Sympathetic Nerves and Myocyte Necrosis
More Than Meets the Eye

John M. Canty, Jr, James A. Fallavollita

The left ventricle is richly supplied with sympathetic nerves, which are spatially localized next to cardiac myocytes in a manner that permits the rapid transmission of autonomic signals via the release of norepinephrine. Previous investigation in the heart has largely focused on the local release and reuptake kinetics of norepinephrine in conjunction with its downstream receptor-mediated events. Nevertheless, accumulating data indicate that the crosstalk between myocardial sympathetic nerves and cardiac myocytes appears to be much more complex. In addition to the co-release of other vasoactive peptides such as neuropeptide Y, sympathetic nerves can also modulate the expression of trophic factors such as nerve growth factor (NGF) and are a potential source of nitric oxide (NO) production via the neuronal NO synthase. Collectively, these can have diverse chronic effects on target tissues such as the heart, which could alter endogenous free radical scavenging mechanisms in pathophysiological states as well as the expression of ion channels involved in depolarization and repolarization. The loss of this crosstalk could alter the myocyte response to ischemia.

In this issue of Circulation Research, Huang and colleagues provide provocative in vivo experimental data to support the notion that the sympathetic nerves modulate oxidant-mediated injury to the heart after ischemia. Chronically instrumented swine with regional sympathetic denervation were subjected to short-term hibernation using a 40% reduction in blood flow for 90 minutes followed by reperfusion for 4 days. Although flow and function were similarly matched during ischemia, the regionally denervated heart developed greater myocardial stunning than the innervated heart. Furthermore, while short-term hibernation was a reversible insult in the innervated heart, denervated animals developed histological evidence of neutrophil infiltration and micronecrosis when examined 4 days after reperfusion. This was accompanied by enhanced 3-nitrotyrosine activation in myocytes examined 1 hour after reperfusion. Protein nitration and micronecrosis in myocytes could be prevented with the free radical scavenger N-2-mercaptopropionyl glycine (MPG) or chronic administration of N-nitro-L-arginine (L-NA). The authors conclude that the denervated heart is subjected to increased stunning and microinfarction (with normal triphenyl tetrazolium chloride [TTC] staining) through a mechanism that involves NO and/or reactive oxygen species. These findings contrast with those previously reported by this group after total coronary occlusion in the regionally denervated heart of the dog where there was no effect of denervation on infarct size. Possible reasons for this are the fact that the heart was not reperfused in the prior study, or the reliance on TTC staining to assess infarction in the previous study may have underestimated micronecrosis.

As the authors point out, the effects of sympathetic innervation on the development of myocyte necrosis and stunning after ischemia highlight the importance of studying the complexity of cell-cell interactions in intact in vivo models versus isolated heart preparations that, by their nature, are acutely denervated. Although denervation is an attractive mechanism to resolve differences regarding the effects of NO on myocardial injury between in vivo and in vitro studies, there are several features unique to the present experiments that may limit extrapolation of the findings to acute isolated heart preparations. First, the topical phenol technique evaluated chronic denervation versus the acute denervation encountered in the isolated heart. Tissue norepinephrine levels and the norepinephrine uptake-1 mechanism remain intact in acutely denervated hearts, and additional time would be required for any chronic myocyte alterations related to denervation to develop. Second, the isolated heart has both sympathetic and parasympathetic denervation while epicardial phenol results in selective sympathetic denervation. Even though the heart is not under tonic parasympathetic control, it is plausible that the neural response to ischemia could differ from that when the heart is regionally denervated. For example, there is evidence that sympathetic nerves modulate the production of NGF from cardiac parasympathetic nerves. Thus, although sympathetic denervation modulates the myocyte response to ischemia in this model, it will be essential to determine whether similar effects occur in the acutely denervated heart.

A surprising observation that is unique to this model of ischemia is the apparent dissociation between normal TTC staining, which is the standard to assess irreversible injury in studies of total coronary occlusion, and pathological quantitation of micronecrosis and inflammation. This difference was not trivial since 10% of the risk area of the denervated heart (predominantly subendocardial) had micronecrosis when evaluated 4 days after ischemia. Previous studies have demonstrated excellent correlation between acute and late TTC and pathology in models of total coronary occlusion.
This provocative finding raises new questions as to what the actual gold standard should be for demonstrating the effects of interventions on irreversible myocyte injury and may be particularly germane to oxidant injury and the manipulation of NO production. Unfortunately, the present study sheds limited insight into the mechanism or time course of myocyte injury. While evidence of increased peroxynitrite-related protein nitration was seen in myocytes evaluated after 1 hour, no data were provided to show if micronecrosis was already present at this time or whether it was a delayed effect of reperfusion due to neutrophil/monocyte cell entry. Another key question is whether the micronecrosis ultimately leads to a scar of similar magnitude or whether the fibrosis is inconsequential. Models of apoptosis-induced myocyte injury have substantial myocyte loss with only small amounts of connective tissue replacement. For example, in chronic hibernating myocardium, Lim et al found extensive myocyte apoptosis-induced loss (≈35%) and compensatory cellular hypertrophy in hibernating myocardium in the face of trivial increases in fibrosis (≈3%) and normal TTC staining. Important areas for future study include determining whether myocyte damage after short-term hibernation predominantly represents necrosis or apoptosis and determining whether it leads to a comparable magnitude of replacement fibrosis and myocyte loss in the healing phase.

How do sympathetic nerves protect the heart from stunning and micronecrosis? The data show that sympathetic denervation leads to increased peroxynitrite production from superoxide anion and NO in cardiac myocytes. Since this was prevented by L-NA or MPG, there is either an increase in NO availability in some compartment of the myocardium or increased superoxide anion (eg, via reduced antioxidant mechanisms or enhanced superoxide production). One possibility is that the degeneration of sympathetic nerves after phenol creates a proinflammatory state due to macrophage infiltration associated with Wallerian degeneration. Data regarding the effects of chronic sympathetic denervation on myocardial antioxidant mechanisms are lacking, but catecholamines may be an oxidative stress, and the loss of norepinephrine in the denervated heart could downregulate endogenous oxidant defense mechanisms. It is also plausible that neurotrophins could modulate myocardial oxidant defense mechanisms. Although the effects of cardiac denervation are unknown, myocardial NGF levels are reduced in disease states associated with the loss of sympathetic nerve terminals and tissue catecholamine depletion such as heart failure and diabetes, which are associated with increased oxidant stress. In neurons, NGF withdrawal leads to apoptosis via an increase in peroxynitrite from a reduction in superoxide dismutase and stimulation of neuronal NO synthase, which may play a role in some neurodegenerative diseases. NGF is also released from cardiac parasympathetic nerve terminals, and its production is reduced after sympathetic denervation. Further studies to evaluate the role of sympathetic nerves on myocardial oxidant defenses will be necessary to determine the causal relation of these mechanisms in myocyte injury.

Finally, how can the paradoxical beneficial effects of inhibiting NO synthase in the denervated heart be reconciled with the previous study showing that L-NA is detrimental in the innervated heart? One possibility is that the findings are related to pharmacologically induced alterations in oxidant stress after a 3-day administration of L-NA. Chronic NO synthase inhibition decreases endothelial NO, increases endothelial superoxide anion, and increases local angiotensin production. If similar effects occur with L-NA, the down-regulation in oxidant defense mechanisms could increase free radical–mediated stunning and infarction. There could also be a proinflammatory component to injury because chronic Nω-nitro-L-arginine methyl ester (L-NAME) increased expression of monocyte chemoattractant protein-1 in as few as 3 days. While speculative, the lack of a detrimental effect after L-NA in the denervated heart suggests an important role of increased norepinephrine release from central sympathetic tone or local angiotensin in producing the oxidative stress accompanying chronic NOS inhibition.

Sympathetic nerves are exquisitely sensitive to what is ordinarily believed to be reversible ischemia. Thus, these findings are potentially clinically relevant since regional inhomogeneity in sympathetic innervation is seen in patients subjected to acute ischemia (with minimal infarction) as well as swine with chronic hibernating myocardium. Like all good experimental studies, Huang and colleagues have raised more new questions from those they have answered, and further studies will be necessary to understand the full implications of their findings in patients and other experimental models.

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References


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