Characteristics of anomalous coronary artery anatomy and implications of ischemia

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Chapter 9

The role of the axonal guidance cue Sema3A in innervation of the postnatal heart in health and disease

Semaphorin3A in the innervation of the postnatal heart

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Abstract

During cardiac development the heart is innervated by the autonomic nervous system. After development, neurons of the autonomic nervous system have limited capacity for growth and regeneration. However, in the past decades, it has become clear that cardiac nerves can regenerate after cardiac damage. An excessive amount of re-innervation, so-called sympathetic hyperinnervation, may render patients vulnerable to ventricular arrhythmias and heart failure. Several studies have investigated axonal guidance cues as mediators of cardiac innervation. Axonal guidance cues direct neuronal growth of the axon and play a significant role in the regeneration and remodelling of cardiac autonomic innervation after cardiac damage. This review focusses on current literature regarding the axonal guidance cue group of semaphorins and their function in the healthy and diseased postnatal heart. In light of cardiac innervation, most studies focus on semaphorin 3A (SEMA3A), whereas less is known about the function of the other semaphorin classes. SEMA3A is a neuronal repellent and is associated with a decrease in the density of sympathetic neurons in the heart. Its decline in expression after myocardial infarction plays a role in the development of sympathetic hyperinnervation and the subsequent increased risk of ventricular arrhythmias. In congestive heart failure the opposite occurs: an increase in SEMA3A expression underlies decreased nerve density that may also serve as a substrate for ventricular arrhythmias. Although literature on their role in cardiac innervation is still relatively scarce, semaphorins, in particular SEMA3A, seem relevant candidates to consider when exploring options to modulate pathological innervation patterns in cardiovascular disease.

Introduction

Development of cardiac autonomic innervation starts around embryonic day E12.5 in mice and arises predominantly from the neural crest cell lineage, a multipotent stem cell population (1). After birth, neurons of the autonomic nervous system are considered to have limited capacity for growth and regeneration. However, in the past decades, it has become clear that cardiac nerves are able to regenerate after cardiac damage and overload, such as myocardial infarction (MI) and heart failure (2,3). An excessive amount of re-innervation, so-called sympathetic hyperinnervation, may render patients vulnerable to ventricular arrhythmias and heart failure (4-8). Especially after MI, there is still a high risk of ventricular arrhythmia and sudden cardiac death despite advances in surgical and percutaneous interventions over the past decades. Ventricular arrhythmia and sudden cardiac death have classically been attributed to re-entry phenomena caused by heterogeneous conduction in the infarction border zone (4). In the past decades, a multitude of reports has emerged that relates sudden cardiac death after MI also to sympathetic hyperinnervation, the increased and disorganized nerve regrowth that occurs after initial nerve degradation due to ischemic damage (4-7,9,10). We have recently shown innervation heterogeneity, in particular in the border zone, that persisted late after MI with reperfusion, potentially contributing to the arrhythmogenicity observed after MI (11).

Multiple neurotrophic factors and axonal guidance cues are involved in the development and regeneration of cardiac autonomic innervation. Among the most important of neurotrophic factors are those belonging to the neurotrophin family, such as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) (12). While neurotrophic factors primarily influence growth and survival of developing nerves, the directed guidance towards their intended destination is provided by axonal guidance cues. Axonal guidance cues have been classified into 4 different classes: semaphorins, netrins, ephrins, and slits (13) (**Figure 1**). These cues function by either attracting or repelling the axonal growth cone (the growing motile end of the axon), depending on the specific factors and receptors at play. For example, netrin 1 attracts axons by binding to the axonal receptor Deleted in Colorectal Cancer (DCC) (14), while semaphorins act as repulsive cues for developing axons (15).

Research focusing on the function of the different neurotrophic factors and axonal guidance cues in regeneration of innervation after (ischemic) damage of the heart is ongoing, and the mechanisms by which they work are slowly being unravelled. For this review, a non-systematic literature review was performed using PubMed as search engine. Relevant articles were selected from the results query (**Online supplemental file**[1], https://doi.org/10.1016/j.cjca.2024.12.030) and new literature was derived from the selected articles. New literature was also sought to complement the discussion where appropriate. As a result, this review offers an overview of current knowledge regarding the distinct functions of semaphorins, in particular the function of semaphorin 3A (SEMA3A) in the innervation of the postnatal heart

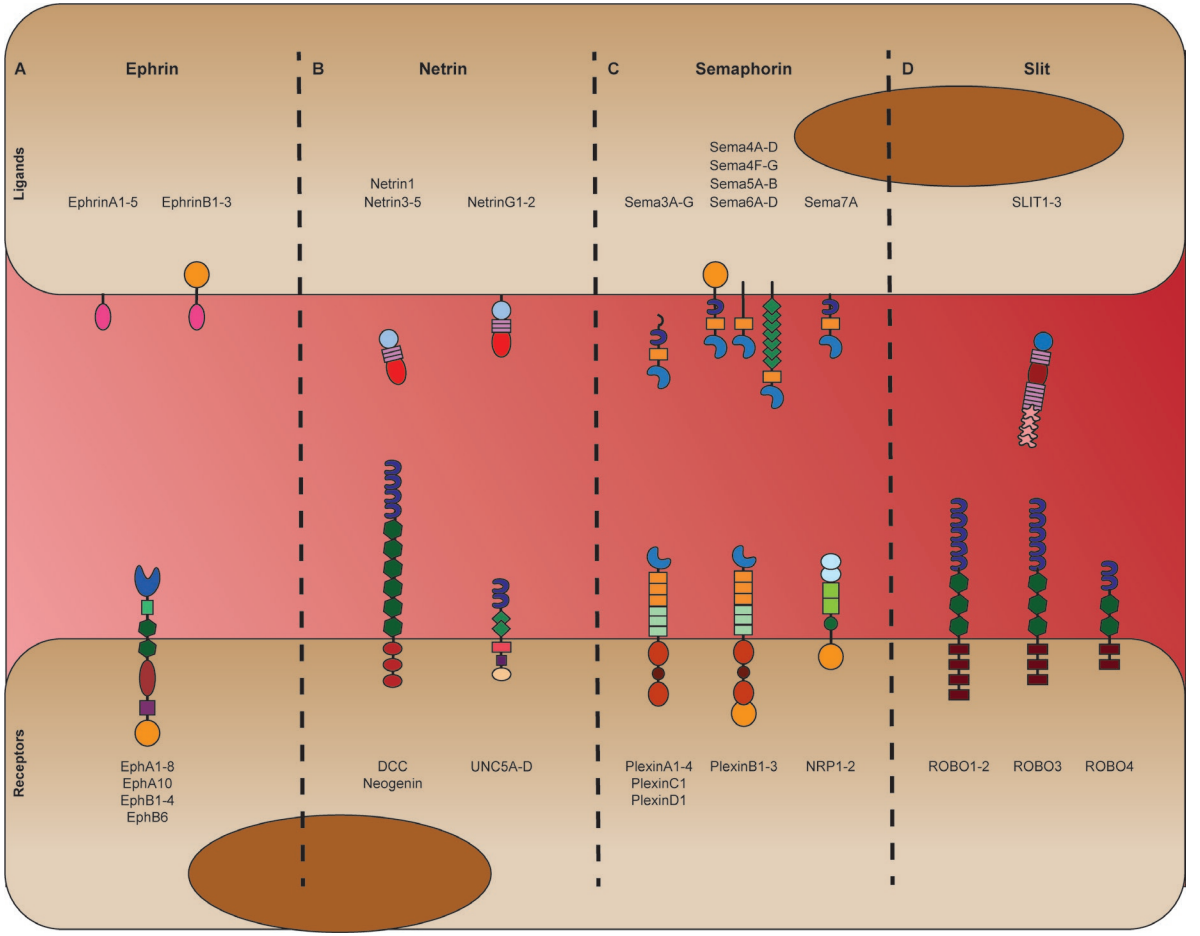

Figure 1. The 4 classes of axonal guidance cues and their receptors. DCC: Deleted in Colorectal Cancer, UNC5: Uncoordinated5, ROBO: Roundabout

in health and disease, and identifies knowledge gaps to provide steppingstones for future research.

The semaphorin family

The semaphorin family was first studied in the 1980s in vitro (16-18). Research initially focused on a protein called Collapsin, which is responsible for growth cone collapse during nerve growth. Over time, more than 30 proteins from the same family were discovered. The proteins in this family are now known as semaphorins. Semaphorins are divided into 8 classes, of which only classes 3 to 7 are found in vertebrate species (**Figure 2**). Semaphorin class 3 are secreted proteins. Class 4-7 are membrane bound through either a transmembrane domain (class 4-6) or a GPI-anchor (class 7).

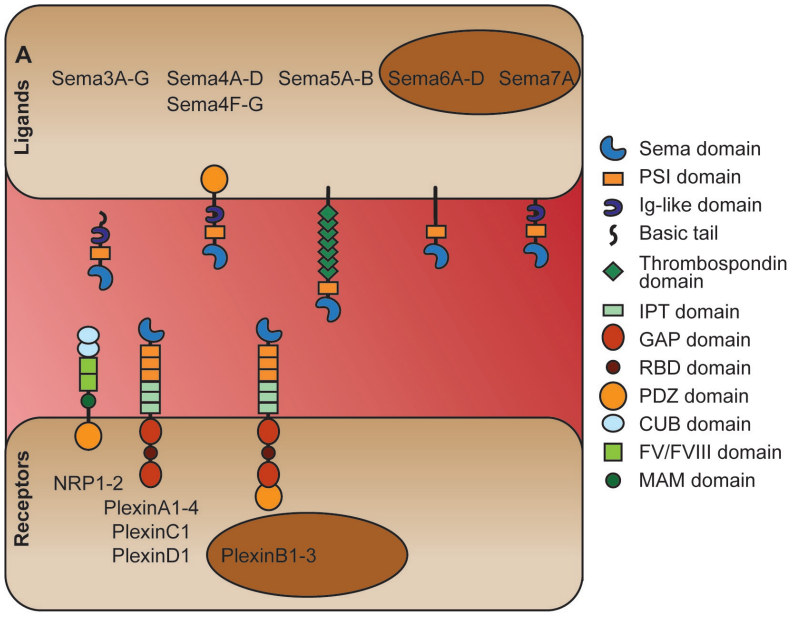

Figure 2. Semaphorin ligands and their receptors with their specific domains.

In particular, SEMA3A has been studied in relation to cardiac innervation prenatally and postnatally in health and disease. SEMA3A belongs to the class 3 semaphorins and is composed of a signal peptide for cellular secretion, a SEMA domain (involved in dimerization and receptor binding to plexins/neuropilins), an Immunoglobulin-like (Ig) domain, a PSI domain (domain found in plexins, semaphorins and integrins necessary for receptor binding to neuropilins) and a basic tail (**Figure 2**) (19). Several studies have highlighted the importance of SEMA3A in the developing as well as in

the adult central and peripheral nervous systems. However, SEMA3A has also been shown to be involved in the maturation and homeostasis of multiple other organs, i.e. in (blood and lymphatic) vessel maturation and in maintaining bone homeostasis (20-22).

In neuronal development, Sema3A operates as a neuronal chemorepellent. In cardiac embryonic development, semaphorins, in particular semaphorins class 3, are involved in neuronal development, but also in anatomical development during E8.5 to E12.5 of murine embryogenesis (23). In this phase cardiac looping occurs and chambers and in- and outflow tracts are developing. Semaphorins exert their function by controlling the migration of neural crest cells and progenitors of the second heart field to form the ventricular outflow tract. Sema3A is mainly involved in the development of the cardiac innervation (24).

Semaphorin3A receptors

The primary semaphorin receptors are the membrane bound plexin receptors, which are divided into four classes (A, B, C, D) based on their structural homology (**Figure 2**). Plexins are large single-pass transmembrane proteins containing a semaphorin domain extracellularly, to bind the semaphorin molecules, and a long intracellular tail. The extracellular domain of plexins contains one SEMA domain followed by three PSI and three transcription factor (IPT) repeats (25). The intracellular domain is highly conserved throughout the plexin family and binding of semaphorins to plexin receptors can induce signalling via the intracellular GTPase Activating Protein (GAP domain) and Rho GTPase Binding Domain (RBD domain) of the plexin receptors (26,27). As the activity and availability of small GTPases, which are key regulators in many cellular processes such as cytoskeletal dynamics, can be directly controlled by these domains, plexin signalling can hereby regulate for example cell morphology, cell migration, and cell proliferation (15,28). Before interacting with class A plexins (PLXNA1-4) the class 3 secreted semaphorins, except for SEMA3E, need interaction with a co-receptor NRP1 or NRP2 (21,29). These neuropilins are single-pass transmembrane glycoprotein receptors.

Of interest is NRP1. NRP1 acts as a co-receptor for SEMA3A, while all plexin A receptors transduce the SEMA3A activated signal into the cell. The receptor PLXNA4 and its co-receptor NRP1, which are expressed by postganglionic sympathetic neurons of the peripheral nervous system, mediate the repulsive guidance cue of SEMA3A upon forming a complex together (30-34) (**Figure 3**). The formed ligand-receptor complex interacts with the cytoskeleton of the axonal growth cone and mediates growth cone collapse (35). Due to its important function in the development and growth of the autonomic nervous system, SEMA3A has also become of interest for its potential involvement in the function of the cardiac conduction system and the development of (pathological) cardiac innervation (36).

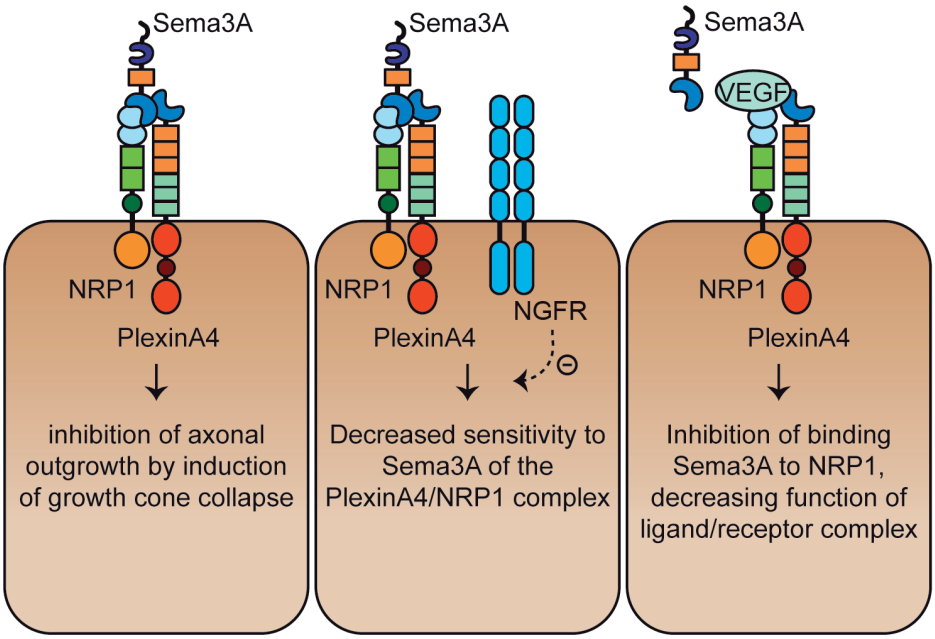

Figure 3. Schematic representation of SEMA3A's mechanisms of action

Development of the neuronal system is closely related to the development of the vasculature. SEMA3A and vascular endothelial growth factor (VEGF) competitively bind to the receptor NRP1 that can be found on endothelial cells of the growing vessels. SEMA3A executes a repellent function regarding vessel outgrowth, similar to its function in neuronal development (37-39). SEMA3A is also required for proper innervation of the blood vessels. This effect is dose-dependent. Vessels expressing high levels of SEMA3A favour PLXNA1/NRP1 signalling that results in chemorepellent cues limiting outgrowth of sympathetic neurons and vascular innervation. In contrast, low levels of SEMA3A favour VEGFR2/NRP1 signalling, resulting in chemoattractive cues for sympathetic outgrowth and vascular innervation (40).

Current literature on the functions of Sema3A in the healthy and diseased heart and its potential implications for therapeutic possibilities are discussed in the following paragraphs. An overview of the relevant articles discussed below, is provided in **Table 1**.

SEMA3A is expressed in and is crucial for adequate functioning of murine cardiac innervation

The cardiac expression of Sema3a mRNA and SEMA3A protein has primarily been studied in mice (36,41) and rats (42-45). SEMA3A protein has been detected in stellate ganglia (44,46), but no mRNA has been shown, therefore it is not clear if

Table 1. Literature on SEMA3A in postnatal cardiac innervation

Reference	Species	Healthy/ disease model	Semaphorin modulation	Main findings
Ieda et al., 2007 (36)	Mice	Healthy	Sema3alacZ/+, Sema3a–/–, cardiac TG Sema3a	In vivo, SEMA3A expression is inversely proportional to cardiac sympathetic innervation. Cardiac sympathetic nerves express NRP1.
Marko et al., 2008 (32)	Rats	Healthy	SEMA3A protein	Ex vivo, VEGF suppresses SEMA3A axonal repellent effect, possibly by binding to NRP1.
Lorentz et al., 2010 (33)	Mice	Healthy	NGFR-/-	Ex vivo, absence of NGFR makes adult sympathetic axons more vulnerable to inhibition by Sema3a by breaking apart NRP1/plexin receptor complex
Sun et al., 2011 (42)	Rats	Isoproterenol-induced heart failure	WT	In vivo, SEMA3A cardiac expression is increased upon CHF, this increased expression of SEMA3A may partially explain sympathetic denervation in CHF.
Wen et al., 2011 (45)	Rats	Myocardial infarction	Sema3a adenovirus injection MI border zone	In vivo, SEMA3A can enhance hemodynamics, reduce infarct size, and prevent ventricular arrhythmias and SCD caused by MI.
Maden et al., 2012 (48)	Mice	Healthy	Nrp1-/-, Sema3a-/-, Sema3f-/-, Sema3a-/-Sema3f-/-, conditional Nrp1-/-	To support appropriate sympathetic nervous system function in adulthood, NRP1 transmits SEMA3A signals in the sympathetic lineage, in vivo.
Hofmann et al., 2013 (63)	Humans	Syndromic short stature	Biallelic mutations in SEMA3A resulting in 80% loss of SEMA3A expression	Patient with biallelic mutations in SEMA3A shows postnatal short stature with relative macrocephaly, camptodactyly, patent foramen ovale, and several minor anomalies.

Reference	Species	Healthy/ disease model	Semaphorin modulation	Main findings
Nakano et al., 2013 (62)	Humans	Unexpected cardiac arrest	SEMA3A nonsynonymous polymorphism (I334V, rs138694505A.G)	Polymorphism of SEMA3AI334V diminishes the cardiac sympathetic innervation gradient and partially contributes to the aetiology of unexpected cardiac arrest.
Hu et al., 2016 (43)	Rats	Myocardial infarction	Myocardial injection of lentiviral Sema3a shRNA, IV SEMA3A protein	In vivo, SEMA3A improves heart function after MI by regulating electrical activity.
Yang et al., 2016 (46)	Rats	Myocardial infarction	Sema3a adenovirus injection left stellate ganglion	Sema3a overexpression in the LSG reduces the induction of ventricular arrhythmias following MI by inhibiting neuronal remodelling.
Hao et al., 2018 (58)	Mice	Myocardial infarction	Gas5 cardiac adenovirus injection pre-MI	In vitro, GAS5 binds to Sema3a RNA, resulting in down-regulation of SEMA3A. In vivo, increased GAS5 reduces infarct size by inhibiting cardiomyocyte apoptosis after MI.
Ge et al., 2020 (41)	Mice	Healthy	WT	Fresh isolated myocardium tissue contains higher levels of SEMA3A compared to cultured myocardium tissue.
Wang et al., 2021 (44)	Rats	Myocardial infarction	WT	Chinese decoction (YQHX) increases SEMA3A protein levels but does not effectively inhibit neural regeneration.
Kurokawa et al., 2023 (71)	Mice	Isoproterenol-induced heart failure	IV SEMA3A protein	Administration of SEMA3A in CHF suppresses sympathetic innervation outgrowth and reduces myocardial remodelling.
Wagner et al., 2023 (61)	Mice	Aging	microRNA-145	Hearts of old mice show more SEMA3A expression. MicroRNA-145 overexpression increases SEMA3A expression. Sema3a overexpression induces denervation.

SEMA3A is also produced by the neurons themselves. The presence of SEMA3A in the ganglia could also be the result of retrograde transport of SEMA3A from the axon terminal in the heart to the cell body, e.g. in response to cardiac damage. Of note, SEMA3A is also synthesized in the Purkinje fibres of the cardiac conduction system after birth (36), as well as in cultured human atrial endocardial cells (47). SEMA3A is of relevance for the sympathetic patterning and function of the heart (8,36,47). Cardiac-specific overexpression of Sema3a results in diminished sympathetic innervation and attenuation of the sympathetic epicardial-to-endocardial gradient of the myocardium. In contrast to wild-type mice, Sema3a knockout mice show decreased subepicardial and increased subendocardial nerve density, reducing the ventricular transmural innervation gradient (36). The disturbed epicardial-to-endocardial gradient in mice with over- or under-expression of Sema3a make mice more prone to ventricular arrhythmias. Mice with decreased expression of SEMA3A in the atrial subendocardium are more susceptible to atrial fibrillation. In the atria of TGF-β transgenic mice and atrial fibrillation patients, increased fibrosis of the atrial subendocardium can be found, accompanied by decrease in SEMA3A expression in the same region. This is due to increased levels of TGF-β that activates microRNA-181b which in its turn decreases SEMA3A expression. TGF-β transgenic mice were more prone to develop atrial fibrillation than wild-type mice. In patients with atrial fibrillation, increased serum TGF-β and microRNA-181b levels were found and SEMA3A expression was significantly decreased in the atrial subendocardium compared to patients with sinus rhythm. The atrial innervation pattern in mice and patients was not reported (47).

Overexpression of Sema3a and the resulting innervation disturbances, result in sudden cardiac death of mice due to ventricular tachycardia as shown by Ieda et al. (36). In Sema3a overexpression, it is proposed that the diminished innervation results in catecholamine supersensitivity as demonstrated by increased expression of the adrenergic receptor β1 by 1.5 fold in Sema3a transgenic mice as compared to wild type mice. Also, Sema3a transgenic mice showed action potential duration lengthening due to disturbed repolarization currents. The action potential duration lengthening was inversely proportional to the sympathetic innervation density (36). Both mechanisms increase the risk of ventricular arrhythmias.

In Sema3a knock-out mice, upon norepinephrine stimulation, spontaneous ventricular extrasystoles were provoked, but no sustained ventricular arrhythmias (36). Sema3a knock-out mice show also disturbed heart rate regulation with spontaneous slowing of the heart rate (sinus bradycardia). These abnormalities in heart rate could not be explained by histological changes in the cardiac conduction system; Sema3a knock-out animals show no abnormalities in the composition of the sinoatrial and atrioventricular nodes and His bundles. However, in the absence of Sema3a, malformations were detected in the stellate ganglia, and sympathetic neurons were found in dislocated positions bilateral to the vertebrae instead of accumulated as organized ganglia. Basal sympathetic activity was found lower than

in wild type mice. Most likely, the disturbed functioning of the ganglia leads to inappropriate input to the sinoatrial node causing sinus bradycardia (36).

Loss of Sema3a in mice is not as severe as loss of the co-receptor Nrp1 in mice, which is embryonically lethal (36,48). The fact that loss of Sema3a is less severe than loss of Nrp1, can be explained by the fact that for members of the semaphorin class 3, except for SEMA3E, NRP1 acts solely as a coreceptor to execute their function (34). It also suggests that Sema3a loss could be (partly) compensated by other semaphorin class 3 members. Similar to knock down of Sema3a, in Sema3f knock-out mice ganglia dislocation has also been observed (23,48). This similarity in phenotype suggests that SEMA3A and SEMA3F may work in synergy to modulate ganglion development, function and neurite guidance. In mice lacking Nrp1 in the neural crest cell lineage, the epicardial-to-endocardial innervation gradient is lost and sinus bradycardia is registered on their electrocardiogram (48), as has also been observed in Sema3a knock-out mice (36). Taken together, the SEMA3A-NRP1 pathway is essential for proper development and functioning of cardiac autonomic innervation, which, in turn, is required for proper functioning of elements of the cardiac conduction system.

Disruptors of SEMA3A signalling

Most research on the SEMA3A pathway focuses on its receptor PLXNA4 and co-receptor NRP1, or potential co-operators like SEMA3F. Less research has been done on other agents that might interfere with the SEMA3A pathway. However, several studies have been conducted on the low-affinity NGF receptor (NGFR) and VEGF as potential disruptors of the SEMA3A pathway (**Figure 3**).

NGFR acts as a common receptor for neurotrophins such as (pro)NGF and BDNF and its activation has been linked to multiple biological processes like cell survival, axonal growth, degeneration, and repulsion (49,50). The impact of NGFR relies highly on different parameters, such as the ligand, co-receptor, and cell type. Therefore, the resulting signalling mediated by NGFR can either promote or inhibit axonal outgrowth. For instance, NGFR activation by BDNF reduces axonal growth (51), but when NGFR interacts with a PLXNA4/NRP1 complex, it inhibits SEMA3A's axonal repellent effect on skin sensory neurons (52). This PLXNA4/NGFR complex seems to decrease the neuron sensitivity to SEMA3A (**Figure 3**).

Mice lacking Ngfr have abnormal sympathetic innervation in the left ventricle, leading to reduced innervation of the subendocardium and septum (33). Electrocardiogram analysis has revealed increased premature ventricular contractions in Ngfr knock-out mice. Furthermore, in vitro experiments on stellate ganglia explants have shown decreased axonal outgrowth in Ngfr knock-out ganglia, which is attributed to heightened sensitivity to SEMA3A. This increased sensitivity to SEMA3A results in an uneven innervation between ventricles, potentially increasing ventricular arrhythmia risk (33). These findings emphasize the role of NGFR in left ventricular sympathetic innervation and cardiac function.

Next to NGFR, VEGF can also disrupt the SEMA3A pathway, as VEGF competitively binds to the receptor NRP1 thereby blocking SEMA3A to bind to NRP1 (**Figure 3**). Indeed, several studies suggest that VEGF and its receptors support sympathetic innervation (40,53). Marko et al. (32) proposed that SEMA3A inhibition by VEGF reduces growth cone collapse in sympathetic neurons, leading to increased innervation. In addition, VEGF has also been shown to influence sympathetic nerve growth in foetal hearts (54). Of note, as a strong cardiac-neurovascular interface has been shown (37), the reverse is also of interest. For example, in a mouse model with pressure overload-induced heart disease, SEMA3A was found to be upregulated in microvascular endothelial cells. Knockdown of SEMA3A in microvascular endothelial cells resulted in greater capillary density and less fibrosis of the heart compared to control. These results suggest that SEMA3A plays a role in the loss of microvasculature and cardiac remodelling in cardiac hypertrophy. A concomitant factor in microvascular rarefaction seems to be that SEMA3A impairs the adaptive response by competitively binding to NRP1 and thus counteracting the effects of VEGF (55).

SEMA3A expression and functional consequences after myocardial infarction and heart failure

After MI, sympathetic hyperinnervation has been described in a myriad of animal and human models (4-6,9-11,56,57). The precise mechanisms are still unknown and SEMA3A has emerged as an intriguing candidate for investigating its potential role in myocardial remodelling and hyperinnervation following MI.

Sema3A expression changes upon myocardial infarction and in heart failure

Several studies showed a decrease in the expression of Sema3A in the heart after acute injury. Wang et al. (44) used male rats and induced MI by ligating the left anterior descending coronary artery. They reported decreased mRNA and protein expression of SEMA3A at 4 weeks after MI in the rat myocardium of the border zone of the infarcted area and in the stellate ganglia compared to sham-operated rats. Hao et al. (58) also observed a decrease in mRNA and SEMA3A protein at 24 hours after MI in mice. In contrast, Hu et al. (43) reported an increase of SEMA3A protein levels from 1 to 5 weeks after MI in the MI border zone in the rat myocardium when compared to sham-operated rats.

In accordance with previously reported downregulation of Sema3a after myocardial damage (44,58), an in vitro study by Ge et al. (41) noted that in mice Sema3a expression was higher in freshly isolated healthy myocardium than in cultured myocardium, which was considered to represent more damaged myocardial tissue. Additional quantification of Sema3a mRNA levels in myocardium co-cultured with epicardium-derived cells (EPDCs) showed a decline of Sema3a expression as compared to cultures without EPDCs, potentially explaining the increase in neurite

outgrowth in the presence of EPDCs. The decline in Sema3aA expression could explain the occurrence of sympathetic hyperinnervation, as this would diminish the amount of axonal repellence and growth cone collapse and could thus induce overgrowth of newly forming and regenerating nerves (**Figure 4**).

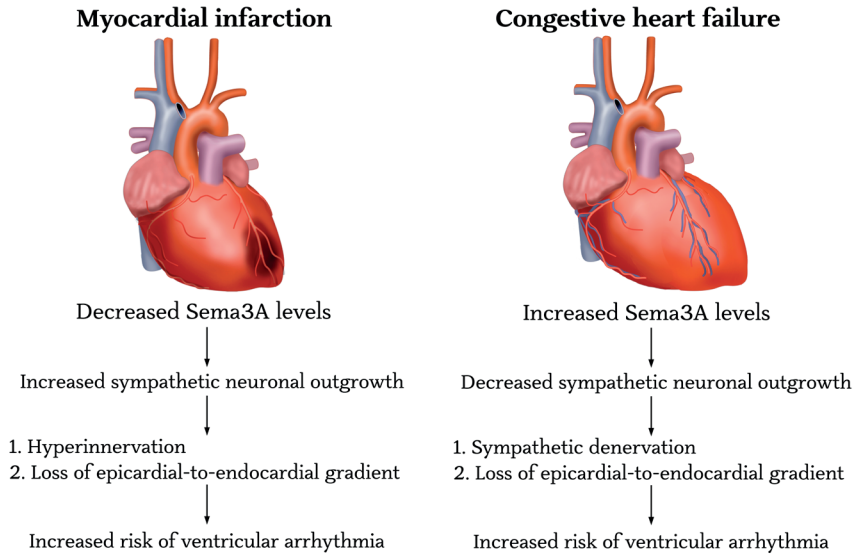

Figure 4. Functional consequences on cardiac innervation.

Cardiac autonomic dysfunction is also part of the spectrum of adverse sequelae of congestive heart failure (CHF). As a response to reduced cardiac output, in the early stages of CHF, the sympathetic nervous system becomes more activated, leading to an increase of heart rate and contractility to compensate for the loss of ventricular function. Over time, the sympathetic nerve density of the ventricular myocardium decreases, potentially rendering patients more prone to (lethal) ventricular arrhythmias (**Figure 4**) (42,59,60). SEMA3A levels have been measured at 30 and 60 days after heart failure induction in rats by a 2-day consecutive injection of isoproterenol, showing significantly increased mRNA and protein levels of SEMA3A at both time points compared to control. Immunohistochemical analysis showed higher intensity and more diffusely scattered SEMA3A expression than in the control group in which SEMA3A was predominantly located in the endocardium (42). Likewise an increase in SEMA3A plasma levels and protein expression was found in hearts of angiotensin II induced cardiac overload in a cardiac hypertrophy mouse model. These increased SEMA3A levels were also found in human cardiac tissue from

hypertrophy hearts compared to control hearts, and in plasma of patients with heart failure with preserved ejection fraction (55).

A similar process seems to take place in the aging heart. In a mouse study by Wagner et al. (61), it was found that 18-month old mice had a significantly reduced left ventricular nerve density when compared to 3-month old mice and showed a higher expression of SEMA3A. Denervation was more pronounced in the myocardial and subendocardial regions than in the epicardial region. Senescent endothelial cells stimulate the release of SEMA3A, thus stimulating the denervation of the surrounding (cardiac) tissue. Another mechanism contributing to this process is microRNA-145, which depresses SEMA3A protein levels. Due to reduction of microRNA-145 levels in aging hearts, SEMA3A expression is increased in the heart. The older mice showed less heart rate variability, decreased diastolic left ventricular function and were more susceptible to ventricular arrhythmias (61).

Sema3A polymorphisms linked to sudden cardiac arrest in human

In humans, idiopathic ventricular fibrillation has been linked to genetic variations in SEMA3A. Nakano et al. (62) have investigated SEMA3A variants in patients with unexplained cardiac arrest, i.e. ventricular fibrillation, that is not caused by any recognized disease or electrical abnormality. Nakano et al. sequenced the SEMA3A gene in 83 Japanese patients diagnosed with unexplained cardiac arrest and 2958 healthy controls. They identified in 16% of the patients a non-synonymous single nucleotide polymorphism (SNP) on exon 10 of the SEMA3A gene (heterozygote), compared to only 6% in controls. Patients with the SNP showed loss of the epicardial-to-endocardial innervation gradient and significantly more often had sinus bradycardia. These results were consistent with findings in mice with loss of Sema3a (36,48). In vitro testing of the functional consequence of the SNP indicates that the SNP does not influence the size or expression of SEMA3A protein, but it does reduce SEMA3A's repellent effect (62). As the SNP is located in exon 10 of the SEMA3A gene, which codes part of the SEMA domain crucial for binding to plexins/neuropilins, it can explain the reduced repellent capacity of the mutated SEMA3A. A case report investigating a SEMA3A biallelic mutation causing a defect in SEMA3A function showed the multiple effects of a SEMA3A genetic defect in humans (63). Their patient showed short stature, skeletal deformities and a persistent foramen ovale, consistent with previous phenotypes found in mice with Sema3a knockout. However, no anomalies in cardiac innervation patterns were discovered in this case.

Another study reported the function of SEMA3A as a potassium channel blocker (the Kv4.3 channel) and suggested a potential link to Brugada syndrome, that makes patients prone to sudden cardiac death. An increase in current over this potassium channel has been identified as the pathogenic basis of some forms of Brugada syndrome. Boczek et al. (64) identified 2 very rare SEMA3A mutations in 2 patients diagnosed with Brugada syndrome that suppress the function of SEMA3A as blocker

of the Kv4.3 channel. However, in these 2 patients it is unknown whether their innervation pattern is also abnormal (64).

Taken together, current literature indicates that SEMA3A dysfunction leads to inappropriate cardiac nerve patterning and increases the risk of ventricular arrhythmias as well as potential sinus node disease due to an abnormal innervation state. Phenotypically, the defect influences cardiac innervation, but may also lead to congenital deformities of the skeleton and heart, suggesting that SEMA3A is an heterogenous guidance cue that works on different organ systems.

Clinical implications

SEMA3A is involved in a wide array of cardiac autonomic functions, both in health and disease and sustains a narrow balance for adequate functioning of the cardiac sympathetic nervous system. This provides prospects but also challenges for the possibilities of implementing SEMA3A in therapeutic strategies. As far as we are aware, there a currently no running trials involving SEMA3A and heart disease (clinicaltrials.gov, visited 18-11-2024). However, SEMA3A does present as a promising protein to use or modulate the implications of cardiovascular disease (for example MI) or to attenuate cardiac disease progression. Multiple studies have already started to explore the therapeutic possibilities of SEMA3A and are discussed below.

Modulating sympathetic hyperinnervation after MI to diminish the risk of deadly ventricular arrhythmias is a much researched therapeutic topic. Yang et al. (46) showed that inducing overexpression of Sema3a can attenuate nerve sprouting in post-MI ganglia and in the peri-infarct area of the heart. The researchers induced overexpression of Sema3a in the left stellate ganglion (LSG) of rats one week after MI by intra-LSG injection of an adenovirus encoding Sema3a. Overexpression of Sema3a in the left stellate ganglion down-regulated nerve sprouting in post-MI ganglia and in the peri-infarct area of the heart, quantified by Growth-associated protein 43 (GAP43) expression. GAP43 is a protein promoting growth cone formation in the developing and adult nervous system and is found in (re)generating nerves (65,66). Of interest, GAP43 has also been demonstrated to contribute to sympathetic remodelling in the post-MI heart by enhancing sympathetic nerve sprouting (67,68). Another study reported on the use of the traditional Chinese medication Yiqi Huoxue Decoction, a plant-based decoction composed of Astragalus membranaceus, Angelica sinensis, Ligusticum chuanxiong Hort, Panax notoginseng, and Panax ginseng, in relation to post-MI innervation. Treatment with Yiqi Huoxue Decoction after MI resulted in increased SEMA3A levels in the LSG and reduced levels of GAP43 in the LSG (44). The diminished GAP43 expression (and concomitant diminished expression of tyrosine hydroxylase (TH), a marker for sympathetic innervation) in the LSG, upon the increased level of SEMA3A in the LSG, was also found in the heart itself (44). These results are in line with the results of Yang et al. where Sema3a was overexpressed in LSG (46).

Intravenous or intracardiac administration of SEMA3A also decreased the sympathetic nerve density (as observed by decreased expression of GAP43 and TH) in the border zone (43,69), while knockdown of Sema3a in the infarcted area of the heart resulted in an increased TH positive nerve density in the infarcted border zone, as shown by Hu et al (43). TH is a marker of cardiac sympathetic innervation, but it does not necessarily indicate whether the nerves are functional. The level of norepinephrine indicates how the cardiac sympathetic nervous system is functioning. Hu et al. (43) noticed that a decrease in TH was accompanied by a decrease in norepinephrine in rats treated with SEMA3A and vice versa. The levels of the parasympathetic nerve marker choline acetyltransferase and its functional marker acetylcholine were unaffected by SEMA3A levels. Next, functionally it has been shown that treatment with SEMA3A, intravenous or overexpression in LSG significantly reduced the inducibility of ventricular arrhythmias during electrophysiological examination in multiple studies, while Sema3a knock-down in the infarcted area of the heart in rats showed significantly increased inducibility of these arrhythmias (43,45,46). The administration of SEMA3A in the border zone also significantly increased heart rate variability and reduced infarct size (45). A higher heart rate variability is associated with a balanced input from the sympathetic and parasympathetic nervous systems and a sign of a normally functioning autonomic nervous system (70).

Kurokawa et al. (71) studied in a CHF mice model also the effects of SEMA3A treatment by intravenous injection of SEMA3A protein at day 7 and day 11 after start of isoproterenol to induce heart failure. In the SEMA3A treated mice, less TH positive nerve fibres were found compared to the untreated CHF mice and myocardial contractility and normal cardiac conduction were maintained.

Also for atrial fibrillation SEMA3A poses as a possible treatment target. Lai et al. (47) treated transgenic TGF-β mice with an inhibitor of microRNA-181b (antagomir-181b) to counteract the effects of microRNA-181b. This resulted in increased SEMA3A expression and less fibrosis of the atria compared to a scrambled control group. The treated mice were less prone to develop atrial fibrillation. Lai et al. (72) also studied the effect of naringin, an active flavanone glycoside found in citrus fruits and a known modulator of multiple pathophysiological risk factors for cardiovascular disease. Human atrial endocardial cells were treated with TGF-β or TGF-β in a low dose combined with naringin. Cells treated with naringin showed increased expression of SEMA3A. Oral administration of naringin to transgenic TGF-β mice showed less fibrosis of the atrial subendocardium than untreated animals and an increase in SEMA3A expression in the subendocardium. Also, naringin rendered these mice less vulnerable to the development of atrial fibrillation upon electrical stimulation (72).

Another potentially grateful therapeutic target is the aging heart, as aging causes decline in innervation as well as increased SEMA3A levels in the heart (61). Wagner et al. (61) treated 18 to 19 month-old mice with 5 mg/kg dasatinib and 50 mg/kg quercetin for 2 months to reverse the aging-process by reducing the number

of senescent cells. After 8 weeks no significant differences were seen anymore between the old and young mice regarding diastolic function, heart rate variability and ventricular arrhythmia vulnerability.

Taken together, the results from various studies indicate that SEMA3A is a valuable option to explore in the context of the treatment of cardiac disease. Increased presence of SEMA3A actively reduces cardiac hyperinnervation after MI by lowering the number of functioning sympathetic fibres and thus reduces the risk of ventricular arrhythmias and infarct size. Also in CHF, atrial fibrillation and aging the modulation of SEMA3A levels, directly or indirectly, shows promise for the future in which treatment to reverse the effects of these (disease) processes seems feasible.

Implications for other axonal guidance cues potentially affecting cardiac innervation

Our literature search revealed a scarcity of research on other axonal guidance cues in the context of cardiac innervation. For netrins and slits no articles related to postnatal cardiac innervation were found. However, they have been studied for innervation in other organs.

Previous studies have demonstrated a role of netrin-1 as a neuronal guidance cue in the development of the nervous system and in repair processes such as facilitating vagal nerve repair after partial hepatectomy and in adrenergic nerve remodelling in lung fibrosis (73,74). Next to netrin-1 also netrin-4 (NTN4) is of significance in the development of the nervous system and other organ systems (75). Recently, we found expression of NTN4 after myocardial ischemia in the epicardial cardiac layer at 3 days post-MI (Koppel et al, unpublished data). Studies investigating NTN4 and its specific role in postnatal cardiac repair are scarce, but NTN4 has been described in e.g. Meniere's disease, corneal damage, and was found to improve neurological outcomes after ischemic cerebral stroke (76-78). Increased nervous conduction velocities were found in a hind limb ischemia model in mice injected with NTN4 compared to control animals, suggesting a role of NTN4 in the regeneration of nerve fibres after ischemic injury (79,80). Though consensus exists about the fact that NTN4 has a function in neuronal development and axonal outgrowth, the exact mechanisms and binding receptors are still disputed (80,81). The function of NTN4 during neurogenesis and regeneration is considered concentration-dependent: low concentrations act as axonal attractant and high concentrations act as axonal repellent through respectively the unc-5 netrin receptor B and DCC netrin 1 receptor (38,82). Its role in (re)generation of the cardiac autonomic nervous system pre- and postnatally is yet to be investigated.

Slit proteins (SLIT1, SLIT2, SLIT3) were also reported to act as an important axonal repellent in the developing and adult nervous system, but have also a role in cardiac morphological development (83,84). Defects in signalling of SLIT-Roundabout guidance receptors resulted in multiple congenital heart deformations in zebrafish, drosophila and mice, including among others valve deformities and ventricular septal

defects (85). This indicates that slit proteins, as well as netrins, are valuable candidates for further research to understand the processes underlying the modulation of pre- and postnatal cardiac innervation.

Another group of axonal guidance cues of potential interest are the ephrins. EphrinB2 (EFNB2) has been reported to be involved in both the developing and adult nervous system. Wang et al. (86) revealed a role of EFNB2 as an axonal repellent cue modulating sympathetic hyperinnervation. Upregulation of Efnb2 resulted in reduced sympathetic sprouting and a decreased incidence of ventricular arrhythmias. EFNB2 is also involved in cardiovascular development. Adams et al. (87) showed that Efnb2 knock-out mice embryos were not viable, indicating a critical role of EFNB2 in embryonic development. In the adult heart, EFNB2 has been shown relevant for cardiac tissue architecture by modulating intercellular gap junctional communication between cardiomyocytes (88). EFNB2 also contributes to angiogenesis following MI by exerting a pro-angiogenic effect when coupled to the EPH receptor B4 (89). These results make EFNB2 also an axonal guidance cue to be considered for further study in the context of post-MI cardiac nervous remodelling.

Conclusions

This review provides an overview of the current knowledge regarding the functions of SEMA3A in the postnatal heart in health and disease (**Table 1**), highlighting the importance of SEMA3A in post-natal cardiac innervation. SEMA3A functions through a PLXNA4/NRP1 receptor complex to exert its repellent effect on axonal outgrowth, which plays a crucial role during cardiac development in the formation of the epicardial-to-endocardial innervation gradient. SEMA3A function is also modulated by other factors, as SEMA3A works synergistically with its plexin and NRP receptor to ascertain correct gangliogenesis and neurite guidance (**Figure 3**). In addition, in the presence of the NGF receptor NGFR, cardiac sensitivity to SEMA3A is decreased and results in anomalous patterning of the innervation (**Figure 3**). Another important modulatory factor is VEGF. VEGF prevents SEMA3A from attaching to NRP1 and thereby inhibits SEMA3A's repellent effect on axonal growth (**Figure 3**).

SEMA3A is also involved in cardiac remodelling after MI and in congested heart failure. Studies investigating SEMA3A post-MI have demonstrated its ability to decrease the infarct zone and amount of sympathetic hyperinnervation (**Figure 4**). Cardiac SEMA3A expression is decreased in post-MI myocardial tissue and as such influences the occurrence of sympathetic hyperinnervation. In addition, defects in the Sema3a gene that cause a reduction in its repellent effect, increase the risk of unexplained cardiac arrest. In congestive heart failure denervation was observed as a result of increased SEMA3A expression. The results from these studies show that a reduction in the function of SEMA3A increases the risk of ventricular arrhythmias and indicate that the administration of SEMA3A might be a potential treatment to counteract cardiac hyperinnervation and diminish the risk of potentially lethal

arrhythmias in different diseases. However, a disbalance in the other direction should be prevented, as was shown in a congestive heart failure model that caused increased susceptibility to ventricular arrhythmias due to decreased sympathetic innervation and cardiac remodelling (42).

To conclude, SEMA3A is a relevant factor to consider while studying regeneration and remodelling of the cardiac sympathetic nervous system after cardiac damage and overload, such as MI and in congestive heart failure. Other axonal guidance cues of potential relevance, such as NTN4, Slits, and EFNB2, are to date scarcely studied but may be of equal interest in the context of cardiac innervation in health and disease. Although the functions of SEMA3A are gradually being unravelled in rodents, data on the implications in humans is still scarce. The identified gaps in literature of the current review open avenues for future research regarding pathophysiology and treatment of cardiac sympathetic hyperinnervation.

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