Development, Maturation, and Transdifferentiation of Cardiac Sympathetic Nerves

Kensuke Kimura, Masaki Ieda, Keiichi Fukuda

Abstract: The heart is electrically and mechanically controlled as a syncytium by the autonomic nervous system. The cardiac nervous system comprises the sympathetic, parasympathetic, and sensory nervous systems that together regulate heart function on demand. Sympathetic electric activation was initially considered the main regulator of cardiac function; however, modern molecular biotechnological approaches have provided a new dimension to our understanding of the mechanisms controlling the cardiac nervous system. The heart is extensively innervated, although the innervation density is not uniform within the heart, being high in the subepicardium and the special conduction system. We and others showed previously that the balance between neural chemoattractants and chemorepellents determine cardiac nervous development, with both factors expressed in heart. Nerve growth factor is a potent chemoattractant synthesized by cardiomyocytes, whereas Sema3a is a neural chemorepellent expressed specifically in the subendocardium. Disruption of this well-organized molecular balance and innervation density can induce sudden cardiac death due to lethal arrhythmias. In diseased hearts, various causes and mechanisms underlie cardiac sympathetic abnormalities, although their detailed pathology and significance remain contentious. We reported that cardiac sympathetic rejuvenation occurs in cardiac hypertrophy and, moreover, interleukin-6 cytokines secreted from the failing myocardium induce cholinergic transdifferentiation of the cardiac sympathetic system via a gpi130 signaling pathway, affecting cardiac performance and prognosis. In this review, we summarize the molecular mechanisms involved in sympathetic development, maturation, and transdifferentiation, and propose their investigation as new therapeutic targets for heart disease. (Circ Res. 2012;110:325-336.)

Key Words: cardiac sympathetic nervous system ■ cardiac innervation patterning ■ nerve growth factor ■ cardiac rejuvenation ■ cholinergic transdifferentiation ■ IL-6 cytokines

Heart tissue is extensively innervated via the autonomic nervous system, which comprises sympathetic and parasympathetic nerves. The cardiac sympathetic nervous system (SNS) uses norepinephrine (NE) as a neurotransmitter and increases the heart rate (chronotropic) and conduction velocity (dromotropic), as well as myocardial contraction (inotropic) and relaxation (lusitropic). Sympathetic innervation density, which is highest in the subepicardium and central conduction system, is stringently regulated in the heart. Regional differences in sympathetic innervation correspond to different areas of influence over cardiac function that cooperate to effectively control cardiac performance. Despite the clinical importance of cardiac innervation, little is known about the developmental and regulatory mechanisms underlying sympathetic innervation patterning.

Cardiac innervation density is altered in pathological hearts, such as after myocardial infarction (MI), in which the cardiac nerves undergo Wallerian degeneration, followed by neurilemmal cell proliferation and axonal regeneration, resulting in heterogeneous innervations. Sympathetic nerve sprouting and unbalanced innervation density cause lethal arrhythmia through ion channel modulation in cardiomyocytes. On the other hand, sympathetic efferent neural tone is upregulated in failing heart, leading to excessive SNS activation and pathophysiological effects such as myocardial damage, depressed cardiac performance, and fatal arrhythmia. The continuous SNS activity also results in depleted cardiac NE content, probably due to NE spillover, as well as insufficiency of NE reuptake and sympathetic neuronal loss.

Recent molecular biotechnological advances have provided us with new insights into the mechanisms underlying cardiac SNS abnormalities at play in congestive heart failure (CHF). This article therefore reviews current understanding of the molecular mechanisms regulating cardiac sympathetic development, maturation, and transdifferentiation, focusing...
on the crosstalk between humoral factors derived from cardiomyocytes and sympathetic neurons. Such mechanisms should now be investigated as new therapeutic targets for heart disease.

Development of the Cardiac Nervous System

The heart is innervated by sympathetic, parasympathetic, and sensory nerves derived from neural crest cells. Trunk neural crest cells migrate and form sympathetic ganglia by midgestation, subsequently proliferating and differentiating into mature neurons.11,12 The cardiac sympathetic nerves extend from the sympathetic neurons in stellate ganglia, which are located bilateral to the thoracic vertebra. Sympathetic nerve fibers project from the base of the heart into the myocardium, and are located predominantly in the subepicardium of the ventricle (Figure 1).13,14 The central conduction system, which includes the sinoatrial node, atroventricular node, and His bundle, is abundantly innervated compared with the working myocardium.14–17 We and others have reported that this regional difference in cardiac sympathetic innervation is highly conserved among mammals.14,15,18,19

In contrast to sympathetic neurons, parasympathetic neurons are derived from cardiac neural crest cells, which migrate into the developing heart and participate in septation of the outflow tract into the aorta and pulmonary trunk, development of aortic arch arteries, and the formation of cardiac ganglia.20–23 The cardiac parasympathetic nerves extend from the parasympathetic neurons in cardiac ganglia, which are located in the base of both atria (Figure 1). Conventional wisdom until recently was that, unlike sympathetic nerves, parasympathetic innervation was scarce in the left ventricle (LV).24 However, Ulphani et al25 recently demonstrated that parasympathetic nerve fibers innervate both atria and ventricles, with higher nerve density on the ventricular endocardium, but greater nerve thickness on the epicardium. In addition, the right ventricle (RV) was more densely innervated than the LV, whereas the LV endocardium was more densely innervated than the RV endocardium.

Cardiac sensory neurons, which are located in the dorsal root ganglia, are derived from trunk neural crest cells, similar to sympathetic neurons. The cardiac sensory nervous system is responsible for pain perception and for initiating a protective cardiovascular response during myocardial ischemia. The sensory signals are conducted through cardiac afferents, primarily thinly myelinated A-fibers and unmyelinated C-fibers that project to the upper thoracic dorsal horn via dorsal root ganglia (Figure 1).26,27 Although the importance of neural control of cardiac performance is well recognized, the molecular mechanisms that regulate cardiac innervation during development were only recently elucidated.

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Non-standard Abbreviations and Acronyms

<table>
<thead>
<tr>
<th>Abbreviation</th>
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<tr>
<td>AF</td>
<td>atrial fibrillation</td>
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<td>ChAT</td>
<td>choline acetyltransferase</td>
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<td>CHF</td>
<td>congestive heart failure</td>
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<td>CNS</td>
<td>central nervous system</td>
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<td>CNTF</td>
<td>ciliary neurotrophic factor</td>
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<td>CRAMP4</td>
<td>collapsin response mediator protein 4</td>
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<td>ET-1</td>
<td>endothelin-1</td>
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<td>GAP43</td>
<td>growth-associated protein 43</td>
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<td>GDNF</td>
<td>glial cell line–derived neurotrophic factor</td>
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<td>GDNFα</td>
<td>GDNF family receptor α</td>
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<td>IL</td>
<td>interleukin</td>
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<td>LIF</td>
<td>leukemia inhibitory factor</td>
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<td>LV</td>
<td>left ventricle</td>
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<td>MI</td>
<td>myocardial infarction</td>
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<td>NE</td>
<td>norepinephrine</td>
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<td>NFAT</td>
<td>nuclear factor of activated T-cell</td>
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<td>nerve growth factor</td>
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<td>NT-3</td>
<td>neurotrophin-3</td>
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<td>NT-4/5</td>
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<td>PAF</td>
<td>paroxysmal AF</td>
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<tr>
<td>RV</td>
<td>right ventricle</td>
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<tr>
<td>SNS</td>
<td>sympathetic nervous system</td>
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<tr>
<td>TH</td>
<td>tyrosine hydroxylase</td>
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<tr>
<td>Trk</td>
<td>tropomyosin-receptor kinase</td>
</tr>
<tr>
<td>TRPC3/6</td>
<td>transient receptor potential channel, canonical 3 and 6</td>
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Figure 1. Anatomy and distribution of the cardiac nervous system. The cardiac sympathetic nerves (blue) extend from sympathetic neurons in the stellate ganglia, which are located bilateral to the thoracic vertebra. The cardiac parasympathetic nerves (red) extend from parasympathetic neurons in the cardiac ganglia, which are located in the base of both atria. The sensory nerves (green) project to the upper thoracic dorsal horn via dorsal root ganglia. The inset indicates the transverse section of the heart and demonstrates the distribution of sympathetic nerve fibers within the left ventricle.
Neurotrophic Factors Are Critical Chemoattractants for Cardiac Sympathetic Innervation

The growth-cone behavior of nerves is modulated by coincident signaling from neural chemoattractants and chemorepellents synthesized in the innervated tissue. Neurotrophic factors play critical roles as chemoattractants in the peripheral organs and central nervous system (CNS) and are classified into the following groups: (1) the neurotrophin family, which includes nerve growth factor (NGF), brain-derived neurotrophic factor, neurotrophin-3 (NT-3), and neurotrophin-4/5 (NT-4/5), which bind to the tyrosine kinase receptors, tropomyosin-receptor kinase (Trk), with high affinity, and to the TNF receptor, p75, with lower affinity; (2) the glial cell line–derived neurotrophic factor (GDNF) family, which includes GDNF, neurturin, and artemin, which bind to the GDNF family receptor α (GFRα) and RET.28–34

NGF is the prototypic member of the neurotrophin family and has been studied extensively. The levels of NGF expression within innervated tissues including heart correspond approximately to the levels of sympathetic innervation density. Indeed, sympathetic ganglion volume is reduced by 80% at postnatal day 3 in mice with a disruption of the NGF gene,35 whereas in mice lacking the NGF receptor TrkA, no neurons remain at postnatal day 9.30,36 Deletion of a single copy of the NGF gene results in a 50% reduction in sympathetic neurons, whereas overexpression of NGF in the heart causes cardiac hyperinnervation and hyperplasia in stellate ganglia neurons.37,38 Together, these results demonstrate the importance of NGF in sympathetic neuronal development via its role in preventing cell apoptosis. In addition to being required for sympathetic neuron survival, NGF mediates axonal growth and synapse formation during development. Glebova et al39 reported that NGF is required for complete peripheral innervation of sympathetic ganglia targets in vivo independently of its requirement for neuronal cell survival in NGF and Bax double-knockout mice, whereas Sharma et al40 demonstrated that target-derived NGF is necessary and sufficient for synapse formation within sympathetic ganglia via its retrograde actions. These results confirm the importance of NGF for regulating sympathetic neuron development and cardiac innervation.

Despite such demonstrated importance of NGF in sympathetic neural development, the upstream molecules that regulate NGF expression in vivo remained unknown.41 We found that endothelin-1 (ET-1) specifically upregulates NGF expression in primary cultured cardiomyocytes among several cardiac hypertrophic factors.37 In addition, NGF expression and cardiac sympathetic innervation were reduced in ET-1–deficient mouse hearts, but not in the hearts of angiotensinogen-deficient mice. In ET-1–deficient mice, the sympathetic stellate ganglia also exhibited excessive apoptosis and neuronal loss.12,37 Moreover, we found that cardiac-specific overexpression of NGF in ET-1–deficient mice rescued the sympathetic nerve retardation. These findings together indicated that ET-1 is a key regulator of NGF expression in cardiomyocytes, and that the ET-1/NGF pathway is critical for sympathetic innervation in the heart.37 In contrast to this NGF upregulation by ET-1, mechanical stretch and α-1-adrenergic stimulation downregulated NGF via activation of the calcineurin–NFAT pathway and subsequent stimulation of myocytes, resulting in decreased neurite outgrowth. Mean-while, inhibition of the myocyte calcineurin–nuclear factor of activated T-cells (NFAT) pathway increased neurite outgrowth of cardiac sympathetic neurons in vitro. Further in vivo studies are needed to confirm the role of calcineurin–NFAT/NGF signaling in the developing cardiac sympathetic nervous system.

Although the requirement of NGF as a final target-derived survival factor for sympathetic neurons is well established in many tissues, the relative contribution of factors released by intermediate targets, such as the vasculature, to growth and survival of these neurons was less clear.35,43 Many sympathetic axons grow in close proximity to blood vessels, an intermediate target that express abundant NT-3, en route to their final targets including heart.12,44 Kuruvilla et al45 found that the related neurotrophins NGF and NT-3 signal through the same receptors, TrkA and p75, to coordinate distinct stages of sympathetic neuron development. NT-3 derived from vascular smooth muscle cells activates TrkA on sympathetic axons, allowing for rapid and robust axonal extension along the vasculature at times when p75 levels are low.46 As axons approach target organs including heart, and begin to acquire target-derived NGF, the ensuing retrograde NGF/TrkA signaling promotes survival, anabolic responses, and expression of p75. The increase in p75, in turn, diminishes axonal responsiveness of TrkA to NT-3, enabling target-derived NGF to become the dominant axonal growth factor.46,47 Kuruvilla et al45 proposed that this hierarchical and finely tuned neurotrophin signaling facilitates the extension of sympathetic axons from intermediate targets into heart.

GDNF Signaling Regulates Parasympathetic Innervation and NGF Signaling Is Critical for Sensory Innervation in the Heart

Parasympathetic nerve development is, at least in part, dependent on GDNF signaling. Hiltunen et al48 demonstrated that signaling activity by GDNF family members and their receptors GFRα2 and Ret is necessary for parasympathetic innervation of the heart. Ret-deficient mice exhibited a reduced volume of cardiac ganglia and cholinergic denervation of the ventricular conduction system. Moreover, adult Gfra2 knockout mice showed reduced cholinergic innervation by 40% in their ventricles and by 60% in the ventricular conduction system. The cardiac SNS was not disrupted in either of these knockout mice. These findings indicated that GFRα2/Ret signaling is specifically required for normal cholinergic innervation of heart.48 In contrast to certain cranial parasympathetic ganglia, which are completely absent in Ret-deficient mice,49 the number of neurons in cardiac ganglia were only reduced by half, and the cholinergic innervation was present, although at a decreased level, in the ventricular conduction system. These results suggested that regulatory mechanisms of parasympathetic innervation could differ among tissues and that other neurotrophic factors may contribute to the survival of the cardiac ganglion neurons in GFRα2/Ret-deficient mice.48,50
Intriguingly, Rana et al. recently implicated NGF as an important growth-inducing factor for cultivated cardiac parasympathetic nerves and NT-3 was identified as an important acetylcholine-releasing factor in vitro. Further in vivo studies should therefore be undertaken to reveal the role of these neurotrophic factors in the developing cardiac parasympathetic nervous system.

In contrast to somatic tissues, visceral organs such as heart are believed to be rich in autonomic efferent innervation, but poor in nociceptive afferent nerves.25 Zahner et al.53 reported that vanilloid receptor-1–immunopositive sensory nerves are enriched in the epicardium, but scarce in the myocardium. We also reported that cardiac sensory innervation is rich both at epicardial sites and in the ventricular myocardium, and that sensory innervation increases with development.54,55 In our screen of several neurotrophic factors, we found that cardiac sensory nerves develop in parallel with NGF synthesized in the heart.55,56 Cardiac nociceptive sensory nerves that are immunopositive for calcitonin gene–related peptide, the dorsal root ganglia, and the dorsal horn are markedly retarded in NGF-deficient mice, whereas cardiac-specific overexpression of NGF rescues these deficits. Thus, NGF synthesis in the heart is critical for the developing cardiac sensory nervous system.45 We and others also found reduced NGF in diabetic hearts which might explain the cardiac sensory denervation and neuropathy reported in diabetic patients.55,57–59

Sema3a Is a Critical Neural Chemorepellent and Determines Sympathetic Innervation Patterning in the Heart

As discussed above, neurotrophic factors play critical roles as chemoattractants in cardiac nerve development. In contrast, the neural chemorepellent that induces growth-cone collapse and repels nerve axons in heart remained unidentified. Sema3a is a Class 3 secreted semaphorin that was cloned and identified as a potent neural chemorepellent and directional guidance molecule for nerve fibers in the skin.60–62 However, it was not known whether cardiomyocytes produce Sema3a, and, if so, whether this protein affects sympathetic neural patterning and cardiac performance.

We found that Sema3a is strongly expressed in the developing heart at E12 and gradually reduced with development.19 By analyzing Sema3a knocked-in lacZ mice, we found Sema3a expression in the subendocardium, but not in the subepicardium of the atria and ventricles, in contrast to the epicardial-to-endocardial gradient of sympathetic innervation (Figure 2).19,63 These results indicated that Sema3a acts with the opposite kinetics and distribution pattern of expression to those of sympathetic innervation in developing hearts, implicating Sema3a as a negative regulator of cardiac innervation. Sema3a knockout mice also showed disrupted sympathetic innervation patterning and malformation of the stellate ganglia that extend sympathetic nerves to the heart. Cardiac-specific Sema3a-overexpressing mice had reduced sympathetic innervation and attenuation of the epicardial-to-endocardial innervation gradient. These results indicated that cardiomyocyte-derived Sema3a plays critical roles in cardiac sympathetic innervation patterning by inhibiting neural growth.

Importantly, the cardiac-specific Sema3a-overexpressing mice died suddenly and sustained ventricular tachyarrhythmias, which were easily induced by epinephrine administration and programmed electric stimulation (Figure 2). The Sema3a-overexpressing mice also had upregulated β-adrenergic receptor density due to denervation supersensitivity and prolonged action potential duration, both of which can augment triggered activity in cardiomyocytes.19,64–68 Thus, the highly organized innervation patterning mediated by Sema3a is critical for the maintenance of arrhythmia-free hearts.

Crosstalk Between Neurotrophin and Semaphorin Signaling in Nerve Development

Since cardiomyocyte-derived NGF acts as a chemoattractant, it is possible that the balance between NGF and Sema3a synthesized in the heart determines cardiac sympathetic innervation patterning. The growth-cone behavior of somatic sensory axons is modulated by coincident signaling between NGF and Sema3a,69,70 both of which are expressed within the developing spinal cord and influence the pathway guidance of sensory axons during development. Sema3a is specifically expressed in the ventral half of the spinal cord and mediates NGF-responsive sensory axons to terminate at the dorsal part of the spinal cord.60,71 In addition, targeted inactivation of Sema3a disrupts neural patterning and projections in the spinal cord, further highlighting the importance of Sema3a signaling for the directional guidance of nerve fibers.63,72 However, the interactions of NGF and Sema3a signaling is further complicated, as the low-affinity NGF receptor p75 can...
partner with the Sema3a receptors neuropilin-1 and plexin A4 to attenuate their combined ability to repel growing axons.73 Lorentz et al.24 found that the subendocardium innervation of adult p75 knockout ventricles was disrupted with the left ventricle essentially devoid of sympathetic nerve fibers, whereas innervation density of the subepicardium was normal. This neural patterning defect is similar to that seen in mice overexpressing Sema3a, such that the sympathetic axons lacking p75 are highly sensitive to Sema3a-mediated inhibition of neurite outgrowth. The heterogeneous innervation was also associated with altered cardiac β1-adrenergic receptor expression and sensitivity, and a significant increase in spontaneous ventricular arrhythmias, which were also observed in Sema3a-overexpressing mice. These results suggested that as sympathetic neurons are innervating the heart, p75, a receptor for neurotrophins, acts to blunt the repulsive effects of Sema3a from the subendocardium, thereby allowing axonal arborization and orchestrating a highly organized epicardial-to-endocardial innervation gradient in the ventricle.75

**Sympathetic Dysfunction in Heart Failure**

The pathology of heart failure involves various abnormalities in sympathetic terminals and sustained sympathetic activation accompanied by decreased NE levels in the myocardium.7,76 These effects have been attributed to increased NE turnover and spillover,8,9,77,78 and to malfunctions in the reuptake of NE (uptake 1).9,79–82 In addition, downregulation of tyrosine hydroxylase (TH), the rate-limiting enzyme for NE synthesis in innervated neurons,10,83,84 and anatomic denervation it- was also associated with decreased NE levels in the myocardium.7,76 The pathology of heart failure involves various abnormalities in sympathetic terminals and sustained sympathetic activation accompanied by decreased NE levels in the myocardium.7,76 These effects have been attributed to increased NE turnover and spillover,8,9,77,78 and to malfunctions in the reuptake of NE (uptake 1).9,79–82 In addition, downregulation of tyrosine hydroxylase (TH), the rate-limiting enzyme for NE synthesis in innervated neurons,10,83,84 and anatomic denervation itself10,85,86 were also implicated in the synaptic terminal abnormalities. In addition to the documented abnormalities, many unexplained events remain including the downregulation of TH despite sympathetic activation, as described above, such that the pathophysiological features are not yet fully understood. In an experimental animal model of heart failure, TH downregulation was not associated with quantitative changes in sympathetic density, suggesting that the abnormalities observed were mainly attributable to functional changes.87

Some researchers speculate that the decreased NE uptake is produced by NE-derived oxidative stress.88–90 In contrast, Backs et al.91 demonstrated that endogenous ET-1 impairs cardiac NE uptake function via the ETA receptor in an experimental heart failure model. In practical terms, this finding demonstrated that antioxidant vitamins and ETA antagonism improve NE uptake impairment in failing heart.79,81

**Involvement of the CNS**

There is mounting evidence that sympathetic efferent neuronal activity is increased in CHF.92–93 High plasma NE concentrations reflect the severity of such increased activity and thus provide an independent risk factor of poor prognosis in patients with CHF.6,94 On the other hand, administering β-adrenergic receptor blockers improves cardiac performance and reduces cardiac mortality.95

One of the mechanisms proposed to explain sympathetic activation in heart failure involves abnormalities in arterial and cardiopulmonary baroreceptors. Signals received by baroreceptors are transmitted to the CNS via afferent nerves and then further transduced via efferent nerves to suppress sympathetic activity.96 However, elevated Na\(^{+/}\)/K\(^{+}\)-ATPase activity in baroreceptors during heart failure appears to hyperpolarize the membrane and decrease receptor sensitivity, thereby prohibiting sympathetic suppression.97 Therefore, the known Na\(^{+/}\)/K\(^{+}\)-ATPase inhibitor, digitals glycosides, might be cardioprotective via recovery of baroreceptor function.98 Mechanisms mediated by the chemoreceptor reflex that senses hypoxic and hypercapnic conditions could also be involved in sympathetic activation,99 as could the cardiac sympathetic afferent reflex.100 Because sympathetic activation in heart failure cannot be explained solely by these autonomic mechanisms regulating hemodynamics, CNS involvement remain a subject of investigation.101 In particular, it is becoming clear that after MI, circulating angiotensin II induces an initial neuronal activation of circumventricular organs, resulting in increased in aldosterone biosynthesis/release in the forebrain, which in turn sustains the activation of hypothalamic nuclei.102 The decreased nitric oxide production in the brain also play important roles in central sympathetic activation.103,104 In addition, activation of a small G-protein Rho/Rho-kinase pathway in the brain stem was recently shown to modulate sympathetic activation in heart failure.105 Considering its relationship with the sympathetic nerves, the involvement of the CNS in sympathetic dysfunction is of considerable interest and further studies in this area are anticipated in the future.106

**The Role of NGF**

Although sympathetic tone from the CNS is augmented, the status of stellate ganglia that relay axonal information from CNS to the heart is critical for cardiac performance. Nuclei in the stellate ganglion modify the synthesis of sympathetic neurotransmitters under the influence of humoral factors derived from heart. For example, cardiac hypertrophic factors such as angiotensin II,107 ET-1,108 leukemia inhibitory factor (LIF),24 NGF,38,83,85 other growth factors,109 and cytokines110 are augmented in failing heart, influencing sympathetic activity, function, plasticity, and phenotype. Complex crosstalk among these neurohumoral factors is thus believed to modulate cardiac sympathetic activation and the pathology of heart failure.

Although Hasan et al.111,112 reported that peri-infarct inflammatory cells, including macrophages and myofibroblasts, play a major role in producing NGF after nerve sprouting and sympathetic hyperinnervation after MI, NGF is the most important cardiomyocyte-secreted humoral factor for maintaining sympathetic homeostasis.113,114 The complex of NGF and its tyrosine kinase receptor, TrkA, is retrogradely transported via the axon to the stellate ganglion nucleus,115 where it plays important roles in neuronal differentiation and survival, and in synaptogenesis.30 In addition, NGF expression in target tissues has been implicated in the regulation of sympathetic nerve fiber density.116 We further demonstrated using a rat model of right ventricular hypertrophy that upregulated ET-1 mRNA in the right ventricle was associated with elevated expression of NGF protein and increased...
sympathetic nerve density in the right ventricle.\(^{83}\) Despite ET-1 being a known cardiac hypertrophic growth factor and NGF recently identified as a cardiac prosurvival factor,\(^ {117,118}\) these results implicated the ET-1/NGF pathway as important for sympathetic innervation in both normal heart development and in pathological cardiac hypertrophy (Figure 3). It therefore seems likely that NGF derived from cardiomyocytes protects against apoptosis as an autocrine prosurvival factor,\(^ {117,118}\) while simultaneously acting as a paracrine neurotrophic factor for cardiac sympathetic innervation. In other words, once cardiomyocytes become apoptotic, sympathetic innervation is no longer indispensable. On the other hand, in an animal model of severe CHF induced by long-term exposure to a high plasma concentration of NE, we and others clearly showed a decline in myocardial expression of NGF and decreased sympathetic density.\(^ {85,86}\) Thus, NGF derived from target organs also acts as an anatomic modulator of sympathetic nerves.

Although the mechanism regulating NGF expression in CHF is not yet fully understood, recent data indicated that NGF expression could be suppressed by applying mechanical stretch stimulation to the myocardium via the transient receptor potential channel, canonical 3 and 6 (TRPC3/6)/calcineurin-nuclear factor of activated T-cells (NFAT) pathway. NGF expression is also attenuated by NE via \(\alpha_1\)-adrenergic receptors and calcineurin-NFAT pathways. Finally, cardiac hypertrophic factor LIF secreted from stressed cardiomyocytes causes sympathetic rejuvenation and cholinergic transdifferentiation via two receptor molecules, LIF receptor (LIFR) and gp130.

Using a rat model of heart failure, Kreussler et al.\(^ {122}\) reported that NGF directly injected into the stellate ganglia improved the NE uptake function, and, in turn, cardiac performance. This outcome accompanying NGF supplementation is quite reasonable because, as we mentioned above, cardiac NGF expression is decreased in decompensated heart. However, we now know that sympathetic cellular function, also involved in NE synthesis and NE reuptake, is already attenuated in the early stages of CHF\(^ {123}\) and compensated cardiac hypertrophy\(^ {83}\) in the absence of increased plasma NE levels. We previously demonstrated that hypertrophic hearts from monocrotaline-treated rats showed upregulated NGF in the myocardium, cardiac sympathetic hyperinnervation along with decreased activity of enzymes involved in TH and an insufficiency of NE reuptake, all indicating a deterioration in sympathetic cellular function.\(^ {83}\) Although the sympathetic hyperinnervation could be explained by the upregulation of NGF, it is equally possible that other, locally derived factors being upregulated in the hypertrophic cardiomyocytes could induce the sympathetic cellular dysfunction.
Interleukin-6 Family Cytokines

It is widely known that hypertrophic cardiomyocytes reactivate a fetal phenotype gene pattern, manifested by phenotypic changes in contractile proteins, regulated proteins such as SERCA-2a, secretory proteins such as brain natriuretic peptide, and energy production molecules. These effects in turn depress functions including contraction and relaxation velocity and induce lowered ATP production accompanied by increased cardiomyocyte volume, which is referred to as rejuvenation of cardiomyocytes. Although the precise mechanisms underlying this rejuvenation remain unclear, autocrine/paracrine factors from cardiomyocytes are definite candidates. Indeed, we recently reported that rejuvenation of sympathetic nerves (hyperplasia, ion channels, regulated proteins such as SERCA-2a, secretory proteins such as brain natriuretic peptide, and energy production molecules) might play a crucial role in the depressed sympathetic function. This induces secretion of NE from sympathetic terminals in the adrenal gland and peripheral vessels, resulting in increased plasma NE concentrations. In contrast, NGF expression is downregulated in the myocardium, whereas LIF and cardiotrophin-1 (CT-1) upregulate the expression of parasympathetic markers, such as choline acetyltransferase (ChAT) and the choline transporter (CHT), via glycoprotein 130 (gp130) signaling, thereby inducing cholinergic transdifferentiation. In addition to NE spillover, attenuated TH expression and decreased NE reuptake, in turn, results in a decline in myocardial NE concentrations. t-NE indicates tissue-NE; p-NE, plasma-NE.

Figure 4. Crosstalk between the myocardium and cardiac sympathetic nerves mediated by humoral factors. Under normal conditions, synaptic input from fibers of central origin is received first by the stellate ganglia in the sympathetic chain, the predominant site for sympathetic nerve supply to the heart, and then transmitted to the cardiac myocytes via secretion of norepinephrine (NE) from the cardiac sympathetic nerve terminals. Conversely, in hypertrophic hearts, ET-1 produced by hypertrophied myocardium induces NGF expression, leading to hyperinnervation. LIF, also produced in hypertrophied heart, upregulates polysialylated neural cell adhesion molecule expression in the stellate ganglia and sympathetic nerve fibers, thereby inducing sympathetic rejuvenation. This results in decreased expression of tyrosine hydroxylase (TH), an enzyme involved in NE synthesis, as well as a decline in reuptake of NE into the sympathetic terminals, which, in turn, results in decreased myocardial tissue NE concentrations. In heart failure, chemoreceptors and baroreceptors transmit signals, such as those associated with decreases in blood pressure (BP), cardiac output (CO), and the partial pressure of oxygen, to the brain, thereby activating sympathetic function. This induces secretion of NE from sympathetic terminals in the adrenal gland and peripheral vessels, resulting in increased plasma NE concentrations. In contrast, NGF expression is downregulated in the myocardium, whereas LIF and cardiotrophin-1 (CT-1) upregulate the expression of parasympathetic markers, such as choline acetyltransferase (ChAT) and the choline transporter (CHT), via glycoprotein 130 (gp130) signaling, thereby inducing cholinergic transdifferentiation. In addition to NE spillover, attenuated TH expression and decreased NE reuptake, in turn, results in a decline in myocardial NE concentrations. t-NE indicates tissue-NE; p-NE, plasma-NE.
hers secreted by the failing myocardium. Among IL-6 cytokines, LIF, CT-1, cardiotrophin-like cytokine, and CNTF are known as cholinergic differentiation factors; however, we found that LIF and CT-1 are markedly upregulated in failing heart. Although sweat glands are innervated by catecholaminergic sympathetic nerves at birth, the switch of neurotransmitter from catecholamine to acetylcholine occurs gradually during development. Sympathetic neuron-specific gene targeting of gp130, an IL-6 cytokine family receptor, revealed that sympathetic nerves do not undergo cholinergic differentiation in the sweat gland. Studies in transgenic mice overexpressing LIF in pancreas also showed that the catecholaminergic characteristics decline, whereas the cholinergic characteristics increase. These results indicate that LIF/gp130 signaling plays an essential role in cholinergic neurotransmitter switching in sympathetic nerves. We therefore shed some light on the pathological significance of these findings in the failing heart, on the basis of in vitro studies showing that the addition of LIF to cultured sympathetic neurons induces cholinergic differentiation (Figure 4). Although the precise mechanism underlying cholinergic differentiation in cardiac sympathetic nerves remains largely unknown, Apostolova et al provided evidence that the chromatin architecture protein, Sath2, which is induced downstream of the gp130 cytokines, could control the neurotransmitter switch in sweat gland-innervated neurons. This finding raised the possibility that an epigenetic modification is essential for sympathetic plasticity induced by target-derived humoral factors.

Together, these results indicate that IL-6 family cytokines secreted from the failing myocardium act as negative modulators of sympathetic function by reactivation and cholinergic differentiation via a gp130 signaling pathway, possibly affecting cardiac performance and prognosis.

Plasticity of Cardiac Sympathetic Neurons
As discussed sympathetic and parasympathetic neurons share a common developmental origin, namely, neural crest cells. Therefore, sympathetic neurons could acquire properties of parasympathetic nerves through a process of cellular remodeling by dedifferentiation, transdifferentiation, or both. The diverse potential of sympathetic neurons in terms of plasticity (adaptability to changes in the environment) is implied by the functional changes to cardiac sympathetic nerves in heart failure. The humoral factor–mediated anatomic changes in sympathetic nerve density seen after heart failure and MI are considered one aspect of cardiac sympathetic plasticity displayed in disease states. Although it is difficult to explain such neuronal plasticity using a single mechanism, we speculate that the expression ratio of cardiac-derived NGF (sympathetic activator) to LIF (sympathetic depressor) could determine sympathetic properties in pathological heart.

It remains controversial whether cardiac sympathetic differentiation induced by gp130–mediated cytokines in CHF is a favorable or unfavorable event for cardiac performance and prognosis. Data indicate that vagal activation or stimulation improves cardiac function and prevents remodeling, which, in turn, improves the prognosis for heart failure and MI, whereas the situation is something reversed for the activation of “native” parasympathetic neurons. In contrast, we found significantly improved survival rate and ventricular function in reference mice compared with SNS-specific, gp130-deficient mice, suggesting a protective role for the transdifferentiation seen in hypoxia-induced right ventricular failure mice, whereas this is a case of cholinergic differentiation of “former” sympathetic neurons. As mentioned above, because the “switched” sympathetic nerves acquire functional cholinergic activity, we believe the transdifferentiation might be in conformity with the beneficial case for augmented “native” parasympathetic activity in CHF. Further investigations are needed to clarify this requirement in various types of heart failure.

Sympathetic Influence on Atrial Fibrillation
Previous reports showed that adrenergic nerves were more numerous in the atrium than in the ventricle, and some studies have demonstrated that there were more cholinergic nerves than adrenergic nerves in the atrium than in the ventricle. AF is the most common type of cardiac arrhythmia, causing substantial mortality. The autonomic nervous system, and particularly the adrenergic/cholinergic balance, has a profound effect on the occurrence of AF. Heart rate variability studies indicated an increased level of adrenergic rather than cholinergic activity preceding the onset of AF after coronary artery bypass grafting. Bettoni et al showed that primary adrenergic activity followed by an abrupt change to cholinergic activity generated paroxysmal AF (PAF), whereas Tan et al reported that simultaneous sympathovagal discharges preceded the onset of PAF in ambulatory canines. These studies indicate that a combined sympathetic and vagal tone could facilitate the generation of AF. However, the precise molecular mechanism underlying the occurrence and maintenance of AF remains unknown. Rapid atrial pacing could produce sustained AF, resulting in atrial nerve sprouting and sympathetic hyperinnervation. Conversely, atrial sympathetic hyperinnervation with electric and structural remodeling could lead to PAF. NGF expression was recently shown to be more abundant in sympathetic nerves than in atrial myocytes and high-rate electric stimulation of sympathetic nerves contributed to nerve sprouting with augmented expression of neuronal NGF, targeting the TrkA receptor via autocrine/paracrine signaling. Interest-

Conclusions
In developing heart, cardiac sympathetic innervation patterning is strictly controlled by the balance between neural chemoattractants and chemorepellents. NGF is a potent chemoattractant, and ET-1 regulates NGF expression in cardiomyocytes. The ET-1/NGF pathway modulates nerve sprouting and plays critical roles in sympathetic nerve development. On the other hand, calcineurin-NFAT activation by mechanical stretch/α-1-
adrenergic stimulation attenuates NGF expression, causing nerve denervation. Sema3a, a neural chemorepellent, and neurtrophilic receptor, p75, can achieve the sympathetic epicardial-to-endocardial innervation gradient via different mural expression levels in the heart. Disruption of the sympathetic innervation pattern may eventually lead to fatal arrhythmia, in both diseased and developing hearts. Better understanding of the mechanisms of cardiac sympathetic innervation patterning represents an important approach for future development of therapies to avoid sudden cardiac death.

In failing heart, cardiac sympathetic effenter activity is increased to comply with the afferent reflex requirement. However, insufficient sympathetic cellular function, involved in decreased NE uptake and synthesis, induces cardiac malperformance. Various causes are proposed to underlie these sympathetic abnormalities, and recent biotechnological advances have revealed the role of cardiac sympathetic plasticity, characterized by mechanisms such as rejuvenation and cholinergic differentiation. Induced by IL-6 cytokines derived from the failing heart, such plasticity could lead to the sympathetic cellular dysfunction. These findings expand our mechanistic understanding of the cardiac nervous system in pathological heart, and we believe that further investigation of this field could yield novel therapeutic targets for heart disease.

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Disclosures
None.

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Kimura et al
New Insights Into Cardiac Sympathetic Nerves 335


