the NCC, which originate from the first three somites up to the mid-otic placode in the rostral section of the embryo, corresponding to neural tube rhombomeres 6, 7 and 8 at the posterior rhombencephalon region (Kirby, Gale & Stewart, 1983; Maschhoff & Baldwin, 2000; Kirby, 2002). CNCs are essential to numerous processes during cardiac development. This includes the modifications of the pharyngeal arch arteries and the cardiac outflow tract to separate the pulmonary trunk from the ascending aorta to form two separate circulations. CNCs also form smooth muscles and pericytes of the great arteries as well as the neurons responsible for the cardiac conduction system. They also contribute to signalling for conotruncus elaboration (Brown & Baldwin, 2006). The CNCs undergo epithelial-to-mescenchyme transformation (EMT) and migrate to the third, fourth and sixth pharyngeal pouches before undergoing further migration to the developing heart, thus providing mesenchymal cells to the developing heart and large vessels (Maschhoff & Baldwin, 2000; Hamburger & Hamilton, 1992). It appears that these CNCs enter the heart both from the arterial and venous poles (Poelmann, Mikawa & Gittenberger-de Groot, 1998; Poelmann & Gittenberger-de Groot, 1999). The subpopulation entering the venous pole of the heart settle in cardiac regions related to the conduction system such as the atrioventricular (AV) node area, the retroaortic root bundle, the bundle of His, the left and right bundle branches and the right ventricular ring bundle, as well as the regions of the atrioventricular cushions. However, it seems that the CNCs do not form the final conduction system, as they then go into apoptosis and therefore fail to differentiate and survive. However, the exact timing of arrival, their apoptosis and the changes of the electropysiological properties within the heart, affect the conduction system's last phase of development. Altough the conduction system development might be affected by the CNCs, it is not a NCC derivative but a derivative from cardiac myogenic precursors (Cheng et al., 1999). This might be due to separation of the central conduction system from the myocardium by the apoptotic CNCs (Poelmann & Gittenberger-de Groot, 1999). CNCs entering the arterial pole migrate to areas related with the outflow tract and its septation, semilunar valves, smooth muscle of the great vessels and the ganglia around them (Poelmann et al., 1998). CNCs use both adhesion molecules such as integrins to interact with the ECM and molecules such as gap junctions alpha 1 connexin (Cx43) and cadherins to communicate with each other (Lo, Waldo & Kirby, 1999). CNCs also require non-canonical Wnt11 signalling in order to form filopodia and lamellipodia thus allowing migration (De Calisto, Araya, Marchant, Riaz & Mayor, 2005).

Mesenchyme derived from CNCs in the pharyngeal arches differentiate into connective tissue that stabilises the great arteries.

It also forms a sheath around the persisting aortic arch arteries, which make up the aortopulmonary septation located at the conal cushions final fusion site. They also populate the cardiac ganglia, which are entirely made up of CNC both the neurons and supporting cells (Kirby et al., 1983; Maschhoff & Baldwin, 2000; Waldo, Lo & Kirby, 1999). Other CNC-derived cells in the pharyngeal arches form the smooth muscle layer that surrounds the pharyngeal arch arteries as they remodel into the aortic arch, the ductus arteriosus and the proximal carotid arteries (Kirby & Waldo, 1995; X. Jiang, Rowitch, Soriano, McMahon & Sucov, 2000). In fact, CNC have the ability to form three types of progenitors. Firstly, stem cells (CNC-SC), which rise to smooth muscle, neurons, chondrocytes, Schwann cells, pigment cells and are capable of self renewal. In addition, CNCs

also forms fate-restricted cells (CNC-RC) and smooth muscle lineage (CNC-smC), which are committed to smooth muscle formation (Sieber-Blum, 2004). However, it is still not known what is the exact role of the CNCs during the asymmetric remodelling of the aorta (Snider, Olaopa, Firulli & Conway, 2007).

Two important gene products required for the development of the heart are NT-3, belonging to the neurotrophin family of neurotrophin-4/5 (NT-4/5), NGF and brain derived neurotrophic factor (BDNF), as well as TrKC, belonging to the Trk tyrosine kinase receptors family (Sieber-Blum, 2004). NT-3 binds to TrkC and leads to recruitment, docking and phosphorylation of signal proteins, which in turn result in activation of pathways that regulate proliferation, neuronal differentiation as well as survival. (Sieber-Blum, 2004; Barbacid, 1994). Unlike other Trk receptors, TrkC is highly expressed in non-neuronal tissues including the heart and vascular smooth muscle cells (Tessarollo et al., 1993; Donovan, Hahn, Tessarollo & Hempstead, 1996). This wide expression of TrkC suggest that it might have a pleiotropic function while the embryo is developing, such as mitogenic function in cardiomyocytes during cardiac looping and ventricular trabeculation (Tessarollo et al., 1993; Lin et al., 2000).

Another important protein is the norepinephrine transporter (NET). NET is a presynaptic reuptake protein and is expressed in adult brain's neuroepithelium, DRG, spinal nerves sympathetic nervous system and the locus cereleus of the brainstem (Amara, 1995; Galli, Blakely & DeFelice, 1996). However, NETs were also found in embryos of birds and mammals, suggesting a broader functional spectrum than that in the adult organism (Ren, Pörzgen, Youn & Sieber-Blum, 2003). It is in fact expressed by NCCs during their late migration when it is in the region of the notochord. Its function is to promote norarenergic differentiation using maternal norepinephrine that crosses the placenta rather than a transporter as in the adult neurons (Rothman, Gershon & Holtzer, 1978; Zhang et al., 1997). Importantly, NETs in the embryo are not only expressed in neuronal cells but also in non-neuronal cells including the cardiovascular system, such as the heart's epicardium and myocardium including the trabeculae, the endothelium of the aorta and veins and the NCCs at the cardiac outflow tract (Ren et al., 2003).

A summary of the neural crest migration, as well as the important controling factors are shown in Fig. 2.

2.5 Branchial Arch Vessel Remodelling to Produce the Great Vessels

Before the aortic arch is remodelled to its definite left sided arch structure, the outflow tract was a single vessel that branched bilaterally and symmetrically into the third, forth and sixth aortic arches within the pharyngeal arches. The remodelling happens using a programmed process of asymmetrical obliteration and preservation of specific arches, with the third arch arteries becoming the common carotids, the fourth arch arteries forming the distal part of the brachiocephalic artery and the proximal right subclavian artery while the sixth arch arteries develop the proximal part of the pulmonary arteries and the ductus arteriosus (Snider et al., 2007). Such a remodelling process requires interaction among the pharyngeal arch arteries endothelium, the surrounding smooth muscle derived from the NCCs and the mesenchyme, as well as the endoderm (Le Lièvre & Le Douarin, 1975; Yanagisawa et al., 1998; Wendling, Dennefeld, Chambon & Mark, 2000). Studies show that the remodelling of the aortic arch depends on a threshold requirement of colonising NCCs (Stoller & Epstein, 2005).

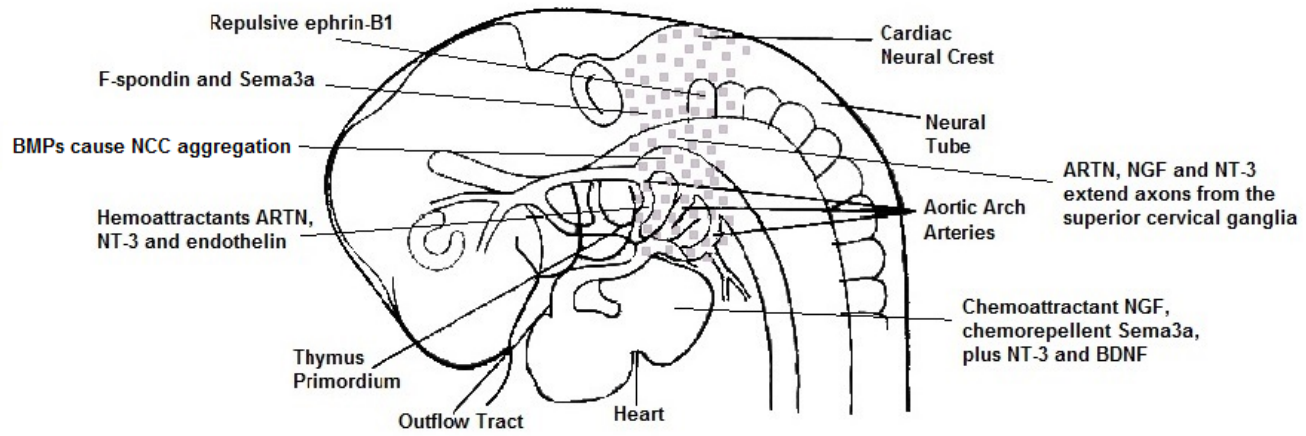

Figure 2: Neural crest cell migration and the main factors involved.

2.6 Cardiac Sympathetic Growth Occurs Along the Coronary Veins

In recent years numerous investigators have been trying to understand the different potential mechanisms of how the sympathetic nerves grow into the heart. From the anatomical congruence between sympathetic nerves and coronary vessels it is clear that sympathetic axons in the heart, like in other organs, follow blood vessels (Nam et al., 2013).

Sympathetic development in the developing heart of mice commences at embryonic day 13.5, when the axons extend along the developing large coronary veins in the dorsal subepicardium of the ventricles. By embryonic day 15.5, these axons spread throughout the dorsal surface and consequently penetrate the dorsal myocardium, while the others reach the ventral subepicardium. It has been demonstrated in many in vitro and in vivo studies that during angiogenesis and coronary vein recruitment, epicardium-derived VSMCs transiently release NGF. This directs the extension of sympathetic axons along the developing coronary veins. Later VSMCs of the developing coronary arteries in the myocardium start to secrete NGF which casues the sympathetic axons to penetrate the myocardium (Nam et al., 2013). This transient period of NGF expression may lead to the explanation as to why axons do not innervate veins despite following them during periods of remodelling.

As coronary vein remodelling is completed, the expression of NGF by the subepicardial VSMCs is downregulated, while its expression by the myocardial VSMCs commences during the period of arterial remodelling. This step leads to the extension of the sympathetic axons into the myocardial cell layer, correctly distributing throughout the myocardium before finally innervating the cells (Nam et al., 2013).

By contrast to the above model, other studies demonstrated that in the developing limb skin, the arteries align with sensory nerves and the sympathetic axons extend along the arteries from the sympathetic ganglion (Luff, 1996; Mukouyama, Shin, Britsch, Taniguchi & Anderson, 2002; Glebova & Ginty, 2005). Nam et al. (2013) provided a detailed mechanism, however it does not explain how the spatiotemporal changes in NGF expressed by the VSMCs in the coronary veins during sympathetic development is controlled. This mechanism requires precise localisation of NGF expression which is crucial to cardiac innervation. There is other research in progress regarding the involvement of other signals and cues that are present in the final target innervation of the myocardium (Ieda et al., 2007).

2.7 Growth of Cardiac Sensory/Afferent Fibres

The heart transmits sensory information to the central nervous system such as nociceptive signals through both sympathetic and parasympathetic vagal afferent fibres (Hua et al., 2004). In a study in cats, it was shown that the cardiac afferent sensory fibres from the heart passed through the stellate ganglion as the efferent fibres do and through the dorsal root ganglia ipsilaterally from C8 to T9, where the cell bodies are (Kuo, Oravitz & DeGroat, 1984). It is interesting to speculate whether in humans (as in rats below, assuming that the sensory nerves follow the sympathetics), any such sensory fibres pass back to the superior and middle cervical ganglia and enter the dorsal root ganglia at higher cervical nervel levels as this may help explain referred cardiac pain to the shoulder tip, neck and the angle of the jaw (innervated by C4, C3 and C2 dermatomes respectively). The cardiac sympathetic afferent fibres are known to be more dense between the T2 and T6 segments located within the Lissauer's tract and lamina 1 of the dorsal horn's lateral border and then extending to terminate into lamina 5 and 7 near the intermediolateral nucleus region. This uniform pattern of grey matter termination, which contains the spinothalamic fibres involved in autonomic nociception and reflexes, is also seen in other visceral sympathetic afferents in the lower thoracic and lumbar segments (Kuo et al., 1984). The vagal afferents for nociception travel and synapse in the nucleus tractus solitarius (NTS) of the medulla and go on to excite cells of the upper cervical spinothalamic tract C1–C3, which might explain the referred pain to these dermatomes (Chandler, Zhang & Foreman, 1996; Foreman, 1999).

Another study on mice hearts showed that the cardiac afferent fibres, which are found on the epicardial surface of the heart and travel through the DRG, contains the vanilloid receptor 1 (VR-1). However, these VR-1 containing neurons are scarce in the myocardium. It was found that these VR-1 containing afferent fibres are essential for the sympatho-excitatory reflex during a myocardial ischemia, which reflex is the increase of cardiac sympathetic activity when the cardiac sympathetic afferent fibers are stimulated (Zahner, Li, Chen & Pan, 2003). A study by Bennett, Dmietrieva, Priestley, Clary and McMahon (1996) found that sensory neurons in viscera express mainly calcitonin gene-related peptide (CGRP) and trkA. TrkA is sensitive to the much required factor, NGF during the development of afferent fibres (Ieda et al., 2006). Sensory neurons in the heart develop later than autonomic motor neurons. In mouse hearts, at the dorsal ventricular subepicardium, autonomic motor neurons are present by day E15.5, while sensory neurons start to appear on day E18.5. Also, the autonomic motor neurons were found to follow and interact with the coronary veins in the subepicardium during cardiac development, whilst other studies show that sensory neurons follow arteries (Nam et al., 2013; Mukouyama et al., 2002)

2.8 Laterality of Innervation and any Influence on Cardiac Pain Syndromes

It is known that the heart is supplied bilaterally by sympathetic fibres from the superior, middle, inferior cervical and the upper thoracic paravertebral sympathetic ganglia (Vegh et al., 2016). However, is there any difference in the laterality of supply of the different parts of the myocardium, and if so can this relate to patterns of ischaemic cardiac pain? A study using Diamidio Yellow retrograde tracer injected into the ventricles of rats in order to label the stomata of the postganglionic nerves in the sympathetic chain shows that the ventricles of the heart are innervated by sympathetic postganglionic neurons bilaterally, but with more left stellate cell bodies innervating the left ventricle and more right stellate cell bodies innervating the right ventricle (Pardini, Lund & Schmid, 1989). The majority of the supply is originating from the middle cervical-stellate (MC-S) ganglion complex with around 92% of labelled cells. (Stellate ganglia are a complex fusion of the lower cervical and first thoracic ganglia) Lesser supply is coming from the superior stellate ganglion and the upper thoracic ganglia. After performing unilateral MC-S ganglionectomies the investigators showed that both atria and the left ventricle are supplied bilaterally by the MC-S complex. On the other hand, the right ventricle receives most of its noradrenaline from the left MC-S ganglion complex, meaning that the left ganglion is supplying all chambers (Pardini et al., 1989).

Studies have shown that electrocardiographic (ECG) changes following unilateral right stellate ganglionectomy were similar to those following left (Yanowitz, Preston & Abildskov, 1966). However, the left stellate ganglion fibres innervate primarily the posterior ventricular surface. On the other hand, sympathetic tone is removed from the anterior surface following unilateral right stellate ganglionectomy (Yanowitz et al., 1966). The identity of the anterior surface of the heart is largely the right ventricle and right atrium and a bit of the interventricular septum and apex of the left ventricle, suggesting a distinction in innervation to left and right ventricles by left and right stellate ganglia. There is also a functional distinction of the left and right sympathetic innervation. Randall and Rohse (1956) found that left stellate stimulation produced mainly inotropic changes (mainly innervating the muscle which feels ischaemic pain), while right stellate stimulation produced both inotropic and chronotrophic changes.

Looking at these older studies, if the sensory afferent nerves from each side follow the sympathetic innervation to each individual sympathetic chain before entering the spinal cord via the dorsal nerve root, the majority of the ventricles would be supplied by nerves entering the left side of the spinal cord. Since most cardiac ischaemia occurs in the thicker left myocardium, this (as well as the considerable left stellate innervation of the right ventricle myocardium) may explain why referred pain to the left arm (lateralising pain) in myocardial infarction and ischaemia far exceeds the presentation of right arm pain. This provides interesting hypotheses for further study.

3 Conclusion

It is clear that there has been extensive research conducted on the development of the sympathetic innervation of the heart. This review highlights areas for further research, which include the exact site of synthesis and function of NGF in cardiac innervation, identification of the chemorepellants which induce cone growth collapse in the heart, and the exact role of the CNCs during the asymmetrical remodelling of the aorta. Although this study collated work based on animals, the information gathered can give a better idea and understanding of how what happens in human subjects when it comes to this complex process. The review attempts to give insights into possible explanations of referred pain distribution in clinical cardiology, based on our understanding of human embryology and anatomy.

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