Congenital Deficiency of Nitric Oxide Synthase 2 Protects Against Endotoxin-Induced Myocardial Dysfunction in Mice

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**Background**—Sepsis can be complicated by severe myocardial dysfunction and is associated with increased nitric oxide (NO) production by inducible NO synthase (NOS2). To investigate the role of NOS2 in endotoxin-induced myocardial dysfunction in vivo, we studied wild-type and NOS2-deficient mice.

**Methods and Results**—Serial echocardiographic parameters of myocardial function were measured before and at 4, 7, 16, and 24 hours after an endotoxin challenge. Seven hours after challenge with either endotoxin or saline, systemic and left ventricular pressures were measured, and the first derivative of left ventricular developed pressure (dP/dt), slope of the end-systolic pressure–dimension relationship (SlopeLVESPDP), and time constant of isovolumic relaxation (τ) were calculated. Endotoxin challenge in wild-type mice decreased left ventricular fractional shortening, velocity of circumferential shortening, dP/dt max, SlopeLVESPDP, and dP/dt min and increased time constant τ. Endotoxin-induced myocardial dysfunction was associated with increased ventricular NOS2 gene expression and cGMP concentrations. Seven hours after endotoxin challenge, NOS2-deficient mice had greater fractional shortening, dP/dt max, and SlopeLVESPDP than did endotoxin-challenged wild-type mice. Measures of diastolic function, dP/dt min and time constant τ, were preserved in endotoxin-challenged NOS2-deficient mice. After endotoxin challenge in wild-type mice, early (3-hour) inhibition of NOS2 with L-6-(1-iminoethyl)lysine hydrochloride prevented, whereas later (7-hour) inhibition could not reverse, endotoxin-induced myocardial dysfunction.

**Conclusions**—These results suggest that NOS2 is required for the development of systolic and diastolic dysfunction in murine sepsis. *(Circulation. 2000;102:1440-1446.)*

**Key Words:** echocardiography ▪ heart failure ▪ inflammation ▪ inhibitors ▪ sepsis

During sepsis, patients can have decreased left ventricular (LV) ejection fraction, ventricular dilation, increased cardiac output, and decreased systemic vascular resistance.1 Although myocardial depression of sepsis has been well described, its pathogenesis is incompletely understood. During infection, bacterial mediators, such as endotoxin, can activate a cascade of endogenous cytokines. Subsequently, endotoxin and cytokines stimulate the production of large amounts of nitric oxide (NO), which appears to be a mediator of cytokine-induced myocardial depression.2–5

Incubating rat myocytes with inflammatory mediators increases NO production and decreases their contractile response to β-adrenergic agents.2 Furthermore, inhibitors of NO synthesis reverse the negative inotropic effects of cytokines in rat and guinea pig myocytes in culture and in hamster papillary muscle.2–5 In dogs, intracoronary injection of interleukin-1β bound to microspheres produced myocardial depression and increased cardiac NO production and formation of peroxynitrite, a potentially toxic product of the reaction between NO and superoxide.6 Others, however, have questioned the role of NO in cytokine-induced myocardial depression. Inhibition of NO synthesis failed to improve myocardial depression in tumor necrosis factor–challenged dogs and cats.7,8 Thus, the role of NO in sepsis-induced myocardial depression is incompletely defined.

Another area of controversy is the source of increased NO during cytokine-induced myocardial depression. Several studies have suggested that NO synthase 3 is the source of NO in cytokine-induced myocardial impairment,3,4 whereas others have proposed that increased expression of the inducible isoform of NO synthase (NOS2) contributes to cytokine-induced myocardial dysfunction.2,9–11

In the present study, we investigated the role of NOS2 on endotoxin-induced myocardial depression in vivo. Cardiac function was studied in mice with and without a congenital deficiency of NOS2 before and after endotoxin challenge. We
also examined the ability of selective pharmacological inhibition of NOS2 to prevent or reverse endotoxin-induced myocardial dysfunction. After endotoxin challenge, wild-type mice developed profound systolic and diastolic myocardial impairment. In contrast, in NOS2-deficient mice, endotoxin-induced myocardial dysfunction was attenuated. Furthermore, specific pharmacological inhibition of NOS2 with L-N^6-(1-iminoethyl)lysine hydrochloride (L-NIL) in wild-type mice, when provided early after endotoxin challenge (3 hours), could prevent endotoxin-induced myocardial dysfunction, whereas when given later (7 hours), L-NIL could not reverse it. Our results suggest that NOS2 is an important mediator of murine endotoxin-induced myocardial dysfunction.

Methods

Experimental Subjects
After approval by the Massachusetts General Hospital Subcommittee on Research Animal Care, we studied SV129/B6F1 wild-type mice (F1-generation of the parental strains of NOS2-deficient mice, SV129 and C57BL/6, Jackson Laboratories, Bar Harbor, ME) and NOS2-deficient mice with an SV129 and C57BL/6 background, generously provided by Dr Carl Nathan (Cornell University Medical School, New York, NY) and by Dr John Mudgett (Merck Research Laboratories, Whitehouse Station, Rahway, NJ). In supplemental studies, NOS2-deficient mice, backcrossed 10 generations onto a C57BL/6 background (C57BL/6-Nos2^tm^lauc), N10-backcross generation, Jackson Laboratories, Bar Harbor, ME) and wild-type C57BL/6 mice were studied. Mice were aged 2 to 5 months and weighed 18 to 30 g. Sex was matched among groups.

Experimental Protocol

Serial Echocardiographic Measurements
Echocardiographic measurements were obtained in wild-type (n=20) and NOS2-deficient (n=21) mice before and 4 and 7 hours after endotoxin challenge. In a subset of wild-type (n=6 at 16 hours and n=4 at 24 hours) and NOS2-deficient (n=6) mice, echocardiographic measurements were obtained at 16 and 24 hours after endotoxin challenge. At time 0, *Escherichia coli* 0111:B4 endotoxin (50 mg/kg, DIFCO Laboratories) was injected intraperitoneally.

Invasive Hemodynamics
Invasive measurements of systemic and LV pressures were obtained 7 hours after saline or endotoxin challenge in wild-type (n=11 and n=9, respectively) and NOS2-deficient (n=12 and n=10, respectively) mice. Additionally, C57BL/6 wild-type mice and C57BL/6-Nos2^tm^lauc mice were studied 7 hours after saline or endotoxin challenge (n=5 for each group).

L-NIL Treatment
Wild-type mice were injected intraperitoneally with L-NIL (5 mg/kg, A.G. Scientific), a selective inhibitor of NOS2, either early (3 hours, n=6) or late (7 hours, n=6) after endotoxin challenge. Serial echocardiograms were performed at 4 and 7 hours after saline or endotoxin challenge. Invasive hemodynamics were measured 7 hours (4 hours after L-NIL administration) after saline (n=4) or endotoxin (n=5) challenge. In a subset of wild-type mice, invasive hemodynamics were measured 7 hours after saline (n=4) or endotoxin (n=8) challenge, and measurements were repeated 0.5 hours after administration of L-NIL at 7 hours.

Serial Echocardiographic Measurements
Echocardiography was performed by using a 13-MHz ultrasound probe (Sequoia, Acuson) in sedated mice (ketamine, 0.05 mg/g IP) as previously described. LV end-diastolic diameter, LV end-systolic diameter, heart rate, and ejection time were measured on M-mode echocardiograms. Fractional shortening (FS), ejection time, and velocity of circumferential shortening corrected for heart rate (V_cfc) were calculated as previously described.

Invasive Hemodynamics
Mice were anesthetized with ketamine (0.1 mg/g), xylazine (0.01 mg/g), and pancuronium (0.002 mg/g) intraperitoneally. Tracheostomy, arterial catheterization, and mechanical ventilation were performed as previously described. A 1.4F high-fidelity pressure catheter (Millar Instruments) was advanced into the LV via the carotid artery. The first derivative of the developed LV pressure (dP/dt) was calculated by differentiation of the digitized analog LV pressure tracing (Windaq, Dataq Instruments). The time constant of LV isovolumic relaxation (τ) was calculated by using the method of Weiss et al. LV end-systolic internal diameter and LV systolic pressure were recorded simultaneously by using echocardiography and micromanometer measurements, as described by Williams et al. Reductions in end-systolic internal diameter and systolic pressure were produced by transient mechanical occlusion of the inferior vena cava through a small laparotomy. Five to 6 end-systolic pressure–dimension points were generated in each animal, a regression line was determined, and slope and intercept were calculated.

Ventricular NOS2 mRNA
Ventricular mRNA was extracted by the guanidine isothiocyanate–cesium chloride method. RNA (10 μg) was fractionated in formaldehyde agarose gels and transferred to nylon membranes. Membranes were hybridized initially with a 3P-labeled 0.3-kb mouse NOS2 cDNA probe (nucleotides 3101 to 3552) and subsequently with a 15-fold excess of a 3P-labeled oligonucleotide complementary to rat 18S RNA.

Ventricular cGMP Concentrations
Ventricles were homogenized with 10% trichloroacetic acid and centrifuged. Supernatants were extracted with water-saturated ether, and cGMP concentrations were measured by use of a radioimmunoassay (Biomedical Technologies Inc). Trichloroacetic acid–precipitable protein was quantified by a Bradford assay. Tissue cGMP levels are expressed as picomoles of cGMP per milligram trichloroacetic acid–precipitated protein.

Ventricular Nitrotyrosine Immunohistochemistry
Cardiac tissue, cut at the midventricular level, was frozen in 2-methylbutane chilled with liquid nitrogen. Sections (10 μm) were reacted with a rabbit polyclonal anti-nitrotyrosine antibody (Upstate Biotechnology). Bound antibody was detected with the use of goat anti-rabbit IgG antibody linked to horseradish peroxidase. As a negative control, sections were incubated with 2% BSA in PBS instead of the primary antibody. As a positive control, sections were reacted with 10 mmol/L peroxynitrite (Upstate Biotechnology) before reaction with the primary antibody.

Statistical Analysis
All data are expressed as mean±SEM. Differences between groups were determined by ANOVA for repeated measurements. When significant differences were detected by ANOVA, a post hoc Fisher test was used. A value of P<0.05 indicated a significant difference.

Results

Baseline Studies and Clinical Effects of Endotoxin
Baseline studies of wild-type and NOS2-deficient mice showed similar mean values for all echocardiographic and invasive hemodynamic measurements (Figures 1 and Tables 1 and 2). The changes in echocardiographic and invasive hemodynamics produced by endotoxin were similar in SV129B6F1 and C57BL/6 wild-type strains and in NOS2-deficient mice with an SV129/B6F1-hybrid background and
NOS2-deficient mice backcrossed for 10 generations on a C57BL/6 background (data not shown). After endotoxin challenge, animals manifested weakness, lethargy, piloerection, and diarrhea. At 24 hours after endotoxin challenge, mortality was similar between wild-type (10 [62%] of 16) and NOS2-deficient (6 [40%] of 15) mice.

**Serial Echocardiography in Sedated Mice**

To determine the time course of endotoxin-induced myocardial dysfunction, echocardiography was performed serially. After endotoxin challenge, FS and V\textsubscript{e}/c decreased in both wild-type and NOS2-deficient mice. Endotoxin-induced cardiac dysfunction was evident at 4 hours, pronounced at 7 hours, and gradually returned to near baseline values at 24 hours after challenge ($P<0.05$ at 4, 7, and 16 hours; Table 1 and Figure 1). However, 4 and 7 hours after endotoxin challenge, NOS2-deficient mice had a greater FS than did wild-type mice (52±2% versus 44±3% at 4 hours and 39±3% versus 26±2% at 7 hours, both $P<0.05$; Table 1 and Figure 1). In addition, 4 and 7 hours after endotoxin challenge, V\textsubscript{e}/c was greater in NOS2-deficient mice than in wild-type mice (3.4±0.2 versus 2.8±0.2 circumferences per second at 4 hours and 2.3±0.2 versus 1.6±0.2 circumferences per second at 7 hours, $P<0.05$; Table 1).

After endotoxin challenge, wild-type and NOS2-deficient mice had decreases in heart rate and increases in ejection time that were evident at 7 hours, more pronounced at 16 hours, and returned toward baseline by 24 hours ($P<0.05$, Table 1).

**Invasive Hemodynamics and Echocardiography in Anesthetized Mice**

To further characterize endotoxin-induced changes in myocardial function, we measured systemic and LV pressures with simultaneous M-mode echocardiography to generate heart rate–independent and load-independent parameters of cardiac function. Seven hours after challenge, FS, dP/dt\textsubscript{max}, and dP/dt\textsubscript{min} were markedly decreased in endotoxin-treated wild-type compared with saline-treated wild-type mice (all $P<0.05$, Table 2 and Figure 2). Additionally, 7 hours after challenge, endotoxin-treated wild-type mice had decreased mean systemic arterial pressure ($P_{SA}$), LV end-systolic pressure ($P_{LVES}$), and increased LV end-diastolic pressure ($P_{LVED}$) compared with saline-treated wild-type mice ($P<0.05$, Table 1).

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**TABLE 1. Serial Echocardiographic Measurements Before and After Endotoxin Challenge in Sedated Wild-Type and NOS2-Deficient Mice**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>4 h</th>
<th>7 h</th>
<th>16 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, bpm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Wild-type</td>
<td>449±11</td>
<td>464±13</td>
<td>434±12</td>
<td>292±12*</td>
<td>306±19*</td>
</tr>
<tr>
<td>NOS2-deficient</td>
<td>454±18</td>
<td>495±15</td>
<td>451±12</td>
<td>326±20*</td>
<td>365±21*</td>
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<td>LVID(ED), mm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild-type</td>
<td>3.3±0.1</td>
<td>3.5±0.2</td>
<td>3.7±0.2</td>
<td>3.5±0.3</td>
<td>3.2±0.3</td>
</tr>
<tr>
<td>NOS2-deficient</td>
<td>3.1±0.1</td>
<td>3.1±0.1</td>
<td>3.6±0.1</td>
<td>3.5±0.7</td>
<td>3.4±0.5</td>
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<tr>
<td>FS, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild-type</td>
<td>55±2</td>
<td>44±3*</td>
<td>26±2*</td>
<td>44±4*</td>
<td>45±7*</td>
</tr>
<tr>
<td>NOS2-deficient</td>
<td>59±1</td>
<td>52±2*†</td>
<td>39±3*†</td>
<td>39±2*†</td>
<td>40±4*†</td>
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<td>Ejection time, ms</td>
<td></td>
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<td></td>
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<tr>
<td>Wild-type</td>
<td>57±2</td>
<td>57±1</td>
<td>69±3</td>
<td>81±5*</td>
<td>74±9*</td>
</tr>
<tr>
<td>NOS2 deficient</td>
<td>54±2</td>
<td>55±2</td>
<td>66±3</td>
<td>93±7*</td>
<td>69±11*</td>
</tr>
<tr>
<td>V\textsubscript{e}/c, circ/s</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild-type</td>
<td>3.6±0.2</td>
<td>2.8±0.2*</td>
<td>1.6±0.2*</td>
<td>2.6±0.3*</td>
<td>2.7±0.6*</td>
</tr>
<tr>
<td>NOS2-deficient</td>
<td>4.1±0.2</td>
<td>3.4±0.2†</td>
<td>2.3±0.2†</td>
<td>1.9±0.2*</td>
<td>2.2±0.3*</td>
</tr>
</tbody>
</table>

Values are mean±SEM. HR indicates heart rate; LVID(ED), LV end-diastolic diameter; circ/s, circumferences per second. ANOVA for repeated measures with Fisher post hoc comparison was used.

* $P<0.05$ vs baseline; † $P<0.05$ vs wild type.
2). In contrast, dP/dt_{max}, dP/dt_{min}, P_{SA}, P_{LVES}, and P_{LVED} did not differ in NOS2-deficient mice challenged with endotoxin or saline. Seven hours after challenge, endotoxin-challenged NOS2-deficient mice had lower FS than did saline-challenged NOS2-deficient mice (42±3% versus 49±1%, P<0.05). However, 7 hours after endotoxin, NOS2-deficient mice compared with wild-type mice had a greater FS (42±3% versus 32±4%) and a higher dP/dt_{max} and dP/dt_{min} (all P<0.05, Table 2 and Figure 2).

The slope of the LV end-systolic pressure–diameter relationship (Slope_{LVESPD}), a load-independent measure of systolic function, was shifted downward in endotoxin-challenged (79±22 mm Hg/mm) compared with saline-challenged (144±9 mm Hg/mm) wild-type mice (P<0.01, Figure 3A). In contrast, the Slope_{LVESPD} did not differ in NOS2-deficient mice challenged with endotoxin (113±11 mm Hg/mm) or saline (136±7 mm Hg/mm, Figure 3B). After endotoxin challenge, the Slope_{LVESPD} was higher in NOS2-deficient mice than in wild-type mice (113±11 versus 79±22 mm Hg/mm, P<0.05; Figure 3A and 3B).

The heart rate–independent and load-independent measurement of diastolic function, time constant τ, was similar in wild-type and NOS2-deficient mice after saline administration. However, 7 hours after endotoxin challenge, the τ value was markedly impaired in wild-type mice but not in NOS2-deficient mice treated with endotoxin (P<0.05, Table 2).

### Inhibition of NOS2 with L-NIL
To determine whether pharmacological inhibition of NOS2 alters cardiac function, wild-type mice were treated with L-NIL. In saline-treated wild-type mice, L-NIL when administered early (at 3 hours) or late (at 7 hours) did not alter myocardial function or hemodynamics, as assessed by echocardiography or invasive hemodynamic measurements (data not shown). However, when L-NIL was given 3 hours after endotoxin challenge, FS and V_{ch} decreased less in L-NIL-treated wild-type mice than in untreated mice (P<0.05 at 4 hours and 7 hours after endotoxin challenge, Table 1 and Figure 1). In wild-type mice treated with L-NIL at 3 hours and studied 7 hours after endotoxin challenge, there were no changes in P_{SA}, P_{LVES}, P_{LVED}, dP/dt_{max}, dP/dt_{min}, Slope_{LVESPD}, and time constant τ compared with those values in saline-treated wild-type mice (Table 2 and Figure 3C). However, 7 hours after endotoxin challenge, L-NIL–treated (at 3 hours) wild-type mice had a higher dP/dt_{max}, dP/dt_{min}, and Slope_{LVESPD} and lower τ values than did endotoxin-challenged wild-type mice not treated with L-NIL (all P<0.05, Table 2 and Figure 3A and 3C).

To determine whether inhibition of NOS2 activity reverses endotoxin-induced cardiac dysfunction once it is established, echocardiograms and invasive hemodynamics were obtained in wild-type mice 7 hours after endotoxin challenge and again 30 minutes after L-NIL administration. When administered late (7 hours), L-NIL did not alter hemodynamic and echocardiographic parameters (data not shown).

### Ventricular NOS2 Gene Expression and cGMP Concentrations
To examine whether endotoxin-induced myocardial dysfunction in wild-type mice was associated with increases in
ventricular NOS2 expression, we measured cardiac NOS2 mRNA and cGMP concentrations. After saline challenge (control, Figure 4A), ventricles of wild-type mice had undetectable levels of NOS2 mRNA. In contrast, NOS2 gene expression was markedly increased in mice challenged with endotoxin compared with saline-challenged mice. Ventricular cGMP levels were increased in a time-dependent manner in endotoxin-challenged wild-type mice compared with saline-challenged mice (Figure 4B). Treatment with L-NIL 3 hours after endotoxin prevented the endotoxin-induced increase in ventricular cGMP levels.

**Figure 3.** $L_{ves}$–LV end-systolic diameter relationship measured 7 hours after saline (dashed line) or endotoxin (solid line) challenge in NOS2+/+ mice (A), NOS2−/− mice (B) and L-NIL–treated (at 3 hours) NOS2+/+ mice (C). Slopes were generated by reducing LV end-systolic diameter by transient occlusion of inferior vena cava (in panel A, slopes differ comparing endotoxin versus saline challenge [P<0.05]).

**Figure 4.** A, Endotoxin challenge increased ventricular NOS2 gene expression in wild-type mice. RNA extracted from ventricles of saline-treated wild-type mice (control) and wild-type mice at 4 and 7 hours after endotoxin challenge was fractionated on formaldehyde-agarose gels, transferred to nylon membranes, and hybridized with a mouse NOS2 cDNA probe and with an oligonucleotide complementary to rat 18S RNA. B, Changes in ventricular cGMP concentrations after saline (open bar and gray bar) or endotoxin (filled bars) challenge and L-NIL treatment (at 3 hours, gray and hatched bars) in wild-type mice (n=4 for all groups). At 4 hours and 7 hours, cGMP levels increased in endotoxin-challenged compared with saline-challenged mice (*P<0.01 vs saline; ‡P<0.05 vs 4 hours). Administration of L-NIL had no effect on cGMP levels after saline challenge (P=NS); however, it prevented cGMP increases in endotoxin-challenged mice (†P<0.05).

**Effect of Endotoxin on Ventricular Nitrotyrosine Immunoreactivity**

To determine whether endotoxin-induced myocardial dysfunction in wild-type mice was associated with increased formation of peroxynitrite, nitrotyrosine immunoreactivity in ventricular sections of wild-type mice 7 hours after endotoxin or saline challenge was assessed. No increase in ventricular nitrotyrosine immunoreactivity was detected in endotoxin- or saline-challenged wild-type mice.

**Discussion**

Despite evidence that NO produced in response to cytokines may mediate decreases in cardiac contractility during sepsis or endotoxemia, there are no data firmly establishing a role for NOS2 in the pathogenesis of endotoxin-induced myocardial impairment. Most studies implicating NO in endotoxin- or cytokine-induced myocardial depression have been performed in vitro and have used NO synthase inhibitors that are not isoform specific.2–5,11,17,18 Unfortunately, these agents can inhibit all 3 NO synthase isoforms and may affect coronary perfusion and heart rate and produce a series of side effects (neurotoxicity, hypertension, or hepatotoxicity).7,19,20 In the present study, we took advantage of mice congenitally deficient in the gene encoding NOS2, thereby avoiding the incomplete selectivity of pharmacological inhibitors of NOS.
Using congenitally NOS2-deficient mice, we demonstrate a clear role for NOS2 in endotoxin-induced myocardial dysfunction.

After endotoxin challenge, wild-type mice developed decreases in FS and V_{cfc} that were pronounced at 7 hours and improved by 24 hours. However, endotoxin-induced decreases in FS and V_{cfc} were less marked in NOS2-deficient mice than in wild-type mice (Table 1 and Figure 1). Seven hours after endotoxin challenge, wild-type mice exhibited profound systolic dysfunction manifested by a decrease in dP/dt_{max} (Table 2 and Figure 2) and a downward shift of Slope_{LVESPD} (Figure 3A) as well as diastolic impairment, indicated by decreases of dP/dt_{min} and increases in time constant \( \tau \) (Table 2). In contrast, NOS2-deficient mice were protected from endotoxin-induced changes in systolic and diastolic function (Table 2 and Figure 3A and 3B). Our observations differ from those of Nicholson et al.,21 who, using echocardiography and lower doses of endotoxin in mice, observed that endotoxin induced an increase in cardiac output in wild-type mice but not in NOS2-deficient mice. Possibly, differences in the models and measurements obtained, as well as the higher dose of endotoxin used in the present study causing profound myocardial dysfunction, permitted us to identify differences between wild-type and NOS2-deficient mice.21

It is of interest that in NOS2-deficient mice 7 hours after endotoxin administration, echocardiographic measurements revealed a modestly decreased FS and V_{cfc} but that heart rate–independent and load-independent hemodynamic measurements showed no evidence of systolic cardiac dysfunction (dP/dt_{max} and Slope_{LVESPD}). It is unlikely that differences in the echocardiographic and invasive hemodynamic parameters are attributable to differences in the anesthetics used, inasmuch as echocardiographic measurements in sedated animals and in anesthetized animals were similar 7 hours after saline or endotoxin challenge. FS and V_{cfc} depend on changes in heart rate, preload and afterload, as well as on myocardial contractility. In contrast, dP/dt_{max} and Slope_{LVESPD} are relatively independent of heart rate and load conditions. Therefore, the differences in echocardiographic and invasive hemodynamic parameters of cardiac function measured in NOS2-deficient mice challenged with endotoxin might reflect the different dependence of these measurements on preload and afterload conditions.

The inhibition of NOS2 with L-NIL at 3 hours after endotoxin challenge prevented endotoxin-induced decreases in FS, dP/dt_{max} and the downward shift of Slope_{LVESPD}, as well as decreases of dP/dt_{min} and increases in time constant \( \tau \) (Figures 1 through 3). Although early inhibition of NOS2 prevented subsequent endotoxin-induced ventricular dysfunction, L-NIL did not reverse established myocardial dysfunction when administered 7 hours after endotoxin challenge. These findings are in agreement with reports that S-\textit{methylisothiourea} (a relatively selective inhibitor of NOS2) partially prevented endotoxin-induced myocardial dysfunction in rats when administered at the time of endotoxin challenge but not when given after myocardial dysfunction had been established.22 Taken together, these findings suggest that inhibition of NOS2 activity cannot reverse endotoxin-induced ventricular dysfunction after it is established.

Our findings strongly suggest that a product of NOS2 contributes to endotoxin-induced myocardial dysfunction. NOS2 produces large quantities of NO, which reacts with various targets in myocardial cells. One such target, soluble guanylate cyclase, augments intracellular levels of the second messenger cGMP. Ventricular cGMP concentrations increased in wild-type mice challenged with endotoxin (Figure 4B). Moreover, treatment with L-NIL at 3 hours after endotoxin prevented the accumulation of cGMP (Figure 4B) and the development of myocardial dysfunction. Therefore, it is conceivable that expression of NOS2 produces myocardial dysfunction via cGMP-dependent mechanisms, including reduced myofilament sensitivity to calcium via activation of cGMP-dependent protein kinase, decreased activity of L-type calcium channels, and stimulation of cGMP-sensitive phosphodiesterases, thereby increasing cAMP degradation.23–25

Superoxide, also a product of NOS2, could have contributed to endotoxin-induced cardiac dysfunction, inasmuch as it has been shown to have a role in interleukin-1\(\beta\)-induced myocardial dysfunction.6 In addition, NO can also interact with superoxide and generate peroxynitrite. Peroxynitrite can react with a variety of proteins participating in contractile function, leading to the formation of nitrotyrosine.26 In the present study, nitrotyrosine was not detected in the hearts of wild-type mice 7 hours after endotoxin. These findings differ from those of Oyama et al.,6 who reported that 2 days after intracoronary administration of interleukin-1\(\beta\) in dogs, myocardial nitrotyrosine levels, assessed by high-performance liquid chromatography, correlated with the degree of ventricular dysfunction. Possible explanations for these differing findings are that high-performance liquid chromatography is more sensitive than immunohistochemistry or that prolonged exposure to endotoxin and/or cytokines is needed before detectable myocardial nitrotyrosine accumulates.

Interestingly, although genetic deficiency and pharmacological inhibition of NOS2 protected mice against endotoxin-induced myocardial dysfunction, they did not alter endotoxin-induced mortality. Our results are similar to earlier reports demonstrating that NOS2-deficient mice are not protected from endotoxin-induced death.27 These findings suggest that endotoxin challenge causes mortality in these mice by mechanisms independent of myocardial dysfunction and that other organ systems may be more vulnerable to endotoxin injury.

In summary, using mice congenitally lacking the gene for NOS2, we have demonstrated the role of NOS2 in endotoxin-induced myocardial dysfunction. Selective pharmacological inhibition of NOS2 activity with L-NIL prevented, but did not reverse, endotoxin-induced myocardial dysfunction. These findings suggest a potential strategy for the pharmacological prevention of sepsis-induced myocardial dysfunction.

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