Nitric Oxide Synthases in the Failing Human Heart
A Doubled-Edged Sword?

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The dose makes the poison.
Paracelsus, 1493–1541

Nitric oxide (NO) is a free radical gas and is readily diffusible; it has a very short half-life, lasting only seconds. NO is synthesized from L-arginine by the catalytic reaction of different isoforms of nitric oxide synthases, including the neuronal, type 1 isoform (nNOS [NOS1]); the inducible, type 2 isoform (iNOS [NOS2]); and the endothelial, type 3 isoform (eNOS [NOS3]). nNOS and eNOS are constitutively expressed enzymes and are regulated predominantly at the posttranslational level, whereas in most cell types, iNOS can be essentially expressed in response to appropriate stimuli. Small amounts of NO, produced by nNOS and eNOS, are involved in signal events that regulate neurotransmission and vascular tone. Because the activity of nNOS and eNOS is triggered, it is transient. Much larger concentrations of NO are usually provided by iNOS in cells during infection and inflammation; however, because iNOS is independent of stimulating agonists and Ca2+, its activity is sustained. Both nNOSs and eNOSs are constitutively expressed in the myocardium; nNOS is expressed in nerve endings involved in the neurotransmission of norepinephrine, and eNOS is expressed in endothelial cells, endocardial cells, and cardiomyocytes. In disease states associated with infection, inflammation, or cytokine activation, the expression of iNOS can be clearly demonstrated in the heart, including in the cardiomyocytes. The ubiquitous distribution of NO synthases within the myocardium and the versatile actions of NO make this molecule unique in the diverse cardiac effects that depend on its concentration and the spatial and temporal activity of its isoforms. The versatility of NO is largely related to its capability to react with oxygen, superoxide, and transition metals. Each of the products of these reactions, NOx, peroxynitrite (OONO–), and metal-NO adducts, support additional reactions. In fact, metal- and thiol-containing proteins serve as major target sites for NO, including ion channels (ie, K(Ca) channel), receptors (ie, NMDA receptor), enzymes (ie, guanylate cyclase) and transcription factors (ie, nuclear factor-kB). An important physiological target of NO is the heme protein soluble guanylyl cyclase. NO activates guanylyl cyclase by interacting with its heme, and NO generates cGMP from GTP, which results in the relaxation of smooth-muscle cells. Although the importance of endothelium-derived NO for the regulation of vasomotor tone is established, the physiological role of NO for cardiac function and structure provided by the constitutively expressed isoforms of NO synthases remains incompletely understood. Removal of endocardium or endothelium modulates cardiac contraction, suggesting that the NO released from endothelial and endocardial cells modulates cardiac contraction. These experimental observations have been supported by clinical studies in which stimulating endothelial release of NO by substance P resulted in similar effects in the human heart in vivo. NO generated from nNOS within neurons controls norepinephrine release in the heart and, thereby, may affect cardiac function and perfusion. The presence of a nNOS isofrom on the sarcolemmal reticulum of cardiomyocytes raises the possibility that nNOS modulates calcium transport. eNOS activity in cardiomyocytes mediates the attenuation of L-type calcium current and the contractility of these cells by muscarinic cholinergic agonists of β-adrenergic stimulation. Conversely, eNOS expression is downregulated by sustained elevation of cAMP. Beyond the functional role of nNOS and eNOS in the heart, the expression of iNOS in cardiomyocytes (and, subsequently, the production of NO) occurs in response to cytokines. In fact, the expression of iNOS in cardiomyocytes seems to be, in part, responsible for the cardiodepressant effects of cytokines and endotoxin, ie, attenuation of the myocardial inotropic response to β-adrenergic stimulation. Notably, additional factors such as insulin and sufficient L-arginine transporter capacity seem to be required for NO production by iNOS.

Several studies have evaluated the effects of NO and the role of NO synthases in the human heart. NO inhibits the positive inotropic response to β-adrenergic stimulation in patients with left ventricular (LV) dysfunction in vivo and patients with severe heart failure in vitro. Blockade of cardiac NO synthesis by Nω-monomethyl-L-arginine (L-NMMA) recovers β-stimulation responsiveness in patients with high levels of cardiac iNOS activity, suggesting that the response to β-adrenergic stimulation is related to the endogenous cardiac generation of nitric oxide by iNOS (rather than eNOS). Thus, cardiac iNOS activity, as seen in patients with severe heart failure and probably in other conditions associated with myocardial inflammation or
systemic cytokine activation (such as septic shock), attenuates responsiveness to β-adrenergic stimulation. The negative-inotropic effect of NO during β-adrenergic stimulation has been related to cGMP-mediated inhibition of Ca\(^{2+}\) influx by means of L-type sarcolemmal voltage-dependent Ca\(^{2+}\) channels. Thus, besides reduced β1-receptor density and increased levels of G-proteins, cardiac generation of NO contributes to the attenuated response elicited by β-adrenergic stimulation in the failing heart by acting by means of a cGMP-dependent mechanism. In addition, high cardiac iNOS activity is associated with an early onset of relaxation.

Although it is clear that chronic heart failure is associated with iNOS expression and activity in the failing human heart, the spatial and temporal expression and activity of iNOS remain controversial. In patients with dilated cardiomyopathy, iNOS expression in cardiomyocytes colocalizes with the expression of tumor necrosis factor-α. However, iNOS activity may also (or predominantly) be related to the density of infiltrating macrophages. The findings of de Belder et al. suggested that iNOS activity (as determined by independence from calcium) is confined to patients with dilated cardiomyopathy. However, significant expression of iNOS occurs in patients with ischemic cardiomyopathy, implying that iNOS expression in the myocardium is a result of heart failure per se rather than related to its cause.

Although some studies have suggested that the expression and activity of eNOS is diminished in the failing human heart, others have observed increased levels.

The study by Heymes et al. in this issue of Circulation may provide an explanation of these conflicting observations by showing that LV gene expression of eNOS is related to the extent of LV dysfunction in patients with dilated cardiomyopathy. A similar relationship was observed for the gene expression of iNOS. Although the present in vivo study assessed mRNA rather than protein levels, the functional assessment of cardiac function and the relationships between gene expression levels and stimulated cardiac function are consistent with the notion that the expression of eNOS and iNOS may be related to the severity of LV dysfunction. In addition, measuring NO production by human LV microvessels has shown reduced NO production in patients with end-stage heart failure. Thus, the spatial and temporal expression of eNOS and iNOS in the human failing heart seems to be quite heterogeneous and depends on the severity of LV dysfunction. These observations are consistent with experimental studies showing that after an early rise, cardiac NO production diminishes after the transition to cardiac failure.

The interpretation of Heymes et al. of the findings of their study is provocative. In the present study, a significant relationship was observed between eNOS/iNOS expression and indices of LV function. The stimulated endothelial release of NO (induced by substance P) resulted in a rightward shift of the end-diastolic pressure-volume relation. Heymes et al. concluded from these findings that the observed correlations between eNOS/iNOS gene expression and indices of cardiac output are due to a myocardial action of NO, consistent with experimental findings suggesting a cGMP-dependent reduction of myofilament response to calcium. Thus, cardiac production of NO may play a beneficial role in LV dysfunction by maintaining the Frank-Starling mechanism. Although supported by experimental data in normal isolated animal hearts, this conclusion represents an assumption based on correlations rather than direct proof. Nevertheless, it is possible that the transient release of low doses of NO by eNOS both from endothelial cells and myocytes may play an important role in modulating load-dependent relaxation, cardiac metabolism, and respiration and autoregulation of myocardial perfusion. In this respect, it is noteworthy that the onset of cardiac decompensation in pacing-induced heart failure was associated with reduced cardiac NO production, probably secondary to endothelial dysfunction. Thus, it may well be that impaired NO production by eNOS is involved in the pathophysiology of cardiac decompensation. However, the beneficial effects of NO derived from eNOS and iNOS on central hemodynamics, as suggested by the present study, should not be taken as evidence that NO in general is beneficial for the myocardium. The more, the better is probably not true. This may be particularly true for sustained high levels of NO production by iNOS within cardiomyocytes.

**How Much of NO Is Good or Bad for the Myocardium?**

NO and cGMP induce a concentration-dependent biphasic contractile response; low doses of NO cause a positive inotropic response, and higher doses are associated with negative inotropic actions.

NO acts a bifunctional regulator of apoptosis. Physiologically relevant levels of NO seem to suppress the apoptotic pathway at multiple levels and by several pathways, i.e., by inhibition of caspase activity by S-nitrosylation. However, higher levels of NO may overwhelm cellular protective mechanisms and exert proapoptotic and cytotoxic effects. NO synthase can generate superoxide and NO in L-arginine–depleted cells, leading to peroxynitrite-mediated cytotoxic injury. Similarly, high production of NO and superoxide may also result in the formation of peroxynitrite. Notably, the extent of cardiomyocyte apoptosis by cytokine-induced production of NO is modulated by the availability of oxygen free radicals and by alterations in the cellular balance of Bak and Bcl-xL, which respond specifically to individual cytokines.

NO released from endothelial eNOS inhibits myocardial oxygen consumption by interfering with mitochondrial electron transport, and the loss of this regulatory function may be involved in the development of heart failure (and is associated with increased myocardial oxygen consumption); however, sustained high levels of mitochondrial NO can induce apoptosis by destabilizing mitochondria through deenergization and/or by inducing Ca\(^{2+}\) release followed by Ca\(^{2+}\) cycling.

Thus, the spatial distribution of production and (transient versus sustained) levels of NO may determine...
whether NO plays a beneficial role in the failing heart. In addition, the spatial expression and activity of radical scavenging systems such as superoxide dismutase, catalase, and glutathione reductase may represent important defense mechanisms that would protect NO from its inactivation, including its path across the vessel wall by extracellular sodium dismutase. In this respect, increased radical formation has been previously shown to emerge in the failing human heart. Interestingly, iNOS mediates opposing effects in models of acute and chronic cardiac rejection. Inos promotes short-term but prevents long-term rejection, as demonstrated in wild-type versus iNOS knockout mice. In short-term rejection, iNOS-deficient animals showed reduced inflammatory cells (lymphocytes and macrophages) and myocardial damage; however, iNOS-deficient animals showed more parenchymal (myocyte) destruction in the late phase of rejection. Taken together, the regulation of NO production by eNOS and iNOS within the myocardium and the cellular source (ie, cardiomyocytes, endothelial cells, and macrophages) is probably crucial.

**How Are eNOS and iNOS Regulated in the Myocardium and in the Failing Human Heart?**

Although eNOS is constitutively expressed in cardiomyocytes and endothelial cells, mechanical stress can upregulate myocardial [27] and endothelial eNOS. Moreover, frequency-dependent activation of eNOS in ventricular myocytes has been reported. In fact, NO production is significantly reduced in the quiescent state after cardiac arrest, implying that NO production by the human myocardium is coupled with the inotropic state of the heart. Both β-blockers and angiotensin-converting enzyme inhibitors increase eNOS expression, and such a mechanism may contribute to the beneficial effects of these drugs in cardiovascular disease, in particular, heart failure. In contrast, iNOS gene expression is driven by the activation of cytokines, such as tumor necrosis factor-α and interleukin-6, whose expression and release increases after increased wall stress. NO production by iNOS may result in growth inhibition of ventricular myocytes, apoptosis, and cytotoxic effects related to peroxynitrite and other toxic products. As a result of these detrimental effects, LV wall stress may increase. In contrast to eNOS, mechanical strain suppresses iNOS in cardiac myocytes, which is one explanation for the diminished gene expression of iNOS in patients with more severely depressed cardiac function.

Carefully designed clinical studies such as the article by Heymes et al are needed to elucidate the relative roles of iNOS and eNOS activity in patients with heart failure. Unfortunately, the selective modulation of iNOS by pharmacological interventions in patients remains elusive at present. However, both angiotensin-converting enzyme inhibitors and β-blockers seem to upregulate the (presumably beneficial) eNOS activity in the myocardium. Importantly, numerous interventions improve endothelial function and NO bioavailability in patients with various cardiovascular disorders, including heart failure, and substance P, which releases NO from the endothelium, increased stroke volume and work in patients with LV dysfunction. If, indeed, low levels and transient release of NO by eNOS are beneficial for the heart with reduced LV function, interventions that enhance endothelial function and NO availability (ie, by increasing endothelial eNOS) may also have a beneficial effect on myocardial function and/or the prevention of cardiac decompensation. This hypothesis should be tested in controlled clinical trials.

**References**


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