Endogenous Basal Nitric Oxide Production Does Not Control Myocardial Oxygen Consumption or Function (43977)

JOHN D. SADOFF,* PETER M. SCHOLZ,*,[1] AND HARVEY R. WEISS†

Departments of Surgery, and Physiology and Biophysics,† Heart and Brain Circulation Laboratory, University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School, New Brunswick, New Jersey 08903-0019*

Abstract. **Previous studies from our laboratory have shown that an extrinsic nitric oxide (NO) donor (i.e., nitroprusside) caused vasodilatation and negative inotropy by activating guanylate cyclase and increasing myocardial cyclic GMP. We tested the hypothesis that endogenous myocardial NO production would limit myocardial oxygen consumption and function *in vivo*. We used the NO synthase inhibitors N^G-nitro-L-arginine methyl ester (L-NAME) and N^G-monomethyl-L-arginine (L-NMMA) in nine open-chest anesthetized mongrel dogs. Either L-NAME (6 mg/kg) or L-NMMA (3 mg/kg) were infused into the left anterior descending coronary artery (LAD). The circumflex (CFX) coronary artery region served as a control. Regional segment work was calculated as the integrated product of local force (miniature transducer) and segment shortening (ultrasonic crystals). Local myocardial O_2 consumption was determined using an ultrasonic LAD flow probe and local arterial-venous O_2 content difference (oximetry). Cyclic GMP levels were obtained via a radioimmunoassay. Both L-NAME and L-NMMA caused a local decrease in coronary blood flow (LAD flow: 80 ± 8 to 69 ± 7 ml/min/100 g [means ± SEM]) and increased O_2 extraction (9.1 ± 0.6 to 10.2 ± 0.7 ml O_2/100 ml). However, this led to no change in local O_2 consumption. LAD segment force was not altered (12.1 ± 0.7 to 11.6 ± 0.9 g), nor was the percent shortening changed (10.8 ± 1.8% to 10.0 ± 1.4%) by L-NAME or L-NMMA, leading to no net change in segment work. Myocardial cyclic GMP levels were not different in a comparison between the LAD (1.7 ± 0.4 pmoles/g) and control (1.7 ± 0.2) regions with either L-NAME or L-NMMA. We conclude that blockade of endogenous NO production with L-NAME and L-NMMA is sufficient to cause vasoconstriction in the heart of anesthetized dogs. However, this dose did not lead to alteration in local myocardial function, O_2 consumption, or cyclic GMP levels.** [P.S.E.B.M. 1996, Vol 211]

Nitric oxide (NO) is produced in vascular endothelial cells as well as several other types of cells. NO is produced from L-arginine by at least two separate nitric oxide synthases (NOS; inducible and constitutive) and is an endogenous stimulator of soluble guanylate cyclase in vascular smooth muscle cells (1). This, in turn, leads to increased intracellular levels of cyclic GMP and subsequently to changes in intracellular calcium concentrations, thereby causing vascular smooth muscle relaxation and vasodilatation (2–4). Basal NO levels are sufficient to relax vascular smooth muscle in heart and other organs (3, 5, 6). Nitrate donors are often administered in clinical situations to cause vasodilatation and thus relieve hypertension and angina pectoris.

Several studies have explored the effects of extrinsic NO donors or NO synthase inhibitors on isolated hearts and myocytes (2, 5, 7, 8). Most studies report negative inotropic effects or reduced O_2 consumption with NO (2, 9, 10, 11), although Weyrich *et al.* (8) reported that physiologic concentrations of NO had no effect on myocytes. One study observed direct myocardial depressive effects from intracoronary nitroprusside given to humans undergoing heart catheter-

[1] To whom requests for reprints should be addressed at Department of Surgery, UMDNJ-Robert Wood Johnson Medical School, One Robert Wood Johnson Place, New Brunswick, NJ 08903-0019.

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ization (12). Another group (13) further defined the NO pathway by showing differences between the two NO synthases (inducible and constitutive) in patients with dilated cardiomyopathy. These negative myocardial inotropic or O_2 consumption effects are related to NO stimulation of myocyte guanylate cyclase (7, 12, 14). We have shown that stimulation of guanylate cyclase via extrinsic nitrate administration leads to negative myocardial inotropic effects *in vivo* (15). Administering guanylate cyclase inhibitors such as methylene blue results in positive myocardial inotropic changes (16). Other investigators have also shown that increases in intracellular levels of cyclic GMP result in negative inotropic effects or reduced myocardial O_2 consumption (12, 14, 17). In several studies, blockade of endogenous NO did not affect myocardial O_2 consumption or function *in vivo* or *in vitro* (8, 18–20). This would imply that endogenous NO production is not sufficient to affect myocardial cyclic GMP levels, since exogenous NO does increase cyclic GMP and reduce myocardial function.

The present study was designed to test the hypothesis that basal endogenous myocardial NO production, in addition to its vascular relaxation effect, is a major controller of myocardial cyclic GMP production and limits myocardial oxygen consumption and function. It is known that the arginine analogs N^G-nitro-L-arginine methyl ester (L-NAME) and N^G-monomethyl-L-arginine (L-NMMA) are potent inhibitors of NO production (1, 4, 21). In addition, L-NMMA is known to inhibit the release of NO from endothelial cells (1). L-NMMA was included as an additional blocking agent because L-NAME may also act as a muscarinic antagonist and we wished to prove that this did not affect our results (22). We directly injected the NOS inhibitors L-NAME and L-NMMA into the left anterior descending coronary (LAD) artery, while using the region perfused by the circumflex coronary (CFX) artery as a functional control. Simultaneous local measurements of developed force and shortening were performed, and tissue samples were taken from LAD and CFX regions for determination of cyclic GMP content. If NOS inhibitors affect myocardial function or O_2 consumption, they should reduce myocardial cyclic GMP levels. We used methylene blue, a guanylate cyclase inhibitor, to demonstrate that lowering cyclic GMP had functional consequences for the heart. The results demonstrate that blockade of basal endogenous NO production does lead to vascular effects in the heart of anesthetized dogs, but does not change local myocardial function, oxygen consumption, or cyclic GMP levels.

Materials and Methods

This study was performed in 12 adult mongrel dogs of either sex that weighed between 22 and 29 kg. Each dog was anesthetized with intravenous sodium pentobarbital (30 mg/kg), intubated, and ventilated with a volume ventilator (Bennett Ventilator). Arterial blood gases and O_2 saturation were maintained within the physiological range throughout the experiment by controlling respiratory rate, tidal volume, and FiO_2. These were monitored periodically using arterial blood samples that were analyzed for pH, PO_2, and PCO_2 on a Radiometer ABL30 blood gas analyzer. Hemoglobin concentration and O_2 saturation were measured using a Radiometer OSM3 hemoximeter.

Limb electrodes were placed for electrocardiographic monitoring. A 20-gauge angiocatheter was inserted percutaneously into a peripheral vein for administration of fluid and anesthetic. A polyethylene catheter was introduced into the right femoral vein. The left femoral artery was cannulated for arterial blood samples. Micromanometers (Millar MPC500) were introduced into the left ventricle through an apical stab wound and through the right femoral artery into the ascending aorta. These were used to measure left ventricular and aortic blood pressures, respectively. The heart was exposed through a left thoracotomy at the fifth intercostal space.

The left anterior descending coronary artery (LAD) and its first or second diagonal branch were exposed. An ultrasonic flow probe (Transonic, Ithaca, NY) was placed around the LAD just distal to the second diagonal branch. The diagonal branch was cannulated and used for injection of nitric oxide synthase inhibitors and Evans blue dye. In addition, a left anterior descending coronary vein was cannulated for venous blood sampling. Ultrasonic dimension crystals (5 MHz, Triton sonomicrometer) were inserted to a depth of 4–6 mm and approximately 1 cm apart on the anterior surface of the left ventricle supplied by the LAD. The position of the crystals was midway between the base and apex, in the short axis of the heart. Miniature force gauges (Warren Research Products, Charleston, SC) were sewn into the myocardium just adjacent to the length crystals in the area supplied by the LAD and on the postero-lateral surface of the heart supplied by the circumflex (CFX) branch of the left coronary artery. The force gauges were placed in the short axis of the heart, in series with the length crystals at a depth of 4–6 mm. The force transducers were connected to a Wheatstone bridge that was balanced and calibrated prior to the experiment.

Lead II electrocardiogram (ECG), global hemodynamics (left ventricular and aortic pressures), and regional functional measurements (segment length and force) were continuously monitored on a Gould ES1000 multichannel digital electrostatic recorder. The signals were simultaneously digitized at a sampling frequency of 200 Hz over 10-sec intervals (Data Translation DT2801) and acquired on a computer for

analysis. The digitized data were analyzed using the algorithm of ensemble averaging of all consecutive heart beats. The parameters measured were aortic blood pressure, left ventricular pressure, segment force, and length. The parameters calculated were heart rate, the first derivative of the left ventricular pressure (dP/dt_{max}), peak force, percent segment shortening, and total regional myocardial segment work. Regional myocardial segment work was calculated by determining the area under the force-length loop during the averaged beat. This was accomplished by multiplying each measured value for force by its corresponding change in length, and integrating all positive values over the interval of the averaged heart beat (23).

Regional myocardial O_2 consumption was calculated using the Fick principle from blood flow (ultrasonic transducer), O_2 extraction, arterial and venous O_2 saturations, and hemoglobin data (blood samples) from the LAD region. The area of blood flow distribution was confirmed prior to the end of the experiment by injecting Evans blue dye into the LAD. Blood flow was expressed per unit weight.

Transmural biopsies were taken from the LAD and CFX regions using a high speed suction biopsy drill (Alko Diagnostics, Holliston, MA) to determine cyclic GMP levels. The drill excises a 3.5-mm plug and freezes it immediately in methylbutane cooled by liquid nitrogen. The heart was excised at the end of each experiment and frozen in liquid nitrogen for later analysis. The biopsy and heart samples were later warmed to 0°C and homogenized in ethanol using a Brinkmann Polytron placed in an ice bath. The homogenate was centrifuged at 30,000*g* for 15 min in a Sorvall RC-5B centrifuge. The supernatant was recovered. The pellet was resuspended in 1 ml of 2:1 ethanol:water and centrifuged as before. The combined supernatants were evaporated to dryness in a 60°C bath under a stream of nitrogen gas. The final residue was dissolved in 1.5 ml of assay buffer (0.05 *M* sodium acetate, pH 5.8, containing sodium azide). Cyclic GMP levels were determined using a radioimmunoassay (Amersham, Arlington Heights, IL). This assay measures the competitive binding of ^{125}I–cyclic GMP to a cyclic GMP–specific antibody. After construction of a standard curve, cyclic GMP levels were determined directly from the counts in picomoles per gram of tissue wet weight.

After all manipulations were completed, the animal was allowed to stabilize for approximately 15 min. Baseline physiologic and blood gas measurements were then recorded. Biopsies were taken from LAD and CFX regions (stored in liquid nitrogen), and repeat physiologic and blood gas measurements were obtained. A saline vehicle was infused into the coronary catheter as a 0.5-ml bolus, and measurements were recorded. Next, acetylcholine (ACH) was infused via the cannulated branch of the LAD as a 1-μg bolus. Along with a saline control, acetylcholine was infused before and after nitric oxide synthase blockade to assess the adequacy of blockade. After a 10-min recovery period, one of the nitric oxide synthase inhibitors N^G-nitro-L-arginine methyl ester (L-NAME, $n = 5$) or N^G-monomethyl-L-arginine (L-NMMA, $n = 4$) was infused into the LAD artery via the cannulated LAD diagonal branch over 2 min for a total dose of 6 or 3 mg/kg, respectively. The CFX artery region served as a functional control. Following infusion of one of the agents, repeat physiologic and blood gas measurements were obtained, ACH was again infused, and after an appropriate recovery time, Evans blue dye was injected into the cannulated diagonal branch of the LAD. The heart was rapidly excised at the artrioventricular ring and frozen in liquid nitrogen.

Three additional dogs received methylene blue (a guanylate cyclase inhibitor). Methylene blue was infused at 1 mg/kg/min via the cannulated branch of the LAD. This dose has been found to increase significantly local regional work (16). The response to methylene blue typically began 2–3 min later. Systemic hemodynamic and regional function parameters were recorded including LAD and CFX shortening and force. Infusion was continued for 15 min, at which time the heart was excised and frozen as described above.

A repeated measure analysis of variance (ANOVA) was used to compare variables measured in the experimental and control regions. Analysis was made to determine if there were differences within a region in response to treatment, or between regions in each experimental stage. Data were collected separately for L-NAME and L-NMMA but have been combined because there were no differences noted between the two treatment groups. The methylene blue data were analyzed by paired Student *t* test. A P value of <0.05 was accepted as significant. All values are expressed as mean ± SEM. All experiments were conducted in accordance with the *Guide for the Care of Laboratory Animals* (DHHS Publication No. 85–23, revised 1985) and were approved by our institution's Animal Care and Use Committee.

Results

The effects of intracoronary infusion of L-NAME and L-NMMA on global hemodynamic measurements in anesthetized dogs are depicted in Table I. There were no significant changes in heart rate, systolic blood pressure, diastolic blood pressure, mean blood pressure, left ventricular pressure, left ventricular end diastolic pressure, or left ventricular dP/dt_{max}. Arterial blood gases and pH were maintained within the physiological range (PaO_2: 83 ± 3 and 81 ± 4 mm Hg; $PaCO_2$: 40 ± 1 and 39 ± 2 mm Hg; pH: 7.34 ± 0.02 and 7.33 ± 0.02; before and during NOS blockade, respectively).

Table I. Effects of Intracoronary L-NAME or L-NMMA on Global Hemodynamics

	Control	L-NAME/L-NMMA
Heart rate (bpm)	159 ± 3	158 ± 4
Systolic blood pressure (mm Hg)	120 ± 7	123 ± 6
Diastolic blood pressure (mm Hg)	89 ± 6	95 ± 7
Mean blood pressure (mm Hg)	103 ± 7	108 ± 7
Left ventricular pressure (mm Hg)	115 ± 6	120 ± 6
Left ventricular dP/dt_{max} (mm Hg/sec)	2390 ± 158	2183 ± 135
Left ventricular end diastolic pressure (mm Hg)	4.3 ± 0.7	4.4 ± 0.7

Note. Values are expressed as mean ± SEM.

Table II indicates the effect of intracoronary L-NAME or L-NMMA on regional myocardial mechanics. Left anterior descending (LAD) coronary artery segment force, percentage shortening, and end diastolic length were all unchanged, leading to no net change in segment work (Fig. 1, top panel). The circumflex (CFX) control region also had no change in segment force due to intracoronary infusion of L-NAME or L-NMMA. In addition, there were no significant differences between LAD and CFX regions either before or after treatment.

Table III summarizes the effect of L-NAME and L-NMMA on LAD blood flow, myocardial venous O_2 saturation, and myocardial arterial-venous O_2 content difference. The weight of the LAD perfusion field was 24.7 ± 1.1 g. Both L-NAME and L-NMMA caused local vasoconstriction. LAD blood flow significantly decreased from 80 ± 8 to 69 ± 7 ml/min/100 g. In addition, the local myocardial arterial-venous O_2 content difference increased from 9.0 ± 0.6 to 10.2 ± 0.6 ml O_2/100 ml. This was accompanied by a drop in the venous O_2 saturation from 55% ± 3% to 50% ± 3%. Local myocardial O_2 consumption remained unchanged after nitric oxide synthase blockade (Fig. 1, bottom panel).

Table II. Effects of Intracoronary L-NAME or L-NMMA on Regional Myocardial Mechanics

	Control	L-NAME/L-NMMA
LAD (experimental)		
Peak force (g)	12.1 ± 0.7	11.6 ± 0.9
% shortening	10.8 ± 1.8	10.0 ± 1.4
End diastolic length (mm)	12.3 ± 0.9	11.8 ± 0.8
CFX (control)		
Peak force (g)	10.6 ± 1.7	10.8 ± 1.3

Note. Values are expressed as mean ± SEM. LAD, left anterior descending coronary artery; CFX, circumflex coronary artery.

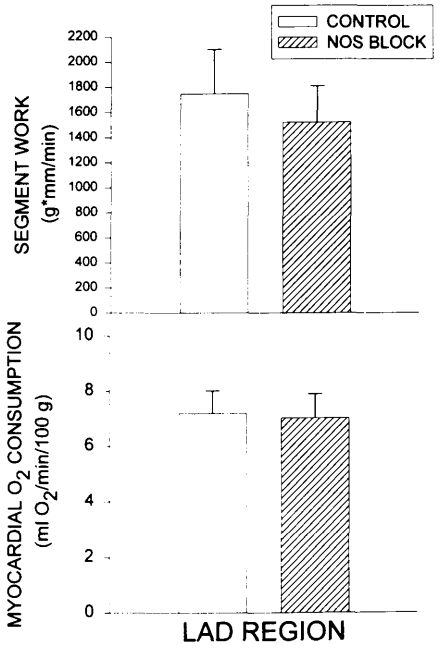

Figure 1. Effect of nitric oxide synthase blockade with L-NAME or L-NMMA on regional myocardial segment work (top panel) and local myocardial O_2 consumption (bottom panel).

Table III. Effects of Intracoronary L-NAME or L-NMMA on Regional Myocardial Oxygenation

	Control	L-NAME/L-NMMA
Venous oxygen saturation (%)	55 ± 3	50 ± 3[a]
LAD blood flow (ml/min/100 g)	80 ± 8	69 ± 7[a]
Arterial-venous O_2 content difference (ml O_2/100 ml)	9.0 ± 0.6	10.2 ± 0.6[a]

Note. Values are expressed as mean ± SEM. LAD, left anterior descending coronary artery.
[a] $P < 0.05$ versus control.

Figure 2 illustrates the effects of intracoronary L-NAME and L-NMMA on local myocardial cyclic GMP levels in the LAD and CFX (control) regions. No changes in cyclic GMP were noted in either the LAD or CFX regions after L-NAME or L-NMMA were administered. Also, there were no significant differences in cyclic GMP levels in a comparison between the LAD (1.7 ± 0.4 pmoles/g) and CFX (1.7 ± 0.2 pmoles/g) regions after blockade.

To test the effectiveness of nitric oxide synthase blockade, acetylcholine (ACH) was injected as a 1-μg bolus via the LAD diagonal branch catheter both before and after L-NAME/L-NMMA blockade (Fig. 3). Prior to blockade, ACH caused a significant 2-fold increase in LAD flow (+19.8 ± 1.9 ml/min). In contrast, after L-NAME and L-NMMA were infused, LAD flow

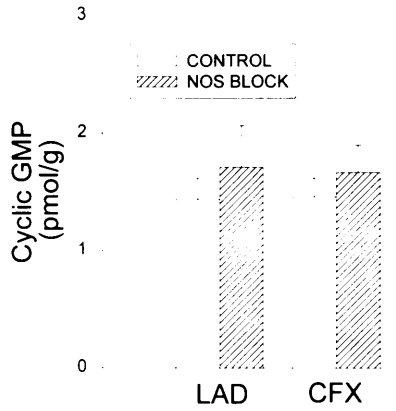

Figure 2. Effect of nitric oxide synthase blockade with L-NAME or L-NMMA on cyclic GMP content of a treated (LAD) and a remote (CFX) left ventricular region.

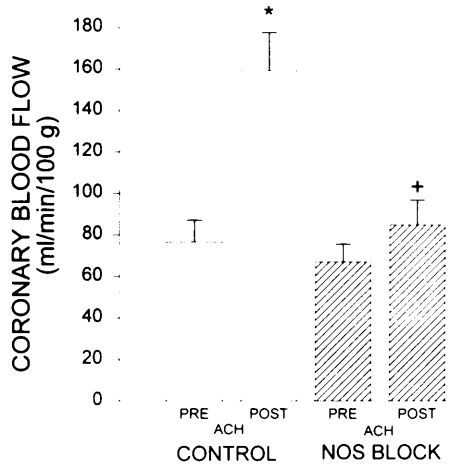

Figure 3. Effect of an intracoronary injection of 1 μg of acetylcholine on left anterior descending coronary artery blood flow before and after nitric oxide synthase blockade. *Significantly different from pre-acetylcholine. + Significantly different from acetylcholine before nitric oxide synthase blockade.

was not significantly increased following the bolus of ACH (+4.4 ± 1.4 ml/min).

Three additional animals were given an intracoronary infusion of methylene blue, which inhibits soluble guanylate cyclase. Cyclic GMP levels in LAD versus CFX (control) regions were significantly different after infusion of methylene blue into the LAD region: 1.0 ± 0.4 pmoles/g LAD vs 1.4 ± 0.4 pmoles/g CFX ($P < 0.05$). Infusion of methylene blue increased LAD force from 9.4 ± 1.2 to 11.0 ± 1 g, while CFX force showed no significant changes (9.9 ± 1.5 to 9.4 ± 1.7 g). Percent shortening was not affected, but overall LAD segment work increased significantly from 1149 ± 204 to 1348 ± 301 g · mm/min. Regional blood flow and arterial-venous O_2 content difference were not significantly affected. Myocardial O_2 consumption also was not significantly affected in either LAD (7.0 ± 1.1 to 8.2 ± 1.6) or CFX (6.4 ± 0.9 to 7.4 ± 0.8 ml O_2/min/100 g) regions. Methylene blue did not alter heart rate, systolic blood pressure, diastolic blood pressure, left ventricular pressure, and left ventricular dP/dt_{max}.

Discussion

The results of this study demonstrated that in the *in vivo* hearts of anesthetized dogs, blockade of basal nitric oxide production via intracoronary injection of the arginine analogs N^G-nitro-L-arginine methyl ester and N^G-monomethyl-L-arginine was not associated with changes in cardiac output, blood pressure, heart rates, myocardial force, shortening, myocardial regional work, myocardial cyclic GMP, or myocardial oxygen consumption. This NOS blockade does, however, lead to local myocardial vascular effects. Furthermore, the observed vascular effects in combination with the marked reduction of vasodilatory response to intracoronary injection of acetylcholine following L-NAME and L-NMMA infusion confirm that these agents did block nitric oxide production.

Nitric oxide is present in many tissues and is capable of relaxing smooth muscle of the vascular system, gut, airway, and urinary tract (1, 3, 4). Nitric oxide production leads to increased cyclic GMP levels in smooth muscle (1, 24). Endothelium-derived NO is an endogenous stimulator of soluble guanylate cyclase (1, 24), thereby directly causing increases in cyclic GMP, smooth muscle relaxation, and vasodilatation. In our study, we observed significant vasoconstriction as a direct result of NO reduction. This was represented by the decreased LAD blood flow and the concomitant increased oxygen extraction. Additionally, we confirmed endothelial NO blockade by showing markedly decreased vasodilatation in response to acetylcholine after L-NAME and L-NMMA. This blockade was also shown in a similar study by Smith and Canty (21). Blockade of intrinsic nitric oxide production does not always alter basal coronary blood flow (20). Agents that decrease vascular cyclic GMP levels have been shown to cause vasoconstriction (25). The opposite is true for drugs that increase cyclic GMP leading to vasodilatation (4, 26).

In myocytes, altering cyclic GMP levels also appear to affect metabolism and function. Negative inotropic responses as well as reduced O_2 consumption have been demonstrated in response to both endogenous and extrinsic NO production, to guanylate cyclase stimulation, and to subsequent increases in cyclic GMP *in vitro* (2, 9, 10, 11, 14). Previous studies from our laboratory and others have shown that inhibition or stimulation or guanylate cyclase lead to positive and negative *in vivo* myocardial inotropic effects, respectively (14–16, 25). Increasing myocyte cyclic GMP by inhibiting cyclic GMP-phosphodiesterase leads to decreased myocardial oxygen consumption (11). Other investigators have also shown increases in

myocardial cyclic GMP to have negative metabolic and inotropic effects on the myocardium in both isolated hearts and heart muscle (9, 10 27). However, increased cyclic GMP may also result in positive responses via its actions on intracellular calcium, protein kinases, and other second messengers (e.g., cyclic AMP) (14, 24, 28). It appears that negative inotropic responses or reduced O_2 consumption predominate under most circumstances. From the present results, it appears that basal endogenous NO production is not sufficient to affect the myocardium.

Most studies have reported that extrinsic NO or enhanced NO production leads to increased cyclic GMP and reduced myocardial function or O_2 consumption (2, 7, 10, 12, 15). This occurs in intact hearts, isolated hearts, muscle strips, and myocytes. However, administration of physiological concentrations of exogenous NO has been reported not to depress the inotropic state of myocytes or isolated rat or cat hearts (8). There is evidence that nitric oxide synthases exist and that the L-arginine–NO pathway is constitutively present in myocytes (6, 7, 13, 29). Although we have demonstrated vascular NOS blockade, we have not determined whether myocyte NOS was also blocked by L-NAME or L-NMMA. It is possible that endogenous myocardial NO along with endothelium-derived NO may interact in regulating cardiac function. Endothelium-derived NO has been shown to attenuate myocyte contraction in isolated guinea pig myocytes (2). In isolated rat hearts, nitric oxide blockade with L-NMMA resulted in vasoconstriction-induced ischemia with subsequent depression of myocardial mechanical function (5). It would appear that ischemia was a major factor leading to depression of myocardial function in this study. Grocott-Mason *et al.* (30) also reported that endogenous nitric oxide production had important functional effects on isolated ejecting hearts.

In the present study, we found that intracoronary infusion of NOS blockers did not cause any significant changes in myocardial mechanics, regional myocardial oxygen consumption, or myocardial cyclic GMP levels. We used an intracoronary infusion of the NOS blockers to maximize local and minimize global effects. The lack of change at the myocardial level of the second messenger cyclic GMP may explain the observed lack of functional or metabolic changes. Kirkeboen *et al.* (18) reported that NOS blockade did not alter local myocardial O_2 consumption in the pig heart. When myocardial NO levels are enhanced, it attenuated dobutamine-induced increases in contractility in the dog heart (31). The differences in results between the lack of effect of endogenous basal nitric oxide production on myocardial function in the *in vivo* heart and isolated heart and myocyte preparations could be due to species variation or differences in the experimental preparations. There are differences in background adrenergic tone between isolated myocyte and heart preparations and the acute, open-chest, anesthetized canine model. However, the lack of effect is probably not related to the barbituate anesthesia we used, as Gurevicius *et al.* (19) found no change in myocardial O_2 consumption in dogs with NOS blockade using fentanyl and midazolam anesthesia.

If the basal level of nitric oxide production was sufficient to affect myocytes *in vivo,* blockade of NOS should lead to a decrease in myocardial cyclic GMP levels (4, 14). This change in cyclic GMP would change myocardial function and/or metabolism under a variety of circumstances (2, 7, 10, 12, 15). We found no effect of NOS blockade on myocardial cyclic GMP levels and also no metabolic changes. In the three dogs administered methylene blue, we did find a reduction in cyclic GMP leading to an increased functional effect. Thus, in this preparation changes in cyclic GMP are capable of changing myocardial function. Our study suggests that endogenous basal production of NO is not sufficient to alter myocardial function. When myocardial NO production is enhanced, it may play some role in inotropy in the dog heart (31).

In summary, intracoronary L-NAME and L-NMMA injection in normal anesthetized mongrel dogs produced significant NO blockade, as evidenced by coronary vasoconstriction and increased myocardial O_2 extraction. In addition, we confirmed NO blockade by observing the near abolition of the vasodilatory effects of cholinergic stimulation. This nitric oxide synthase blockade led to no significant changes in global hemodynamics, cardiac mechanics, myocardial O_2 consumption, or myocardial cyclic GMP levels. Thus, it appears that endogenous vascular and myocardial nitric oxide production is insufficient to control local myocardial cyclic GMP levels, and therefore is not a major controller of myocardial oxygen consumption or function *in vivo* under basal conditions.

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