Deficiency of Neuronal Nitric Oxide Synthase Increases Mortality and Cardiac Remodeling After Myocardial Infarction

Role of Nitroso-Redox Equilibrium

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**Background**—Neuronal nitric oxide synthase (NOS1) plays key cardiac physiological roles, regulating excitation-contraction coupling and exerting an antioxidant effect that maintains tissue NO-redox equilibrium. After myocardial infarction (MI), NOS1 translocates from the sarcoplasmic reticulum to the cell membrane, where it inhibits β-adrenergic contractility, an effect previously predicted to have adverse consequences. Counter to this idea, we tested the hypothesis that NOS1 has a protective effect after MI.

**Methods and Results**—We studied mortality, cardiac remodeling, and upregulation of oxidative stress pathways after MI in NOS1-deficient (NOS1<sup>−/−</sup>) and wild-type C57BL6 (WT) mice. Compared with WT, NOS1<sup>−/−</sup> mice had greater mortality (hazard ratio, 2.06; P=0.036), worse left ventricular (LV) fractional shortening (19.7±1.5% versus 27.2±1.5%, P<0.05), higher LV diastolic diameter (5.5±0.2 versus 4.9±0.1 mm, P<0.05), greater residual cellular width (14.9±0.5 versus 12.8±0.5 μm, P<0.01), and equivalent β-adrenergic hyporesponsiveness despite similar MI size. Superoxide production increased after MI in both NOS1<sup>−/−</sup> and WT animals, although NO increased only in WT. NADPH oxidase (P<0.05) activity increased transiently in both groups after MI, but NOS1<sup>−/−</sup> mice had persistent basal and post-MI elevations in xanthine oxidoreductase activity.

**Conclusions**—Together these findings support a protective role for intact NOS1 activity in the heart after MI, despite a potential contribution to LV dysfunction through β-adrenergic hyporesponsiveness. NOS1 deficiency contributes to an imbalance between oxidative stress and tissue NO signaling, providing a plausible mechanism for adverse consequences of NOS1 deficiency in states of myocardial injury. (Circulation. 2005;112:3415-3422.)

**Key Words:** myocardial infarction ■ nitric oxide synthase ■ heart failure ■ receptors, adrenergic, beta

**Neuronal nitric oxide synthase (NOS1) participates in several critical physiological cardiac processes. This isoform, located within the cardiac sarcoplasmic reticulum (SR) under physiological conditions, facilitates the SR Ca<sup>2+</sup> cycle that is central to excitation-contraction coupling.**<sup>1,2</sup> In addition, NOS1 constrains the activity of cardiac xanthine oxidoreductase (XOR), a major source of O<sub>2</sub>− in the heart, with which it forms a protein-protein interaction.<sup>3,4</sup> This in turn contributes to maintaining tissue balance between the production of reactive nitrogen species (RNS) and reactive oxygen species (ROS), the nitroso-redox balance.<sup>3–6</sup> After myocardial infarction (MI) and in failing myocardium due to dilated cardiomyopathy, NOS1 alters its subcellular localization and translocates to the sarcolemma,<sup>7,8</sup> where it exerts an inhibitory effect on β-adrenergic activity. The latter has been theorized to be maladaptive and a contributor to left ventricular (LV) dysfunction.<sup>9</sup> On the other hand, NOS1 translocation to the sarcolemma and subsequent attenuation of β-adrenergic contractility could also be adaptive, essentially acting as a postreceptor β-adrenoreceptor antagonist. Here we tested the hypothesis that the presence of NOS1 in the myocardium is adaptive after MI and approached this prediction by studying the effects of MI on mice with homozygous deletions for NOS1 (NOS1<sup>−/−</sup>) and respective wild-type (WT) mice.

**Animal Model**

We studied young (2 to 3 months, n=91) male and female C57BL/6 (WT; Jackson Laboratories, Bar Harbor, Me) and transgenic mice with homozygous deletions for NOS1 (n=97) bred on a C57BL/6 background (originally bred in the laboratory of Dr Mark Fishman, Massachusetts General Hospital, Boston, Mass<sup>10</sup>). The institutional animal care and use committee of Johns Hopkins University School of Medicine approved all protocols and experimental procedures.
Induction of MI was performed as previously described, with minor modifications. In brief, MI was surgically induced under general anesthesia (0.02 mg/g etomidate IP and 12 μg SC buprenorphine) and mechanical ventilation (150 breaths per minute; Minivent 845, Harvard Apparatus). The heart was exposed via a left thoracotomy. A 7-0 polypropylene monofilament suture (Ethicon) was tied around the left anterior descending coronary artery 1 to 2 mm from the tip of the left atrium (LA). Sham animals underwent similar surgery, but the left anterior descending coronary artery suture was not tied. There were no significant differences in age, sex, or body weight between the 4 subgroups at the time of surgery (Table 1).

Echocardiography
We performed transthoracic echocardiography with a Sonos 5500 echocardiography unit equipped with a 15-MHz linear-array transducer (Philips) in anesthetized (2% isoflurane inhalation) mice. At least 5 consecutive beats were recorded in a short-axis view at the level of the papillary muscles. M-mode echocardiography was used to measure cardiac dimensions (left ventricular diameters in diastole [LVDd] and systole [LVDs] and LA diameter) and LV fractional shortening (FS). LVFS in percent was calculated as \((\text{LVDd} – \text{LVDs}) / \text{LVDd}\) × 100.

Hemodynamics
Intact heart hemodynamic analysis was performed 4 weeks after surgery by using miniaturized pressure-volume catheterization as previously described. A 4-electrode pressure-volume catheter (SPR-719, Millar Instruments Inc) was inserted into the right carotid artery in the anesthetized animal (IP 200 μg etomidate, 30 mg urethane, and 20 μg morphine) and advanced into the LV.

Histopathology
Excised hearts were weighed and processed according to routine histological procedures. Five-micron sections were cut and stained with Masson’s trichrome and hematoxylin-eosin. Infarct size was determined by methods previously described as the total infarct circumference divided by total LV circumference (in percent). Infarct size was also measured 48 hours after surgery. Excised hearts were retroperfused with phosphate-buffered saline after aorta cannulation, followed by 5 mL of 1% 2,3,5-triphenyltetrazolium at 540 nm. Standards were made by serial dilutions of sodium nitrite.

TABLE 1. Morphological Characteristics

<table>
<thead>
<tr>
<th></th>
<th>WT Sham</th>
<th>NOS1−/− Sham</th>
<th>WT MI</th>
<th>NOS1−/− MI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, d</td>
<td>70.6±1.6 (21)</td>
<td>75.9±3.0 (22)</td>
<td>81.4±4.7 (44)</td>
<td>72.0±1.8 (48)</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>16/5</td>
<td>13/9</td>
<td>33/11</td>
<td>38/10</td>
</tr>
<tr>
<td>Weight, g</td>
<td>25.90±0.9 (21)</td>
<td>25.9±0.7 (22)</td>
<td>27.0±0.6 (23)</td>
<td>25.0±0.5 (23)</td>
</tr>
<tr>
<td>Heart weight, mg</td>
<td>144.1±7.2 (21)</td>
<td>140.2±6.0 (20)</td>
<td>187.4±5.7 (33)*</td>
<td>197.0±9.6 (23)*</td>
</tr>
<tr>
<td>Heart weight/body weight, mg/g</td>
<td>5.5±0.1 (21)</td>
<td>5.4±0.1 (22)</td>
<td>7.0±0.2 (33)*</td>
<td>7.9±0.4 (23)*</td>
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</tbody>
</table>

Numbers in parentheses indicate number of animals.

* P<0.001 in relation to sham; † P<0.01 between MI groups.

NOS1 Measurement
NO production in heart homogenates was determined by evaluation of its oxidation product (nitrite) measured by the Griess reaction (nitrite colorimetric assay kit, Cayman Chemical). The Griess reagent was composed of 2% sulfanilamide in 1 mol/L H₃PO₄ and 0.2% sulfanilamide in water. Nitrite was quantified colorimetrically at 540 nm. Standards were made by serial dilutions of sodium nitrite.

NADPH Oxidase Activity
NADPH-dependent superoxide (O₂⁻) production was measured in heart homogenates with lucigenin (5 μmol/L)-enhanced chemiluminescence (β-NADPH, 300 μmol/L; room temperature) on a microplate luminometer (PerkinElmer). In brief, proteins were diluted in modified Krebs-HEPES buffer, and NADPH and lucigenin were added to wells just before reading. Chemiluminescence readings were expressed as integrated light units per milligram protein. Experiments were also performed in the presence of the flavoprotein inhibitor diphenyleneiodonium (10 μmol/L) or allopurinol (100 μmol/L) or the NOS inhibitor N⁷-nitro-L-arginine methyl ester hydrochloride (100 μmol/L).

Western Blotting
Whole-heart proteins were prepared, and Western blot analysis was performed as described. The blots were incubated with primary monoclonal anti-xanthine dehydrogenase/XO antibody (1:1000, NeoMarkers Inc), anti-p47phox antibody (1:500, Upstate), anti-p22phox antibody (1:500, Santa Cruz Biotechnology), anti-endothelial NOS (NOS3) antibody (1:1000, BD Transduction Laboratories), anti-p67phox antibody, or anti-gp91phox antibody (1:500, BD Transduction Laboratories). A monoclonal anti-glyceraldehyde 3-phosphate dehydrogenase antibody (1:100 000, Research Diagnostic Inc) was used separately as a normalizer. Afterward, the membranes were incubated for 1 hour with peroxidase-conjugated chicken anti-rabbit or anti-mouse IgG antibodies (Santa Cruz Biotechnology) in 1:5000 dilutions. Bands were visualized by chemiluminescence (SuperSignal substrate kit, Pierce) and quantified with NIH Image software.
Quantification of mRNA
Fluorescence-based, real-time, quantitative polymerase chain reaction was used to determine the relative expression of cardiac mRNA. Total RNA was isolated, cDNA was synthesized, and each sample was run in duplicate on a GeneAmp 7900 sequence detection system (Applied Biosystems) and analyzed with SDS 2.0 software (Applied Biosystems) as we have already described. All samples were run in triplicate and repeated with glyceroldehyde 3-phosphate dehydrogenase as the normalizer. The primer sequences are described in Table I of the online-only Data Supplement.

Statistics
Data were expressed as mean±SEM. Survival data were analyzed with Kaplan-Meier survival curves and the log-rank test. Relative risks were computed from Cox proportional-hazard models. Echo-cardiographic and hemodynamic data within groups were analyzed by 1-way ANOVA, followed by Student-Newman-Keuls post hoc analysis, and between-group analyses were done by 2-way ANOVA with an interaction term for treatment group. The other parameters were analyzed by 1-way ANOVA, followed by Student-Newman-Keuls post hoc analysis. The null hypothesis was rejected at P<0.05.

Results
Survival
We first examined the consequence of NOS1 deficiency on mortality after MI. The mortality in infarcted NOS1−/− mice (45.8%, 22 of 48) was significantly higher compared with WT mice (25.0%, 11 of 44; hazard ratio of 2.06; 95% confidence interval, 1.05 to 4.41, P=0.036). Both MI groups had reduced survival in relation to their respective sham-operated controls (1P=0.014 between WT subgroups and P=0.0001 between NOS1−/− subgroups). There was no significant difference in survival after sham operation between WT (n=21) and NOS1−/− (n=22) mice. Abbreviations are as defined in text.

Hemodynamics and β-Adrenergic Responsiveness
We next evaluated the effects of NOS1 deficiency on cardiovascular hemodynamics and β-adrenergic responsiveness. Characteristic impairments in preload, contractility (dP/dtmax, [dP/dt/IP]), and diastolic function (dP/dt_int, Tau) were induced by MI in both strains (P=0.05, Table 2). Diastolic relaxation was further impaired in NOS1−/− with respect to WT mice after MI, as there was a significantly higher isovolumic relaxation time constant (P=0.05) in NOS1−/− than in WT mice after MI (Table 2). There were no differences in other hemodynamic parameters between the 2 MI groups (Table 2).

β-Adrenergic responsiveness was investigated by analyzing isoproterenol-induced increases in dP/dt_max. β-Adrenergic

Figure 1. Survival curves after MI in WT and NOS1−/− mice. Animals were followed up for 60 days. Post-MI survival was significantly reduced in NOS1−/− (n=48) vs WT mice (n=44, P=0.036). Both MI groups had reduced survival in relation to their respective sham-operated controls (1P=0.014 between WT subgroups and P=0.0001 between NOS1−/− subgroups). There was no significant difference in survival after sham operation between WT (n=21) and NOS1−/− (n=22) mice. Abbreviations are as defined in text.

Figure 2. Cardiac performance after MI. A, Echocardiographic M-mode measurements of cardiac dimensions (LA diameter, LVDd, and LVDs) and LVFS at baseline and after MI in WT and NOS1−/− mice. □, indicates sham-operated WT (n=21); ○, sham-operated NOS1−/− (n=22); □, infarcted WT (n at baseline = 44; by 1 week, 36; and by 2 and 3 weeks, 33); and ○, infarcted NOS1−/− (n at baseline = 48 and by 1, 2, and 3 weeks, 23). 1P<0.001 versus baseline (time 0); 1P<0.05 between infarcted WT and NOS1−/− mice. B, Isoproterenol-induced increase in dP/dt_max was blunted in NOS1−/− compared with WT sham-operated mice (1P<0.01). The response to isoproterenol was also blunted after MI in WT mice compared with sham-operated controls (1P<0.01). The response to isoproterenol in NOS1−/− mice after MI was reduced versus NOS1−/− sham (1P<0.01). Basal dP/dt_max was suppressed after MI in both strains (1P<0.05). □ Indicates sham-operated WT (n=11); ○, sham-operated NOS1−/− (n=6); □, infarcted WT (n=12); and ○, infarcted NOS1−/− (n=5). Abbreviations are as defined in text.
Table 2. Hemodynamic Measurements 4 Weeks After MI

<table>
<thead>
<tr>
<th></th>
<th>WT Sham (n=10)</th>
<th>NOS1−/− Sham (n=10)</th>
<th>WT MI (n=20)</th>
<th>NOS1−/− MI (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, bpm</td>
<td>535.7±20.6</td>
<td>547.3±19.2†</td>
<td>482.2±9.4</td>
<td>507.3±19.1</td>
</tr>
<tr>
<td>LVEPS, mm Hg</td>
<td>91.6±4.0</td>
<td>90.4±4.1</td>
<td>87.7±2.2</td>
<td>88.8±3.5</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td>5.32±0.85</td>
<td>5.58±1.42</td>
<td>9.68±1.42*</td>
<td>13.34±1.97*</td>
</tr>
<tr>
<td>dP/dtmax, mm Hg/s</td>
<td>11550±770</td>
<td>11780±757</td>
<td>7548±462*</td>
<td>7809±871*</td>
</tr>
<tr>
<td>dP/dtimin, mm Hg/s</td>
<td>−897±464</td>
<td>−9570±540</td>
<td>−6549±336*</td>
<td>−6367±616*</td>
</tr>
<tr>
<td>Tau, ms</td>
<td>4.8±0.2</td>
<td>4.5±0.3</td>
<td>6.5±0.3*</td>
<td>7.8±0.7†</td>
</tr>
<tr>
<td>(dP/dt)/IP, 1/s</td>
<td>215.3±12.2</td>
<td>236.2±12.2</td>
<td>152.3±7.6*</td>
<td>150.7±12.9*</td>
</tr>
</tbody>
</table>

HR indicates heart rate; LVEPS, LV end-systolic pressure; LVEDP, LV end-diastolic pressure; and IP, instantaneous developed pressure. Other abbreviations as in text.

*p<0.05 between MI and sham-operated groups; †p<0.05 in relation to MI WT mice.

Cardiac Remodeling and MI Size

The different cardiac performance variables after MI between strains were associated with differences in the degree of cardiac remodeling and hypertrophy. Although heart weight index was increased in both MI groups in relation to sham (P<0.001), heart weight index was higher (P<0.01) in infarcted NOS1−/− compared with infarcted WT mice (Figure 3A, Table 1). At the cellular level, the increase in width of residual LV myocytes after MI was higher (P<0.01) in NOS1−/− than in WT mice (Figure 3B through 3D). Finally, MI size was analyzed at both 48 hours and 4 weeks after surgery and was similar between groups at both time points (Figure 3E and 3F).

Nitroso-Redox Balance

We next examined the consequences of NOS1 deficiency on changes induced by MI in RNS and ROS production to test the hypothesis that NOS1 deficiency contributes to an imbalance in tissue NO-redox formation. Oxidative fluorescence microtopography with the fluorescent probe DHE (orange staining) demonstrated increased O₂− production of similar degrees in myocardial tissue slices obtained from both strains 3 days after MI (Figure 4A). Panels combining DHE with the NO-specific probe DAF (green staining) demonstrated a greater increase in NO production after MI in WT compared with NOS1−/− mice (Figure 4B). There was also more DAF staining in WT sham than in NOS1−/− sham mice (Figure 4B) and a stronger DHE staining in NOS1−/− sham than in WT mice (Figure 4A). Nitrite levels in heart homogenates were similar between sham groups but were increased significantly after MI in WT but not in NOS1−/− mice (P<0.05; Figure 4C). Taken together, these results demonstrate that in WT mice, there is a concomitant increase in O₂− and NO production induced by MI, whereas in NOS1−/− mice, increased O₂− is not accompanied by increased NO production.

XOR and NADPH Oxidase Activities

We examined possible sources for the increased ROS production after MI by analyzing the activity and abundance of XOR and NADPH oxidase. At baseline, XOR activity was higher in sham-operated NOS1−/− than in sham-operated WT mice (P<0.05), as previously demonstrated. Three days after MI, XOR activity increased by 4.2-fold in WT mice (P<0.05), achieving a similar level to that in NOS1−/− infarcted mice, but by 4 weeks after MI, this XOR activity returned to basal values. In NOS1−/− mice, XOR activity, already elevated at baseline (sham), had a nonsignificant

Figure 3. Cardiac hypertrophy and MI size by 4 weeks after MI. The increases in heart weight indices (A) and in cellular width (B) after MI were significantly higher in NOS1−/− compared with WT controls. Light photomicrographs of sections with hematoxylin-eosin staining, demonstrating surviving myocytes from infarcted WT (C) and NOS1−/− (D) mice. Light photomicrographs of sections with Masson’s trichrome staining (E) and triphenyltetrazolium staining (F), demonstrating similar infarct sizes in WT and NOS1−/− mice at 4 weeks and 48 hours after surgery, respectively. *P<0.001 between MI and sham-operated groups; †P<0.01 between infarcted WT and infarcted NOS1−/− mice. Abbreviations are as defined in text.
increase 3 days after MI and remained elevated at 4 weeks after MI (Figure 5A).

NADPH oxidase-dependent \( \text{O}_2^- \) production was similar between sham-operated WT and NOS1\(^{-/-} \) mice and increased by \( \approx 3 \)-fold \((P<0.05)\) in both strains by 3 days after MI. By 4 weeks after MI, the increase in NADPH oxidase activity declined in both groups to values similar to those found in their respective sham-operated controls (Figure 5B). NADPH oxidase-dependent \( \text{O}_2^- \) production was abolished by diphenyleneiodonium in all groups but was not affected by N\(^{-}\)-nitro-L-arginine methyl ester or allopurinol (see online-only Data Supplement Figure I).

Expression and Abundance of XOR and NADPH Oxidase
We investigated whether the increased \( \text{O}_2^- \) production after MI could be attributed to differences in the expression and/or abundance of XOR and/or NADPH oxidase. Cardiac XOR protein abundance was 4-fold increased in WT \((P<0.01)\) and 3-fold increased in NOS1\(^{-/-} \) mice \((P<0.01)\) in relation to their respective sham groups 3 days after MI. Cardiac XOR protein abundance (Figure 6A) and mRNA expression (Figure 6B) were similar among WT and NOS1\(^{-/-} \) infarcted and sham-operated mice 4 weeks after surgery, similar to our previous description at baseline.\(^a\) Overall, at 4 weeks after MI, there was a similar upregulation in NADPH oxidase subunits (gp91\(^{phox} \), p22\(^{phox} \), p47\(^{phox} \), and p67\(^{phox} \)) mRNA expression in WT and NOS1\(^{-/-} \) mice (for specific details, see Data Supplement). However, the cardiac protein abundance of NADPH oxidase subunits was similar among the groups (see online-only Data Supplement Figure II).

Expression and Abundance of NOS Isoforms
The mRNA expression of NOS1, inducible NOS (NOS2), and NOS3 was investigated at 4 weeks after MI. Whereas NOS2 and NOS3 mRNA expression was similar among the groups (see online-only Data Supplement Table II), NOS1 mRNA expression was abolished in NOS1\(^{-/-} \) mice. Despite similar mRNA levels, cardiac NOS3 protein abundance was higher in NOS1\(^{-/-} \) infarcted mice than in sham \((P<0.01)\) and infarcted \((P<0.05)\) WT mice (Figure 7).

Discussion
The major new finding of this study is that NOS1 exerts a protective role after MI. In comparison with controls after
MI, NOS1<sup>−/−</sup> mice had higher mortality, worse cardiac performance, accentuated remodeling, and increased macroscopic and myocyte cardiac hypertrophy. There was an imbalance between RNS and ROS production after MI in NOS1<sup>−/−</sup> mice due in large part to a failure of NO production to increase, coupled with a persistently augmented XOR activity within the myocardium. Interestingly, NADPH oxidase upregulation after MI was not different in NOS1<sup>−/−</sup> compared with WT. Together these findings support a protective role for NOS1 activity within the myocardium after myocardial injury.

Nitroso-Redox Balance

Our findings are in agreement with an accumulating body of work supporting specific and spatially confined roles for NOS isoforms within the heart. NOS3 is found at the cell membrane, where NO derived from NOS3 inhibits the L-type Ca<sup>2+</sup> channel and attenuates -adrenergic myocardial contractility, and NOS1 localizes to the SR and mitochondria, where NO derived from NOS1 facilitates SR Ca<sup>2+</sup> cycling and enhances myocardial contractility stimulated by either catecholamines or increased heart rate. A recent new insight into NOS spatial confinement derives from observations that NOS1 translocates from the SR to the cell membrane in various states of experimental and human LV dysfunction. This translocation has been previously postulated to play a pathophysiological role in heart failure, contributing to β-adrenergic hyporesponsiveness.

We have shown that the absence of NOS1 in cardiac tissue generates disequilibrium between the production of ROS and NO after MI. Increased ROS production after MI in both strains can be attributed to higher activity of both XOR and NADPH oxidases. Notably, XOR activity was elevated at baseline in NOS1<sup>−/−</sup> relative to WT mice and remained persistently elevated, whereas WT mice exhibited a transient elevation that was restored to normal by 4 weeks after MI. The increased XOR activity in both strains at 3 days after MI can be attributed to XOR upregulation. Importantly, the increased levels of XOR activity in NOS1<sup>−/−</sup> both at baseline and at 4 weeks after MI were independent of XOR abundance, because protein levels were similar across the samples. This phenomenon is in accordance with previous findings from our group and others that showed increased XOR activity in NOS1<sup>−/−</sup> mice, resulting in higher ROS production despite similar levels of XOR protein abundance.

The mechanisms involved in increased XOR activity are complex and probably include NOS1-mediated posttranslational modification (eg, S-nitrosylation) of XOR. Increased XOR activity, with unchanged XOR protein or mRNA, has been demonstrated after hypoxia exposure in cultured human bronchial epithelial cells and in bovine aortic endothelial cells. Furthermore, in rat pulmonary endothelial cells, p38 kinase phosphorylation mediates hypoxia-induced increases in XOR activity, a mechanism also implicated in increased XOR activity in NOS1<sup>−/−</sup> mice. The possibility that the NO-redox milieu influences responsiveness to the phosphorylation cascades is also consistent with recent data. Additional considerations for potential signaling interactions are that XOR itself may catalyze NO formation from nitrite reduction and that XOR can be deactivated during this reaction. However, maximal XOR-mediated NO formation occurs under anaerobic conditions. Under normoxic conditions, there is still XOR-mediated NO formation, mostly with NADH as the electron donor, and the reaction is also highly dependent on pH, with maximal NO formation at pH 5.

The increase in NADPH oxidase activity described here is ascribed to upregulation of NADPH subunits at the transcrip-
tional level. The increase in gp91phox and in p22phox mRNA expression after MI has been previously shown.24,25 Here we have expanded this observation to other NADPH oxidase subunits (p47phox and p67phox). NO production also increased substantially after MI in WT mice but not in NOS1−/− mice. This reinforces the importance of NO-derived from NOS1 in the pathophysiology after MI, a fact made relevant by previously described increases in NOS1 abundance and activity after MI.8,9,26 Thus, the absence of NOS1 within the myocardium after MI inhibits an increase in NO production that, together with augmented O2− production, creates disequilibrium in the balance between RNS and ROS pathways, which could affect numerous biological signaling processes.5,6

Importantly, NO inhibits both XOR3,4 and NADPH oxidase,27–29 but here we have demonstrated a specific interaction between NO derived from NOS1 with XOR and not with NADPH oxidase. We have shown similar NADPH oxidase activities in the presence and absence of NOS1 within the myocardium both at baseline and after MI. On the other hand, the cardioprotection against deleterious effects of MI obtained by NOS3 gene delivery in rats was associated with reduced O2− production and NADPH oxidase activity.29 The findings are consistent with the known spatial localization of NADPH oxidase to myocyte cell membranes30 and XOR to the SR in proximity to NOS3 and NOS1, respectively.

The absence of NOS1 was associated with higher mortality and worse cardiac performance after MI, not attributable to increased MI size. Indeed, the disequilibrium between RNS and ROS production after MI could contribute to these findings. Increased ROS production accentuates mortality and worsens cardiac performance after MI; in this regard, antioxidant strategies such as overexpression of glutathione peroxidase,31 probucol,32 and XO inhibition with allopurinol33 have all increased survival and improved cardiac performance in rodents after MI. The more accentuated diastolic dysfunction seen after MI in NOS1−/− mice can also be a secondary effect of the more exaggerated cardiac hypertrophy seen in these animals after MI.

Cardiac Structure and Function After MI
Cardiac hypertrophy induced by MI was worse in the absence of NOS1. Here, too, an NO-redox imbalance likely contributed to the enhanced hypertrophy seen in NOS1−/− infarcted mice. Oxidative stress (mediated by both NADPH oxidase35 and XO35) is linked to cardiac hypertrophy, whereas NO is reported to be antihypertrophic.36 Moreover, the use of allopurinol decreased the MI-induced cardiac hypertrophy in WT mice.34

The response to β-adrenergic stimulation was blunted after MI in WT mice and was also blunted in both NOS1−/− groups compared with WT controls. This last observation agrees with previous findings from our group1 and others37 that NOS1−/− mice are hyporesponsive to isoproterenol, which is attributable to the lack of NOS1-mediated potentiation of SR calcium release, induced possibly by S-nitrosylation of ryanodine receptors.1,6

Previously, NOS3 had been implicated as playing a cardioprotective role after MI, as demonstrated by studies showing that NOS3−/− mice exhibit increased mortality after MI.38 Conversely, NOS3 overexpression improves survival39 and limits LV dysfunction and remodeling after MI.39,40 On the other hand, NOS3 expression and activity have been shown to be suppressed after MI in rats,8 whereas its expression is upregulated in human heart failure.41 Despite the increased NOS3 reported in NOS1−/− animals, they still had worse survival and remodeling. It is important to consider that under pathological conditions, NOS3 upregulation may represent a futile compensatory mechanism without increased NO production42 and the possibility of NOS3-mediated OS. Here, the increased NOS3 abundance in NOS1−/− infarcted mice was not associated with increased nitrite levels. These findings illustrate the importance of both constitutive NOS isoforms as mediators of cardiac protection after MI.

Limitations
Potential limitations to our work include the fact that we have not used true littermate controls. However, the colonies of mice used were bred in parallel in the same environment in our laboratory. We have observed highly consistent cardiovascular phenotypes in our knockout and WT animals with regard to cardiac inotropy, lusitropy, and basal function.1,2,4,44 Nevertheless, we cannot completely exclude the possibility that intercolony variations could contribute to different post-MI outcomes between the strains studied.

In summary, disruption of the normal NOS1 pathway leads to increased mortality and reduced cardiac performance after MI. Increased ROS production attributable largely to persistent upregulation of XOR after MI was not accompanied by a concomitant augmented NO production in NOS1−/− mice. Thus, NOS1 plays a specific cardioprotective role after MI that encompasses both an antioxidant effect and a postreceptor β-adrenoreceptor–blocking role.

Together these data highlight the isoform-specific roles played by NOS1 and NOS3 as cardioprotective agents after MI and have important therapeutic implications for strategies designed to manipulate the NO-redox balance in the failing or infarcted heart.

Acknowledgments
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