nNOS Gene Deletion Exacerbates Pathological Left Ventricular Remodeling and Functional Deterioration After Myocardial Infarction

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Background—The neuronal isoform of nitric oxide synthase (nNOS) has been implicated in the regulation of basal and $\beta$-adrenergic inotropy in normal and chronically infarcted hearts. Furthermore, myocardial nNOS expression and activity increase in failing hearts, raising the possibility that nNOS may influence left ventricular (LV) remodeling progression and functional deterioration after myocardial infarction (MI)

Methods and Results—We compared LV remodeling at 1, 4, and 8 weeks after MI in nNOS-knockout mice (nNOS−/−) and their wild-type (WT) littermates matched for infarct size by using a highly accurate 3-dimensional echocardiographic technique. Basal LV hemodynamics and the inotropic response to dobutamine infusion (4 and 16 ng · g$^{-1}$ · min$^{-1}$) were also evaluated 8 weeks after MI. Sham-operated nNOS−/− mice showed enhanced basal LV contractility ($P<0.03$ versus WT, as evaluated by preload-recruitable stroke work) but an attenuated inotropic response to dobutamine infusion ($P<0.01$ versus WT). Both basal and $\beta$-adrenergic LV relaxations were significantly impaired in nNOS−/− mice. Survival after MI did not differ between groups. However, nNOS−/− mice developed a faster and more severe LV dilation compared with WT mice ($P<0.05$ for both end-systolic and end-diastolic volume indices). WT mice maintained a positive inotropic response to dobutamine 8 weeks after MI. In contrast, infarcted nNOS−/− mice responded to dobutamine with a dramatic fall in LV contractility ($P<0.01$ for preload-recruitable stroke work).

Conclusions—nNOS plays a crucial role in preventing adverse LV remodeling and maintaining myocardial $\beta$-adrenergic reserve after MI. Taken together, our findings suggest that upregulation of myocardial nNOS in infarcted hearts may be an important adaptive mechanism.

Key Words: heart failure ▪ hemodynamics ▪ nitric oxide synthase ▪ receptors, adrenergic, beta ▪ remodeling

The progression of left ventricular (LV) remodeling after myocardial infarction (MI) results from the failure of initial compensatory mechanisms to preserve LV function and morphology over time. Several factors have been implicated in this maladaptive process, which ultimately leads to heart failure. Of these, impaired myocardial Ca$^{2+}$ handling and neurohumoral activation have been shown to influence disease progression and survival after MI.$^{1,2}$ During the last 10 years, experimental evidence has indicated that constitutive nitric oxide (NO) production may play an important part in fine-tuning the myocardial response to stress and $\beta$-adrenergic stimulation.$^{3,4}$ However, selective gene deletion of the most common cardiac NO synthase (NOS) isoform (the “endothelial” NOS, or eNOS) has not produced consistent effects on LV remodeling after MI.$^{5,6}$ Furthermore, eNOS expression and activity have been found to be significantly suppressed in the failing myocardium,$^{7-9}$ suggesting that the eNOS-mediated regulation of cardiac function and $\beta$-adrenergic responses may be reduced in myocardial disease.

In 1999, Xu et al.$^{10}$ located a neuronal-like NOS (nNOS, or NOS1) to the sarcoplasmic reticulum of several mammalian species, including humans. Although it is now accepted that nNOS-derived NO regulates myocardial inotropy and relaxation,$^{11-15}$ the mechanisms underlying these actions and their potential relevance to myocardial disease states are still debated. It is intriguing that, in contrast with eNOS, nNOS expression and activity are significantly increased in the hypertrophic$^{16}$ or failing$^{7,9}$ rat myocardium and in failing human hearts,$^{8}$ suggesting that nNOS may play an active role...
in the myocardial stress response. This hypothesis has not been fully tested; however, Bendall et al. have recently reported that short-term systemic nNOS inhibition enhances the LV response to β-adrenergic stimulation in chronically infarcted rats, suggesting that nNOS-derived NO may act as a natural “brake” against the detrimental effects of excessive sympathetic stimulation. Nevertheless, it remains unclear whether nNOS upregulation in the diseased myocardium is an adaptive or pathogenic mechanism. To address this question, we compared LV remodeling after MI in nNOS-knockout (nNOS−/−) mice and their wild-type (WT) littermates by using a highly accurate 3-dimensional (3D) echocardiographic imaging protocol and evaluated LV hemodynamics and the inotropic response to dobutamine infusion 8 weeks after MI.

**Methods**

**Animal Model**

We used the progeny of heterozygous breeding pairs of mice with targeted disruption of exon 2 of nNOS. Expression of alternatively spliced RNA forms can still be detected in the brains of these mice, wherein they account for ~5% of NOS catalytic activity. These nNOS spliced variants lack the membrane-anchoring PDZ domain and are localized to the cytosolic fraction; hence, their ability to contribute to nNOS-mediated physiological effects is uncertain.

Tail DNA samples were harvested for genotyping by polymerase chain reaction, as described previously. Male and female nNOS−/− mice and their WT littermates (age, 2 to 4 months) underwent ligation of the left anterior descending coronary artery to induce MI, as previously described. All protocols were in accordance with the Home Office Guidance on the Operation of Animals (Scientific Procedures) Act, 1986 (HMSO).

**3D Echocardiography**

Mice were imaged at weeks 1, 4, and 8 after MI or sham surgery under isoflurane anesthesia. A detailed description and validation of our 3D echocardiographic technique, including determination of infarct size, have been recently published. A single echocardiographer blinded to mouse genotype performed all image acquisitions and analyses.

**Invasive Hemodynamics**

Immediately after the final 8-week echocardiographic study, a 1.4F micromanometer conductance catheter (SPR-839, Millar Instruments Inc) was advanced from the right carotid artery into the LV and positioned under echocardiographic guidance. The right jugular vein was cannulated with stretched polythene tubing for intravenous infusions; anesthetic was reduced to 1.5% isoflurane, after which a micromanometer conductance catheter (SPR-839, Millar Instruments Inc) was advanced from the right carotid artery into the LV and positioned under echocardiographic guidance. The right jugular vein was cannulated with stretched polythene tubing for intravenous infusions; anesthetic was reduced to 1.5% isoflurane, after which a 15-minute period of equilibration was allowed to elapse.

A laparotomy was performed to allow alteration of loading conditions by transient occlusion of the inferior vena cava at the level of the diaphragm. LV pressure-volume loops were recorded before and after occlusion at baseline and during dobutamine infusions (4 and 16 ng · g−1 · min−1). Measurements were recorded on a PowerLab 4SP data recorder (AD Instruments).

Three calibration steps were undertaken for conversion of relative volume units into LV volumes: (1) An IV bolus injection of 5 µL of hypertonic saline (30%) was used to derive the parallel conductance; (2) the regression formula was calculated for catheter-derived volume measurements plotted against known volumes from 6 wells of fresh heparinized blood; and (3) catheter-derived LV volume measurements were calibrated according to those obtained by 3D echocardiography, thereby allowing calibration of end-diastolic and end-systolic volumes (EDV and ESV, respectively) in addition to stroke volume.19

LV systolic pressure was assessed by end-systolic elastance (Ees, mm Hg/µL) and preload-recruitable stroke work (PRSW, mm Hg). PRSW has been found to be independent of LV volumes and calibration corrections, and as such, it is regarded as a robust index of LV inotropy in vivo. LV afterload was assessed by arterial elastance (mm Hg/µL), LV preload by end-diastolic pressure (mm Hg), and relaxation by dP/dt max (mm Hg/s); the time constant of isovolumic relaxation (τ, ms) was also determined.

**Tissue Histology and Immunoblots**

Hearts from sham-operated and infarcted nNOS−/− and WT mice were fixed in formalin, dehydrated, embedded in paraffin, and cut into 5-µm sections before being stained with Masson’s trichrome. Fibrosis was measured as the percentage of pixels that stained blue for collagen from 40 fields of view per heart with the aid of NIH ImageJ software (version 1.33). The investigator performing the analysis was blinded to mouse genotype. Average data reflect the results from 4 hearts from each group.

Immunoblots were performed with antibodies against nNOS (monoclonal SC-5302, Santa Cruz or polyclonal 160870, Cayman Chemical), eNOS (Transduction Laboratories), caveolin 3 (Cav-3, Santa Cruz), and heat-shock protein 90 (Santa Cruz) in LV homogenates from infarcted and sham-operated nNOS−/− and WT mice, as described previously. Band density was quantified by laser densitometry with Fluor Chem 8800 software and normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH), Preliminary experiments were performed to obtain the correct linear range for quantification in Western blot analyses.

**Statistical Analysis**

Comparisons were made between nNOS−/− and WT mice that were matched for infarct size (>23% by 3D echocardiography; equivalent to an infarct size of 30% by histology). Two-way repeated-measures ANOVA was used to test between-group differences in LV remodeling over time and in the response to dobutamine, after applying Bonferroni’s correction for multiple comparisons. When a significant interaction was found (P<0.05), differences between groups were analyzed for each time point. Multilevel modeling (in SPSS 12.0.2) was used in some comparisons. The Kaplan-Meier method was used to construct survival curves, which were then compared by the log-rank test, with correction for multiple comparisons. Results from Western blot analyses were evaluated by Student’s t test. Data are shown as mean±SEM.

**Results**

Survival and Parametric Data

 Coronary ligation or sham surgery was carried out in 122 mice (62 nNOS−/− mice, 44 females and 18 males; and 60 WT mice, 37 females and 23 males). All mice survived sham surgery (8 nNOS−/− and 7 WT mice). Survival after MI did not differ between nNOS−/− and WT mice for either males or females (Figure 1), but males had a significantly higher mortality compared with females (P<0.01) because of a
TABLE 1. Lung, LV, and Right Ventricle Weights in Sham-Operated and Infarcted nNOS<sup>-/-</sup> Mice and WT Littermates 8 Weeks After Surgery

<table>
<thead>
<tr>
<th></th>
<th>Sham WT</th>
<th>Sham nNOS&lt;sup&gt;-/-&lt;/sup&gt;</th>
<th>P*</th>
<th>MI WT</th>
<th>MI nNOS&lt;sup&gt;-/-&lt;/sup&gt;</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung wt/body wt, mg/g</td>
<td>5.2±0.6</td>
<td>5.9±0.2</td>
<td>NS</td>
<td>6.8±0.4†</td>
<td>6.7±0.2†</td>
<td>NS</td>
</tr>
<tr>
<td>LV wt/body wt, mg/g</td>
<td>2.8±0.1</td>
<td>3.2±0.1</td>
<td>0.04</td>
<td>3.8±0.1†</td>
<td>3.7±0.1†</td>
<td>NS</td>
</tr>
<tr>
<td>RV wt/body wt, mg/g</td>
<td>0.9±0.04</td>
<td>0.9±0.03</td>
<td>NS</td>
<td>1.2±0.1†</td>
<td>1.2±0.1†</td>
<td>NS</td>
</tr>
</tbody>
</table>

Table 1. Lung, LV, and Right Ventricle Weights in Sham-Operated and Infarcted nNOS<sup>-/-</sup> Mice and WT Littermates 8 Weeks After Surgery

wt indicates weight; RV, right ventricle; and NS, not significant. Other abbreviations are as defined in text.

*P for comparisons between WT and nNOS<sup>-/-</sup> mice.
†P<0.05 for comparisons between sham-operated and infarcted mice within each group.

3D Echocardiography

To evaluate LV remodeling over time in each group, we reconstructed the ESV and EDV in WT and nNOS<sup>-/-</sup> mice at 1, 4, and 8 weeks after coronary ligation or sham surgery. The time-dependent changes in ESV and EDV indices after surgery (ESVI and EDVI, respectively, in μL per g of body weight) are shown in Figure 2A and 2B. There was no significant difference in heart rate between nNOS<sup>-/-</sup> and WT mice (Table 2). There was a tendency for body weight to increase during follow-up (1.2±0.7 g), which did not differ between groups.

Sham-Operated Mice

The LV EDVI did not differ between groups, whereas the ESVI was slightly smaller in nNOS<sup>-/-</sup> mice (P<0.01, Figure 2A). Consequently, LV ejection fraction was higher in sham-operated nNOS<sup>-/-</sup> mice (P=0.001) at each time point (Table 2). No differences in LV volumes over time were seen in sham-operated mice from either group (Figure 2A and 2B).

Infarcted Mice

As expected, infarcted mice from both groups had a significantly higher LV ESV and EDV compared with their sham-operated controls (Figure 2A and 2B, P<0.0001). However, LV dilation was significantly greater in nNOS<sup>-/-</sup> mice compared with their WT littermates, as indicated by their greater ESVI (P<0.02) and EDVI (P<0.05). Differences in LV remodeling between nNOS<sup>-/-</sup> and WT mice became more dramatic when ESV and EDV in infarcted mice were expressed as relative changes from measurements taken in their respective sham-operated controls at the same time point. As

Figure 2. Time-dependent changes in LV volumes (ESVI and EDVI are ESV and EDV, respectively, normalized for body weight) in sham-operated or infarcted nNOS<sup>-/-</sup> mice and WT littermates. ESVI was slightly smaller in sham-operated nNOS<sup>-/-</sup> mice compared with WT littermates (§P<0.01, A), LV dilation was significantly greater in infarcted nNOS<sup>-/-</sup> mice vs WT, as indicated by their greater ESVI (P<0.02, A) and EDVI (P<0.05, B). C and D show the relative increase over time in ESV and EDV in infarcted mice. LV remodeling was more accentuated in infarcted nNOS<sup>-/-</sup> mice at all time points (P<0.01 for ESVI and **P<0.05 for EDV) compared with infarcted WT littermates with the same infarct sizes. Abbreviations are as defined in text.
shown in Figure 2C and 2D, LV dilation was more accentuated in infarcted nNOS−/− mice than in WT littermates at all time points (P<0.01 for ESV and P<0.05 for EDV).

Invasive Hemodynamic Assessment

Sham-Operated Mice

Under basal conditions, sham-operated nNOS−/− mice showed a higher LV end-systolic pressure-volume relation compared with WT littermates, as evaluated by LV PRSW and Ees (Table 3). However, the LV inotropic response to β-adrenergic stimulation with dobutamine was significantly blunted in nNOS−/− mice. Figure 3A and 3B show representative LV pressure-volume loops from 1 sham-operated WT (A) and 1 nNOS−/− (B) mouse under basal conditions and during dobutamine infusion. As shown in this example, the slope of the end-systolic pressure-volume relation (ie, the LV Ees) increased to a lesser extent in nNOS−/− mice during dobutamine infusion (average respective increase with 4 and 16 ng · g−1 · min−1 dobutamine, 26 ±1% and 35 ±1% in nNOS−/− mice versus 42 ±1% and 197 ±5% in WT; P<0.001 between groups). Mean values of PRSW shown in Figure 4A confirmed a blunted inotropic response to dobutamine in nNOS−/− mice (P<0.0001 between groups).

Sham-operated nNOS−/− mice also showed a significantly impaired LV relaxation when compared with WT littermates, as indicated by a prolonged τ both under basal condition and during dobutamine infusion (Table 3 and Figure 5A, P<0.01) and by a reduced LV dP/dtmin (Table 3).

Infarcted Mice

As expected, LV systolic function was significantly impaired in infarcted mice from both groups compared with sham-operated controls (Table 3).

As observed in the sham-operated group, infarcted nNOS−/− mice had a higher basal LV systolic performance than WT mice (Table 3 and Figure 4B). However, whereas infarcted WT mice still showed a modest increase in LV contraction in response to dobutamine, the nNOS−/− mice had a negative inotropic response to β-adrenergic stimulation 8 weeks after MI. As illustrated in Figure 3C, LV pressure-volume loops of a representative WT mouse 8 weeks after MI were shifted to the left in response to dobutamine, whereas the Ees slope showed a modest increase (on average, by 43 ±2% and 71 ±2% with dobutamine at 4 and 16 ng · g−1 · min−1, respectively). In contrast, infarcted nNOS−/− mice, the LV pressure-volume relation was shifted to the right in response to dobutamine (Figure 3D), and the Ees slope decreased (−6 ±1% and −7 ±1% in response to dobutamine at 4 and 16 ng · g−1 · min−1, respectively; P<0.02 versus WT). The ESVI increased in response to dobutamine infusion in the infarcted nNOS−/− mice (from 4.4 ±0.3 μL/g under basal conditions to 5.4 ±0.4 and 5.5 ±0.6 μL/g during 4 and 16 ng · g−1 · min−1 dobutamine infusion, respectively), whereas adrenergic stimulation tended to decrease ESVI in WT mice (from 3.1 ±0.3 μL/g at baseline to 2.8 ±0.4 and 2.9 ±0.4 μL/g during 4 and 16 ng · g−1 · min−1 dobutamine infusion, respectively; P<0.05 versus nNOS−/−).

In keeping with these findings, mean values of LV PRSW (Figure 4B) showed a significant reduction in LV inotropy in both groups 8 weeks after MI and confirmed the decrease in LV systolic function in response to β-adrenergic stimulation in infarcted nNOS−/− mice. In contrast, infarcted WT mice maintained a significant dose-dependent increase in LV inotropy in response to dobutamine (P<0.01, Figure 4).

<table>
<thead>
<tr>
<th>TABLE 2. LV Ejection Fraction and Heart Rate at 1, 4, and 8 Weeks After MI or Sham Surgery in nNOS−/− Mice and WT Littermates</th>
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<tbody>
<tr>
<td><strong>WT</strong></td>
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<tr>
<td><strong>Sham</strong> (n=7)</td>
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<tr>
<td>LVEF, %</td>
</tr>
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<td>HR, bpm</td>
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EF indicates ejection fraction; HR, heart rate. Other abbreviations are as defined in text.

*P<0.05 between sham-operated WT and nNOS−/− mice at the same time point. EF and HR did not differ between infarcted WT and nNOS−/− mice.

<table>
<thead>
<tr>
<th>TABLE 3. Basal Hemodynamic Parameters 8 Weeks After MI or Sham Surgery</th>
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<tr>
<td><strong>Sham Operated</strong></td>
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<tr>
<td><strong>WT</strong></td>
</tr>
<tr>
<td>HR, bpm</td>
</tr>
<tr>
<td>PRSW, mm Hg</td>
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<tr>
<td>Ees, mm Hg/μL</td>
</tr>
<tr>
<td>LVESP, mm Hg</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
</tr>
<tr>
<td>τ, ms</td>
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<tr>
<td>dP/dtmin, mm Hg/s</td>
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</table>

HR indicates heart rate; ESV, end-systolic pressure; EDV, end-diastolic pressure; and NS, not significant. Other abbreviations are as defined in text.

*P<0.05 compared with WT or nNOS−/− mice.
†P<0.05 vs sham-operated WT mice.
‡P<0.05 vs sham-operated nNOS−/− mice.
LV diastolic function was significantly impaired in infarcted mice compared with sham-operated mice; however, neither \( E_{\text{es}} \) nor LV \( \frac{dP}{dt_{\text{max}}} \) differed significantly between infarcted \( nNOS^{-/-} \) mice and WT mice at baseline (Table 3) or during dobutamine infusion (Figure 5B). Indices of LV preload (end-diastolic pressure) or afterload (arterial elastance) did not differ between \( nNOS^{-/-} \) and WT mice under any experimental conditions (data not shown).

**Myocardial Collagen Fraction and Quantitative Immunoblotting**

The LV collagen fraction did not differ between sham-operated \( nNOS^{-/-} \) mice and WT mice (5.0 ± 1.2% versus 5.1 ± 0.4% in \( nNOS^{+/+} \); \( P=0.92 \)). Eight weeks after MI, myocardial fibrosis had increased in the remote LV myocardium of both groups of mice (\( P<0.01 \) versus sham-operated controls); however, the increase in myocardial fibrosis was less in \( nNOS^{-/-} \) mice compared with WT mice (\( P=0.02 \); data not shown). Eight weeks after MI, myocardial fibrosis had increased in the remote LV myocardium of both groups of mice (\( P<0.01 \) versus sham-operated controls); however, the increase in myocardial fibrosis was less in \( nNOS^{-/-} \) mice compared with WT mice (\( P=0.02 \); data not shown).

**Figure 3.** Representative LV pressure-volume loops of a sham-operated (A, B) or an infarcted (C, D) WT and \( nNOS^{-/-} \) mouse. As illustrated by this example, \( E_{\text{es}} \) increased to a lesser extent in \( nNOS^{-/-} \) mice (B) compared with WT (A) in response to dobutamine (Dob) infusion (4 and 16 ng · g⁻¹ · min⁻¹). Note that dobutamine caused a modest increase in \( E_{\text{es}} \) and a reduction in ESV in the infarcted WT mouse (C). In contrast, in infarcted \( nNOS^{-/-} \) mice, dobutamine caused a reduction in \( E_{\text{es}} \) and an increase in ESV. Other abbreviations are as defined in text.

**Figure 4.** A, Mean values of LV PRSW showed a higher basal inotropy in \( nNOS^{-/-} \) mice and a blunted inotropic response to dobutamine in this group compared with WT mice (\( P<0.0001 \) between groups). B, LV inotropy was significantly reduced in both groups 8 weeks after MI, although PRSW remained greater in \( nNOS^{-/-} \) mice under basal conditions. Infarcted WT mice maintained a significant dose-dependent increase in PRSW in response to dobutamine. In contrast, infarcted \( nNOS^{-/-} \) mice showed a reduction in LV inotropy in response to \( \beta \)-adrenergic stimulation \( P<0.01 \) between groups. *\( P<0.05 \) between WT and \( nNOS^{-/-} \) mice for each time point. Abbreviations are as defined in text.
collagen fraction was significantly less in nNOS\(^{-/-}\) mice than in WT mice matched for infarct size (13.8±0.7\% versus 9.7±0.5\% in nNOS\(^{-/-}\), \(P<0.005\); Figure 6A and 6B).

As shown in Figure 6C, there was a significant increase in Cav-3 protein expression in LV homogenates from nNOS\(^{-/-}\) and WT mice 8 weeks after MI (Cav-3 to GAPDH ratio: In WT mice, 0.76±0.08 in sham versus 3.07±0.4 after MI; in nNOS\(^{-/-}\) mice, 0.80±0.07 versus 3.09±0.4; \(P<0.005\) for both, \(n=4\) in each group). Heat-shock protein-90 protein expression tended to be higher in the infarcted myocardium; however, this difference did not reach statistical significance in either group. In agreement with previous findings,7,9 nNOS protein level was significantly raised in LV homogenates from infarcted WT mice (nNOS to GAPDH ratio, 0.40±0.14 in shams versus 0.50±0.01 after MI; \(P<0.05\), \(n=4\) in each group). However, there was no significant difference in the LV protein expression of eNOS in sham-operated or infarcted nNOS\(^{-/-}\) and WT mice (\(n=4\)).

**Discussion**

The key findings of our study are as follow: (1) By performing a highly accurate sequential assessment of LV volumes in infarcted mice, we have demonstrated that adverse LV remodeling after MI was significantly more severe in nNOS\(^{-/-}\) mice than in WT littermates with identical infarct sizes; (2) basal LV inotropy was significantly greater in nNOS\(^{-/-}\) mice versus WT, both after sham surgery and after MI; (3) whereas WT mice maintained a positive (albeit reduced) inotropic response to \(\beta\)-adrenergic stimulation 8 weeks after MI, infarcted nNOS\(^{-/-}\) mice responded to IV dobutamine with a significant dose-dependent fall in LV systolic function, which could not be attributed to arrhythmias or to differences in LV preload, afterload, or chronotropic response between groups; (4) LV relaxation was significantly impaired in sham-operated nNOS\(^{-/-}\) mice both under basal conditions and during dobutamine infusion. However, these differences were no longer present 8 weeks after MI. A significant attenuation in the development of interstitial fibrosis in the remodeled LV myocardium of infarcted nNOS\(^{-/-}\) mice may have accounted for these findings; and (5) the protein expression of nNOS in LV homogenates was significantly raised in infarcted WT mice compared...
with sham-operated, control mice, whereas there was no difference in eNOS protein level between sham-operated or infarcted nNOS−/− mice and WT mice. Taken together, these findings demonstrate that nNOS plays an important role in myocardial remodeling and β-adrenergic responses after MI.

We have developed a quantitative and highly accurate 3D echocardiographic technique that allows fast and highly reproducible evaluation of LV volumes in normal and infarcted mice without the need for geometric assumptions.18 LV volumes and infarct sizes calculated by using this technique are virtually identical to those obtained by magnetic resonance imaging,18 with the added advantage that 3D echocardiography is faster (4 minutes) and requires a very light anesthetic protocol (1.25% to 1.5% isoflurane), thus causing minimal cardiodepressant effects.24 Using this technique, we have clearly demonstrated that adverse LV remodeling after MI was more severe in nNOS−/− mice than in WT littermates that were matched for age, sex, and infarct size. In nNOS−/− mice, the infarcted LV underwent faster and progressive dilation during the 2-month follow-up. Conversely, in WT mice, the remodeling process appeared to plateau at ∼4 weeks after MI, as described previously.25 The mechanisms underlying these findings are still unclear; however, myocardial nNOS upregulation in the chronically infarcted myocardium may prevent adverse LV remodeling and functional deterioration by acting as a natural “brake” against the detrimental effects of excessive adrenergic stimulation.9 Our immunoblots confirmed that nNOS was upregulated in LV homogenates from infarcted WT mice; however, in contrast with data in the failing rat or human myocardium,7–9 we did not observe a significant reduction in LV eNOS protein expression after MI, probably reflecting a less severe heart failure phenotype in mice. Furthermore, it has recently been reported that xanthine oxidase–mediated generation of reactive oxygen species is increased in the LV of nNOS−/− mice,15–26 providing a further putative mechanism for adverse remodeling in this group.

We observed a higher LV ejection fraction and a smaller LV ESVI in sham-operated nNOS−/− mice at all time points. Invasive assessment of LV function confirmed an enhanced basal LV inotropy in nNOS−/− mice compared with WT controls, as shown by their higher LV PRSW and Ees after both sham surgery and MI. We have previously reported that selective gene deletion or pharmacological inhibition of nNOS results in increased myocyte contraction that is driven by greater Ca2+ entry via the sarcoplasmic L-type Ca2+ channels.14 suggesting that nNOS–mediated regulation of myocardial Ca2+ fluxes may account for the enhanced basal LV inotropy in nNOS−/− mice in vivo.

As shown previously,12,22 LV β-adrenergic reserve was blunted in nNOS−/− mice in vivo. This finding cannot be easily explained by our in vitro data, because we found that contraction remained greater in LV myocytes isolated from 2- to 4-month-old nNOS−/− mice in the presence of both low (2 nmol/L)14 and high (1 μmol/L)27 concentrations of isoproterenol. Under different experimental conditions, Barouch et al12 have reported a trend toward an increased inotropic response to isoproterenol at concentrations <10−10 mol/L in nNOS−/− myocytes with no further increase in contraction at higher concentrations. Discrepancies between data obtained in LV myocytes and in vivo have been reported before; specifically, the inotropic response to β-adrenergic stimulation has been found to be enhanced in eNOS−/− mice in vivo but not in isolated myocytes (eg, see Gyurko et al21 and Han et al28), suggesting that the effects of eNOS-derived NO on myocardial contraction may be predominantly paracrine and require intact endothelial membranes. Importantly, it is well established that nNOS-derived NO modulates autonomic transmission in both the central nervous system and peripheral nerves.4 Specifically, nNOS−/− mice have been shown to have an impaired vagal control of heart rate and an elevated sympathetic nerve activity.29–31 It is possible, therefore, that the latter may have contributed to the enhanced basal inotropy and the reduced β-adrenergic reserve in nNOS−/− mice in vivo, as well as to the more severe post-MI LV remodeling in this group. Similarly, myocardial production of reactive oxygen species is known to increase in failing hearts via upregulation of the activity of NAD(P)H oxidases32,33 and possibly xanthine oxidase.34 Because nNOS gene deletion has been found to be associated with increased xanthine oxidase activity in LV homogenates,15–26 it is possible that stimulation of free-radical production by this enzyme in response to β-adrenergic stimulation15 might lead to a reduction in myofilament Ca2+ sensitivity,15 energetic imbalance,26 and LV dysfunction.16

Interestingly, our hemodynamic studies also showed impaired basal and β-adrenergic LV relaxation in sham-operated nNOS−/− mice. Although the presence of mild LV hypertrophy in nNOS−/− mice may have contributed to these findings, we have previously observed similar results in younger nNOS−/− mice in vivo,32 before LV hypertrophy could be demonstrated. Impaired relaxation is also seen in isolated LV myocytes from nNOS−/− mice, which also exhibit a slower decay of the Ca2+ transient compared with WT myocytes,14 suggesting that nNOS-derived NO may affect cytoplasmic Ca2+ removal, possibly by regulating sarcoplasmic reticulum Ca2+-ATPase activity. However, 8 weeks after MI, LV τ was prolonged to a similar extent in both groups of mice, indicating that, under these conditions, factors such as LV fibrosis and scar formation may be more important than sarcoplasmic reticulum Ca2+ uptake in determining the rate of LV isovolumic relaxation. Indeed, we showed that the increase in interstitial fibrosis in the remodeled LV myocardium of infarcted animals was significantly attenuated in nNOS−/− mice compared with WT mice with similar infarct sizes. A similar finding has been recently reported in the LV myocardium of eNOS−/− mice 9 weeks after aortic banding,37 suggesting that constitutive NOS may play a part in the development of myocardial fibrosis in chronically stressed hearts, probably by contributing to the formation of pro-oxidant molecules via enzyme “uncoupling.”37,38
Conclusions

In summary, our data unequivocally show that nNOS deletion significantly accelerates adverse LV remodeling and functional deterioration after MI and suggest that nNOS upregulation in the chronically infarcted and failing myocardium may be a protective mechanism aimed at maintaining myocardial Ca\(^{2+}\) homeostasis and \(\beta\)-adrenergic reserve.

Acknowledgments

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Disclosure

None.

References


**CLINICAL PERSPECTIVE**

It is now well established that both the endothelial (eNOS) and the neuronal (nNOS) isoforms of nitric oxide synthase are constitutively present in the ventricular myocardium of several mammalian species, including humans. Although it is now accepted that nNOS-derived NO regulates myocardial inotropy and relaxation, the mechanisms underlying these actions and their potential relevance to myocardial disease states are still debated. It is intriguing that, in contrast with eNOS, nNOS expression and activity are significantly increased in failing hearts, suggesting that nNOS may play an active role in the myocardial stress response. To test this hypothesis, we compared left ventricular (LV) remodeling after myocardial infarction (MI) in nNOS-knockout mice (nNOS-/-) and their wild-type littermates (WT) by using a highly accurate 3-dimensional echocardiographic imaging protocol. We then evaluated LV hemodynamics and the inotropic response to dobutamine infusion 8 weeks after MI. Our data demonstrate that adverse LV remodeling after MI was significantly more severe in nNOS-/- mice than in WT littermates with identical infarct size. Furthermore, whereas WT mice maintained a positive (albeit reduced) inotropic response to β-adrenergic stimulation 8 weeks after MI, infarcted nNOS-/- mice responded to dobutamine with a significant dose-dependent fall in LV systolic function that could not be attributed to arrhythmias or to differences in LV preload, afterload, or chronotropic response between groups. Taken together, our findings indicate that nNOS plays a crucial role in preventing adverse LV remodeling and maintaining myocardial β-adrenergic reserve after MI and suggest that upregulation of myocardial nNOS in infarcted hearts may be an important adaptive mechanism.
nNOS Gene Deletion Exacerbates Pathological Left Ventricular Remodeling and Functional Deterioration After Myocardial Infarction
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