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Sympathetic nerve sprouting, electrical remodeling and the mechanisms of sudden cardiac death

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Abstract

The purpose of this article is to review the nerve sprouting hypothesis of sudden cardiac death. It is known that sympathetic stimulation is important in the generation of sudden cardiac death. For example, there is a diurnal variation of sudden death rate in patients with myocardial infarction. Beta blockers, or drugs with beta blocking effects, are known to prevent sudden cardiac death. It was unclear if the cardiac nerves in the heart play only a passive role in the mechanisms of sudden death. To determine if nerve sprouting and neural remodeling occur after myocardial infarction, we performed immunocytochemical studies of cardiac nerves in explanted native hearts of transplant recipients. We found that there was a positive correlation between nerve density and a clinical history of ventricular arrhythmia. Encouraged by these results, we performed a study in dogs to determine whether or not nerve growth factor (NGF) infusion to the left stellate ganglion can facilitate the development of ventricular tachycardia (VT), ventricular fibrillation (VF), and sudden cardiac death (SCD). The results showed that augmented myocardial sympathetic nerve sprouting through NGF infusion plus atrioventricular (AV) block and MI result in a 44% incidence (four of nine dogs) of SCD and a high incidence of VT in the chronic phase of MI. In contrast, none of the six dogs (with AV block and MI) without NGF infusion died suddenly or had frequent VT episodes. Based on these findings, we propose the nerve sprouting hypothesis of ventricular arrhythmia and SCD. The hypothesis states that MI results in nerve injury, followed by sympathetic nerve sprouting and regional (heterogeneous) myocardial hyperinnervation. The coupling between augmented sympathetic nerve sprouting with electrically remodeled myocardium results in VT, VF and SCD. Modification of nerve sprouting after MI may provide a novel opportunity for arrhythmia control.

Keywords: Autonomic nervous system; Infarction; Ion channels; Remodeling; Sudden death; Ventricular arrhythmias

1. Introduction

The nerve sprouting hypothesis of sudden cardiac death states that MI results in nerve injury, followed by sympathetic nerve sprouting and regional myocardial hyperinnervation. The coupling between augmented sympathetic nerve sprouting with electrically remodeled myocardium results in VT, VF and SCD. Modification of nerve sprouting after MI may provide a novel opportunity for arrhythmia control. In this article we will review the evidence in support of the nerve sprouting hypothesis of sudden cardiac death.

2. Cardiac nerves

The first descriptions of cardiac innervation were made in the mid-19th century by Ludwig [1] and Bidder [2] in

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studies of the frog's heart. Since then, the autonomic innervation of the vertebrate heart has been studied extensively [3]. Hearts are innervated by both sympathetic and parasympathetic nerves. Cardiac sympathetic innervation includes nerves from both cervical and thoracic sympathetic ganglia. The postganglionic efferent fibers in the sympathetic cardiac nerves arise in the sympathetic trunk ganglia. Therefore, the sympathetic nerves do not make synaptic connections within the heart. The sympathetic innervation to the ventricles follows a course along the common pulmonary artery into the plexus supplying the main left coronary artery. The sympathetic nerves are distributed to the myocardium in superficial epicardial layers. They penetrate the myocardium along with the coronary arteries [4,5]. The parasympathetic innervation to the heart comes from the vagus nerve. The efferent component of the vagal branches to the heart includes preganglionic fibers. In contrast to sympathetic innervation, the parasympathetic nerves do make synaptic connections with ganglion cells in the cardiac plexus or intracardiac ganglia. Like other peripheral nerves [6], many cardiac nerves have a sheath of Schwann. However, there are also nonmyelinated fibers in the heart.

Recent advancements in immunocytochemical techniques have allowed investigators to stain the Schwann cells using the antibody to the S100 protein [7,8] and to stain the autonomic nerves using antibodies against nerve-specific markers such as neurofilament, synaptophysin (SYN), tyrosine hydroxylase (TH), protein gene product 9.5, and many others [9–13]. Chow et al. [14] studied the effects of death on the immunofluorescence of autonomic nerves supplying the human ventricular myocardium. Percutaneous myocardial samples were obtained up to 5–11 days after death. The authors showed that immunocytochemical techniques could be used on human hearts to detect cardiac nerves up to 6 days after death [14]. Those studies indicate that it is possible to study the cardiac nerves using immunocytochemical techniques in animal models and in human autopsy specimens. Using these techniques, Marron et al. [11] examined the entire endocardial and epicardial surfaces of infant and adult hearts obtained postmortem and at transplantation. Nerve fiber terminals were identified in the atrial endocardium, epicardium, and coronary sinus. These terminals displayed immunoreactivity for tyrosine hydroxylase, neuropeptide Y, and the general neural marker protein gene product 9.5. Acetylcholinesterase activity was detected in $<5\%$ of endocardial terminals and in no epicardial terminals arising from myelinated fibers. A distinct population of terminals was demonstrated to arise from nonmyelinated fibers in the endocardial plexus of the atria and left ventricle and were predominantly acetylcholinesterase-positive. These data indicate that autonomic nerve fibers are widely distributed in the human myocardium. Nerve fibers containing acetylcholine and catecholamines may be important in regulating both the mechanical and the electrical activity of the heart. Table 1 shows a summary of the stains used in our laboratory.

3. Left and right functional asymmetry of cardiac innervation

Cardiac sympathetic innervation shows significant functional asymmetry [15–20]. Stimulating right sympathetic nerves results in an increased heart rate due to the activation of sinus node. It also decreases vulnerability to arrhythmia. In contrast, stimulating left sympathetic nerves results in a prominent increase of blood pressure and a significant increase of ventricular vulnerability to fibrillation. While right stellate ganglion stimulation is characterized by an increase of heart rate and left stellate ganglion stimulation an increase of contraction and blood pressure, this functional asymmetry is not absolute. Brandys et al.

Table 1
Comparison among different stains

Stain	Specific for	Utility[a]
S100 protein	Schwann cells	Most useful stain for old, formalin-fixed human cardiac tissues
Tyrosine hydroxylase	Sympathetic nerves	To identify sympathetic nerves or structure that contains tyrosine hydroxylase
Acetylcholinesterase	Parasympathetic nerves	To identify parasympathetic nerves and intracardiac parasympathetic ganglia
Synaptophysin	Nerve synapses	To identify cardiac nerves
Neurofilament	Nerve axons	To identify cardiac nerves
Protein gene product 9.5	Nerves	To identify cardiac nerves
Growth associated protein 43 (GAP43)	Growth cones of sprouting axons	To identify growing nerves. Expected to be positive in nerve sprouting. The nerves that stain positive for GAP43 may not be mature enough to express tyrosine hydroxylase

[a] Two major factors that may affect the results: (1) antibody specificity. For example, rabbit anti-cow S100 antibody that was extremely useful in staining paraffin-fixed human cardiac tissue blocks often failed to stain canine cardiac tissues. (2) Overfixation with formalin may result in loss of antigenicity with tissue processing (for example, use of formalin-fixed paraffin embedded tissues rather than frozen sections). We use 4% formalin for 45–60 min, followed by storage in 70% alcohol for best results.

[21] stimulated 96 sites of right stellate ganglion and showed that in 6% there was pure heart rate response, 9% force response and 79% of mixed (both heart rate and contraction force) responses. In contrast, stimulation of 69 sites of left stellate ganglion resulted in 0% pure heart rate responses, 62% generalized force response and 23% of mixed responses. Similarly, the antiarrhythmic effects of electrical stimulation of right stellate ganglion are not reproduced in all experimental models. For example, Cardinal et al. [22] performed electrical stimulation of right and left stellate ganglia in dogs with complete AV block created by formaldehyde injection. They reported that both right and left stellate ganglion stimulation could result in VT and VF in that model.

Left and right sympathetic imbalance has been implicated in the sudden infant death syndrome (SIDS). Schwartz [23] suggested that prolongation of the QT interval in SIDS [24] may depend on developmental alterations in cardiac sympathetic innervation. Because the right and left sympathetic nerves may occasionally develop at different rates, it leads to an imbalance that might be arrhythmogenic [25].

4. Nerve sprouting after myocardial infarction

Following division, crushing, interference of blood supply, or other means of injury to a nerve, the peripheral nerves undergo Wallerian degeneration [6], which may be followed by neurilemma cell proliferation and axonal regeneration. The axonal regeneration is initially slow, but accelerates to reach a constant rate by the third day after injury [26,27]. However, the rate may vary depending on the injury type, age, and species. Estimates of the rate of regeneration vary between 1 and 3 mm/day [26,27]. In unconditioned rat sciatic nerve, for example, the regeneration rate is 3.2 mm/day [27]. The regeneration effort is triggered by the reexpression of nerve growth factor (NGF) or other neurotrophic factor genes in the non-neural cells around the site of injury [28]. For example, there is a significant increase of NGF activity in the Schwann cells of the sympathetically-denervated rat iris [29]. It has also been demonstrated that Interleukin-1 released by macrophages plays a role in the augmented local production of NGF after peripheral nerve injury [30]. The NGF originated from non-neural cells then results in nerve sprouting during which the neural cells produce sprouts in an effort to reinnervate the end organ. Some of the spouts may form functional synapses and permanently alter the physiological environment. This sequence of events may account for the etiology of injury-related epilepsy, which is associated with abnormal nerve sprouting in the central nervous system after brain injury [31,32].

Because nerves in the ventricles may be injured during myocardial ischemia [33], it is reasonable to expect that nerve sprouting also occurs after MI. Compatible with this expectation, necrotic injury to the rat myocardium results in denervation followed by proliferative regeneration of Schwann cells and axons [34]. Abnormal patterns of neurilemma proliferation have been documented in infarcted human and rat hearts [7,8]. Using I-123-metaiodobenzylguanidine scanning, both denervation and reinnervation have been demonstrated in humans and dogs after MI [35–37]. The functional significance of reinnervation after MI is unclear. Vracko and co-workers [7,8] suggested that abnormal patterns of myocardial innervation may cause ventricular arrhythmia. However, no data were presented to support that hypothesis. On the other hand, in patients with cardiac transplantation, reinnervation is associated with significant improved exercise performance determined by heart rate, anaerobic threshold and oxygen uptake [38]. These data suggest that nerve sprouting occurs after MI and cardiac transplantation, and that it may contribute to the increased hemodynamic performance of the surviving myocardium. However, excessive nerve sprouting may result in abnormal patterns of myocardial innervation and may potentially increase cardiac arrhythmia.

5. Animal models of sudden cardiac death

There are a number of experimental models [17,19,39–43] in which ventricular tachyarrhythmias have been provoked in the presence or absence of structural alterations imposed on the myocardium. However, the investigators induced ischemia or provided artificial ‘triggers’ such as electrical stimulation or drugs, to induce VT or VF. If these perturbations were not performed, the probability of SCD in dogs after MI was low. For example, Hunt et al. [44] reported a study in which 97 dogs were subjected to LAD ligation and followed for up to 4 months. Spontaneous SCD occurred in only three (3%) of these dogs. Vos and co-workers [43,45] reported a series of studies in dogs with chronic complete AV block. These dogs had an increased vulnerability to ventricular arrhythmia during *d*-sotalol administration. However, without drugs, the incidence of spontaneous SCD was $<15\%$ (1/7 in one study) [45]. The only high-yield SCD model in which VT occurred spontaneously [46] was in dogs with inherited ventricular arrhythmia. This latter model may not be applicable to the vast majority of patients whose vulnerability to SCD develops after suffering from MI. Recently, Marian et al. [47] reported a transgenic rabbit model for human hypertrophic cardiomyopathy caused by a common point mutation in the β-myosin heavy chain gene, R400Q. There is a high mortality rate (44%) in that model; many deaths are sudden. However, there was no electrocardiogram documentation of the cardiac rhythm, and the increased incidence of SCD was not related to MI or coronary occlusion. To date, there exists no high-yield

animal model in which SCD caused by VF occurs spontaneously in chronic MI [18–20].

6. Electrical and anatomical remodeling after MI

Because most SCDs occurs in patients with coronary artery diseases and MI, the electrical and anatomical remodeling created by infarction may play an important role in the pathogenesis of VF and SCD. In experimental models, permanent occlusion of the LAD causes the development of transmural anteroseptal infarction [40]. The surviving rim of myocardial fibers (few to several hundred cells thick) on the epicardial surface of a transmural infarct is known as the epicardial border zone (EBZ) [48]. The EBZ has been shown to be the frequent site of origin of inducible VT/VF 1 week after LAD occlusion (healing phase of MI) [49,50]. In the first week after LAD occlusion, myocardial remodeling leads to increased connective tissue, collagen and edema between the epicardial muscle bundles, resulting in non-uniform anisotropy [48]. Active membrane properties of EBZ are altered. Peak L-type inward calcium current is reduced in myocytes isolated from the EBZ [51], and the recovery of the fast inward sodium current is delayed [52]. The changes in active membrane properties lead to altered excitability and post-repolarization refractoriness in the EBZ, promoting conduction block and reentry either during premature stimulation or during rapid pacing [49,50,52]. Electrical remodeling is not limited to the EBZ. Significant changes may also occur in non-infarcted myocardium. Qin et al. [53] demonstrated that MI might result in hypertrophy, action potential duration (APD) prolongation and marked heterogeneity of the time course of repolarization in the non-infarcted myocardium. These changes could be explained by the reduction of transient outward K^+ currents. Aimond et al. [54] subjected young rats to left coronary ligature. After 4–6 months, they studied enzymatically dissociated ventricular cells. They found that long-term coronary ligation resulted in a significantly altered L-type calcium current, including reduced peak amplitude and a slower inactivation. They also found a significantly altered K^+ current in post-MI cells. These findings indicate that MI results in significant electrical remodeling in both the EBZ and other non-infarcted myocardium [55].

7. Electrical remodeling due to complete AV block

Significant alterations of heart rate can result in electrical remodeling and increased vulnerability to ventricular arrhythmia. Vos et al. [43] first reported that, when challenged with *d*-sotalol, dogs with chronic AV block and ventricular hypertrophy may develop torsade de pointes (TdP) VT. These proarrhythmic effects were due to the electrical remodeling that occurs after complete AV block [45,56]. The electrical remodeling includes downregulation of delayed rectifier K^+ currents IKs and IKr in the myocardium, which delays repolarization, prolongs the QT interval, and thereby predisposes the myocardium to the acquired TdP [57,58]. While AV block and bradycardia cause electrical remodeling, it is interesting to note that rapid pacing, which results in heart failure, can also result in electrical remodeling, including downregulation of I(to) and action potential prolongation [42]. CsCl, which inhibits repolarizing K^+ currents that are downregulated during heart failure, results in a larger prolongation of APD in these dogs than in normal controls.

8. The interaction between nerve sprouting (neural remodeling) and electrical remodeling

β-Adrenergic stimulation is known to increase ionic current through L-type calcium channels (ICa), IKs, and chloride channels [ICl(Ca) and ICl-cAMP] [59,60]. The increased IKs tends to shorten APD while the increased ICa tends to prolong APD. In the normal canine ventricles in vivo, sympathetic stimulation results in shortening of APD and decreasing dispersion of refractoriness [61], implying that there is a net increase of outward repolarizing currents IKs and ICl versus the inward current ICa. However, the same is not true when IKs is suppressed or downregulated [62]. In the perfused canine tissues, chromanol 293B (a specific IKs blocker) prolongs the QT interval and APD_{90} of all myocardial cells but does not widen the T wave or induce TdP. Isoproterenol in the continued presence of chromanol 293B abbreviated the APD_{90} of epicardial and endocardial cells but not that of the M cells, resulting in widening of the T wave and a dramatic accentuation of dispersion of repolarization. Spontaneous as well as programmed electrical stimulation-induced TdP was observed only after exposure to the IKs blocker and isoproterenol. The IKs is also abnormal in patients with type I congenital long QT syndrome (LQT1) [63–65]. During exercise testing, these patients had diminished chronotropic response and paradoxical prolongation of QT interval [66]. Epinephrine may induce TdP, while β-blocker therapy and left-sympathectomy are antiarrhythmic in patients with LQT1 [65,67]. Kadish et al. [68] reported that in patients with a history of polymorphic VT in response to type Ia antiarrhythmic agents, exercise testing at drug-free state can result in paradoxical lengthening of the QT interval. In contrast, only one of the 11 control patients had QT lengthening with exercise. Half of the patients in that study had coronary artery diseases. These data suggest that sympathetic stimulation is arrhythmogenic if the IKs is genetically abnormal (such as in the congenital long QT syndrome), inhibited by pharmacological agents, or downregulated. Sympathetic stimulation in these ventricles may increase the dispersion of repolarization and the TdP arrhythmia. The mechanism is most

likely related to a large augmentation of residual IKs in epicardial and endocardial cells but not in M cells, in which IKs is intrinsically weak [62]. This hypothesis may explain the mechanism by which an abnormally prolonged QT interval is a predictor of cardiovascular mortality after MI [69].

If an interaction between sympathetic stimulation and reduction of IKs is important in cardiac arrhythmogenesis and SCD, then left stellectomy, which removes or reduces cardiac innervation, should be effective in preventing cardiac arrhythmia. Compatible with this hypothesis, left stellectomy in general have been shown to be antiarrhythmic. In both human patients and in animals, left stellectomy is effective in the prevention of SCD after MI [70], which had acquired abnormalities of potassium channels due to electrical remodeling. In type I congenital long QT syndrome, the abnormal potassium channel activity was genetic rather than acquired. However, left stellectomy was also useful (although not 100% effective) in preventing the recurrence of cardiac events [67]. These results show that an interaction between abnormal electrophysiological substrate and sympathetic stimulation is important in cardiac arrhythmogenesis. Removing cardiac sympathetic nerves might be partially effective in preventing SCD.

While many studies have been performed on the mechanisms of electrical remodeling after MI, there is little information available on the importance and arrhythmogenic potentials of sympathetic nerve sprouting after MI. To demonstrate the importance of nerve sprouting on arrhythmogenesis, we studied the explanted native hearts of human transplant recipients. We also performed a prospective study to determine whether or not augmented sympathetic nerve sprouting increases the incidence of SCD in a canine model. Here we provide a brief summary of these studies.

9. Relationship between regional cardiac hyperinnervation and ventricular arrhythmia in humans

We [71] studied 53 native hearts of transplant recipients. History was reviewed to determine the presence (group A) or absence (group B) of ventricular arrhythmias (including both VT and a history of sudden cardiac death). Immunocytochemical staining for S100 protein, neurofilament protein, tyrosine hydroxylase and protein gene product 9.5 was performed to study the distribution and the density of sympathetic nerves. A total of 30 patients had documented ventricular arrhythmias including unsustained or sustained ventricular tachycardia and SCD. Regional increase of sympathetic nerves was observed around the diseased myocardium and blood vessels in all 30 hearts. Fig. 1 shows an example of positive S100 protein staining of cardiac nerves (arrows) between normal (N) and scar tissues (SC). The density of nerve fibers was significantly higher in group A patients than in group B patients. We conclude that there is an association between a history of spontaneous ventricular arrhythmia and an increased density of sympathetic nerves in patients with severe heart failure. These findings suggest that abnormally increased post-injury sympathetic nerve density may be in part responsible for the occurrence of ventricular arrhythmia and SCD in these patients.

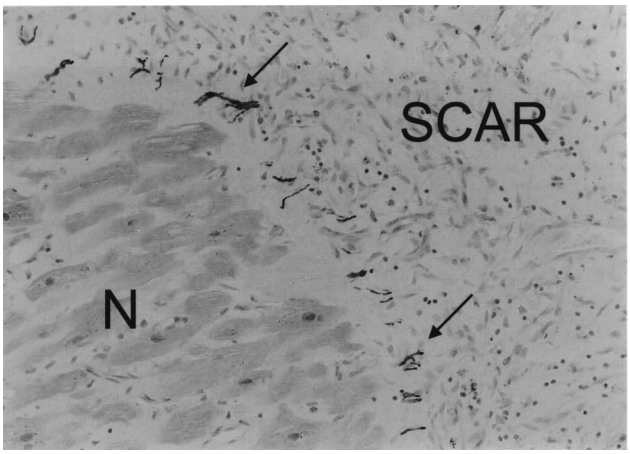

Fig. 1. Cardiac nerves in humans. Nerve twigs (arrows) were seen between normal myocardium (N) and scar (SC) tissues.

10. Nerve sprouting and sudden cardiac death in dogs

We [72] hypothesized that induction of increased sympathetic nerve sprouting by nerve growth factor (NGF) infusion to the left stellate ganglion in dogs with complete AV block [45,56] and MI may increase the frequency of ventricular arrhythmia and SCD. The augmented sympathetic nerve sprouting would provide increased sympathetic innervation to the myocardium. The AV block and MI would result in electrical remodeling. An interaction between nerve sprouting (neural remodeling) and electrical remodeling may result in a high-yield animal model of spontaneous SCD after MI. The purpose of the present study was to test this hypothesis.

The first step was to prove that NGF infusion to the left stellate ganglion could result in nerve sprouting. In three normal dogs, we infused NGF to the left stellate ganglion via an Alzet osmotic pump over a period of 2 weeks. Immunocytochemical studies showed that this method successfully induced sympathetic nerve sprouting in the ventricles. We then proceeded with the experiments in which dogs underwent the following procedures: AV block was induced by radiofrequency catheter ablation of the AV junction. MI was created by ligation of the LAD below the first diagonal branch. An implantable cardioverter-defibrillator (ICD) was implanted to detect VT and VF. In nine dogs with MI and complete AV block, we also infused

nerve growth factor (NGF) to the left stellate ganglion via an osmotic pump (experimental group). Another six dogs with MI and complete AV block but without NGF infusion served as controls. Immunocytochemical staining revealed greater sympathetic nerve sprouting in the experimental group than in the control group.

All dogs showed spontaneous VT after MI (phase-1 VT). Spontaneous VT reappeared 13.1±6.0 days after surgery (phase-2 VT). The frequency of phase-2 VT was significantly higher in the experimental group than in the control group. In the experimental group, the occurrence of phase-2 VT shows a significant ($P<0.05$) diurnal variation with the peak incidence in the morning to early afternoon. Four dogs in the experimental group but none in the control group died suddenly of spontaneous VF. In an additional six dogs we created MI without producing AV block or infusing NGF. All six dogs showed histological evidence of sympathetic nerve sprouting 48±5 days later. However, none of these six dogs died suddenly.

It is interesting to note that the nerve density in patients with ventricular arrhythmia ($19.6\pm11.2/mm^2$) [71] overlaps with the nerve density in the experimental group of the present study. Also, in dogs with MI only (without AV block and NGF infusion), there is also evidence of nerve sprouting. These results suggest that cardiac nerve sprouting may occur after MI even without exogenous NGF. Infusion of NGF accelerated and intensified the magnitude of nerve sprouting, resulting in a high incidence of SCD. Another interesting finding is that NGF infusion to the left stellate ganglion in normal dogs resulted in nerve sprouting but did not result in ventricular arrhythmia or SCD. In the control group of dogs, electrical remodeling induced by AV block and MI also failed to result in SCD. An interaction between nerve sprouting (neural remodeling) and electrical remodeling is needed to create a high-yield canine model of VT, VF, or SCD.

As stated above, the only other canine model of SCD in which VF occurs spontaneously was the German shepherds with inherited ventricular arrhythmias [46,73]. In that model, heterogeneous sympathetic innervation was consistently identified [74]. Furthermore, there was a heterogeneous increase of beta-receptor density. Isolated anteroseptal M cell preparations of afflicted dogs studied with microelectrodes showed abnormal lengthening, rather than shortening of action potential duration in response to isoproterenol infusion [75]. These results imply that in the German shepherd model of SCD, the mechanisms of death might also be related to an interaction between heterogeneous sympathetic innervation and abnormal electrical substrate, most likely due to the presence of abnormal potassium channels [76].

11. Clinical implications

Nerve sprouting hypothesis explains the efficacy of β-blockers in the prevention of SCD [77,78]. It may also explain the results of some clinical trials. For example, sotalol, a drug with β-blocking effects, is known to be effective in preventing ventricular arrhythmias in patients with chronic MI [79]. If the β-blocking activity is removed (such as in *d*-sotalol), then the drug increases mortality in patients with MI [80]. Future development of antiarrhythmic interventions should target not only the electrical remodeling, but also the neural remodeling (sympathetic nerve sprouting and hyperinnervation) after MI.

It is also known that left stellectomy is effective in the prevention of SCD after MI both in animal models and in humans [70,81,82]. These findings are also compatible with the nerve sprouting hypothesis of SCD.

12. Conclusions

We conclude that sympathetic hyperinnervation occurs in some (but not all) patients after MI. There is an association between sympathetic hyperinnervation and ventricular arrhythmia. NGF infusion to the left stellate ganglion in dogs with chronic MI and AV block increases sympathetic nerve sprouting and creates a high-yield model of spontaneous VT, VF, and SCD. The magnitude of sympathetic nerve sprouting may be an important determinant of SCD in chronic MI. These results support the nerve sprouting hypothesis of sudden cardiac death.

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References

[1] Ludwig C. Über die Herznerven des Frosches. Arch Anat Physiol 1848;139.
[2] Bidder F. Ueber functionell verschiedene und räumlich getrennte Nervencentra im Froschherzens. Arch Anat Physiol 1852;163.
[3] Hirsch EF, Jellinik M, Cooper T, Kaiser GC, Barner HC, Borghard-Erdle AM, editors, The innervation of the vertebrate heart, Springfield: Charles C. Thomas, 1970, pp. 1–413.
[4] Zipes DP. Influence of myocardial ischemia and infarction on autonomic innervation of heart. Circulation 1990;82:1095–1104.
[5] Randall WC, Szentivanyi M, Pace JB, Wechsler JS, Kaye MP. Patterns of sympathetic nerve projections onto the canine heart. Circ Res 1968;22:315–323.
[6] Guth L. Regeneration in the mammalian peripheral nervous system. Physiol Rev 1956;36:441–478.

[7] Vracko R, Thorning D, Frederickson RG. Nerve fibers in human myocardial scars. Hum Pathol 1991;22:138–146.
[8] Vracko R, Thorning D, Frederickson RG. Fate of nerve fibers in necrotic, healing, and healed rat myocardium. Lab Invest 1990;63:490–501.
[9] Oki T, Fukuda N, Kawano T et al. Histopathologic studies of innervation of normal and prolapsed human mitral valves. J Heart Valve Dis 1995;4:496–502.
[10] Chow LT, Chow SS, Anderson RH, Gosling JA. Innervation of the human cardiac conduction system at birth. Br Heart J 1993;69:430–435.
[11] Marron K, Wharton J, Sheppard MN et al. Distribution, morphology, and neurochemistry of endocardial and epicardial nerve terminal arborizations in the human heart. Circulation 1995;92:2343–2351.
[12] Gulbenkian S, Saetrum OO, Ekman R et al. Peptidergic innervation of human epicardial coronary arteries. Circ Res 1993;73:579–588.
[13] Wharton J, Polak JM, Gordon L et al. Immunohistochemical demonstration of human cardiac innervation before and after transplantation. Circ Res 1990;66:900–912.
[14] Chow LT, Chow WH, Lee JC, Chow SS, Anderson RH, Gosling JA. Postmortem changes in the immunohistochemical demonstration of nerves in human ventricular myocardium. J Anat 1998;192(1):73–80.
[15] Yanowitz F, Preston JB, Abildskov JA. Functional distribution of right and left stellate innervation to the ventricles. Production of neurogenic electrocardiographic changes by unilateral alteration of sympathetic tone. Circ Res 1966;18:416–428.
[16] Schwartz PJ, Priori SG. Sympathetic nervous system and cardiac arrhythmias. In: Zipes DP, Jalife J, editors, Cardiac electrophysiology: from cell to bedside, Philadelphia, PA: W.B. Saunders, 1990, pp. 330–343.
[17] Schwartz PJ, Vanoli E, Zaza A, Zuanetti G. The effect of antiarrhythmic drugs on life-threatening arrhythmias induced by the interaction between acute myocardial ischemia and sympathetic hyperactivity. Am Heart J 1985;109:937–948.
[18] Janse MJ, Schwartz PJ, Wilms-Schopman F, Peters RJ, Durrer D. Effects of unilateral stellate ganglion stimulation and ablation on electrophysiologic changes induced by acute myocardial ischemia in dogs. Circulation 1985;72:585–595.
[19] Schwartz PJ, Vanoli E, Stramba-Badiale M, De Ferrari GM, Billman GE, Foreman RD. Autonomic mechanisms and sudden death. New insights from analysis of baroreceptor reflexes in conscious dogs with and without a myocardial infarction. Circulation 1998;78:969–979.
[20] Verrier RL, Thompson PL, Lown B. Ventricular vulnerability during sympathetic stimulation: role of heart rate and blood pressure. Cardiovasc Res 1974;8:602–610.
[21] Brandys JC, Hopkins DA, Armour JA. Cardiac responses to stimulation of discrete loci within canine sympathetic ganglia following hexamethonium. J Auton Nerv Syst 1984;11:243–255.
[22] Cardinal R, Scherlag BJ, Vermeulen M, Armour JA. Distinct activation patterns of idioventricular rhythms and sympathetically-induced ventricular tachycardias in dogs with atrioventricular block. Pacing Clin Electrophysiol 1992;15:1300–1316.
[23] Schwartz PJ. Cardiac sympathetic innervation and the sudden infant death syndrome. A possible pathogenetic link. Am J Med 1976;60:167–172.
[24] Schwartz PJ, Stramba-Badiale M, Segantini A et al. Prolongation of the QT interval and the sudden infant death syndrome [see comments]. New Engl J Med 1998;338:1709–1714.
[25] Stramba-Badiale M, Lazzarotti M, Schwartz PJ. Development of cardiac innervation, ventricular fibrillation, and sudden infant death syndrome. Am J Physiol 1992;263:H1514–H1522.
[26] Fu SY, Gordon T. The cellular and molecular basis of peripheral nerve regeneration. Mol Neurobiol 1997;14:67–116.
[27] Sjoberg J, Kanje M. The initial period of peripheral nerve regeneration and the importance of the local environment for the conditioning lesion effect. Brain Res 1990;529:79–84.
[28] Levi-Montalcini R. Growth control of nerve cells by a protein factor and its antiserum. Science 1964;143:105–110.
[29] Rush RA. Immunohistochemical localization of endogenous nerve growth factor. Nature 1984;312:364–367.
[30] Lindholm D, Heumann R, Meyer M, Thoenen H. Interleukin-1 regulates synthesis of nerve growth factor in non-neuronal cells of rat sciatic nerve. Nature 1987;330:658–659.
[31] Sutula T, Cascino G, Cavazos J, Parada I, Ramirez L. Mossy fiber synaptic reorganization in the epileptic human temporal lobe. Ann Neurol 1989;26:321–330.
[32] Sutula T, He X-X, Cavazos J, Grayson S. Synaptic reorganization in the hippocampus induced by abnormal functional activity. Science 1988;239:1147–1150.
[33] Barber MJ, Mueller TM, Henry D, Felten SY, Zipes DP. Transmural myocardial infarction in the dog produces sympathectomy in noninfarcted myocardium. Circulation 1983;67:787–796.
[34] Nori SL, Gaudino M, Alessandrini F, Bronzetti E, Santarelli P. Immunohistochemical evidence for sympathetic denervation and reinnervation after necrotic injury in rat myocardium. Cell Mol Biol 1995;41:799–807.
[35] Stanton MS, Tuli MM, Radtke NL et al. Regional sympathetic denervation after myocardial infarction in humans detected noninvasively using I-123-metaiodobenzylguanidine. J Am Coll Cardiol 1989;14:1519–1526.
[36] Minardo JD, Tuli MM, Mock BH et al. Scintigraphic and electrophysiological evidence of canine myocardial sympathetic denervation and reinnervation produced by myocardial infarction or phenol application. Circulation 1988;78:1008–1019.
[37] Hartikainen J, Kuikka J, Mantysaari M, Lansimies E, Pyorala K. Sympathetic reinnervation after acute myocardial infarction. Am J Cardiol 1996;77:5–9.
[38] Schwaiblmair M, von Scheidt W, Uberfuhr P et al. Functional significance of cardiac reinnervation in heart transplant recipients. J Heart Lung Transplant 1999;18:838–845.
[39] Schwartz PJ. Do animal models have clinical value? Am J Cardiol 1998;81:14D–20D.
[40] Karagueuzian HS, Fenoglio JJ, Weiss Jr. MB, Wit AL. Protracted ventricular tachycardia induced by premature stimulation of the canine heart after coronary artery occlusion and reperfusion. Circ Res 1979;44:833–846.
[41] Echt DS, Griffin JC, Ford AJ, Knutti JW, Feldman RC, Mason JW. Nature of inducible ventricular tachyarrhythmias in a canine chronic myocardial infarction model. Am J Cardiol 1983;52:1127–1132.
[42] Pak PH, Nuss HB, Tunin RS et al. Repolarization abnormalities, arrhythmia and sudden death in canine tachycardia-induced cardiomyopathy. J Am Coll Cardiol 1997;30:576–584.
[43] Vos MA, Verduyn SC, Gorgels AP, Lipcsei GC, Wellens HJ. Reproducible induction of early afterdepolarizations and torsade de pointes arrhythmias by *d*-sotalol and pacing in dogs with chronic atrioventricular block. Circulation 1995;91:864–872.
[44] Hunt GB, Ross DL. Influence of infarct age on reproducibility of ventricular tachycardia induction in a canine model. J Am Coll Cardiol 1989;14:765–773.
[45] Vos MA, de Groot SH, Verduyn SC et al. Enhanced susceptibility for acquired torsade de pointes arrhythmias in the dog with chronic, complete AV block is related to cardiac hypertrophy and electrical remodeling. Circulation 1998;98:1125–1135.
[46] Moise NS, Meyers-Wallen V, Flahive WJ et al. Inherited ventricular arrhythmias and sudden death in german shepherd dogs. J Am Coll Cardiol 1994;24:233–243.
[47] Marian AJ, Wu Y, Lim DS et al. A transgenic rabbit model for human hypertrophic cardiomyopathy. J Clin Invest 1999;104:1683–1692.
[48] Wit AL, Dillon SM, Coromilas J, Saltman AE, Waldacker B. Anisotropic reentry in epicardial border zone in myocardial infarcts. Ann NY Acad Sci 1990;591:86–108.
[49] Dillon SM, Allessie MA, Ursell PC, Wit AL. Influences of anisotropic tissue structure on reentrant circuits in the epicardial border zone of subacute canine infarcts. Circ Res 1988;63:182–206.

[50] Gough WB, Megra R, Restivo M, Zeiler RH, El-Sherif N. Reentrant ventricular arrhythmias in the late myocardial infarction period in the dog: 13 correlation of activation and refractory maps. Circ Res 1985;57:432–445.
[51] Aggarwal R, Boyden PA. Diminished Ca^{2+} and Ba^{2+} currents in myocytes surviving in the epicardial border zone of the 5-day infarcted canine heart. Circ Res 1995;77:1180–1191.
[52] Pu J, Boyden PA. Alterations of Na^{+} currents in myocytes from epicardial border zone of the infarcted heart. A possible ionic mechanism for reduced excitability and postrepolarization refractoriness. Circ Res 1997;81:110–119.
[53] Qin D, Zhang ZH, Caref EB, Boutjdir M, Jain P, El-Sherif N. Cellular and ionic basis of arrhythmias in postinfraction remodeled ventricular myocardium. Circ Res 1996;79:461–473.
[54] Aimond F, Alvarez JL, Rauzier JM, Lorente P, Vassort G. Ionic basis of ventricular arrhythmias in remodeled rat heart during long-term myocardial infarction. Cardiovasc Res 1999;42:402–415.
[55] Pinto JM, Boyden PA. Electrical remodeling in ischemia and infarction. Cardiovasc Res 1999;42:284–297.
[56] Volders PG, Sipido KR, Vos MA, Kulcsar A, Verduyn SC, Wellens HJ. Cellular basis of biventricular hypertrophy and arrhythmogenesis in dogs with chronic complete atrioventricular block and acquired torsade de pointes. Circulation 1998;98:1136–1147.
[57] Volders PG, Sipido KR, Vos MA. Downregulation of delayed rectifier K^{+} current predisposes to acquired torsades de pointes and sudden cardiac death in dogs with chronic complete atrioventricular block [abstract]. PACE 1999;22:II760.
[58] Volders PG, Sipido KR, Vos MA et al. Downregulation of delayed rectifier K(+) currents in dogs with chronic complete atrioventricular block and acquired torsades de pointes. Circulation 1999;100:2455–2461.
[59] Hume JR, Harvey RD. Chloride conductance pathways in heart. Am J Physiol 1991;261:C399–C412.
[60] Zygmunt AC. Intracellular calcium activates a chloride current in canine ventricular myocytes. Am J Physiol 1994;267:H1984–H1995.
[61] Takei M, Sasaki Y, Yonezawa T, Lakhe M, Aruga M, Kiyosawa K. The autonomic control of the transmural dispersion of ventricular repolarization in anesthetized dogs. J Cardiovasc Electrophysiol 1999;10:981–989.
[62] Shimizu W, Antzelevitch C, Cellular basis for the ECG. features of the LQT1 form of the long-QT syndrome: effects of beta-adrenergic agonists and antagonists and sodium channel blockers on transmural dispersion of repolarization and torsade de pointes. Circulation 1998;98:2314–2322.
[63] Sanguinetti MC, Curran ME, Zou A et al. Coassembly of K(V)LQT1 and minK (IsK) proteins to form cardiac I(Ks) potassium channel. Nature 1996;384:80–83.
[64] Barhanin J, Lesage F, Guillemare E, Fink M, Lazdunski M, Romey G. K(V)LQT1 and lsK (minK) proteins associate to form the I(Ks) cardiac potassium current. Nature 1996;384:78–80.
[65] Schwartz PJ, Locati EH, Napolitano C, Priori SG. The long QT syndrome. In: Zipes DP, Jalife J, editors, Cardiac electrophysiology: from cell to bedside, Philadelphia: W.B. Saunders, 1994, pp. 788–811.
[66] Swan H, Viitasalo M, Piippo K, Laitinen P, Kontula K, Toivonen L. Sinus node function and ventricular repolarization during exercise stress test in long QT syndrome patients with KvLQT1 and HERG potassium channel defects. J Am Coll Cardiol 1999;34:823–829.
[67] Schwartz PJ, Locati EH, Moss AJ, Crampton RS, Trazzi R, Ruberti U. Left cardiac sympathetic denervation in the therapy of congenital long QT syndrome. A worldwide report. Circulation 1991;84:503–511.
[68] Kadish AH, Weisman HF, Veltri EP, Epstein AE, Slepian MJ, Levine JH. Paradoxical effects of exercise on the QT interval in patients with polymorphic ventricular tachycardia receiving type Ia antiarrhythmic agents. Circulation 1990;81:14–19.
[69] Okin PM, Devereux RB, Howard BV, Fabsitz RR, Lee ET, Welty TK. Assessment of QT interval and QT dispersion for prediction of all-cause and cardiovascular mortality in American Indians. The Strong Heart Study. Circulation 2000;101:61–66.
[70] Italian Sudden Death Prevention Group, Schwartz PJ, Motolese M, Pollavini G et al. Prevention of sudden cardiac death after a first myocardial infarction by pharmacologic or surgical antiadrenergic interventions. J Cardiovasc Electrophysiol 1992;3:2–16.
[71] Cao J-M, Fishbein MC, Han JB et al. Relationship between regional cardiac hyperinnervation and ventricular arrhythmia. Circulation 2000;101:1960–1969.
[72] Cao J-M, Chen LS, Kenknight BH et al. Nerve sprouting and sudden cardiac death. Circ Res 2000;86:816–821.
[73] Moise NS, Gilmour Jr. RF, Riccio ML. An animal model of spontaneous arrhythmic death. J Cardiovasc Electrophysiol 1997;8:98–103.
[74] Dae MW, Lee RJ, Ursell PC, Chin MC, Stillson CA, Moise NS. Heterogeneous sympathetic innervation in German shepherd dogs with inherited ventricular arrhythmia and sudden cardiac death. Circulation 1997;96:1337–1342.
[75] Sosunov EA, Anyukhovsky EP, Shvilkin A, Hara M, Steinberg SF, Danilo PJ, Rosen MR, Moise NS, Merot J, Probst V, Charpentier F, Legeay Y, Le MH. Abnormal cardiac repolarization and impulse initiation in German shepherd dogs with inherited ventricular arrhythmias and sudden death. Cardiovasc Res 1999;42:65–79.
[76] Merot J, Probst V, Debailleul M, Gerlach U, Moise NS, Le MH, Charpentier F. Electropharmacological characterization of cardiac repolarization in German shepherd dogs with an inherited syndrome of sudden death., abnormal response to potassium channel blockers. J Am Coll Cardiol 2000;36(3):939–947.
[77] The Norwegian Multicenter Study Group. Timolol-induced reduction in mortality and reinfarction in patients surviving acute myocardial infarction. New Engl J Med 1981;304:801–807.
[78] Rarnaot M. Nicotine patches may not be safe. Br Med J 1995;310:663–664.
[79] Mason JW. A comparison of electrophysiologic testing with Holter monitoring to predict antiarrhythmic drug efficacy for ventricular tachyarrhythmias. Electrophysiologic Study versus Electrocardiographic Monitoring Investigators. New Engl J Med 1993;329:445–451.
[80] Waldo AL, Camm AJ, deRuyter H et al. Effect of *d*-sotalol on mortality in patients with left ventricular dysfunction after recent and remote myocardial infarction. The SWORD Investigators. Survival with oral *d*-sotalol [see comments] [published erratum appears in Lancet 1996;348(9024):416]. Lancet 1996;348:7–12.
[81] Schwartz PJ. The rationale and the role of left stellectomy for the prevention of malignant arrhythmias. Ann NY Acad Sci 1984;427:199–221.
[82] Nelson SD, Lynch JJ, Sanders D, Montgomery DG, Lucchesi BR. Electrophysiologic actions and antifibrillatory efficacy of subacute left stellectomy in a conscious, post-infarction canine model of ischemic ventricular fibrillation. Int J Cardiol 1989;22:365–376.