Nitric Oxide Protects Against Pathological Ventricular Remodeling
Reconsideration of the Role of NO in the Failing Heart

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Nitric oxide (NO) is generated by NO synthases (NOSs) that convert l-arginine to l-citrulline in the presence of molecular oxygen, nicotinamide-adenine dinucleotide phosphate, calmodulin, and several cofactors. Three NOS isoforms have been identified: NOS1 (neuronal NOS), NOS2 (inducible NOS), and NOS3 (endothelial NOS). The constitutive isoforms NOS1 and NOS3 display Ca\textsuperscript{2+}-dependent calmodulin binding and are compartmentalized in cardiomyocytes, with NOS1 localizing to the ryanodine receptor of the sarcoplasmic reticulum (SR), and NOS3 localizing to the caveolae of the sarcolemma (SL) in close proximity to β-adrenergic receptors and the L-type Ca\textsuperscript{2+}-channel. NOS2 expression in cardiomyocytes requires induction in response to inflammatory cytokines or biological stress (eg, ischemia and reperfusion). NOS2 is expressed primarily in the cytosol, displays Ca\textsuperscript{2+}-independent activity because of its high calmodulin-binding affinity, and has high capacity for generating NO.

Modulation of cardiac function by NO is complex and multifaceted and is mediated by at least 2 mechanisms: activation of soluble guanylate cyclase with production of the second messenger cGMP and direct protein thiol nitrosylation. NO affects myocardial contraction in a dose-dependent biphasic pattern, with low NO concentrations (as occurs under normal, unstressed conditions) exerting positive inotropic effects and high concentrations exerting negative inotropic effects. Antecedent β-adrenergic stimulation shifts the descending limb of this bimodal curve to the left, such that the negative inotropic effects become more prominent. NO hastens relaxation, shortens contraction duration, and improves diastolic distensibility, and also depresses mitochondrial respiration, thereby altering mechanoenergetic coupling. NO-mediated contractile effects are significantly impacted by spatial confinement and localization of NOS isoforms. Studies in NOS1 and NOS3 null mice have demonstrated that by virtue of isoform-dependent localization of NO release and attendant divergent effects on SR and SL Ca\textsuperscript{2+} flux, each isoform exerts unique, and often opposing, effects on contractility and excitation–contraction coupling. NOS3-derived NO enhances basal contractility and inhibits the β-adrenergic response via its effects on the L-type Ca\textsuperscript{2+} channel and inward Ca\textsuperscript{2+} current. In contrast, NOS1-derived NO diminishes basal contractility but enhances β-adrenergic- and force-frequency responses via effects on SR Ca\textsuperscript{2+} release. The biological response to NO is also influenced by the degree of oxidative stress. For example, whereas NO-mediated depression of mitochondrial respiration protects cells against apoptosis, excessive NO production or oxidative stress results in peroxynitrite generation and a proapoptotic state.

The pathophysiological role of NO in chronic heart failure (HF) is the subject of significant controversy. The prevailing hypothesis that increased NO production in the failing heart contributes to detrimental cardiac remodeling was based on the observations of: (1) NO-mediated depression of inotropy, mitochondrial respiration, and β-adrenergic responsiveness; (2) enhanced NOS2 activity in human HF and improvement of the β-adrenergic response with NOS inhibition; and (3) robust myocardial expression of inflammatory cytokines, potent stimulators of NOS2, in the failing heart. However, these studies did not establish whether NOS2 induction and increased NO represented a causal event, a compensatory response, or an epiphenomenon. Furthermore, this concept appeared to be at odds with a large body of evidence indicating that NO is cardioprotective during acute ischemia and reperfusion injury and has a dual role as both a trigger and mediator of late preconditioning.

Indeed, multiple lines of evidence suggest that NO need not exert detrimental effects in HF. Several studies in both animal models and human ischemic HF have not indicated enhanced NOS2 induction, suggesting that NOS2 expression may be an epiphenomenon related to the temporal stage of disease. Additionally, although NO inhibition in HF improves the response to β-adrenergic stimulation, there is no evidence that NO inhibition improves basal left ventricular (LV) function or the force-frequency response in this setting. In a canine pacing model, myocardial NO content declined significantly during the evolution of cardiac decompensation, together with significant increases in LV diastolic filling pressure (suggesting loss of diastolic distensibility) and the respiratory quotient (indicating a pathological switch in substrate utilization). Similarly, infusion of substance P, an agonist that stimulates NO release, in patients...
with dilated cardiomyopathy resulted in hemodynamic improvement (not decompensation) because of improved diastolic distensibility.20 Lastly, several investigations in human and experimental HF have reported that rather than increased NO, the failing heart is characterized by reduced NO bioactivity, impaired NO-dependent coronary vasodilatation, and decreased NOS3 expression.9,13,17,21,22 Together, these studies argue against the hypothesis of an adverse effect of excessive myocardial NO in HF and instead raise the concept that loss of NO bioactivity, at least in part related to reduced NOS3, contributes to cardiac dysfunction and that NO confers a cardioprotective effect in HF via its effects on oxygen consumption, substrate utilization, diastolic function, and coronary blood flow.

In this issue of Circulation Research, Janssens et al report an important study that supports the hypothesis that NOS3-derived NO provides salutary effects in HF.23 These investigators evaluated the effect of cardiomyocyte-restricted overexpression of human NOS3 on postinfarction LV remodeling. Cardiac-specific NOS3 transgenic (TG) mice displayed a 30-fold increase in cardiac NOS activity, with abundant transgene immunoreactivity, appropriate NOS3 targeting to the caveolae, and no change in gene expression of murine NOS isoforms. Despite markedly increased NOS3 abundance, there was no adverse cardiac phenotype at baseline with regard to hypertrophy or contractile function, although there was blunting of the inotropic response to β-adrenergic stimulation, consistent with previous studies.5,6 Furthermore, NOS3 TG mice were protected against detrimental remodeling after coronary ligation, displaying improved LV systolic and diastolic function and attenuation of LV and myocyte hypertrophy. These data are consistent with a previous study by Jones et al examining postinfarction remodeling in TG mice with systemic NOS3 overexpression. In that study, after coronary ligation, there was improvement of survival, pulmonary edema, and LV shortening fraction in the NOS3 TG mice compared with wild-type, with no baseline cardiac phenotype before infarction. The improved outcome was ascribed to reductions in systemic vascular resistance in the NOS3 TG mice. In contrast, a third study in TG mice with a much higher (≈200-fold) increase in cardiac-specific NOS3 expression revealed no cardiac hypertrophy or histologic abnormality, but did evidence contractile depression in direct relation to the degree of transgene expression attributed to reduced myofilament Ca2+ sensitivity.25

Unfortunately, Janssens et al did not examine the impact of NOS3 overexpression on NO oxidation products in the heart (to provide an index of NOS activity in vivo), nor did they directly evaluate the impact of NOS3 overexpression on cardiac cell apoptosis, oxidative stress, or β-adrenergic signaling, all of which would have provided further mechanistic insights as to the mechanisms of NOS3-mediated protection in the remodeled heart. Nonetheless, when taken in context with studies from other groups demonstrating that genetic ablation of NOS3 worsens LV dilation, hypertrophy, capillary density, and contractile function in postinfarction HF,26 and impairs the beneficial effects of angiotensin converting enzyme inhibitors and angiotensin receptor antagonists on LV remodeling,27 this study adds to the growing evidence that NO, derived from NOS3, can offer long-term cardioprotection in the failing heart. Thus, restoration or enhancement of NOS3 activity may constitute an important therapeutic approach to the amelioration of detrimental remodeling in HF.

It is not clear whether the results of the study of Janssens et al can be generalized to NO derived from other NOS isoforms. In this regard, studies in mice with genetic modulation of NOS2 have revealed conflicting results. Whereas 1 group has reported that mice with cardiac-specific NOS2 overexpression show no detrimental cardiac phenotype despite a 40-fold increase in iNOS activity in vivo,28 again challenging the concept that increased NO generation is a pathophysiological mediator of cardiac remodeling, another group has reported that mice with conditional, lower levels of NOS2 overexpression show higher mortality and atrioventricular block.29 Furthermore, in contradistinction to NOS3 null mice, NOS2 null mice show a modest decrease in apoptosis and a modest improvement in contractile function in postinfarction HF.30,31 findings manifested only in advanced stages of remodeling (4 months after myocardial infarction) and only at high, nonphysiological preload.30 The underlying reasons for these contrasting results between NOS2- and NOS3-derived NO in HF are unclear but may relate to differences in spatial localization, physiological regulation, and NO-generating capacity of these 2 isoforms, and differences in oxidative stress and peroxynitrite generation that favors the induction of apoptosis. Notably, a recent elegant study reported that in sepsis, the cellular source of NOS2 could determine beneficial or detrimental cardiac effects.32 Whereas leukocyte-derived NOS2 exerted damaging effects on myocytes, myocyte-restricted NOS2 expression was protective and actually preserved myocyte β-adrenergic responsiveness, suggesting that the cellular microenvironment, perhaps related to the generation of oxygen-free radicals, significantly influences NO-mediated responses. Whether an analogous balance between myocyte NOS2 and inflammatory cell NOS2 plays an important pathophysiological role in HF and accounts for the conflicting reports in the literature is unknown but certainly warrants further exploration.

In summary, the study by Janssens et al demonstrating that cardiac-specific NOS3 overexpression attenuates postinfarction LV remodeling and hypertrophy adds to a growing number of studies that refute the idea that increased myocardial NO necessarily implies an adverse effect on LV remodeling in HF and underscores the need for a reappraisal of the pathophysiologic role of NO in the failing heart. As is the case with NO-mediated effects on myocardial function, the impact of NO on the biology of failing myocardium is complex and is likely influenced by NOS isoform-specific properties and localization, the degree of oxidant stress, and the cellular source of NO and attendant microenvironment. Importantly, the evidence is growing that under appropriate conditions, rather than being detrimental, NO in general, and NOS3-derived NO in particular, can impart cardioprotective effects in the failing heart.

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References


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