Nitric Oxide Modulates Myocardial Oxygen Consumption in the Failing Heart

YingJie Chen, MD, PhD; Jay H. Traverse, MD; Ruisheng Du, PhD; MingXiao Hou, MD, PhD; Robert J. Bache, MD

Background—Endogenous nitric oxide (NO) has been reported to inhibit oxygen consumption in the normal heart, so that nonselective inhibition of NO synthase (NOS) caused an increase of myocardial oxygen consumption (MVO₂). Although endothelial NOS responses are depressed in congestive heart failure (CHF), inducible NOS (iNOS) may be expressed in failing myocardium.

Methods and Results—This study tested the hypothesis that NOS inhibition would increase MVO₂ in the failing heart. CHF was produced in dogs by use of the rapid ventricular pacing model. In comparison with normal values, animals with CHF had reduced coronary blood flow and MVO₂ at rest, with a blunted response to treadmill exercise. Selective iNOS inhibition with S-methylisothiourea (1.5 mg/kg IC) increased left ventricular systolic pressure and left ventricular dP/dt and caused an increase in MVO₂ at rest and during exercise (P<0.05), with a parallel upward shift in the relationship between MVO₂ and rate-pressure product. In contrast, S-methylisothiourea had no effect on MVO₂ or coronary flow in normal animals, although nonselective NOS inhibition with NG-nitro-L-arginine did cause an increase of MVO₂ in normal and in CHF animals.

Conclusions—The results indicate that endogenous NO can modulate MVO₂ in failing hearts, but unlike the normal heart, this NO appears to be produced, at least in part, by iNOS. (Circulation. 2002;106:273-279.)

Key Words: metabolism ■ myocardium ■ blood flow ■ nitric oxide

Nitric oxide (NO) can modulate mitochondrial respiration by competitive inhibition of oxygen at cytochrome oxidase.¹ Several investigators have reported that blockade of NO production with nonselective NO synthase (NOS) inhibitors leads to significant increases of myocardial oxygen consumption (MVO₂) in the normal heart,²³ although others found no effect⁴ or a decrease in MVO₂.⁵ Three distinct NOS isoforms exist in mammalian cells: neuronal (nNOS), inducible (iNOS), and endothelial (eNOS) isoforms.⁶ nNOS and eNOS are constitutively expressed in many cell types, whereas iNOS is expressed in response to infection, inflammation, or cytokine activation. Circulating and tissue levels of cytokines, including tumor necrosis factor-α, interleukin-2, and interleukin-6, are elevated in patients with chronic heart failure,⁷⁻⁹ and iNOS expression has been found in cardiac myocytes of patients with dilated cardiomyopathy, myocarditis, and ischemic heart disease,¹⁰¹¹ suggesting that iNOS could be a source of NO in the failing heart. Consequently, this study was performed to determine whether selective iNOS inhibition with S-methylisothiourea (SMT) influences MVO₂ of the failing heart at rest or during exercise. The effect of SMT was compared with nonselective NOS inhibition with N⁶-nitro-L-arginine (L-NNA) to determine whether constitutive NOS can also modulate MVO₂ in congestive heart failure (CHF).

Methods

Studies were performed in 21 adult mongrel dogs weighing 20 to 26 kg and trained to run on a treadmill. All experiments were performed in accordance with the “Guiding Principles in the Care and Use of Laboratory Animals” as approved by the council of the American Physiological Society and with prior approval of the University of Minnesota Animal Care Committee.

Surgical Preparation

Animals were anesthetized with sodium pentobarbital (30 to 35 mg/kg), intubated, and ventilated with oxygen-enriched air. A left thoracotomy was performed, and polyvinyl chloride catheters (3.0-mm OD) was inserted into the ascending aorta and the left ventricle (LV). A solid-state micromanometer (Konigsberg Instruments) was inserted into the LV at the apex. A final catheter was introduced into the right atrial appendage and advanced through the coronary sinus until the tip could be palpated at the anterior interventricular vein to allow selective sampling of blood draining the myocardium perfused by the left anterior descending coronary artery (LAD).³ A Doppler velocity probe (Craig Hartley) was positioned on the LAD, and a silicone catheter (0.3-mm ID) was introduced into the LAD distal to the velocity probe. Catheters were tunneled to exit at the base of the neck; catheters were flushed daily...
to maintain patency. Postoperative analgesia was provided with butorphanol, 0.4 μg/kg SC every 4 to 6 hours.

Effect of SMT and L-NNA in the Normal Heart
Selective iNOS inhibition was studied in 8 normal dogs 10 to 14 days after surgery. Resting hemodynamics and coronary blood flow (CBF) were recorded, and 2 mL of blood was withdrawn from the aortic and coronary venous catheters for blood gas analysis. Subsequently, a 2-stage treadmill exercise protocol was begun (stage 1, 6.4 km/h at 0% grade; stage 2, 6.4 km/h at 10% grade). Each exercise stage was 3 minutes in duration; aortic and coronary venous blood samples were withdrawn during the last 30 seconds of each exercise stage. After a 10-minute rest period, the selective iNOS inhibitor SMT was infused into the coronary catheter in a dose of 1.5 mg/kg over a period of 10 minutes. Forty minutes after SMT administration, all measurements were repeated at rest and during exercise.

The effect of nonselective NOS inhibition was examined in 9 normal dogs (3 of which had also been studied with SMT on a different day). After control rest and exercise measurements had been obtained, L-NNA was infused in a dose of 1.5 mg/kg IC over a period of 10 minutes. Forty minutes after L-NNA, all measurements were repeated.

Degree of Blockade of Endothelium-Dependent Vasodilation
In 7 normal dogs, the effects of SMT and L-NNA on endothelium-dependent vasodilation were examined. The increases in CBF produced by acetylcholine (3.75 to 75 μg/min IC) were observed before and after SMT (1.5 mg/kg IC). On a separate day, the response of CBF to acetylcholine was examined in 5 dogs before and after L-NNA (1.5 mg/kg IC).

Production of CHF
CHF was produced by rapid ventricular pacing. After completion of baseline studies, the pacemaker was activated at a rate of 220 bpm; pacing was continued at that rate or adjusted upward to a maximum of 250 bpm on the basis of weekly assessments of hemodynamics 1 hour after deactivation of the pacemaker. CHF was considered to have developed when resting LV end-diastolic pressure (LVEDP) was >20 mm Hg or visual estimation of ejection fraction by 2-D echocardiography was <30%; the mean duration of pacing was 27±3 days.

Effect of SMT After Development of CHF
One hour after the pacemaker was deactivated, measurements were obtained at rest and during exercise at 3.2 km/h, 0% grade and 6.4 km/h, 0% grade. SMT, 1.5 mg/kg IC, was then infused over a period of 10 minutes, and 40 minutes later, rest and exercise measurements were repeated.

Effect of L-NNA After Development of CHF
To study the effect of nonselective NOS blockade in dogs with CHF, L-NNA (1.5 mg/kg IC) was administered to 5 dogs that had previously received SMT, and all measurements were repeated 40 minutes later.

Hemodynamic Measurements
LV pressure was measured with the micromanometer; the first derivative of LV pressure (dP/dt) was obtained via electrical differentiation. Coronary blood velocity was measured with a Doppler flowmeter (Craig Hartley). Data were recorded on an 8-channel recorder.

Myocardial Oxygen Consumption
Po₂, PCO₂, and pH were then measured with a blood gas analyzer (model 113, Instrumentation Laboratory). Hemoglobin was determined by the cyanmethemoglobin method. Hemoglobin oxygen saturation was calculated from the blood Po₂, pH, and temperature by use of the oxygen dissociation curve for canine blood. Blood O₂ content was computed as (hemoglobin × 1.34 × %O₂ saturation) + (0.0031 × Po₂). MVO₂ was calculated as the product of LAD blood flow and the aortic–coronary vein O₂ content difference.

Western Blot
Tissue homogenates of LV myocardium (160 μg) were separated on 8% SDS-PAGE and transferred onto nitrocellulose membrane, followed by routine Western blotting. Endothelial cells from aortas of normal dogs were used as a positive control for eNOS. Anti-iNOS and anti-eNOS antibodies were purchased from BD Transduction Laboratories and Santa Cruz Biotechnology, respectively.

Data Analysis
Hemodynamic variables were measured from the chart recordings. Coronary flow was computed from the Doppler shift as previously described. Statistical analysis was performed by use of 2-way (exercise level and treatment) ANOVA for repeated measures. Comparisons within groups were made by use of 1-way ANOVA followed by Scheffe’s post hoc test. Comparisons between groups were made with Student’s t test with the Bonferroni correction. Significance was accepted at P<0.05. Data are presented as mean±SEM.

Results
Effect of SMT or L-NNA in Normal Dogs
SMT caused no significant changes of aortic pressure, heart rate, LVEDP, or LV dP/dt at rest or during exercise and did not alter CBF or MVO₂ compared with control conditions. The relationships between CBF or MVO₂ and rate-pressure product were unchanged after SMT (Figures 1 and 2, left panels).

Nonselective NOS inhibition with L-NNA caused significant increases of aortic pressure, LV systolic pressure, and LV dP/dtmax, whereas resting coronary flow and MVO₂ were increased by 12% (P<0.05) and 18% (P<0.05), respectively (Table 1). L-NNA caused parallel upward shifts of the relationships between both CBF and MVO₂ with rate-pressure product. L-NNA also caused a significant decrease of coronary venous O₂ tension.

Effect of SMT in Animals With CHF
As shown in Table 2, CHF was associated with increases in resting heart rate and LVEDP and decreases in aortic pres-
sure, LV systolic pressure, LV dP/dt\text{max}, CBF, and MVO\textsubscript{2} (each P<0.05). In dogs with CHF, selective iNOS inhibition with SMT significantly increased aortic pressure, LV systolic pressure, LV dP/dt\text{max}, and rate-pressure product, whereas heart rate and LVEDP were unchanged. Moreover, SMT caused increases in CBF and MVO\textsubscript{2} at rest and during exercise in CHF dogs (P<0.05); this was principally an offset effect with an upward shift in the relationship between rate-pressure product and both CBF (Figure 1, right) and MVO\textsubscript{2} (Figure 2, right).

**Effect of L-NNA in Animals With CHF**

After completion of the SMT measurements, in 5 dogs with CHF, nonselective NOS inhibition with L-NNA was produced. In comparison with SMT, L-NNA caused no further change of LV systolic pressure, aortic pressure, or LV dP/dt\text{max} (Table 3). L-NNA tended to further increase MVO\textsubscript{2} and CBF (Table 3 and Figure 3), although this was not significant.

**Responses to Acetylcholine**

Intracoronary acetylcholine had no effect on heart rate or aortic pressure but caused dose-dependent increases of CBF. SMT had no significant effect on the increase in CBF produced by acetylcholine. In contrast, L-NNA caused 52% mean inhibition of the increase in coronary flow produced by acetylcholine (3.75 to 75 \textmu g/min, P<0.01).

**Expression of iNOS and eNOS**

Western blots of ventricular homogenates were performed in 6 CHF and 6 normal dogs (Figure 4). iNOS was almost undetectable in the normal dogs but was significantly increased in the failing hearts (1.26±0.01 versus 3.50±0.49, P<0.01). eNOS expression was not significantly different between the 2 groups (4.1±0.4 in CHF versus 5.8±0.4 in normal).

**Discussion**

The selective iNOS inhibitor SMT increased MVO\textsubscript{2} and CBF in dogs with CHF but not in normal animals, suggesting that NO produced by iNOS can modulate cardiac function in the failing heart. SMT also increased LV dP/dt\text{max} in dogs with CHF, suggesting an increase of myocardial contractility. The finding that the addition of the nonselective NOS inhibitor L-NNA tended to further increase MVO\textsubscript{2} suggests that NO produced by eNOS or nNOS might also play a role in the failing hearts.

**NO in the Normal Heart**

Nonselective NOS inhibition with L-NNA resulted in modest significant increases of CBF at rest and during exercise in the normal animals.\textsuperscript{12,13} This increase in CBF appeared to result from an increase of MVO\textsubscript{2} with secondary coronary vasodilation.\textsuperscript{14} Inasmuch as coronary sinus PO\textsubscript{2} decreased significantly after L-NNA, indicating increased myocardial oxygen extraction. NO can compete with oxygen at cytochrome oxidase,\textsuperscript{1} and nonselective NOS inhibition has been reported to increase both total body and skeletal muscle oxygen consumption in normal dogs.\textsuperscript{15,16} Conversely, stimulating endogenous NO production with bradykinin or administering an NO donor decreased oxygen consumption in perfused rat hearts, an effect not mediated by cGMP.\textsuperscript{17} In contrast to the above reports, in anesthetized open-chest dogs, blockade of

**TABLE 1. Effects of L-NNA in 9 Normal Dogs**

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>Exercise Stage 1, 6.4 km/h, 0%</th>
<th>Exercise Stage 2, 6.4 km/h, 10%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, bpm</td>
<td>Control</td>
<td>127±6.6</td>
<td>238±8.4*</td>
</tr>
<tr>
<td></td>
<td>L-NNA†</td>
<td>129±5.9</td>
<td>232±8.7*</td>
</tr>
<tr>
<td>Mean aortic pressure, mm Hg</td>
<td>Control</td>
<td>111±2.4</td>
<td>124±3.8*</td>
</tr>
<tr>
<td></td>
<td>L-NNA†</td>
<td>117±2.3†</td>
<td>130±3.6†</td>
</tr>
<tr>
<td>LV systolic pressure, mm Hg</td>
<td>Control</td>
<td>131±3.1</td>
<td>159±4.8*</td>
</tr>
<tr>
<td></td>
<td>L-NNA†</td>
<td>138±2.6†</td>
<td>162±5.0*</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td>Control</td>
<td>2340±160</td>
<td>5150±435*</td>
</tr>
<tr>
<td></td>
<td>L-NNA†</td>
<td>2380±157</td>
<td>5000±421*</td>
</tr>
<tr>
<td>CBF, mL/min</td>
<td>Control</td>
<td>43.4±4.4</td>
<td>74.8±5.9*</td>
</tr>
<tr>
<td></td>
<td>L-NNA†</td>
<td>48.6±3.2†</td>
<td>84.8±6.7†</td>
</tr>
<tr>
<td>CV-P\textsubscript{O2}, mm Hg</td>
<td>Control</td>
<td>19.8±0.5</td>
<td>15.0±0.4*</td>
</tr>
<tr>
<td></td>
<td>L-NNA†</td>
<td>16.7±1.5</td>
<td>12.7±0.7*</td>
</tr>
<tr>
<td>MVO\textsubscript{2}, mL/min</td>
<td>Control</td>
<td>4.9±0.4</td>
<td>11.3±0.9*</td>
</tr>
<tr>
<td></td>
<td>L-NNA†</td>
<td>5.8±0.2†</td>
<td>12.6±1.1†</td>
</tr>
</tbody>
</table>

CBF indicates coronary blood flow; CV-P\textsubscript{O2}, coronary venous oxygen tension.

All values are mean±SE.

\*P<0.05 vs rest; †P<0.05 vs control conditions by 2-way ANOVA.
NO production with Nω-nitro-arginine methyl ester failed to increase MVO₂,
production increases contractile performance. If basal cyto-
Production levels of cGMP, NO can enhance contractile performance by
increasing in the failing heart; the avid reaction of NO with
superoxide would be expected to decrease NO bioavailability.
Furthermore, a change in eNOS localization might cause a
functional alteration despite there being no change in protein
content. For example, although eNOS expression was de-
creased in the endothelium of aortas from rats with CHF,
eNOS expression in aortic smooth muscle was increased,
with no change in total aortic eNOS expression. Such a
change in localization might decrease endothelium-dependent
NO-mediated responses.

In the present study, inhibition of NO synthesis with either
SMT or L-NNA resulted in increases of LV dP/dt max in the
failing myocardium. Furthermore, we observed that nonselective
NOS inhibition increased MVO₂ in dogs with pacing-induced
CHF. This finding raises questions concerning the source of
NO in the animals with CHF and the mechanism for the
increased MVO₂ after blockade of NO synthesis. In the
present study, Western blotting showed no difference in
eNOS protein expression between myocardium from normal
and CHF hearts. Protein expression, however, does not
necessarily correlate with activity. Superoxide production is
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TABLE 2. Effects of SMT on Systemic and Coronary Hemodynamic Data at Rest
and During Graded Treadmill Exercise in 8 Dogs With CHF

<table>
<thead>
<tr>
<th></th>
<th>Before Pacing</th>
<th>After Pacing (Heart Failure)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rest (Normal)</td>
<td>Exercise Stage 1, 3.2 km/h, 0%</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>SMT</td>
<td>Control</td>
</tr>
<tr>
<td>Control</td>
<td>121±8.2</td>
<td>128±5.3</td>
</tr>
<tr>
<td>SMT</td>
<td>137±5.6</td>
<td>168±8.6*</td>
</tr>
<tr>
<td>Mean aortic pressure, mm Hg</td>
<td>Control</td>
<td>111±5.5</td>
</tr>
<tr>
<td>SMT</td>
<td>93±3.1†</td>
<td>100±3.7†</td>
</tr>
<tr>
<td>LV systolic pressure, mm Hg</td>
<td>Control</td>
<td>132±6.4</td>
</tr>
<tr>
<td>SMT</td>
<td>110±2.6†</td>
<td>119±4.2†</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td>Control</td>
<td>5.8±1.7</td>
</tr>
<tr>
<td>SMT</td>
<td>27.6±1.8</td>
<td>32.9±2.3*</td>
</tr>
<tr>
<td>LV dP/dt, mm Hg/s</td>
<td>Control</td>
<td>2516±202</td>
</tr>
<tr>
<td>SMT</td>
<td>1660±39†</td>
<td>2220±144†</td>
</tr>
<tr>
<td>CBF, mL/min</td>
<td>Control</td>
<td>42.0±3.1</td>
</tr>
<tr>
<td>SMT</td>
<td>44.3±8.2†</td>
<td>50.0±8.9†</td>
</tr>
<tr>
<td>CS·Po₂, mm Hg</td>
<td>Control</td>
<td>20.6±2.0</td>
</tr>
<tr>
<td>SMT</td>
<td>23.0±2.9</td>
<td>19.3±3.2</td>
</tr>
<tr>
<td>MVO₂, mL/min</td>
<td>Control</td>
<td>5.2±0.5</td>
</tr>
<tr>
<td>SMT</td>
<td>4.0±0.7†</td>
<td>5.4±0.9†</td>
</tr>
</tbody>
</table>

CBF indicates coronary blood flow; CS·Po₂, coronary venous oxygen tension. All values are
mean±SE.
*P<0.05 vs rest; †P<0.05 vs control conditions by 2-way ANOVA.

NO in CHF

CHF is associated with impaired endothelium-dependent
vasodilation. Moreover, bradykinin caused smaller
decreases of oxygen consumption in myocardial tissue slices
from CHF dogs compared with normal dogs, suggesting
depressed receptor-mediated endothelial NO production.
Administration of an NO donor did inhibit oxygen consump-
tion, indicating that NO can modulate MVO₂ in failing
myocardium. Furthermore, we observed that nonselective
NOS inhibition increased MVO₂ in dogs with pacing-induced
CHF. This finding raises questions concerning the source of
NO in the animals with CHF and the mechanism for the
increased MVO₂ after blockade of NO synthesis. In the
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eNOS protein expression between myocardium from normal
and CHF hearts. Protein expression, however, does not
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increased in the failing heart; the avid reaction of NO with
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eNOS expression in aortic smooth muscle was increased,
with no change in total aortic eNOS expression. Such a
change in localization might decrease endothelium-dependent
NO-mediated responses.

In the present study, inhibition of NO synthesis with either
SMT or L-NNA resulted in increases of LV dP/dtmax in the
dogs with CHF, suggesting an increase of contractility, but
not in normal dogs. It is possible that differences in basal
levels of cytosolic cGMP were responsible for the differing
responses between normal and CHF animals. With low basal
levels of cGMP, NO can enhance contractile performance by
inhibiting cAMP phosphodiesterase, thereby allowing cAMP
to accumulate. Conversely, high basal levels of cGMP
reduce contractility because of activation of cGMP-
dependent protein kinases; in this situation, blocking NO
production increases contractile performance. If basal cyto-
TABLE 3. Effects of SMT and SMT+L-NNA on Systemic and Coronary Hemodynamic Data at Rest and During Graded Treadmill Exercise in 5 Dogs With CHF

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>Exercise Stage 1, 3.2 km/h, 0%</th>
<th>Exercise Stage 2, 6.4 km/h, 0%</th>
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<tbody>
<tr>
<td>Heart rate, bpm</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>132±6.0</td>
<td>172±9.4*</td>
<td>189±10*</td>
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<tr>
<td>SMT</td>
<td>134±5.7</td>
<td>168±10*</td>
<td>195±12*</td>
</tr>
<tr>
<td>SMT+L-NNA</td>
<td>141±7.2</td>
<td>174±13*</td>
<td>196±12*</td>
</tr>
<tr>
<td>Mean aortic pressure, mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>81±1.2</td>
<td>89±2.9*</td>
<td>99±3.2*</td>
</tr>
<tr>
<td>SMT†</td>
<td>94±5.1</td>
<td>99±4.9*</td>
<td>106±5.1*</td>
</tr>
<tr>
<td>SMT+L-NNA†</td>
<td>99±5.0</td>
<td>102±6.3</td>
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<td>LV systolic pressure, mm Hg</td>
<td></td>
<td></td>
<td></td>
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<td>Control</td>
<td>101±1.4</td>
<td>114±4.9*</td>
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<td>SMT†</td>
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<td>Control</td>
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<td>31±3.0*</td>
<td>35±2.7*</td>
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<td>31±2.8</td>
<td>35±3.6*</td>
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<td>27±3.3</td>
<td>31±3.1</td>
<td>36±3.1*</td>
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<tr>
<td>CBF, mL/min</td>
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<td></td>
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<tr>
<td>Control</td>
<td>1450±80</td>
<td>2070±211*</td>
<td>2740±240*</td>
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<tr>
<td>SMT†</td>
<td>1650±45</td>
<td>2230±199*</td>
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<td>1760±113</td>
<td>2250±176*</td>
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<td>CS-P02, mm Hg</td>
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<tr>
<td>Control</td>
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<td>SMT</td>
<td>38.8±5.1</td>
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<td>SMT+L-NNA</td>
<td>41.1±4.7</td>
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<td>MVO2, mL/min</td>
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<td>21±2.2</td>
<td>17±2.9</td>
<td>15±2.1*</td>
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<td>SMT+L-NNA</td>
<td>19±2.8</td>
<td>15±3.8</td>
<td>14±3.4*</td>
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</table>

CBF indicates coronary blood flow; CS-P02, coronary venous oxygen tension. All values are mean±SEM. *P<0.05 vs rest; †P<0.05 vs control conditions by 2-way ANOVA.

solic cGMP levels in the cardiac myocytes were increased in the animals with CHF, then inhibition of NO production would be expected to increase contractile performance and therefore MVO2.

A second possible explanation for an increase of contractility in the dogs with CHF is that removal of an inhibitory effect of NO on mitochondrial respiration might allow ATP production to increase, thereby augmenting energy availability to the contractile apparatus. Tatsumi et al23 demonstrated that interleukin-1β increased NO production in cultured neonatal cardiac myocytes, and this was followed by a decline in cellular ATP concentration and myocyte contractility; these effects were blocked by Nω-monomethyl-L-arginine. In isolated perfused hearts, Kelm et al24 demonstrated that addition of NO to the coronary perfusate caused a decrease of LV developed pressure that was associated with reductions of myocardial ATP and the free-energy change of ATP hydrolysis that was paralleled by decreased MVO2. Washout of NO reversed the high-energy phosphate changes and returned MVO2 and contractile performance to baseline levels. The investigators concluded that NO depressed myocardial energy generation with a secondary decrease of contractile performance. It is possible that NO could similarly impair myocardial energy production in the failing heart.

A third possible explanation for an increase of myocardial contractility after NOS inhibition relates to a decrease in peroxynitrite formed as NO reacts with superoxide. In isolated working rat hearts, cytokine infusion caused a decline in...
contractile function that was associated with increased production of NO and peroxynitrite. The decreased contractile function was abrogated by NOS inhibition with L-NNa, the superoxide scavenger tiron, or the peroxynitrite decomposition catalyst 5,10,15,20-tetrakis-(4-sulfonatophenyl)parphyrinato-iron(III) (FeTPPS). Superoxide production is increased in the failing heart; the simultaneous augmentation of NO production would result in increased formation of peroxynitrite, which could depress contractility. A fourth possible mechanism for the increase in MVO₂ produced by NOS inhibition relates to the contribution of NO to the depressed responsiveness to β-adrenergic stimulation in the failing heart. Nonselective NOS inhibition enhances the inotropic response to catecholamines further increased, in contrast to the increased arterial and LV systolic pressure that would probably have been most prominent during exercise. Data were obtained during control conditions, after administration of selective iNOS inhibitor SMT (1.5 mg/kg), and after nonselective NOS blockade with L-NNa (1.5 mg/kg IC).

Figure 3. Relationship between rate-pressure product and CBF (left) and MVO₂ (right) in 5 dogs with CHF at rest and during exercise. Data were obtained during control conditions, after administration of selective iNOS inhibitor SMT (1.5 mg/kg), and after nonselective NOS blockade with L-NNa (1.5 mg/kg IC).

Finally, it is likely that the increase of MVO₂ resulted in part from the increased arterial and LV systolic pressure that occurred in response to NOS inhibition in animals with CHF. iNOS expression has been reported in skeletal muscle in the absence of CHF. If the increased MVO₂ after NOS inhibition were due to an enhanced catecholamine response, however, then the effect would probably have been most prominent during exercise, when catecholamines are further increased, in contrast to the observed parallel upward shift in the relationship between MVO₂ rate-pressure product.

Figure 4. Western blots of iNOS and eNOS in CHF and normal dogs.

were studied relatively early (on day 11 of pacing) compared with 27±3 days in the present study. It is possible that the longer duration of pacing in the present study would favor increased peripheral iNOS expression.

Limitations of the Study
The increased MVO₂ after SMT might have been partly a result of nonselective inhibition of eNOS or nNOS. The dose of SMT used did not impair the response to the endothelial-dependent vasodilator acetylcholine, however, indicating that the concentration of SMT used did not inhibit agonist-mediated NO production by eNOS. Furthermore, SMT caused no change of coronary flow or MVO₂ in normal dogs. One must consider whether expression of iNOS in CHF animals was not the result of heart failure but rather the result of the surgical procedure and chronic instrumentation. Had this been the case, however, it is likely that similar iNOS expression would have been found in the chronically instrumented animals that did not have CHF. The finding that SMT had no effect on animals without heart failure suggests that the chronic instrumentation alone did not result in iNOS expression in the absence of CHF.

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References


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