Inhibition of NO production increases myocardial blood flow and oxygen consumption in congestive heart failure

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Traverse, Jay H., Yingjie Chen, Mingxiao Hou, and Robert J. Bache. Inhibition of NO production increases myocardial blood flow and oxygen consumption in congestive heart failure. Am J Physiol Heart Circ Physiol 282: H2278–H2283, 2002; 10.1152/ajpheart.00504.2001.—Coronary blood flow (CBF) and myocardial oxygen consumption (MV\textsubscript{O}2) are reduced in dogs with pacing-induced congestive heart failure (CHF), which suggests that energy metabolism is downregulated. Because nitric oxide (NO) can inhibit mitochondrial respiration, we examined the effects of NO inhibition on CBF and MV\textsubscript{O}2 in dogs with CHF. CBF and MV\textsubscript{O}2 were measured at rest and during treadmill exercise in 10 dogs with CHF produced by rapid ventricular pacing before and after inhibition of NO production with \textit{N}\textsuperscript{G}-nitro-L-arginine (L-NNA, 10 mg/kg iv). The development of CHF was accompanied by decreases in aortic and left ventricular (LV) systolic pressure and an increase in LV end-diastolic pressure (25 ± 2 mmHg). L-NNA increased MV\textsubscript{O}2 at rest (from 3.07 ± 0.61 to 4.15 ± 0.80 ml/min) and during exercise; this was accompanied by an increase in CBF at rest (from 31 ± 2 to 40 ± 4 ml/min) and during exercise (both \textit{P} < 0.05). Although L-NNA significantly increased LV systolic pressure, similar increases in pressure produced by phenylephrine did not increase MV\textsubscript{O}2. The findings suggest that NO exerts tonic inhibition on respiration in the failing heart.

Recent evidence suggests that endogenous nitric oxide (NO) can inhibit mitochondrial respiration in a variety of tissues including skeletal muscle (22) and cardiac myocytes (3). In isolated mitochondria, NO reversibly inhibits respiration by competing with oxygen at the Fe/Cu centers of cytochrome oxidase (4). In isolated heart preparations, the addition of authentic NO (20) or endothelium-dependent agonists such as bradykinin (25) significantly decreased oxygen consumption (MV\textsubscript{O}2) and myocardial contractility. Conversely, inhibition of NO production with competitive antagonists of NO synthase (NOS) resulted in significant increases in whole body oxygen consumption (29) and small but significant increases of MV\textsubscript{O}2 and coronary blood flow (CBF) in normal dogs (1, 15).

In dogs with pacing-induced congestive heart failure (CHF), we observed that CBF and MV\textsubscript{O}2 were significantly decreased at rest and during exercise compared with normal animals (32). Insufficient oxygen delivery did not appear to be a limiting factor in the reduced oxygen uptake, because oxygen extraction was not increased and coronary sinus PO\textsubscript{2} was not different from normal. This suggested that energy utilization and therefore oxygen demand is depressed in the failing heart. Because of the known effects of NO in depressing mitochondrial respiration, we undertook the present study to determine whether endogenous NO inhibits MV\textsubscript{O}2 and CBF at rest and during exercise in dogs with pacing-induced CHF. The MV\textsubscript{O}2 and CBF responses were compared to a group of normal dogs previously studied in our laboratory (1).

METHODS

Studies were carried out on 10 adult mongrel dogs (body wt 25–30 kg) in accordance with the position of the American Heart Association on research animal use and were approved by the Animal Care Committee of the University of Minnesota.

Surgical preparation. Animals were anesthetized with pentobarbital sodium (30–35 mg/kg iv), intubated, and ventilated with oxygen-enriched room air. A thoracotomy was performed and heparin-filled polyvinyl catheters (3.0 mm OD) were introduced into the ascending aorta and the left atrium. A similar catheter was advanced through the right atrium into the coronary sinus and positioned in the great cardiac vein to drain venous blood from the distribution of the left anterior descending coronary artery (LAD). A fluid-filled catheter and Konigsberg micromanometer were introduced into the left ventricle at the apex. A Doppler velocity probe was placed around the proximal LAD, and a heparin-filled silicone-rubber catheter (0.3 mm ID) was placed into the artery. An epicardial pacing lead was screwed into the right ventricle. The pericardium was loosely closed, the catheters and electrical leads were tunneled subcutaneously to exit at the base of the neck, and the thoracotomy was repaired. A programmable pacing generator (Medtronic model 5385; Minneapolis, MN) was placed in a subcutaneous pocket and connected to the pacing lead.

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Production of heart failure. One week after surgery, the pacemaker was activated at 220 beats/min and continued at that rate or increased up to 250 beats/min until CHF had developed. Weekly assessments of hemodynamics and CBF were obtained with the dogs standing quietly in a sling in sinus rhythm 1 h after the pacemaker had been deactivated. CHF was deemed to have developed when the resting left ventricular (LV) end-diastolic pressure (LVEDP) was >20 mmHg or when the visual estimation of ejection fraction by echocardiography was <25%.

Effects of Nω-nitro-L-arginine on CBF and \( \dot{MVO}_2 \). On the day of the study, the pacemaker was deactivated and the dog was placed on the treadmill. Resting hemodynamic and CBF measurements were obtained 1 h after pacemaker deactivation. Aortic and coronary venous blood samples (3 ml) were withdrawn and placed on ice for determination of oxygen content. The treadmill was then started at 3.2 km/h and 0% grade. After 3 min of exercise, hemodynamic measurements and blood samples were again withdrawn for blood gas measurements. For six dogs, the treadmill speed was increased to 6.4 km/h; after 3 min, hemodynamic measurements and blood samples were again obtained. During the first exercise stage, radioactive microspheres were injected through the left atrial catheter (n = 9) to determine the transmural distribution of myocardial blood flow. After a 1-h rest period, Nω-nitro-L-arginine (L-NNA, 10 mg/kg) was infused through the left atrial catheter to inhibit NO production. All resting and exercise measurements were repeated 1 h later.

Determination of \( \dot{MVO}_2 \). Blood oxygen content was determined with a blood gas analyzer (Instrumentation Laboratory model 113). Blood oxygen content (in milliliters per 100 milliliters of blood) was calculated as \((0.0136 \times \text{hemoglobin} \times \% \text{oxygen saturation}) + (\text{Po}_2 \times 0.0031)\). \( \dot{MVO}_2 \) in the LAD distribution was calculated as the arteriovenous difference of oxygen content multiplied by CBF.

Determination of myocardial blood flow. Myocardial blood flow was measured with radioactive microspheres in nine animals during the first stage of exercise before and after L-NNA. For each measurement, \(3 \times 10^6\) microspheres (15 \( \mu \)M diameter) were injected through the left atrial catheter. After completion of exercise, the animals were euthanized with pentobarbital sodium. Myocardial specimens were removed from the anterior and posterior regions of the left ventricle, sectioned into four layers from epicardium to endocardium, weighed, and placed into vials for counting.

Effect of L-NNA administration on responses to endothelium-dependent agonist. To assess the ability of L-NNA to inhibit NO-mediated vasodilation, CBF responses to ACh were measured before and after L-NNA administration in three animals with CHF. ACh dissolved in normal saline was infused through the LAD catheter at rates of 3.75 \(10^{-3}\) mg/min (0.15–1.5 ml/min). L-NNA (10 mg/kg iv) was then administered through the left atrial catheter and the responses to ACh were repeated 1 h later.

Effects of phenylephrine on CBF and \( \dot{MVO}_2 \). L-NNA administration resulted in a significant increase in mean aortic pressure that might have increased CBF and \( \dot{MVO}_2 \) independent of NO inhibition. Consequently, in four dogs with CHF, measurements of CBF and \( \dot{MVO}_2 \) were performed while the animals stood quietly in a sling before and after administration of the \( \alpha \)-adrenergic agonist phenylephrine in a dose (8 \( \mu \)g·kg\(^{-1}\)·min\(^{-1}\) iv) that caused an increase in aortic pressure at least as great as that produced by L-NNA infusion.

Data analysis. Heart rate, pressures, and coronary velocity were measured from the strip-chart recordings. LAD flow was calculated from the Doppler frequency shift as previously described (16). Data were compared using two-way

<table>
<thead>
<tr>
<th>Exercise stage</th>
<th>Heart Rate, beats/min</th>
<th>LVEDP, mmHg</th>
<th>( \dot{MVO}_2 ), mmHg/L/min</th>
<th>Rate Pressure Product, mmHg·beats/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest</td>
<td>Control</td>
<td>L-NNA</td>
<td>Control</td>
<td>L-NNA</td>
</tr>
<tr>
<td>0 (n = 10)</td>
<td>90 ± 3</td>
<td>105 ± 5</td>
<td>91 ± 3</td>
<td>107 ± 5</td>
</tr>
<tr>
<td>Exercise stage 1</td>
<td>91 ± 4†</td>
<td>107 ± 4‡</td>
<td>91 ± 3‡</td>
<td>107 ± 4‡</td>
</tr>
<tr>
<td>Exercise stage 2</td>
<td>102 ± 3‡</td>
<td>115 ± 3‡</td>
<td>118 ± 3‡</td>
<td>125 ± 3‡</td>
</tr>
</tbody>
</table>

Values are means ± SE. L-NNA, \( \omega \)-nitro-L-arginine; LV, left ventricular; EDP, end-diastolic pressure. \( \dot{MVO}_2 < 0.05 \text{ vs. control} \); \( \dot{MVO}_2 < 0.05 \text{ vs. control} \); \( \dot{MVO}_2 < 0.01 \text{ vs. exercise stage 1} \).
Table 2. Effects of L-NNA on myocardial oxygen consumption

<table>
<thead>
<tr>
<th></th>
<th>Hemoglobin</th>
<th>Coronary Sinus PO₂, mmHg</th>
<th>MVO₂, ml O₂/min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>L-NNA</td>
<td>Control</td>
</tr>
<tr>
<td>Rest (n = 6)</td>
<td>11.8 ± 1.4</td>
<td>12.5 ± 1.1</td>
<td>23 ± 2</td>
</tr>
<tr>
<td>Exercise stage 1</td>
<td>13.2 ± 0.9</td>
<td>12.3 ± 1.1</td>
<td>18 ± 2†</td>
</tr>
<tr>
<td>Exercise stage 2</td>
<td>13.7 ± 0.9</td>
<td>13.3 ± 1.0</td>
<td>19 ± 2†</td>
</tr>
</tbody>
</table>

Values are means ± SE. MVO₂, myocardial oxygen consumption. *P < 0.05 vs. control; †P < 0.05 vs. rest.

RESULTS

Control hemodynamics and MVO₂. After the development of CHF, resting mean aortic pressure was 89 ± 3 mmHg, mean heart rate was 140 ± 4 beats/min, LVEDP was 25 ± 2 mmHg, and LAD CBF was 31 ± 2 ml/min (Table 1). Exercise resulted in significant progressive increases in heart rate, aortic pressure, and LV systolic pressure. CBF increased to 37 ± 3 ml/min during exercise stage 1 (n = 10; P < 0.01) and to 44 ± 4 ml/min during exercise stage 2 (n = 8; P < 0.05). Resting MVO₂ was 3.1 ± 0.6 ml O₂/min and progressively increased with each stage of exercise (Table 2). Coronary sinus PO₂ was 23 ± 2 mmHg at rest and decreased to 18 ± 2 mmHg during exercise stage 1 (P < 0.05) with no further change during exercise stage 2.

Hemodynamics and MVO₂ after L-NNA administration. L-NNA infusion significantly increased resting mean aortic and LV systolic pressure (see Table 1). Heart rate tended to decrease, but this was not significant. Resting CBF after L-NNA administration was 30 ± 8% higher than during control conditions at a similar rate-pressure product. Similarly, CBF was significantly higher than control during each exercise stage after L-NNA administration (Fig. 1). MVO₂ was significantly greater after L-NNA infusion at rest and during the first stage of exercise compared with control and tended to be greater during exercise stage 2, although this did not achieve statistical significance (Fig. 2). Coronary venous PO₂ tended to be lower after L-NNA administration, which reflects an increase in oxygen extraction.

Transmural myocardial blood flow. Subendocardial and subepicardial blood flow and its ratio (endo/epi) was measured with microspheres in nine dogs during the first stage of exercise before and after L-NNA infusion. During control exercise, the endo/epi flow ratio was 1.34 ± 0.15; this was unchanged during exercise with L-NNA (1.42 ± 0.18).

Effect of L-NNA on responses to endothelium-dependent agonist. During control conditions, intracoronary ACh progressively increased CBF with no significant change in systemic hemodynamics (Fig. 3). L-NNA administration (10 mg/kg iv) resulted in 50–70% inhibition of the increase in CBF produced by ACh.

Effects of phenylephrine on CBF and MVO₂. In four dogs with CHF, phenylephrine increased LV systolic pressure from 87 ± 2 to 116 ± 7 mmHg (P < 0.05) while heart rate decreased from 112 ± 6 to 100 ± 3 beats/min. There was no change in CBF (22 ± 4 vs. 21 ± 3 ml/min) or MVO₂ (2.0 ± 0.3 vs. 2.0 ± 0.7 ml O₂/min) during phenylephrine infusion. These results suggest that the increase in CBF and MVO₂ produced by L-NNA administration was specific to NO inhibition and was not the result of the increase in LV systolic pressure that was produced by L-NNA.

DISCUSSION

In this study, we report for the first time the in vivo effects of NO inhibition on MVO₂ and CBF at rest and exercise.
during exercise in the failing heart. The results demonstrate that inhibition of NO production with L-NNA caused significant increases of CBF and MVO$_2$ and that these effects could not be explained by the increase in LV systolic pressure that is produced by this agent.

Effects of NO on mitochondrial respiration in the normal heart. Borutaite and Brown (3) demonstrated that NO caused reversible inhibition of oxygen consumption in mitochondria isolated from rat hearts. Furthermore, they showed that the rate of oxygen consumption varied with the local NO/oxygen ratio, thus supporting the concept that competitive inhibition exists between NO and oxygen at the level of cytochrome oxidase and that this occurs at physiological concentrations (4). Similarly, N$\text{O}_2$-nitro-L-arginine methyl ester increased oxygen consumption of isolated porcine aortic endothelial cells whereas the receptor-mediated production of NO (bradykinin) significantly reduced respiration (5). In isolated guinea pig hearts, Kelm et al. (20) observed that NO concentrations of 10 nM to 1 μM increased CBF and cGMP without changing LV developed pressure or MVO$_2$. However, at a concentration of 100 μM, NO caused reversible decreases of LV developed pressure and dP/dt (first derivative of LV pressure) as well as a 60% decrease in MVO$_2$. This was accompanied by significant decreases of ATP and phosphocreatine, which suggests that high levels of NO can impair contractile performance by depressing myocardial ATP production in a reversible, dose-dependent manner.

Studies in normal animals have generally (1, 15, 20, 27, 29) but not always (6, 28, 30) reported that inhibition of NO production has a stimulatory effect on oxygen consumption. In normal dogs, L-NNA administration tended to increase MVO$_2$ during treadmill exercise, but this was associated with a small but significant increase in rate-pressure product that could have contributed to the increase in MVO$_2$ (1, 15). Using a lower dose of L-NNA (1.5 mg/kg intracoronary) which did not cause an increase in blood pressure, Ishibashi et al. (15) demonstrated that NOS blockade increased MVO$_2$ during heavy treadmill exercise at rate-pressure products that were similar to control. The findings imply that NOS blockade results in an increase of MVO$_2$ out of proportion to any increase in cardiac work that it may produce.

**Effect of NO on MVO$_2$ in the failing heart.** In a previous study using this experimental model (32), we observed that the development of CHF was accompanied by significant decreases of resting CBF and MVO$_2$, and that CBF and MVO$_2$ failed to increase normally with increasing levels of exercise. The oxygen supply to the failing myocardium did not appear to be a limiting factor, because oxygen extraction did not increase and coronary venous PO$_2$ did not differ from control. To determine whether an increase of external cardiac work produced by NOS inhibition could account for the decreases of CBF and MVO$_2$ that were observed after L-NNA administration, in the present study we examined the response of MVO$_2$ to phenylephrine-induced increases of blood pressure. Increases in LV systolic pressure produced by phenylephrine did not increase MVO$_2$ or CBF. Thus the increased MVO$_2$ produced by L-NNA infusion in the present study could not be ascribed to the increase in LV systolic pressure that it produced. Although our study cannot exclude other effects of L-NNA on internal efficiency, catecholamine responsiveness, or substrate selectivity, the results suggest that endogenous NO exerts some degree of tonic inhibition on MVO$_2$ in the failing heart.

L-NNA caused a parallel upward shift in the relation between MVO$_2$ and rate-pressure product. The increase in MVO$_2$ after L-NNA administration was accompanied by a significant increase in the arteriovenous oxygen difference, which indicates that the failing myocardium was able to increase oxygen extraction. However, there was no further increase in oxygen extraction with exercise, so that the increase in MVO$_2$ during exercise after L-NNA infusion resulted solely from the increased CBF. Thus the increase in oxygen usage secondary to disinhibition of NO effects on respiration was maximal at rest, so that no further increase in oxygen extraction occurred during exercise.

The apparent role for NO in the failing heart is surprising given previous reports that endothelium-dependent NO production and NO-mediated vasodilation are impaired in the peripheral (8, 17, 22) and coronary circulation with CHF (13, 33). Kaiser et al. (17) observed that femoral artery vasodilation to topical ACh was reduced in dogs with pacing-induced CHF whereas responses to nitroglycerine were unchanged compared to normal. Elsner et al. (8), using a similar model of CHF, observed no significant increase in systemic vascular resistance after NO inhibition with L-NNA (5 mg/kg iv), which suggests that NO production is reduced in the peripheral vasculature in heart failure. In contrast, using a higher dose of L-NNA (10 mg/kg), we observed a significant increase in arterial pressure, although we did not measure systemic vascular resistance. Wang et al. (33) observed that the oxidative products of NO (NOx) produced by isolated coronary microvessels in response to endothelium-de-
dependent vasodilators was decreased in dogs with pacing-induced CHF compared to normal dogs. Katz et al. (19), using infusions of L-[15N]arginine, observed that the 24-h excretion of [15N]nitrate was reduced in patients with CHF, which suggests decreased activity of the L-arginine/NO pathway. In contrast, several studies suggest that NO activity is increased in CHF or that basal NO production is maintained whereas agonist-mediated NO production is diminished (2, 7). In myocardium taken from explanted human hearts during transplantation, Loke et al. (23) reported that the endothelium-dependent agonist bradykinin decreased oxygen consumption up to 21% in a dose-dependent manner, which supports an effect of endothelium-derived NO on respiration in failing hearts.

Drexler et al. (7) demonstrated that the decrease in forearm blood flow in response to NG-monomethyl-L-arginine administration was enhanced in patients with CHF, which suggests increased basal NO production. In aortic rings from rats with CHF 8 wk after left coronary artery ligation, Bauersachs et al. (2) observed impaired relaxation in response to ACh, which suggests the development of endothelial dysfunction. Interestingly, these animals demonstrated upregulation of endothelial NOS (eNOS) and soluble guanylate cyclase but reduced formation of cGMP in response to sodium nitroprusside, which the investigators attributed to enhanced NADH-dependent vascular superoxide anion (O$_2^-$) production. In dogs with pacing-induced CHF, Hare et al. (13) also found that eNOS protein was not decreased. These findings suggest that NO production is preserved or even upregulated in CHF, but that its bioavailability may be reduced because of inactivation by O$_2^-$.

Inflammatory cytokines such as interleukin-1 and tumor necrosis factor-α are reported to be increased in patients with CHF and can result in induction of inducible NOS (iNOS) expression in cardiac myocytes and vascular smooth muscle (12). Haywood et al. (14) demonstrated that iNOS was expressed in parallel with ANP in explanted hearts from patients undergoing cardiac transplantation with no expression in normal ventricles. Similar findings were reported by Habib et al. (12) using immunohistochemistry on myocardial biopsy specimens from patients with dilated cardiomyopathy. Previous reports employing the model used in this study have failed to demonstrate significant iNOS in myocardial tissue (13, 21) and cytokine levels are not increased with the development of pacing-induced CHF (26). We observed only a faint iNOS band on Western analysis of myocardial tissue from the failing ventricles of the animals in this study. However, because of the high NO output of iNOS, even modest expression of this protein might account for significant NO production in the failing heart. An additional possible mechanism for the increased MV$\text{O}_2$ after L-NNA administration relates to reports that NO inhibits β-adrenergic responsiveness in failing hearts but not in normal hearts (13, 31). Because exercise is associated with sympathetic activation, inhibition of NO production might augment β-adrenergic effects on myocardial contractility and coronary resistance vessel dilation. Recently, an isoform of NOS localized to the inner mitochondrial membrane has been reported (10, 11) and identified as neuronal NOS (18). Although NO production by the mitochondria has been reported not to be a significant source of NO in the normal heart (9), this isoform is overexpressed in dysfunctional cardiomyocytes from dystrophin knockout mice that are deficient in caveolar NOS (eNOS) (18). NO production by mitochondria-associated NOS has not been studied in failing myocardium produced by rapid ventricular pacing. Because L-NNA used in the present study causes nonselective inhibition of NOS activity, we cannot determine the contribution of the individual isoforms to our results.

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REFERENCES


