Two Faces of Nitric Oxide
Lessons Learned From the NOS2 Knockout

Brian D. Hoit

Nitric oxide synthases (NOSs) comprise a family of enzymes that generate the freely diffusible, free radical gas NO, by the conversion of L-arginine to L-citrulline in the presence of O2 and NADPH. Bioactive derivatives of NO (NOx) are produced depending on the redox state of the cell and the availability of substrates and cofactors. Each of the three NOSs arise from separate genes, but each NOS molecule is composed of a calmodulin binding domain that links a carboxyl terminal flavin-containing reductase domain with an amino terminal heme-containing oxygenase domain. Neuronal NOS (or NOS1) and endothelial NOS (or NOS3) are constitutively active isoforms with an oxygenase domain, whereas inducible NOS (iNOS or NOS2) binds calmodulin tightly, even at physiological [Ca2+]i.

Each of the three NOS isoforms is present in the heart, undergoes posttranslational modification, and is spatially constrained within the cell. NOS1 expression is limited and its physiological role is unclear. The largest sources in the heart of the cytokine (interleukin-1β [IL-1β]), tumor necrosis factor-α [TNF-α], interferon-γ [IFN-γ], interleukin-6 [IL-6]) and lipopolysaccharide-inducible NOS2 are from cardiac myocytes, endothelial cells, and infiltrating macrophages. NOS3 is found in the endocardial and coronary arterial endothelium, myocytes, and specialized cardiac conduction tissue.

Constitutive expression of NO is protective. Vascular effects include vasodilatation, inhibition of platelet adhesion and neutrophil infiltration, prevention of neointimal proliferation, and inhibition of vascular remodeling in heart. Cardiac effects of constitutive NO include regulation of the coronary circulation, heart rate, and contractility. In contrast, iNOS is implicated in the pathophysiology of myocardial dysfunction in several syndromes including the systemic inflammatory response syndrome, inflammatory myocarditis, cardiac allograft rejection, and heart failure. Intracoronary infusion of NO donors enhances diastolic distensibility, augments preload, reduces myocardial oxygen consumption (MV0₂), and prevents downregulation of Ca2+ ATPase, and in late preconditioning, NOS2 is cytoprotective. This seemingly paradoxical behavior may be explained by the amount of NO generated (ie, extensive, unregulated, and unrestricted release of NO may lead to myocardial dysfunction), temporal-spatial intracellular compartmentalization of NO, and the intracellular redox environment, which allows for the modification of NO and alters the balance between cell death and survival. Discrepancies in the literature can also be explained by the experimental model, and the use of NO-donating drugs with pharmacokinetics unlike the endogenous release of NO, and nonspecific NOS inhibitors that have independent hemodynamic effects and toxicities.

NOS2 induction diminishes both basal and catecholamine-enhanced chronotropic and inotropic function in isolated myocytes and intact hearts. NOS inhibition in pacing-induced heart failure in dogs enhances the inotropic response to β-adrenergic stimulation, and endogenous NO attenuates β-adrenergic responses in humans with left ventricular (LV) dysfunction. Mechanisms responsible for these effects are complex and include activation of soluble guanylyl cyclase, inhibition of electron transport, N-nitrosylation of thiol groups, and production of oxidants such as superoxide and peroxynitrite. NO also modulates arrhythmogenesis. Thus, NO derived from myocytes suppresses ouabain-induced arrhythmia via cGMP, NO synthesis may offer protection against ventricular arrhythmia after coronary occlusion, and NOS inhibition potentiates the tachycardia produced by sympathetic stimulation.

NO generated by NOS2 is implicated in apoptosis in a number of cell types, including myocytes, and chronic NO inhibition caused thickening of coronary vascular remodeling and cardiac hypertrophy in vivo. These findings, and those concerning contractility (above), coupled with reports of expression of NOS2 and cytokines that induce NO in failing heart, lend plausibility to the hypothesis that NOS2 expression is responsible for postmyocardial infarction remodeling and heart failure. Indeed, in rats with myocardial infarction (MI), TNF-α, IL-1β, and IL-6 were expressed in the infarct zone acutely and transiently, but at 20 weeks, the cytokine mRNA was increased in the noninfarct zone. Interestingly, in another study, NOS inhibition failed to improve myocardial dysfunction in TNF-α–challenged animals, suggesting that cytokine effects on contractility were independent of NO. In rats studied 12 weeks after MI, isoproterenol responsiveness was unaltered with NOS inhibition and circulating NO was undetected in rats 12 weeks after MI; in the noninfarct zone, NOS2 was absent, whereas TNF-α, IL-1β, and IL-6 mRNA were detected. In this regard, it is interesting that there are no data that clearly

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Implicate NOS2 in the pathogenesis of heart failure in humans. Among this controversy, the study by Sam et al23 in this issue of Circulation Research is both timely and important. In contrast to the reduction in infarct size seen after pharmacological NOS2 inhibition,24 but consistent with other studies in mice with genetic ablation of NOS2,25 MI sizes were similar in wild-type and NOS2 knockout mice. LV remodeling and ventricular systolic function as assessed by developed pressure in the isovolumic heart were similar in wild-type and knockout mice at 1 month. However, at 4 months, LV developed pressure was reduced, the number of TUNEL-positive nuclei greater, and survival worse in the wild-type compared with knockout mice. In addition, NOS2 was detected in the noninfarct myocardium of wild-type mice at 4 months; unfortunately, measurements were not made at 1 month. The authors conclude that NOS2 contributes to decreased contractility, increased myocyte apoptosis in remote myocardium, and reduced survival late after MI. They prudently caution that the data demonstrate only associations, not cause and effect.

Several issues warrant consideration when interpreting the results of this study. First, there were no differences in ventricular remodeling, despite differences in the number of apoptotic cells, contractility, and survival. Whether the Langendorff preparation has adequate sensitivity to detect differences in remodeling is uncertain. Second, effects of NOS2 on ventricular function were profoundly modest. Indeed, NOS2 had no effect on diastolic function, whereas NOS inhibition was shown to impair diastolic function in dogs with pacing-induced heart failure.9 The changes in LV developed pressure were small, occurring at only the highest LV volumes, and despite an association with an increase in the apoptotic index and reduced survival, heart failure was not an outcome; the use of a more sophisticated hemodynamic and/or echocardiographic evaluation of the cardiovascular phenotype would address the concern that the modest difference in LV developed pressure may have no real significance in terms of cardiac function. In contrast, after endotoxin exposure, marked attenuation of LV fractional shortening, velocity of circumferential fiber shortening, and +dP/dt and the time constant of LV relaxation was seen in NOS2 knockout mice compared with controls.6 NOS3 knockout mice had greater LV end-diastolic dimension and mass, but lower fractional shortening, greater depression of dP/dt, and longer τ than did wild-type mice 28 days after MI,26 suggesting that the effects of NO generated from NOS3 had greater effects on post-MI LV remodeling and function than that generated from NOS2. Finally, the late survival advantage of NOS2 knockout mice raises important questions as to the mechanisms of death, considering the absence of heart failure, the modest impairment in contractility, and the effects of NOS on arrhythmogenesis.

Despite these serious limitations, Sam et al23 provide important insights into the role of NOS2 late after MI. Their use of the NOS2 knockout mouse allows for a relatively unambiguous study design and avoids problems related to non-specific NOS inhibitors. NOS2 expression is clearly associated with TUNEL positivity, a modest decrease in systolic function, and an increased mortality rate late after MI. However, the relative importance and causality of NOS2 in the remodeling process awaits a more comprehensive assessment of the NOS2 knockout cardiac phenotype after infarction. Assessment of mice with conditional, cardiac-restricted overexpression of NOS2 may also provide further understanding in that regard.

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