Molecular Mechanisms of Sympathetic Remodeling and Arrhythmias

Running Title: Sympathetic Remodeling and Arrhythmias

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Introduction

A variety of pathological conditions can increase risk for development of ventricular arrhythmias in humans including diabetes, obesity, myocardial infarction (MI), and heart failure. Many of these diseases involve global disruption of the autonomic nervous system, including increased sympathetic drive and parasympathetic withdrawal, but another common factor among these disorders is sympathetic dysfunction within the heart. Treatments that target cardiac sympathetic transmission, including beta blockers and ganglionectomy, prolong life and decrease arrhythmias [1-5].

Sympathetic control of the heart under normal conditions occurs primarily via norepinephrine (NE) acting on β adrenergic receptors (β-AR) to stimulate increases in heart rate (chronotropy), conduction velocity (dromotropy), and contractility (inotropy). These positive effects of sympathetic stimulation allow myocytes to meet increased cardiac demands during stress or exercise, serving to maintain homeostasis. The nervous system adapts to changing conditions, however, and sympathetic neurons undergo structural and functional alterations in response to injury and disease. There are at least four types of sympathetic remodeling that occur during conditions of increased arrhythmia susceptibility: hyperinnervation (increased nerve density), denervation (decreased nerve density), altered neurotransmitter or neuropeptide production, and increased neuronal excitability. Rubart and Zipes proposed a model to explain how these diverse changes in sympathetic transmission might contribute to arrhythmia generation [6], suggesting that inappropriate heterogeneity of NE release within the heart leads to differential electrical remodeling of cardiac myocytes and predisposes the heart to electrical instability. Many studies now support the hypothesis that heterogeneity of noradrenergic transmission increases the risk of arrhythmia, and have identified some of the mechanisms that underlie neuronal remodeling. This review will examine the mechanisms of sympathetic remodeling and will connect neural changes to increased arrhythmia susceptibility. We will focus on ventricular arrhythmias, as atrial arrhythmias were reviewed recently [7].

Hyperinnervation and excess NE release

Regional hyperinnervation was the first type of neural remodeling linked to arrhythmia generation in humans [8, 9]. Areas of sympathetic hyperinnervation, defined as increased nerve fiber density compared to control tissue, have now been identified in many conditions with increased arrhythmia susceptibility including heart failure [9], myocardial infarction [10, 11], spinal cord injury [12], and diet induced obesity[13, 14]. Nerve growth factor (NGF) has been the focus of studies related to cardiac hyperinnervation since NGF stimulates the extension, or sprouting, of sympathetic nerve endings during development and after injury. Cardiac NGF is elevated in animal and human hearts during conditions associated with hyperinnervation [10, 12, 15], and is decreased in conditions that include a loss of functional sympathetic innervation such as late stage heart failure [16] and diabetic neuropathy [17].

Sympathetic axon outgrowth is triggered through activation of the TrkA *(*Tropomyosin receptor kinase A) receptor, which stimulates serine phosphorylation of STAT3 (signal transducer and activator of transcription 3) in addition to activating other signaling pathways. Although TrkA can be activated by both NGF and Neurotrophin 3 during development, NGF is the only ligand that activates TrkA in mature sympathetic neurons, and thus is crucial for maintaining sympathetic neuron health and stimulating axon regeneration. Recent studies indicate that cytokines like Leukemia Inhibitory Factor (LIF) and Cardiotrophin-1 (CT-1), which activate the gp130 receptor, do not stimulate axon growth on their own but are required for maximal NGF-induced sympathetic axon extension [18]. These cytokines stimulate tyrosine phosphorylation of STAT3, and phosphorylation of STAT3 on both serine and tyrosine is required for maximal axon outgrowth [18]. The related cytokine leptin also enhances sympathetic axon outgrowth [19], and may contribute to the cardiac hyperinnervation observed in diet-induced obesity.

Pathological conditions resulting in hyperinnervation within the heart are also associated with increased sympathetic drive from the CNS, and enhanced excitability of post-ganglionic neurons may also contribute to elevated adrenergic transmission in the heart. Neuronal cell size is increased significantly in stellate ganglia removed from humans with ischemic and non-ischemic cardiomyopathy compared to control ganglia [20], and similar changes identified in canines coincide with increased neuronal excitability [21]. Similarly, increased dendrite field size and synapse number contribute to elevated cardiac sympathetic tone in rats following T5 spinal cord transection [22]. Retrograde signaling by NGF regulates synapse formation during development [23] and high NGF likely contributes to increased cell size and synapse formation after injury. NGF may also increase sympathetic excitability by altering sensitivity to inflammatory mediators [24], and by altering neuronal firing properties [25]. Interestingly, statins decrease sympathetic neuron excitability by stimulating dendrite retraction [26], raising the possibility that dampening sympathetic drive via peripheral actions contributes to their therapeutic value in patients with cardiovascular disease.

Functional noradrenergic transmission requires a balance between NE synthesis, release, and reuptake, each of which can be regulated independently [27]. For example, after myocardial infarction newly sprouting sympathetic fibers have low NE content [28], but there is a paradoxical increase in extracellular NE [27]. This can be explained in part by elevated NE synthesis and release in other regions of the heart that are not matched by a similar increase in NE removal [11]. Such functional changes have been identified in patients with heart failure, where increased NE release as well as decreased NE reuptake contribute to a buildup of extracellular NE and excessive activation of β-AR [29].

Myocardial responses to acute hyperinnervation/excess NE

Norepinephrine released from sympathetic nerves activates cardiac β-AR to modulate myocyte repolarization and contractility. Sympathetic nerves are not distributed evenly across the heart, but are most dense near the base of the ventricles. Likewise, the epicardial to endocardial gradient in cardiac action potential duration (APD) [30-32] that is critical for normal activation and repolarization of the left ventricle is regulated by innervation, and disrupting the normal organization of sympathetic nerves in an otherwise healthy heart is arrhythmogenic [33, 34]. Activation of cardiac β-AR modulates myocyte repolarization by altering transmembrane currents and Ca^{2+} homeostasis [35-37]. β-AR stimulated cAMP leads to phosphorylation of proteins involved in excitation-contraction coupling including phospholamban, L-type Ca^{2+} channels, and ryanodine receptors (RyR), resulting in increased sarcoplasmic reticulum (SR) Ca^{2+}-ATPase (SERCA) activity and an increase in SR Ca^{2+} content[38]. Thus, during sympathetic stimulation more Ca^{2+} is released from the SR[39] to activate the myofilaments, increasing contractility, but spontaneous Ca^{2+} release from the SR also becomes more likely [40, 41]. Therefore, the positive inotropic effects of sympathetic stimulation that allow myocytes to meet increased cardiac demands are accompanied by an increased risk for pathological arrhythmias via focal (triggered) mechanisms.

At the cellular level, focal activity during sympathetic activation is likely due to delayed afterdepolarizations (DADs),[42] which are membrane depolarizations occurring during phase 4 of the action potential. The increased cytosolic and SR Ca^{2+} levels that occur during sympathetic activation can lead to SR Ca^{2+} overload (Figure 1), which may result in spontaneous opening of RyRs and Ca^{2+} release that is not in response to an action potential. This leads to Ca^{2+} extrusion from the cytosol via the Na^+/Ca^{2+} exchanger (NCX).[43] NCX is electrogenic, extruding one Ca^{2+} ion (2+) in exchange for 3 Na^+ ions (3+), which produces a net inward current. If the inward current is large enough, the cell membrane depolarizes and a triggered action potential may occur.

At the tissue level, several thousand cells must all experience DADs *simultaneously* in order to generate enough depolarizing current to produce a propagating action potential (called the 'source-sink mismatch').[44, 45] Recent studies revealed that local application of β-AR agonists such as NE or isoproterenol can induce premature ventricular complexes (PVCs) and sustained focal VT in intact hearts,[46-49] providing a mechanistic link between the numerous experimental and clinical investigations that have found regional hyperinnervation accompanied by increased arrhythmia risk. [8] Furthermore, electrophysiological remodeling of cardiac myocytes in response to myocardial infarction or heart failure can cause changes (e.g., increased expression of NCX,[50] increased SR Ca^{2+} leak through RyR,[51] decreased inward rectifying K^+ current,[43] decreased gap junction coupling,[52] and fibrosis) that further increase the likelihood of DADs and arrhythmia generation in response to localized sympathetic stimulation. [45, 46]

Sympathetic stimulation also has effects on ionic currents that impact the ventricular action potential and risk for reentrant arrhythmias. The effects of adrenergic activation on individual ion channels have been reviewed elsewhere[53, 54], but one of the best-known features of β-AR stimulation is an increase in L-type Ca^{2+} current via phosphorylation of the channel (Cav1.2).[55] This effect, by itself, is expected to increase APD, but is counterbalanced by the effect of β-AR on K^+ currents, most notably an increase in I_{Ks}, although I_{Kr} may also be involved.[56, 57] The net effect of NE typically results in a shortening of the APD, a requirement for the heart to beat at faster rates during sympathetic activity. Due to the base to apex gradient in cardiac sympathetic nerves, however, sympathetic activation results in non-uniform changes in APD throughout the ventricle. For example, sympathetic nerve stimulation in a normal rabbit heart led to increased dispersion of repolarization and reversed the direction of the repolarization wavefront.[58]

Administration of the β-AR agonist isoproterenol generated a different set of responses, suggesting that the dramatic changes in repolarization observed with sympathetic nerve stimulation were not due to differences in β-AR distribution or sensitivity, but rather due to the heterogeneous distribution of the nerves. [58] Similar results have been obtained in porcine ventricles, where dramatically different spatial patterns of activation-recovery intervals (ARIs, surrogate measure for APD) and repolarization were observed in response to sympathetic nerve stimulation vs. circulating NE[59, 60]. Thus, even under non-pathological conditions, considerable heterogeneity of sympathetic nerve distribution leads to increased dispersion of repolarization and potential for reentrant arrhythmias. Therefore, in conditions of maladaptive nerve remodeling, significantly greater heterogeneity of APD and repolarization may exist. Indeed, sympathetic stimulation after myocardial infarction not only caused increased dispersion of repolarization compared to controls, but activation and propagation patterns were also altered significantly [61]. This was confirmed in patients with MI in whom reflex sympathetic stimulation caused a 230% increase in dispersion of repolarization compared to patients with structurally normal hearts.[62]

Myocardial responses to chronic hyperinnervation/excess NE

Acute effects of sympathetic activation often occur on a background of remodeled myocardial properties induced by heart failure or MI, but alterations to sympathetic transmission can also lead to chronic remodeling of the myocardium. Sympathetic hyperinnervation and elevated sympathetic tone are key features of many cardiovascular diseases. In these conditions the myocardium becomes less responsive to adrenergic stimulation over time, and simultaneously less capable of maintaining adequate cardiac output, which further increases sympathetic drive from the CNS. The loss of cardiomyocyte responsiveness to adrenergic stimulation is a hallmark of sustained adrenergic stimulation and hyperinnervation [63]. Several factors contribute to this loss of sensitivity, including down-regulation of the receptor itself [64], but an especially important regulator of β-AR activity is the G-protein receptor kinase 2 (GRK2, also known as βARK1). Acutely, GRK2 is activated by PKA in response to adrenergic stimulation and acts to inhibit β-AR activity in a self-contained negative feedback loop. Sustained activation of β-AR in adult mice, however, leads to increased GRK2 expression [64]. A similar increase in GRK2 is seen in canine heart failure, where it is reversed by sympathetic denervation, confirming regulation of GRK2 by sympathetic transmission [65]. Long term activation of β-AR also leads to G-protein uncoupling and a reduction of Gαs protein [66], as well as a reduction of repolarizing K^+ current [67, 68]. Thus, sustained activation of adrenergic receptors leads to adaptations that limit myocyte sensitivity to adrenergic stimulation and alter ion channel expression. Long term treatment with beta blockers blunts many of these adaptations [63] and normalizes myocyte calcium handling [69], contributing to the well-established protective effects of sympathetic blockade [1-4].

Cardiac denervation and axon degeneration

The mechanisms by which too much sympathetic transmission can be toxic for the heart are well-characterized, but the local loss of sympathetic transmission within the heart also contributes to rhythm instability. Regional deficits in sympathetic transmission, identified in patients by imaging the uptake of labeled NE transporter substrates, have been observed in several pathological conditions including myocardial infarction [70, 71], heart failure [72], and Parkinson's Disease [73]. Several recent clinical studies suggest that sympathetic denervation after MI predicts the probability of serious ventricular arrhythmias [74-76], and a detailed electrical mapping study in human hearts revealed that sympathetic denervation of the normal myocardium adjacent to the scar resulted in β-AR agonist super-sensitivity and increased dispersion of repolarization that was arrhythmogenic [62].

Paradoxically, members of the neurotrophin family of growth factors can be involved in the destruction of sympathetic nerves following cardiac injury. While the neurotrophin NGF stimulates TrkA in sympathetic neurons to promote axon maintenance and process outgrowth, its precursor protein ProNGF, which is elevated in the human heart after MI [77], activates the p75 neurotrophin receptor (p75NTR; also called TNF receptor super family 16, TNFRS16), to trigger axon degeneration [78, 79] (Figure 2). Similarly, ProBDNF (Pro Brain Derived Neurotrophic Factor) and BDNF selectively activate p75NTR on sympathetic neurons to stimulate axon degeneration [80]. The Trk tyrosine kinase receptors and p75NTR have opposing actions not just in cardiac sympathetic nerves[81], but also in the coronary vasculature [77] and cardiac myocytes [15, 82]. Thus, ProNGF activation of p75NTR after MI leads to the loss of nerve fibers in viable myocardium [81] as well as microvascular damage and scar extension [77].

While activation of p75NTR can contribute to the loss of cardiac nerves, other factors are involved in sustaining denervation. Chondroitin sulfate proteoglycans (CSPGs) are produced in the cardiac scar after ischemia-reperfusion, where they prevent reinnervation of the border zone and scar [83]. This contrasts with the scar that forms after sustained ischemia, which is devoid of CSPGs [83] and receives sympathetic hyperinnervation [10, 27]. Removing or inhibiting the CSPG receptor protein tyrosine phosphatase receptor sigma (PTPσ) in mice leads to reinnervation of the scar and border zone, restoring normal NE content and β-AR responsiveness to that region of the damaged left ventricle [84]. Consistent with the human studies linking post-MI denervation to arrhythmia risk, restoring innervation throughout the scar and border zone in mouse heart normalizes post-MI calcium handling and decreases arrhythmia susceptibility [84].

Myocardial responses to chronic denervation

Just as sympathetic hyperinnervation can alter the molecular makeup of myocytes, sustained sympathetic denervation has similarly profound effects. One of the best characterized changes is a loss of the transient outward K^+ current I_{to}, which is responsible for the initial repolarization in phase 1 of the action potential. Sympathetic denervation in rat decreases I_{to} by lowering expression of several different K^+ channel subunits, and increases susceptibility to ventricular fibrillation [85, 86]. Decreased I_{to} is also observed in disease states characterized by sympathetic denervation including Chagas disease, diabetic neuropathy, and myocardial infarction [84, 87, 88]. Restoring adrenergic transmission in Chagas animals with NE infusion [89], or promoting sympathetic re-innervation of denervated infarct and border zone tissue [84] reverses the loss of I_{to}.

The consequences of sympathetic denervation are not limited to the transient outward K^+ current. While hyperinnervation increases GRK2, sustained treatment with the beta blocker atenolol in mice[90] and surgical sympathectomy in dogs[65] leads to GRK2 down-regulation. This reduction in GRK2 may play an important role in the β-AR supersensitivity observed following sympathetic denervation, as GRK2 knock out mice exhibit a similar supersensitivity[91]. The absence of GRK2 also alters Ca^{2+} homeostasis by reducing SERCA activity, which leads to reduced SR Ca^{2+} load and increased cytosolic Ca^{2+} levels, thus increasing NCX activity[91]. Increased activity of the electrogenic NCX can initiate DADs, and the adrenergic supersensitivity that accompanies decreased GRK2 increases the likelihood that β-AR stimulation will be sufficient to overcome source-sink mismatch and generate focal arrhythmia[47]. Consistent with this possibility, isoproterenol stimulation of hearts after MI triggers focal arrhythmias that arise from denervated tissue near the infarct, while release of NE from sympathetic nerves in the same hearts does not trigger arrhythmias [84]. Restoration of sympathetic innervation to the scar and border zone of infarcted hearts prevents isoproterenol-induced arrhythmias and abnormal Ca^{2+} handling, confirming a role for denervation induced β-AR super-sensitivity in arrhythmia generation [84]. Sudden cardiac death is most common in the morning [92] when circulating catecholamines are rising rapidly [93], suggesting that high circulating NE and epinephrine trigger arrhythmias in denervated myocardium via activating super-sensitive β-AR signaling pathways. Thus, denervation and hyperinnervation may trigger arrhythmias via similar mechanisms within cardiac myocytes.

Neurotransmitter and neuropeptide production

In addition to the loss or gain of nerve fibers, sympathetic neurons innervating the heart can undergo changes in neurotransmitter and peptide production and release following injury. Sympathetic nerves in the heart produce the peptide co-transmitter neuropeptide Y (NPY), which inhibits release of ACh from cardiac parasympathetic nerves [94] and causes vasoconstriction on the cardiac vasculature [95]. NPY is elevated after MI [96], and high plasma NPY levels in patients with acute ST elevation MI correlate with increased microvascular resistance following reperfusion [97]. NPY is released during periods of high sympathetic drive, and in the context of myocardial infarction high levels of sympathetic activation resulting in NPY release appears to be detrimental for the heart. Over a longer time frame, cardiac damage can lead to changes in neuropeptide and neurotransmitter expression in sympathetic neurons. The best characterized change in sympathetic transmission is a developmental transition from production of NE to ACh due to the actions of gp130 cytokines [98]. Recent studies revealed a similar change in phenotype triggered by cytokines like LIF and CT-1 during heart failure [99]. Stellate ganglia obtained from humans with heart failure also exhibited expression of proteins associated with cholinergic transmission [99], suggesting that cholinergic sympathetic transmission can occur in the human heart. Although the functional consequences of ACh release from sympathetic nerves are unclear, NE and ACh have opposing effects on ventricular action potential duration (NE shortens whereas ACh lengthens). Thus, cholinergic sympathetic transmission may indeed be arrhythmogenic by limiting the adaptation of the action potential duration to increased heart rates during sympathetic activity. Therefore, the functional impact of changes in neurotransmitter phenotype represents an important area for future investigation.

Summary

Interactions between sympathetic neurons and cardiac myocytes can become destructive in pathophysiological conditions, giving rise to electrical instability and increased arrhythmia susceptibility. We have summarized the most common changes that occur in cardiac sympathetic neurons during pathologies associated with increased ventricular arrhythmia risk, and how altered neurotransmission might contribute to arrhythmia generation. Many relevant studies were excluded due to reference limits, but we have tried to cite work from different laboratories who have contributed to our understanding. Interventions that target the sympathetic innervation of the heart have been successful in treating arrhythmias, and our hope is that this review will stimulate the development of new interventions aimed at normalizing sympathetic dysfunction.

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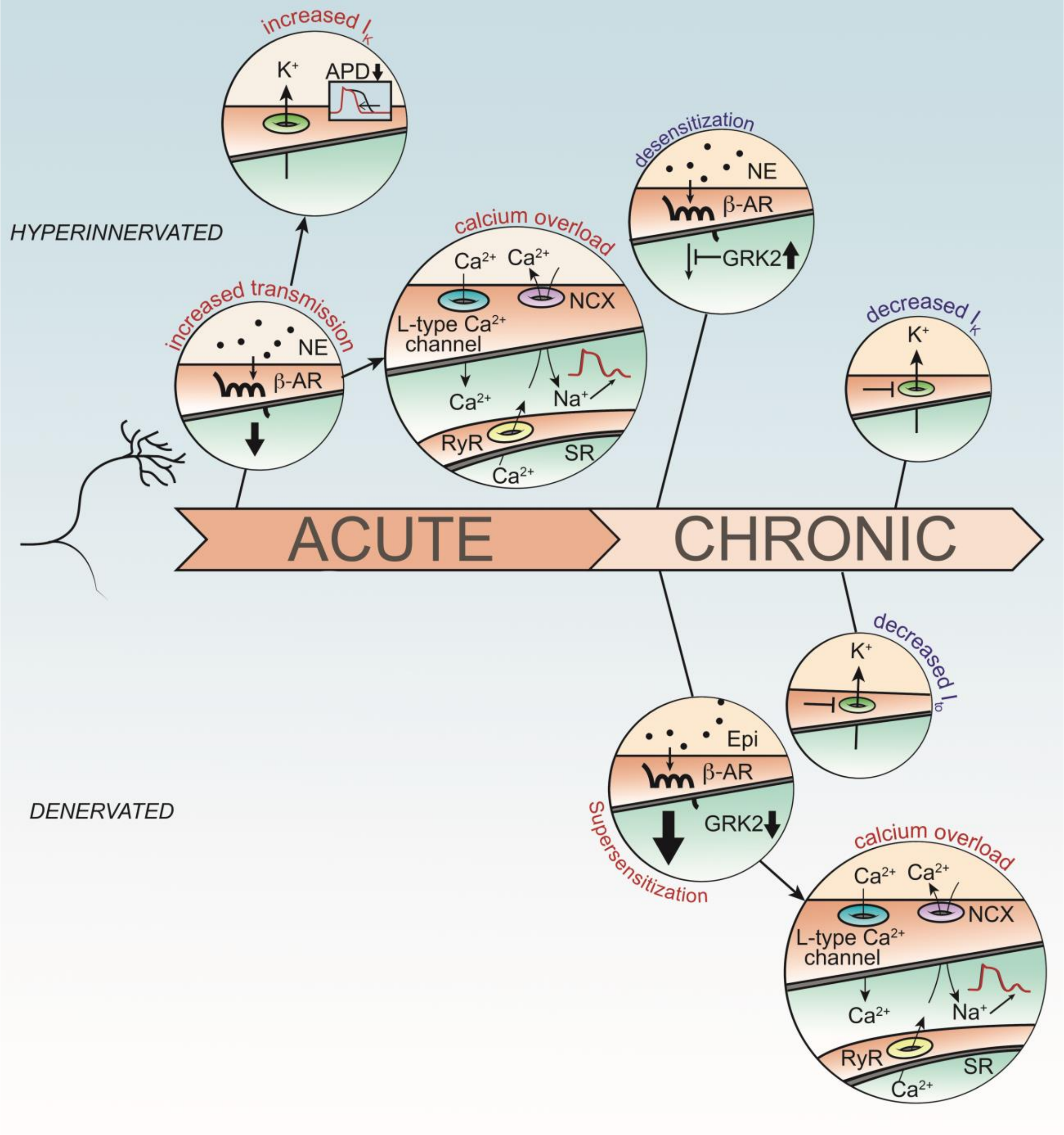
Figure 1.


increased IK
K+
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HYPERINNERVATED
increased transmission
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calcium overload
Ca2+
Ca2+
NCX
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Ca2+
Na+
RyR
SR
Ca2+
desensitization
NE
β-AR
GRK2
decreased IK
K+
ACUTE
CHRONIC
decreased Ito
K+
Epi
β-AR
GRK2
Supersensitization
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SR
Ca2+

Figure 2.

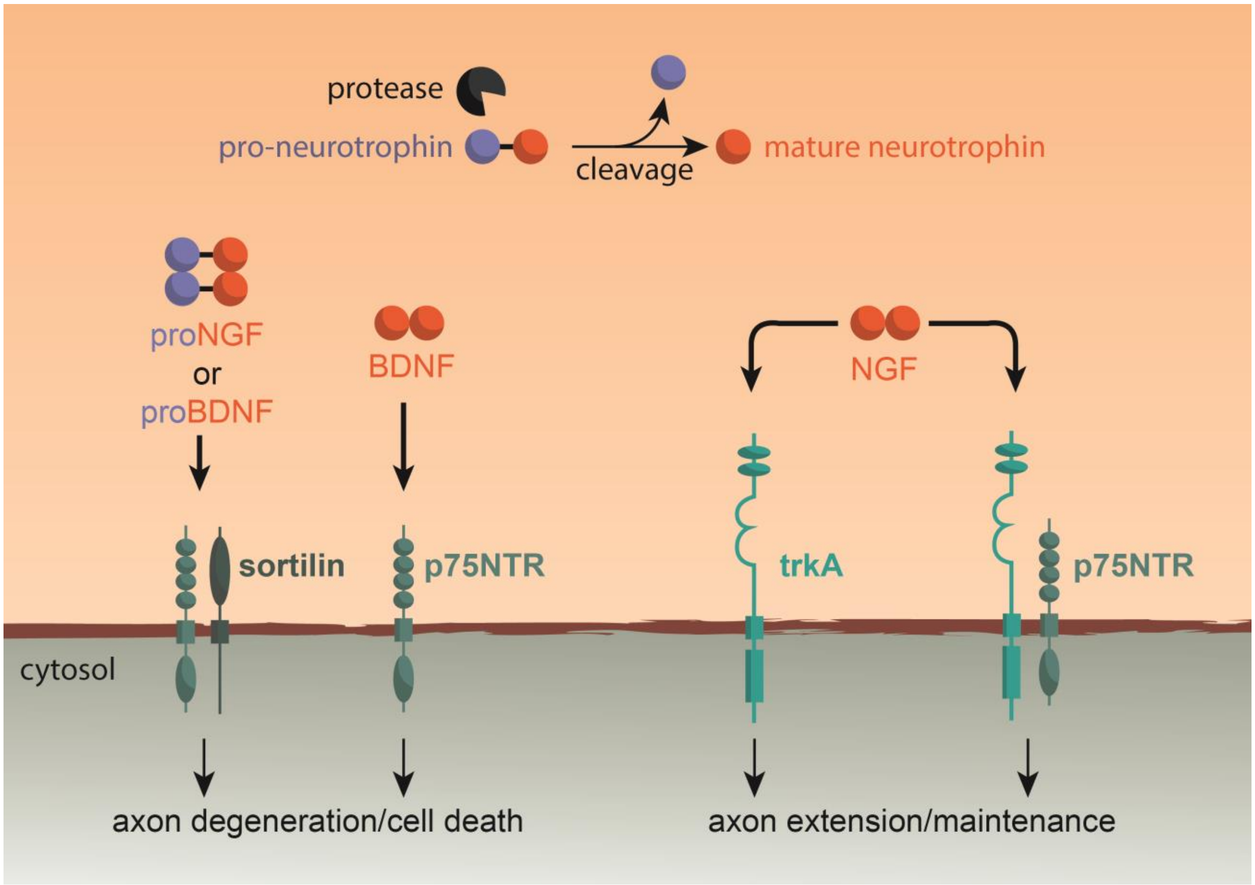
protease
pro-neurotrophin
cleavage
mature neurotrophin
proNGF
or
proBDNF
BDNF
NGF
sortilin
p75NTR
trkA
p75NTR
cytosol
axon degeneration/cell death
axon extension/maintenance

Figure Legends

Figure 1. Hyperinnervation and denervation can both contribute to arrhythmias. Acute hyperinnervation or excess NE (Top) leads to increased activation of β-ARs (beta adrenergic receptors) and subsequent changes in I_k and calcium overload. β-AR signaling increases I_k which shortens action potential duration (APD). At the same time, intracellular Ca^{2+} rises due to enhanced influx via the L-type Ca^{2+} channel and increased release from the sarcoplasmic reticulum (SR). Extrusion of Ca^{2+} from the cytosol via the Na^+/Ca^{2+} exchanger (NCX) produces a net inward current leading to delayed afterdepolarizations (DADs). In contrast, chronic hyperinnervation leads to desensitization of β-AR signaling and decreased I_k. Chronic denervation (Bottom) results in β-AR supersensitivity and decreased I_{to}. Activation of super-sensitive β-AR signaling pathways by circulating epinephrine leads to calcium overload and DADs.

Figure 2. Neurotrophins stimulate different effects in sympathetic neurons via activation of p75NTR and/or TrkA. Pro-Neurotrophins like ProNGF and ProBDNF are processed to mature neurotrophins (NGF, BDNF) by intra- and extra-cellular proteases. Activation of a p75NTR/Sortilin receptor complex by ProNGF or ProBDNF, or activation of p75NTR by BDNF, stimulates axon degeneration in sympathetic neurons. In contrast, NGF signaling via TrkA or a TrkA/p75NTR receptor complex stimulates sympathetic axon maintenance and growth.