Nitric Oxide and Short-Term Hibernation

Friend or Foe?

John M. Canty, Jr

Subendocardial blood flow (as a surrogate of regional oxygen consumption) and myocardial function are closely coupled during acute myocardial ischemia.\(^1,2\) Whereas severe ischemia results in the rapid onset of irreversible injury, viability in the face of moderate levels of acute ischemia can be maintained for several hours.\(^3\) A uniform finding during moderate steady-state ischemia is the fact that the myocardium is able to restore a balance between a limited blood supply and demand by exquisite coupling between local flow and metabolism. Thus, the transient increases in myocardial lactate and reductions in creatine phosphate and ATP at the onset of ischemia gradually return toward normal over a period of hours.\(^4,5\) The intrinsic mechanism by which myocytes are able to metabolically adapt and match function to a reduced level of flow and oxygen delivery has remained elusive, and the phenomenon has become known as “short-term hibernation.”\(^3\)

Nitric oxide (NO) is a potential candidate for such regulation because it is capable of modulating contractile function under a variety of circumstances. It has become increasingly apparent that the biological actions of NO in the heart are quite complex. The effects on contractility vary among experimental preparations, and they are highly concentration-dependent. Part of this variability undoubtedly reflects the complex cellular distribution of nitric oxide synthase (NOS) and the partitioning of isoforms within particular subcellular compartments of the cardiac myocyte. As a result, local cardiac function can be modulated via NO originating from several sources. Coronary flow and shear stress stimulates NOS3 (or endothelial nitric oxide synthase [eNOS]), which produces NO that can diffuse from the vascular endothelium to affect cardiac myocyte function and metabolism in a paracrine fashion. On the basis of this mechanism, it is somewhat counterintuitive that NOS activity and cardiac NO production increase during ischemia.\(^6,7\) One would anticipate that reductions in flow and shear stress should decrease endothelial NO release. The explanation may reside in developing a better understanding of the other sources of NO production in the heart. NO is also synthesized within cardiac myocytes and can modulate cardiac muscle in an autocrine fashion. Recently, both NOS1 (or neuronal nitric oxide synthase [nNOS], localized to the sarcoplasmic reticulum)\(^8\) and NOS3 (localized to the sarcolemma and T-tubules)\(^9\) have been identified in normal cardiac tissue. Finally, in pathophysiological disease states,\(^10\) as well as after reversible ischemia,\(^11\) NOS2 (or inducible nitric oxide synthase [iNOS]) can be induced in the heart. How these myocyte sources of NO production are altered during acute ischemia is currently unknown.

It is also increasingly apparent that NO is a two-edged sword, having a variety of opposing effects that are highly dose-dependent. In terms of myocardial function, high doses of NO depress contractility via activation of guanylate cyclase and the accumulation of cGMP.\(^12\) At low doses, NO increases contractility via a cAMP-dependent mechanism.\(^12\) In addition, NO can also affect cellular proteins that modulate contractile function via direct actions rather than through the generation of cyclic nucleotide intermediaries. NO can directly activate the L-type Ca\(^{2+}\) channel, the Ca\(^{2+}\) release channel, and inhibit the SR Ca\(^{2+}\) ATPase via S-nitrosylation and/or thiol oxidation.\(^13,14\) These direct effects of NO can stimulate cardiac contraction. Finally, NO modulates sympathetic-mediated responses with increased cGMP, attenuating β-adrenergic-mediated production of cAMP to reduce contractility.\(^15\) In terms of metabolism, NO reversibly depresses mitochondrial respiration and decreases basal myocardial oxygen consumption in vitro.\(^16\) In vivo, NO shifts myocardial substrate catabolism from the utilization of free fatty acids to lactate.\(^17\) In light of the complex cellular distribution of NOS and the diverse modulatory effects of NO, it is not surprising that divergent findings have arisen from studies intended to elucidate the effect of NO on myocardial function and metabolism.

In this issue of Circulation Research, Heusch and colleagues\(^18\) report the results of studies designed to evaluate the role of endogenous NO release during short-term hibernation in vivo. Regional ischemia was produced in open-chest anesthetized swine under constant flow conditions. Inhibiting NOS with pharmacological doses of L-nitro-arginine (L-NA) produced a large reduction in baseline regional wall thickening and myocardial work before ischemia but had no effect on resting flow or oxygen consumption, indicating a reduction in mechanical efficiency. During ischemia, myocardial function was also lower after inhibiting NOS, yet the level of oxygen consumption was similar to controls and myocardial substrate utilization shifted from free fatty acids to lactate. Although coronary NO release was attenuated by L-NA, there was no measurable reduction in tissue cGMP. The results indicate

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that regional function and work for any given level of oxygen consumption were lower in comparison to controls and animals where the systemic hemodynamic effects of L-NA were matched by aortic constriction to increase systolic pressure to comparable levels. These observations suggest an important role of myocardial NO in allowing myocardial function to be optimized for any given level of oxygen consumption under control conditions as well as during ischemia. Despite the prominent changes in regional mechanical efficiency, the metabolic changes associated with steady-state perfusion-contraction matching during acute ischemia were not altered. Thus, NO is not responsible for the phenomenon of short-term hibernation. Although these conclusions are appropriate, there are several additional factors to consider in interpreting the data.

Could the findings simply be explained by alterations in afterload? Probably not. First of all, there was a parallel downward shift in the relation between external work and oxygen consumption when data after L-NA administration were compared with data during aortic constriction at a similar level of systolic pressure. Although this does not completely ensure that loading conditions were identical, the relationships between external work and oxygen consumption under control conditions and after aortic constriction were similar. This suggests that small variations in afterload over this range do not shift the relation between external work and oxygen consumption in the absence of L-NA in this preparation. Thus, it is difficult to argue that the reduction in wall thickening could simply be due to afterload changes induced by L-NA.

Were there differences in subendocardial blood flow among the interventions? This is somewhat more difficult to exclude based on the data presented. Although the experiments were performed under conditions of constant flow, there were very likely steep gradients in transmural perfusion in this preparation and measurements of subendocardial perfusion are not reported. Regional function (and presumably oxygen consumption) is most closely coupled to subendocardial perfusion. Because the lower pressure limit of coronary autoregulation will increase after inhibiting NOS, it would have been helpful to exclude the possibility that subendocardial flow was lower after L-NA. More severe subendocardial ischemia after L-NA could have resulted in an apparent reduction in wall thickening and Ca^{2+} responsiveness. Despite this concern, lactate release during ischemia appeared similar in each group, making this a less likely possibility.

Is there something unique about high-dose L-NA administered systemically versus other L-arginine analogs that leads to effects on myocardial function that are perhaps, unique? The dose of L-NA (35 mg/kg), although similar to that used in other laboratories examining the metabolic effects of endogenous NO, is higher than that required to inhibit endothelial-dependent vasodilation via NOS3. Studies using lower intracoronary doses of other L-arginine analogs (e.g., N^\omega-nitro-L-arginine methyl ester [L-NAME] and N^\omega-monomethyl-L-arginine [L-NMMA]) frequently report no effect on myocardial oxygen consumption or function at rest. Other studies have found reductions in regional function after intracoronary L-NAME, but they were associated with a reduction in regional myocardial oxygen consumption. Finally, evaluation of global myocardial function showed that L-NMMA increased oxygen consumption and the slope of the end-systolic pressure volume relation, consistent with improved contractility after inhibiting NOS. Nevertheless, there was no alteration of global contractile efficiency. A plausible explanation for the divergent effects of NO on resting function and oxygen consumption among studies including that of Heusch et al are variations in the extent that different isoforms or compartments of NOS in the heart are blocked. Although all studies have assessed pharmacological efficacy by demonstrating attenuated endothelium-dependent coronary dilation, it is more difficult to show that myocyte NOS isoforms are blocked. This may reconcile the diversity of findings regarding baseline oxygen consumption and function after inhibiting NOS in vivo and is an area that requires further study.

Do the data unequivocally indicate a role for NO in optimizing function during ischemia beyond the L-NA-induced reduction in function produced at baseline? As Heusch et al emphasize, matching between perfusion, contraction, and local metabolism during short-term hibernation continues after inhibiting NO. Although there was a parallel shift in the relation between external work and oxygen consumption after L-NA, relative reductions during ischemia expressed as a percentage of the baseline values were actually quite similar. For example, myocardial oxygen consumption after 10 minutes of ischemia fell to 65% of control, wall thickening to 52% of control, and regional work to 47% of control when afterload was matched by aortic constriction. After L-NA, the myocardial oxygen consumption during ischemia fell to 70% of control whereas wall thickening fell to 47% and regional work to 44% of control. Thus, although inhibiting NO altered baseline myocardial efficiency as reflected by a lower baseline external work and function at a similar level of oxygen consumption, it may not have had any critical importance during ischemia. An alternative interpretation of the data is that the shift in the baseline value of function was responsible for the shift during ischemia.

How can we relate these findings to hibernating myocardium in humans? Although the model of prolonged moderate ischemia or short-term hibernation has provided considerable insight into the acute adaptation to ischemia, it is unlikely to be the route through which viable chronically dysfunctional myocardium develops in patients with chronic coronary artery disease. As the name implies, these adaptations are short-term, and there is increasing evidence that acute moderate ischemia leads to the development of irreversible injury when prolonged beyond several hours. Increasing evidence indicates that viable chronically dysfunctional myocardium in patients develops from chronic repetitive ischemia where contractile dysfunction is initially associated with normal flow (chronic stunning). As coronary flow reserve becomes critically reduced, resting flow decreases in dysfunctional myocardium, consistent with a progression to a state of chronic hibernation. Thus, reduced flow is a result rather than cause of chronic contractile dysfunction in hibernating myocardium.
Finally, although repetitive ischemia with full reflow appears to induce myocardial NO production, it is unclear whether a similar effect occurs in the presence of a chronic critical stenosis. Induction of NOS expression leads to a protective effect that prevents the development of chronic stunning, given that resting myocardial function remains unchanged. Although speculative, it is also plausible that NOS expression is actually downregulated in areas of chronically reduced flow, as in hibernating myocardium. Thus, the chronic contractile dysfunction could partly be related to a reduction in regional NO production in a fashion similar to the acute responses demonstrated by Heusch et al after L-NA. Studies are needed to evaluate NOS expression in the setting of a chronic stenosis and integrate the findings regarding the role of NO during acute ischemia into the context of long-term chronic adaptations to myocardial ischemia.

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References
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