Endothelial Nitric Oxide Synthase Limits Left Ventricular Remodeling After Myocardial Infarction in Mice

Marielle Scherrer-Crosbie, MD*; Roman Ullrich, MD*; Kenneth D. Bloch, MD; Hiroshi Nakajima, MD; Boris Nasseri, MD; H. Thomas Aretz, MD; Merry L. Lindsey, MD; Anne-Claire Vançon, MD; Paul L. Huang, MD; Richard T. Lee, MD; Warren M. Zapol, MD; Michael H. Picard, MD

Background—To investigate the role of endothelial nitric oxide synthase (NOS3) in left ventricular (LV) remodeling after myocardial infarction (MI), the impact of left anterior descending coronary artery ligation on LV size and function was compared in 2- to 4-month-old wild-type (WT) and NOS3-deficient mice (NOS3 –/– ).

Methods and Results—Two days after MI, both strains of mice had a similar LV size, fractional shortening, and ejection fraction by echocardiography. Twenty-eight days after MI, both strains had dilated LVs with decreased fractional shortening and lower ejection fractions. Although the infarcted fraction of the LV was similar in both strains, LV end-diastolic internal diameter, end-diastolic volume, and mass were greater, but fractional shortening, ejection fraction, and the maximum rate of developed LV pressure (dP/dt max) were lower in NOS3 –/– than in WT mice. Impairment of diastolic function, as measured by the time constant of isovolumic relaxation (τ) and the maximum rate of LV pressure decay (dP/dt min), was more marked in NOS3 –/– than in WT mice. Mortality after MI was greater in NOS3 –/– than in WT mice. Long-term administration of hydralazine normalized blood pressure in NOS3 –/– mice, but it did not prevent the LV dilatation, impaired systolic and diastolic function, and increased LV mass that followed MI. In WT mice, capillary density and myocyte width in the nonischemic portion of the LV did not differ before and 28 days after MI, whereas in NOS3 –/– mice, capillary density decreased and myocyte width increased after MI, whether or not hydralazine was administered.

Conclusions—These results suggest that the presence of NOS3 limits LV dysfunction and remodeling in a murine model of MI by an afterload-independent mechanism, in part by decreasing myocyte hypertrophy in the remote myocardium. (Circulation. 2001;104:1286-1291.)

Key Words: echocardiography ■ ventricles ■ hypertrophy

After a myocardial infarction (MI), ventricular remodeling occurs in both the myocardium surrounding the infarcted tissue (border zone) and the myocardium remote from the infarct to preserve cardiac output and limit wall stress. This adaptation involves left ventricular (LV) dilatation, changes in LV shape, myocyte hypertrophy, and interstitial myocardial fibrosis. However, in some cases, progressive LV remodeling after MI leads to a deterioration in contractile function that is associated with increased morbidity and mortality. Therapeutic strategies designed to limit ventricular remodeling after MI in patients can decrease the incidence of congestive heart failure and improve survival.

Nitric oxide (NO) can modulate many of the processes leading to ventricular remodeling. In clinical studies, long-term administration of nitrates (NO-donor compounds) limited LV remodeling after MI. Endothelium-derived NO causes systemic vascular relaxation, thereby reducing cardiac preload and afterload. Recent evidence suggests that NO can increase angiogenesis, decrease cardiac fibrosis, and decrease angiotensin II–induced cardiac myocyte hypertrophy, all of which could limit ventricular remodeling after MI.

NO is synthesized by a family of enzymes termed NO synthases (NOS), which include 3 known isoforms (NOS1, NOS2, and NOS3). Inhibition of various NOS isoforms has led to differing effects on myocardial structure and function. However, most of the available NOS inhibitors are incompletely isoform-specific, potentially leading to uncertainty regarding the role of individual NOS isoforms in cardiac dysfunction and ventricular remodeling after MI.

Received March 29, 2001; revision received May 18, 2001; accepted May 24, 2001.

From the Cardiology Division (M.S.-C., K.D.B., A.-C.V., P.L.H., M.H.P.) and the Cardiovascular Research Center (K.D.B., H.N., P.L.H.) of the Department of Medicine, the Department of Anesthesia and Critical Care (R.U., W.M.Z.), the Department of Pathology (H.T.A.), and the Department of Surgery (B.N.) at Massachusetts General Hospital, and the Cardiovascular Division (M.L.L., R.T.L.) of the Department of Medicine at Brigham and Women Hospital and Harvard Medical School, Boston, Mass.

*The first 2 authors contributed equally to the study.

Correspondence to Marielle Scherrer-Crosbie, MD, Cardiac Ultrasound Laboratory, Massachusetts General Hospital, 32 Fruit Street, Boston, MA 02114. E-mail marielle@crosbie.com

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Circulation is available at http://www.circulationaha.org
To identify the specific role of NOS3 (endothelial NOS) in ventricular remodeling of the noninfarcted myocardium after MI, we compared the changes in LV structure and function in wild-type (WT) and congenitally NOS3-deficient (NOS3–/–) mice after permanent occlusion of the left anterior descending coronary artery (LAD). Furthermore, we investigated the mechanisms by which NOS3 influences ventricular remodeling.

**Methods**

**Experimental Animals**
After approval by the Massachusetts General Hospital Subcommittee on Research Animal Care, we studied 2- to 4-month-old SV129/B6F1 WT mice and NOS3–/– mice with a SV129 and C57BL/6 background. In supplemental studies, NOS3–/– mice, backcrossed 10 generations onto a C57BL/6 background, and WT C57BL/6 mice were studied.

**Experimental Protocol**
MI was produced by ligation of the LAD, as previously described. In additional experiments, NOS3–/– mice were treated with hydralazine (250 mg per liter of drinking water). Hydralazine was started 2 weeks before MI and continued until death. Mice were weighed before and 28 days after MI.

**Serial Echocardiographic Measurements**
Echocardiograms were obtained before and 2, 15, and 28 days after MI, as described previously. Measurements were made by an observer who was blinded to the experimental group (M.S.-C.). In additional studies, echocardiograms were obtained in 5 NOS3–/– mice 28 days after MI, before and 10 minutes after the administration of hydralazine (0.1 mg/kg through the carotid artery). LV end-diastolic volume, end-systolic volume, and ejection fraction (LVEF) were calculated using 3 paraserial short-axis views and the parasternal long-axis view.

**Invasive Hemodynamics**
Hemodynamic measurements were obtained 28 days after MI using a 1.4-French high-fidelity Millar pressure catheter, as described previously.

**Noninvasive Blood Pressure Measurements**
After a fasting period of 1 week, blood pressure and heart rate were measured noninvasively in awake mice (5 WT, 5 NOS3–/–, and 5 NOS3–/+ mice treated with hydralazine) using a tail-cuff recording device (Visitech BP-2000, Visitech Systems).

**Histological Analysis**
The left ventricle was weighed, frozen, and cut along the short axis into 10-μm-thick slices obtained at 1 mm intervals.

**Infarction Size**
After staining with hematoxylin-eosin, the infarcted circumference was traced at each level. Total infarct size was measured by a modification of the method of Pfeffer et al.

**Capillary Density**
Endothelial cells were detected with an antibody directed against platelet/endothelial cell adhesion molecule-1 (Santa Cruz Biotechnology), as described previously. The number of capillaries per square millimeter was counted in regions with transversely-sectioned myocytes in the border zone of the MI and in remote myocardium. Three fields of 100 μm x 100 μm for the border zone and 200 μm x 200 μm for the remote myocardium were analyzed for each mouse at a magnification of 200×. Additional sections were counterstained with hematoxylin, and the number of myocyte nuclei per field was counted.

**Results**

**Serial Echocardiographic Measurements**
LV dimensions and function, examined using echocardiography, were similar in WT and NOS3–/– mice before MI (Table 1). Two days after MI, fractional shortening (FS) and LVEF declined similarly in both strains. Thereafter, both strains of mice demonstrated progressive LV dilatation (P<0.05 for LV end-diastolic volume). However, LV enlargement was greater in NOS3–/– than in WT mice (Table 1 and Figure 1). In WT mice, FS and LVEF did not differ between days 2 and 28 after MI, whereas in NOS3–/– mice, FS and LVEF decreased (P<0.05; Table 1). Parasternal long-axis views of a WT

**TABLE 1. Echocardiogram Analysis**

<table>
<thead>
<tr>
<th></th>
<th>Before MI</th>
<th>Day 2</th>
<th>Day 15</th>
<th>Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LVID_{es}, mm</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>3.3±0.1</td>
<td>3.6±0.1*</td>
<td>3.7±0.1*</td>
<td>3.7±0.1*</td>
</tr>
<tr>
<td>NOS3−/−</td>
<td>3.4±0.1</td>
<td>3.6±0.1</td>
<td>4.2±0.1†</td>
<td>4.4±0.1††</td>
</tr>
<tr>
<td>WT HLD</td>
<td>3.5±0.1</td>
<td>3.7±0.1</td>
<td>4.2±0.2*</td>
<td>4.5±0.2††</td>
</tr>
<tr>
<td><strong>FS, %</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>58±2</td>
<td>38±2</td>
<td>47±2†</td>
<td>44±2*</td>
</tr>
<tr>
<td>NOS3−/−</td>
<td>57±3</td>
<td>38±3†</td>
<td>39±2*</td>
<td>32±3††</td>
</tr>
<tr>
<td>NOS3−/− HLD</td>
<td>58±1</td>
<td>43±3*</td>
<td>42±3*</td>
<td>39±3*</td>
</tr>
<tr>
<td><strong>LVEDV, μL</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>31±2</td>
<td>34±2</td>
<td>38±2†</td>
<td>42±3†</td>
</tr>
<tr>
<td>NOS3−/−</td>
<td>29±2</td>
<td>34±2</td>
<td>55±5†</td>
<td>69±7††</td>
</tr>
<tr>
<td>NOS3−/− HLD</td>
<td>33±1</td>
<td>39±2†</td>
<td>60±6†</td>
<td>73±7††</td>
</tr>
<tr>
<td><strong>LVEF, %</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>62±2</td>
<td>40±3†</td>
<td>44±2*</td>
<td>46±2*</td>
</tr>
<tr>
<td>NOS3−/−</td>
<td>60±1</td>
<td>40±3†</td>
<td>37±3*</td>
<td>32±2††</td>
</tr>
<tr>
<td>NOS3−/− HLD</td>
<td>59±3</td>
<td>38±3†</td>
<td>40±3*</td>
<td>36±3*</td>
</tr>
<tr>
<td><strong>HR, bpm</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>416±14</td>
<td>335±38</td>
<td>434±24</td>
<td>443±28</td>
</tr>
<tr>
<td>NOS3−/−</td>
<td>450±26</td>
<td>355±47</td>
<td>472±23</td>
<td>481±15</td>
</tr>
<tr>
<td>NOS3−/− HLD</td>
<td>432±35</td>
<td>413±46</td>
<td>519±25</td>
<td>476±41</td>
</tr>
</tbody>
</table>

Values are mean±SEM. n=16 for WT mice, n=13 for NOS3−/− mice, and n=11 for NOS3−/− mice treated with hydralazine. LVID_{es} indicates left ventricular end-diastolic internal diameter; FS, fractional shortening; LVEDV, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; HR, heart rate; and NOS3−/− HLD, NOS3−/− mice treated with hydralazine.

*P<0.05 vs day 2; †P<0.05 vs day 2; ‡P<0.05 vs WT.

**Myocyte Size**
Myocyte width was measured in regions of myocardium remote from the MI on slides stained with platelet/endothelial cell adhesion molecule-1 and counterstained with hematoxylin. Twenty measurements were obtained at the level of the nucleus in longitudinally-sectioned myocytes.

**Statistical Analysis**
All data are expressed as mean±SEM. Differences between groups were determined using 2 way ANOVA for repeated measurements. When significant differences were detected by ANOVA, a post hoc Fisher’s test was employed. Analysis of survival after MI was performed with the log-rank test. P<0.05 was considered significant.
mouse and a NOS3–/– mouse at 2 and 28 days after MI are illustrated in Figure 2.

**Hemodynamic Measurements**

Twenty-eight days after MI, mean blood pressure was greater in NOS3 –/– than in WT mice (Table 2). Compared with WT mice, both the maximum rate of developed LV pressure and the maximum rate of pressure decay (dP/dt max and dP/dt min) were less in NOS3 –/– mice, but the time constant of isovolumic relaxation ($t_\infty$) was greater.

Invasive hemodynamic measurements and echocardiographic parameters after MI did not differ in SV129/B6F1 and C57BL/6 WT strains and did not differ in NOS3 –/– mice with a SV129/B6F1-hybrid background and NOS3 –/– mice backcrossed for 10 generations on a C57BL/6 background (data not shown).

**LV Weight and Infarction Size**

Seven days after MI, the infarcted zone represented the same fraction of the LV in WT and NOS3–/– mice (Table 3). This remained true at 28 days after MI (Table 3; $P<0.05$). This was not due to a loss of body weight in NOS3–/– mice, because body weight remained unchanged during the study duration in both strains (Table 3).

**Impact of Reduced Afterload on LV Remodeling in NOS3–/– Mice**

Blood pressure was measured noninvasively in noninfarcted, awake mice; it was greater in NOS3–/– than in WT mice (121±16 versus 103±19 mm Hg; $P<0.05$). Vasodilator treatment for 1 week significantly decreased blood pressure in NOS3–/– mice (96±10 mm Hg) to the level observed in WT mice. Hydralazine treatment did not prevent LV dilatation in NOS3–/– mice subjected to LAD occlusion (Table 1 and Figure 1). Hydralazine treatment attenuated the decrease in FS and LVEF (Table 1). This effect of hydralazine on LVEF was reproduced when the drug was administered in the short-term: in 5 NOS3–/– mice at 28 days after MI, the FS increased from 29±3% to 38±2% by 10 minutes after intra-arterial administration of hydralazine ($P<0.05$), without changes in end-diastolic LV internal diameter and LV end-diastolic volume. Long-term treatment with hydralazine did not improve LV contractile function in NOS3–/– mice at 28 days after MI (Table 2). Compared with WT mice, diastolic function remained impaired in hydralazine-treated NOS3–/– mice, as reflected by a greater value of $t_\infty$ and a lower dP/dt min ($P<0.05$ for both). The infarcted zone represented the same fraction of the LV in hydralazine-treated and untreated NOS3–/– mice, as well as WT mice, but the LV weight/body weight ratio was increased in both groups of NOS3–/– mice ($P<0.05$). There was no change of body weight in hydralazine-treated NOS3–/– mice after MI (Table 3).
LV Capillary Density
Before MI, LV capillary density did not differ in NOS3+/− and WT mice (Table 3). Twenty-eight days after MI, the density of capillaries in the remote myocardium decreased in NOS3+/− but not in WT mice (Table 3). Decreased capillary density was also present in the remote myocardium of hydralazine-treated NOS3+/− mice after MI (Table 3). The density of capillaries was decreased in the infarct border zone 28 days after MI in both strains, but to a lesser extent in WT than in NOS3−/− mice (2.2±0.1 versus 1.8±0.1 × 10³ capillaries/mm²; P<0.05), even after hydralazine treatment (1.9±0.1 × 10³ capillaries/mm²; P<0.05 versus WT).

Myocyte Size
Before MI, myocyte width in the remote myocardium did not differ in WT and NOS3−/− mice (Table 3). Twenty-eight days after LAD occlusion, myocyte width was increased in NOS3−/− mice (Figure 3, whether or not they were treated with hydralazine (Table 3). Myocyte width did not increase in WT mice after MI (Figure 3 and Table 3). The density of myocyte nuclei did not differ in WT and NOS3−/− mice at baseline (1080±59 and 950±33 nuclei/mm² in WT and NOS3−/− mice, respectively), and it decreased in both strains 28 days after MI. The decrease in the density of myocyte nuclei was more marked in NOS3−/− mice and NOS3−/− mice treated with hydralazine (458±39 and 492±36 nuclei/mm², respectively) than in WT mice (735±126 nuclei/mm²; P<0.05 versus both groups of NOS3−/− mice).

Survival After MI
The survival rate after MI was higher in WT than NOS3−/− mice (P<0.01; Figure 4). Five mice (25%) died in the WT group, and 18 mice (58%) died in the NOS3−/− group. The mortality rate at 28 days for NOS3−/− mice treated with hydralazine was 42% (P=0.08 versus WT).

Discussion
Ventricular remodeling is a critical determinant of the prognosis of patients after MI. Understanding the mechanisms regulating ventricular remodeling is likely to lead to new therapeutic approaches to decrease morbidity and mortality after MI. We examined a mouse MI model to investigate the role of a single NOS isoform, NOS3, in modulating ventricular function and remodeling after MI. NOS3 is known to be present in murine myocardium, and we observed that after MI, NOS3 expression was preserved in the noninfarcted myocardium for up to 28 days (data not shown). In NOS3−/− mice, occlusion of the LAD caused greater LV remodeling and more marked LV dysfunction than in WT mice.

Before MI, LV dimensions and systolic function did not differ between WT and NOS3−/− mice, confirming previous reports. Two days after LAD occlusion, LV dimensions increased and systolic function decreased to a similar extent in both strains of mice. However, 28 days after MI, echocardiographic measurements revealed that LV dilatation and systolic dysfunction were more marked in NOS3−/− than in WT mice. Invasive hemodynamics similarly demonstrated that LV systolic and diastolic functions were impaired to a greater extent in NOS3−/− mice than in WT mice. LV hypertrophy, as reflected by LV mass and myocyte width, was greater in NOS3−/− than in WT mice 28 days after MI. Importantly, NOS3 deficiency was associated with increased murine mortality after MI.

Because NOS3−/− mice are known to have an increased systemic blood pressure, we considered the possibility that the increased LV remodeling observed in NOS3−/− mice after MI was attributable to increased afterload. To test this hypothesis, the LV response to LAD occlusion was measured in NOS3−/− mice that were treated long-term with hydralazine. Although systemic blood pressure in hydralazine-treated NOS3−/− mice did not differ from that in WT mice, LV

![Figure 3](image-url) Myocyte width in the noninfarcted myocardium in WT and NOS3−/− mice 28 days after MI. Longitudinally sectioned myocytes are shown at a magnification of 600×. Myocyte nuclei (open arrow) are stained blue. Myocyte width at the level of the nucleus (shown between solid arrows) was increased in NOS3−/− compared with WT mice.
enlargement, systolic and diastolic dysfunction, and LV hypertrophy were more marked after MI in the former. The mortality after MI tended to be greater in hydralazine-treated NOS3–/– than in WT mice, but this difference did not reach statistical significance. After MI, both FS and LVEF were greater in hydralazine-treated NOS3–/– mice than in untreated NOS3–/– mice. This selective improvement was reproduced (P < 0.08).

Values are mean ± SEM. Body weight, LV weight/body weight (LV/BW), and MI size (when applicable) were measured in 5 mice of each strain before MI, 7 mice of each strain 7 days after MI, and 13 WT, 9 NOS3–/– and 11 hydralazine-treated NOS3–/– (NOS3 /H) mice 28 days after MI. Capillary density and myocyte width were measured in a subgroup of 5 WT mice before and 28 days after MI, 5 NOS3–/– mice before and 28 days after MI, and 5 hydralazine-treated NOS3–/– mice 28 days after MI. NA indicates not applicable.

Table 3. Pathological Findings

<table>
<thead>
<tr>
<th>Strain</th>
<th>Body Weight, g</th>
<th>MI Size, % Heart</th>
<th>LV/BW, mg/g</th>
<th>Capillary Density, ×10³/mm²</th>
<th>Myocyte Width, μm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before MI</td>
<td>Day 28</td>
<td>Day 7</td>
<td>Day 28</td>
<td>Before MI</td>
</tr>
<tr>
<td>WT</td>
<td>26 ± 1</td>
<td>27 ± 1</td>
<td>29 ± 9</td>
<td>23 ± 3</td>
<td>3.6 ± 0.1</td>
</tr>
<tr>
<td>NOS3–/–</td>
<td>28 ± 1</td>
<td>28 ± 1</td>
<td>27 ± 10</td>
<td>27 ± 3</td>
<td>3.7 ± 0.1</td>
</tr>
<tr>
<td>NOS3–/–H</td>
<td>24 ± 1</td>
<td>26 ± 1</td>
<td>NA</td>
<td>30 ± 4</td>
<td>NA</td>
</tr>
</tbody>
</table>

Figure 4. Survival of WT mice (solid line), NOS3–/– mice (hatched line), and NOS3–/– mice treated with hydralazine (dotted line). Life span is represented by the Kaplan-Meier method. Survival was decreased in NOS3–/– mice treated with hydralazine compared with WT mice (P < 0.01). There was a trend toward decreased survival in the NOS3–/– mice treated with hydralazine compared with WT mice (P = 0.08).
response to MI, and they suggest new strategies for preventing detrimental LV remodeling in patients after MI.

Acknowledgments

This study was supported by a Research Fellowship from the American Society of Echocardiography, a Scientist Development Grant from the American Heart Association (to M.S.-C.), United States Public Health Service grants HL-42397 (to W.M.Z.) and HL-57172 (to K.D.B.), and a grant from the Gesellschaft der Freunde des Deutschen Herzzentrum Berlin (to B.N.). Dr Bloch is an Established Investigator of the American Heart Association. The authors thank Dr S. Houser for his help with the pathological techniques, Dr J.D. Roberts, Jr, for his advice on immunohistochemistry, and Tatyana Taksir and Rebecca Nowak for their technical assistance.

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_Circulation_. 2001;104:1286-1291
doi: 10.1161/hc3601.094298

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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