Modulation of Coronary Autoregulatory Responses by Nitric Oxide
Evidence for Flow-Dependent Resistance Adjustments in Conscious Dogs

Thomas P. Smith, Jr, John M. Canty, Jr

The present study tested the hypothesis that nitric oxide production in coronary resistance vessels is an important mechanism affecting the regulation of myocardial perfusion in unanesthetized dogs. We inhibited nitric oxide synthesis with the arginine analogue Nω-nitro-L-arginine methyl ester (L-NAME, 10 mg/kg) and maintained the compressive determinants of myocardial blood flow constant by ventricular pacing. L-NAME did not affect resting coronary blood flow and reduced the receptor-mediated increase in flow to intracoronary acetylcholine (100 μg/min 1C) from 143±20% (mean±SEM) under control conditions to 31±10% after L-NAME (P<.001). Coronary autoregulatory relations were determined as steady-state coronary pressure was reduced by inflating a hydraulic occluder. Initial resistance adjustments over the autoregulatory plateau were not affected by L-NAME. Closed-loop autoregulatory gain was 0.84±0.09 under control conditions versus 0.78±0.07 after L-NAME (P=NS). As coronary pressure was reduced further, however, the critical pressure at which myocardial ischemia began (lower autoregulatory break point) increased from 45±3 mm Hg under control conditions to 61±2 mm Hg (P<.001) after L-NAME. In addition, the slope of the coronary pressure-flow relation below the autoregulatory break point was reduced (1.0±0.2 versus 0.58±0.09 mL·min⁻¹·mm Hg⁻¹ after L-NAME, P<.05), reflecting a reduction in the maximal conductance recruitable during ischemia. In concert with the effects of L-NAME on autoregulatory responses during ischemia, peak reactive hyperemic flow to a 30-second coronary occlusion was also reduced (from 200±22 to 166±24 mL/min after L-NAME, P<.01). In contrast, metabolic flow recruitment to a twofold increase in heart rate was not affected by L-NAME. These results indicate that (1) both initial autoregulatory adjustments to reductions in coronary pressure and metabolic flow recruitment are probably mediated by either myogenic and/or metabolic mechanisms and do not require nitric oxide production, and (2) during ischemia, endothelium-dependent production of nitric oxide is an important mechanism responsible for minimizing coronary vascular resistance. Thus, inhibiting nitric oxide production increases the vulnerability of the myocardium to ischemia at reduced perfusion pressure. In pathophysiological states associated with impaired endothelium-dependent vasodilation, the loss of nitric oxide–dependent resistance adjustments may contribute to the functional significance of a coronary stenosis. (Circulation Research 1993;73:232-240)

KEY WORDS • autoregulation • reactive hyperemia • metabolic recruitment • nitric oxide • Nω-nitro-L-arginine methyl ester

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The precise mechanisms responsible for maintaining coronary blood flow constant as coronary arterial pressure is reduced distal to a stenosis are still controversial.1 Several previous studies have investigated the role of metabolic mediators such as adenosine.1–4 Although the local concentrations of metabolic mediators have been inferred to increase when either coronary flow is reduced or myocardial metabolism is increased, subsequent adjustments in coronary vascular tone would cause their concentration to return toward normal once the balance between oxygen delivery and consumption is restored. As a result, the changes in their concentration in the perivascular space are small, and they have been difficult to implicate in controlling steady-state coronary resistance in the absence of sustained myocardial ischemia.4

Recently, studies in isolated coronary resistance arterioles that are uncoupled from metabolic mediators of vascular tone have demonstrated that both myogenic and flow-mediated endothelium-dependent mechanisms may be important in the local regulation of blood flow throughout much of the coronary microcirculation.5 Kuo et al6 demonstrated that these responses compete with one another so that they could permit both local flow and capillary pressure to be regulated very closely. Flow-mediated adjustments in vascular tone in porcine microvessels were dependent on the presence of a functionally normal endothelium5 and could be completely inhibited by blocking nitric oxide production (one of the putative forms of endothelium-
Nitric Oxide and Autoregulation in Conscious Dogs

Smith and Canty

Nitric oxide (NO) plays a crucial role in the regulation of coronary blood flow. It is a potent vasodilator and is produced by the enzyme NO synthase. While NO is synthesized and released in response to various stimuli, it is also affected by metabolic and autoregulatory factors. In this study, we aimed to investigate the effects of NO on coronary blood flow and autoregulation in conscious dogs.

Materials and Methods

Studies were conducted in chronically instrumented dogs. All experimental protocols were performed in accordance with institutional guidelines. A total of 19 mongrel dogs (28 ± 1 [mean ± SEM] kg) were studied.

Experimental Preparation

Animals were fasted overnight and premedicated with Innovar-Vet (0.4 mg/ml fentanyl and 20 mg/ml droperidol, 1 to 3 ml IM). A surgical plane of anesthesia was induced using sodium thiopental (20 mg/kg IV). After endotracheal intubation, anesthesia was maintained with nitrous oxide (=60%), oxygen (=40%), and halothane (=1% to 2%) mixture. Under sterile conditions, a thoracotomy was performed in the fourth left intercostal space. Tygon catheters were inserted into the left atrium and descending thoracic aorta. Pacing leads were sewn onto the right ventricular outflow tract. A micromanometer (P6.5, Königssberg Instruments, Inc, Pasadena, Calif) was inserted into the left ventricular apex through a stab wound. We dissected a 2 to 3-cm length of the proximal left circumflex artery free and instrumented it with a transit-time ultrasonic flow probe (Transonic Inc, Ithaca, NY), hydraulic occluder, and Teflon angioplast placed into the distal coronary artery and constructed as we have previously described.29 A Teflon angioplast was also placed into the main pulmonary artery for drug infusion. To limit the small amount of flow arising from epicardial collaterals that would not be measured by the flow probe, we ligated superficial anastomoses between epicardial branches of the anterior descending and circumflex arteries as well as between the distal right coronary artery and distal circumflex artery as we have previously described.9 At the conclusion of instrumentation, the chest was closed, and the pneumothorax was evacuated. Animals were given prophylactic antibiotics (300 mg streptomycin and 300,000 U IM procaine penicillin) for 3 to 5 days after surgery. Postoperative analgesia (2 mg IM butorphanol, as needed) was given until the animals appeared free of subjective signs of discomfort. Catheters were flushed with saline at regular intervals and filled with heparin (1000 U/mL). Enteric-coated aspirin was begun on the third to fifth postoperative day (325 mg PO every day). The animals were allowed to recover for at least 10 days before studies were conducted with animals in the unanesthetized state. Two separate groups of animals were studied.

Group 1: The Effect of L-NAME on the Coronary Flow Response to Intracoronary Acetylcholine and Steady-State Autoregulatory Response

Group 1 animals (n=10) were fasted overnight and sedated with Innovar-Vet (1 to 3 mL IM). After allowing 30 minutes for the animals to adjust to the laboratory, measurements of systemic and coronary hemodynamics were obtained with the animals lying on their right side. To prevent bradycardia during intracoronary acetylcholine, animals were paced at their spontaneous rate before and after inhibiting nitric oxide production with L-NAME (10 mg/kg IV bolus). After baseline variables were recorded in the resting state, a saline vehicle was infused into the distal coronary catheter at a rate of 1 mL/min. Subsequently, acetylcholine (dilutions of 1, 100, and 300 µg/mL saline) was infused into the distal coronary artery at doses between 0.1 and 300 µg/min (infusion rates, 0.1 to 1 mL/min).

After performing graded acetylcholine infusions, we assessed steady-state autoregulatory relations between distal coronary pressure and flow while maintaining heart rate constant by ventricular pacing. Progressive reductions in distal circumflex pressure were produced by inflating the hydraulic occluder. At least 1 minute was allowed for coronary pressure and mean coronary flow to equilibrate before sampling data. Pressure was reduced in 5 to 10-mm Hg increments over the autoregulatory plateau and in smaller pressure increments below the autoregulatory break point. Once we obtained the control autoregulatory relation, coronary pressure was restored, and regional flow was allowed to return to control values with 60 minutes allowed for recovery. L-NAME was administered, and after 30 minutes, flow responses to graded infusions of acetylcholine and the steady-state autoregulatory relation were repeated.

Group 2: Effects of L-NAME on Myocardial Reactive Hyperemia and Metabolic Flow Recruitment to Pacing

In group 2 animals (n=9), we assessed the effects of inhibiting nitric oxide synthesis on the coronary reactive hyperemic response to a 30-second total coronary occlusion. Seven of the nine animals in this group were part of a separate study that assessed the role of nitric oxide in flow-mediated vasodilation of epicardial conduit arteries in vivo.10 Under control conditions, measurements of peak flow following release of the occlusion as well as the percentage of flow repayment to debt were determined in each of the animals. The percentage of flow repayment to debt was determined by integrating the area under the reactive hyperemic flow curve as described by Coffman and Gregg11: blood flow debt (mL)=control flow rate (mL/min)×occlusion time (minutes); excess flow during reactive hyperemia=total flow volume during reactive hyperemia (mL)−control

derived relaxing factor) with the arginine analogue Nω-monomethyl-L-arginine (L-NMMA).6 If such flow-dependent mechanisms were operative in vivo, the inhibition of nitric oxide synthesis could increase the minimal vascular resistance attainable in the coronary circulation and cause increased vulnerability of the myocardium to ischemia. On the other hand, the local metabolic stimuli to which these vessels are subjected in vivo may be able to compensate for impaired flow-dependent vasodilation when nitric oxide production is inhibited.

The purpose of the present investigation was to examine whether coronary autoregulatory responses were mediated or modulated by flow-dependent nitric oxide production from coronary resistance vessels in vivo. We compared physiological flow adjustments during autoregulation, myocardial reactive hyperemia, and metabolic flow recruitment to pacing before and after inhibiting nitric oxide production with the arginine analogue Nω-nitro-L-arginine methyl ester (L-NAME) in conscious chronically instrumented dogs. The results demonstrate that nitric oxide is an important mediator of physiological flow adjustments during ischemia at reduced pressures distal to a coronary stenosis.

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flow rate (mL/min)×hyperemia duration (minutes); repayment of flow debt (%)=[excess flow during reactive hyperemia (mL)/flow debt (mL)]×100. After flow returned to baseline, we determined the effect of ventricular pacing on metabolic recruitment. Under control conditions, measurements of coronary flow and systemic hemodynamics were obtained during sinus rhythm at rest and after an approximately twofold increase in heart rate (200 beats per minute) produced by ventricular pacing. Ventricular pacing was stopped, and coronary flow was allowed to return to normal, after which L-NAME (10 mg/kg IV) was administered. Since the increase in mean aortic pressure caused the sinus rate to slow, ventricular pacing was implemented at a rate that approximated the sinus rate under control conditions at rest. We assessed the adequacy of nitric oxide inhibition by determining the flow response to intracoronary acetylcholine (100 μg/min IC). The coronary flow responses to transient occlusion and pacing were then repeated.

**Data Analysis**

All data were recorded on a Gould 2800W recorder. Catheters were connected to Statham P23dB transducers for pressure measurement. The zero reference level was taken at the level of the dorsal spine in the right lateral decubitus position in group 1 animals. Group 2 animals were studied while standing in a sling with the zero reference level taken at the midchest. The Königsberg micromanometer was calibrated at the beginning of each study by matching the systolic pressure to that measured simultaneously in the ascending aorta and matching the left ventricular end-diastolic pressure to equal the peak atrial wave on the simultaneously measured left atrial pressure. Hemodynamic data were digitized at a sampling rate of 200 Hz with an analog-to-digital convertor (model DT2801A, Data Translation Inc, Marlboro, Mass) interfaced to an IBM PC AT computer. All results represent the average of a 15-second sampling interval comprising at least 15 cardiac cycles.

Coronary autoregulatory relations were constructed under resting conditions and after L-NAME using the approach schematically illustrated in Fig 1. Closed-loop autoregulatory gain\(^\dagger\) over the autoregulatory plateau was calculated from the slope of the pressure-flow regression relation above the autoregulatory break point using the following equation:

\[
\text{Autoregulatory Gain} = \frac{\Delta F}{\Delta P} = \frac{P_i}{F_i}
\]

where \(\Delta F/\Delta P\) is the slope of the autoregulatory plateau regression relation, \(P_i\) is the resting coronary pressure, and \(F_i\) is the resting coronary flow. Plateau regression relations were determined using points at coronary pressures greater than 50 mm Hg under control conditions and 70 mm Hg after L-NAME to ensure that they were above the lower autoregulatory break point. The autoregulatory relation during ischemia was determined using linear regression of pressure and flow points where flow was reduced by at least 20% below the mean value obtained over the autoregulatory plateau. The lower limit of autoregulation (or autoregulatory break point) was defined as the intersection between the two regression relations. From each autoregulatory relation, pressure and flow points below the autoregulatory break point corresponding to relative flow reductions of 75%, 50%, 25%, and 0% of control were calculated from individual regression relations during ischemia. These were pooled to compare pressure-flow points under control conditions to those following L-NAME. Detailed autoregulatory relations were available for analysis in 8 of the 10 group 1 animals. Two were excluded because peripheral coronary pressure during a total coronary occlusion was greater than 20 mm Hg. Although this criterion was used to identify animals with an unusually high degree of collateralization, the shift in the autoregulatory relations produced by L-NAME was similar.

Data are presented as mean±1 SEM. Hemodynamic data under control conditions and after L-NAME were analyzed using a repeated-measures analysis of variance (ANOVA) with post hoc tests performed using paired \(t\) tests and the Bonferroni correction for multiple comparisons when appropriate. Differences in pressure-flow regression relations under control circumstances and after L-NAME were also determined by ANOVA. Values were considered significant at \(P<.05\).

**Results**

All animals were in good health at the time of the study. Arterial blood gases were as follows: pH, 7.37±0.01; P\(CO_2\), 34±1 mm Hg; and P\(O_2\), 85±1 mm Hg. Hematocrit averaged 36±1%. Resting hemodynamics in group 1 animals before and after L-NAME are summarized in Table 1. Mean left atrial pressure at the beginning of the experiments was 6.5±1 mm Hg. Although L-NAME produced an increase in mean arterial and systolic left ventricular pressure, heart rate did not change since it was maintained constant by ventricular pacing. In addition, left ventricular end-diastolic pressure was not affected by L-NAME. Thus, the compressive determinants of diastolic coronary flow were not affected by inhibiting nitric oxide production.
TABLE 1. Systemic and Coronary Hemodynamics in Group 1 Dogs

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>L-NAME</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (bpm)</td>
<td>107±7</td>
<td>107±7</td>
</tr>
<tr>
<td>Mean aortic pressure (mm Hg)</td>
<td>92±4</td>
<td>124±4*</td>
</tr>
<tr>
<td>LV systolic pressure (mm Hg)</td>
<td>114±5</td>
<td>140±4*</td>
</tr>
<tr>
<td>LV end-diastolic pressure (mm Hg)</td>
<td>8±1</td>
<td>7±1</td>
</tr>
<tr>
<td>Coronary flow (mL/min)</td>
<td>29±4</td>
<td>26±3</td>
</tr>
</tbody>
</table>

L-NAME, Nω-nitro-L-arginine methyl ester; bpm, beats per minute; LV, left ventricular. Values are mean±SEM.
*P<.001 vs control.

Vasodilation to Intracoronary Acetylcholine

The transient response to intracoronary acetylcholine was characterized by an initial increase in flow that, over a period of 1 to 2 minutes, decreased and reached a stable plateau above the resting flow level. Similar transient responses have been reported by Van Winkle and Feigl. Accordingly, our flow responses to intracoronary acetylcholine infusions represent steady-state values obtained after 2 to 3 minutes at each infusion rate and are summarized in Fig 2. The vasodilation to intracoronary acetylcholine was expressed as a percentage and calculated as the change in flow above the resting value. Intracoronary infusion of the saline vehicle produced no significant change in coronary flow. Acetylcholine caused progressive vasodilation that began at doses of 0.1 μg/min IC and reached a maximum at infusion rates of 100 μg/min IC. Systemic hemodynamics other than coronary flow remained unchanged below infusion rates of 100 μg/min. At infusion rates of 300 μg/min under control conditions, systemic recirculation of acetylcholine occurred, causing reductions in arterial pressure and a reflex increase in heart rate. Under control conditions, measurements at infusion rates of 300 μg/min could not be obtained in four animals because of severe systemic hypotension, which caused coronary flow to decrease. After inhibiting nitric oxide production with L-NAME, the vasodilation to intracoronary acetylcholine was attenuated (P<.00001, by ANOVA). Importantly, the peak flow response following L-NAME appeared to plateau at infusion rates of 100 μg/min, reaching a maximum of 31% above the resting values.

Coronary Autoregulatory Responses

Fig 3 summarizes the effects of L-NAME on autoregulatory relations from all of the animals that were studied. There was very little change in coronary blood flow as pressure was reduced over the autoregulatory plateau before as well as after L-NAME. Closed-loop autoregulatory gain averaged 0.84±0.09 at rest versus 0.78±0.07 after L-NAME (hatched triangles). Both the lower autoregulatory break point (arrows) and the relation between pressure and flow during ischemia were shifted to the right after inhibiting nitric oxide production. Flow began to fall when coronary pressure reached 61 mm Hg after L-NAME vs. 45 mm Hg under control conditions. Values are mean±SEM.

Fig 2. Bar graph showing the effect of Nω-nitro-L-arginine methyl ester (L-NAME) on the increase to steady-state infusions of intracoronary acetylcholine. VEH, saline vehicle. Acetylcholine caused progressive vasodilation that increased flow to a maximum of 143% of resting values at an infusion rate of 100 μg/min (estimated arterial plasma concentration, ≈10⁻⁵ M) under control conditions (solid bars). After inhibiting nitric oxide production with L-NAME (hatched bars), the flow increase to acetylcholine was markedly attenuated but not completely abolished. The maximum response obtained was 32% and remained constant as the infusion rate was increased from 10 to 300 μg/min. Values are percent increase from resting flow levels as the mean±SEM.
Myocardial Reactive Hyperemia

On release of a transient coronary occlusion, flow increased by 510±36% of resting values, with an average percentage of flow repayment to debt of 548±102%. In this group of animals, L-NAME reduced the flow increase to intracoronary acetylcholine (100 μg/min) from 108±13 to 56±6 mL/min (P<.001), demonstrating a significant inhibition of nitric oxide synthesis. The effects of inhibiting nitric oxide production on the coronary reactive hyperemic response to a 30-second total occlusion are summarized in Fig 4. Despite an increase in mean arterial pressure, inhibition of nitric oxide production reduced peak reactive hyperemic flow from 200±22 mL/min under control conditions to 166±24 mL/min after L-NAME (P<.01). In addition, the percentage of flow repayment to flow debt was reduced to 240±30% after L-NAME (P<.01 versus the control condition).

Metabolic Flow Recruitment During Ventricular Pacing

The effects of inhibiting nitric oxide production on pacing-induced increases in flow are summarized in Fig 5. Systemic hemodynamics under each condition are summarized in Table 2. Resting flow and heart rate were not affected by L-NAME. Since bradycardia was prevented by ventricular pacing after L-NAME, there was a loss of atrioventricular synchrony, and left ventricular end-diastolic pressure did not change despite an increase in mean arterial pressure. When myocardial oxygen demand was increased by pacing to a similar rate (200±3 versus 197±4 beats per minute), coronary flow increased to 74±8 mL/min under resting conditions and to 73±8 mL/min after L-NAME (P=NS). Thus, inhibiting nitric oxide production did not affect flow recruitment to increases in myocardial metabolism.

Discussion

The results of this study demonstrate that the arginine analogue L-NAME is a potent inhibitor of acetylcholine-induced stimulation of nitric oxide production in conscious dogs. Despite this inhibition, we found variable effects of L-NAME on adjustments in coronary flow to physiological stimuli. L-NAME did not affect initial autoregulatory adjustments in coronary vascular resistance as coronary pressure distal to a stenosis was reduced or resistance adjustments occurring in response to modest increases in demand produced by pacing. In contrast, inhibiting nitric oxide production blunted the coronary reactive hyperemic response, increased the critical coronary pressure at which flow began to fall during autoregulation, and reduced flow at similar coronary pressures below the lower autoregulatory break point. These latter findings suggest that endothelium-dependent production of nitric oxide in coronary resistance vessels is an important mechanism that is involved in regulating myocardial perfusion during ischemia.

Inhibition of Agonist-Induced Vasodilation to Acetylcholine

We confirmed the inhibition of nitric oxide production by assessing the flow response to intracoronary

Table 2. Systemic and Coronary Hemodynamics in Group 2 Dogs

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>Tachycardia</th>
<th>L-NAME</th>
<th>Control L-NAME</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (bpm)</td>
<td>95±3</td>
<td>86±2</td>
<td>200±3</td>
<td>197±4</td>
</tr>
<tr>
<td>Mean aortic pressure (mm Hg)</td>
<td>100±4</td>
<td>130±2*</td>
<td>103±4</td>
<td>128±2*</td>
</tr>
<tr>
<td>LV systolic pressure (mm Hg)</td>
<td>119±5</td>
<td>144±3†</td>
<td>115±5</td>
<td>135±3†</td>
</tr>
<tr>
<td>LV end-diastolic pressure (mm Hg)</td>
<td>9±1</td>
<td>7±1</td>
<td>4±1</td>
<td>3±1</td>
</tr>
<tr>
<td>Coronary flow (mL/min)</td>
<td>42±4</td>
<td>41±4</td>
<td>74±8</td>
<td>73±8</td>
</tr>
</tbody>
</table>

L-NAME, Nω-nitro-L-arginine methyl ester; bpm, beats per minute; LV, left ventricular. Values are mean±SEM (n=9).
*P<.001, †P<.05 vs control.
acetylcholine. The changes in flow we found during sustained infusions of acetylcholine under resting conditions were similar to those that have previously been reported in closed-chest anesthetized dogs by Van Winkle and Feigl.\(^5\) Inhibition of nitric oxide synthesis reversed the flow increase to intracoronary acetylcholine and shifted the coronary flow response curve to the right.\(^6\) In contrast to other L-arginine analogues, the inhibition of agonist-induced vasodilation could not be overcome by increasing doses of acetylcholine as high as 300 \(\mu\)g/min (estimated plasma concentration, \(\approx 10^{-4}\) M). After L-NAME, the flow increase plateaued at a maximum of \(\approx 30\%\) above resting values (versus \(\approx 140\%\) under control conditions) and did not increase further as inflation rates were varied from 100 to 300 \(\mu\)g/min.

Thus, at the dosage we used, L-NAME blocked the nitric oxide synthase enzyme in a way that could not be overcome by agonist-induced stimulation. The persistent attenuation of acetylcholine-induced vasodilation is consistent with the observations of Rees et al,\(^5\) who demonstrated a plateau in the relaxation of isolated aortic rings to acetylcholine (maximum concentration, \(3 \times 10^{-6}\) M) at concentrations of L-NAME that were greater than 1 \(\mu\)M. In addition to demonstrating persistent attenuation of acetylcholine-induced vasodilation, Broten et al\(^6\) also found that L-NAME-induced inhibition of nitric oxide production could not be readily reversed with exogenous L-arginine.

### Modulation of Autoregulatory Responses by Nitric Oxide

Recent studies in isolated coronary resistance vessels that are removed from their metabolic milieu have demonstrated the importance of myogenic and flow-mediated mechanisms of vasodilation. Kuo et al\(^1\) found a transmural variation in the ability of statically pressurized resistance vessels to dilate in response to reductions in distending pressure. Myogenic dilation was maximal at pressures of 60 cm H\(_2\)O for subendocardial arterioles, whereas it persisted to pressures as low as 40 cm H\(_2\)O for subepicardial arterioles. Although the magnitude of diameter changes over the entire range of pressure studied were modest (<10% of resting values), their results demonstrated a potential role for myogenic mechanisms in eliciting autoregulatory resistance adjustments that are independent of the endothelium.\(^1\)

The influence of local flow on coronary tone was quantitatively much more important. In saline-perfused arterioles, flow-dependent vasodilation was demonstrated to occur over a flow range that varied from zero to approximately two times the estimated resting flow value.\(^5\) Flow increased arteriolar diameter by up to 25% of resting values, and the changes approached the maximum passive dilation elicited by nitroprusside. At flows above this range, diameter remained constant and equaled the passive value. Although myogenic responses were independent of the endothelium, flow-mediated vasodilation was completely eliminated after the endothelium was removed.\(^5\)\(^6\) Furthermore, Kuo et al\(^6\) found that incubation of microvessels with L-NMMA completely abolished the reactivity due to changes in flow. These authors concluded that endothelium-dependent nitric oxide production was responsible for flow-mediated vasodilation of coronary resistance vessels. Although interesting, the extent to which this in vitro inhibition could have affected resistance adjustments to physiological stimuli in vivo could not be determined from their data.

We found that inhibition of nitric oxide synthesis with L-NAME in conscious dogs had no effect on resting flow or coronary flow adjustments over the autoregulatory plateau. Initial reductions in coronary pressure continued to be coupled with resistance adjustments that maintained flow nearly constant over the autoregulatory plateau. When pressure was reduced to the lower autoregulatory break point, flow changed by less than 10% under each circumstance. Closed-loop autoregulatory gain was 0.84 under control conditions versus 0.78 after L-NAME. This similarity indicates that the role of nitric oxide production in mediating resistance adjustments over the autoregulatory plateau is probably limited in the canine coronary circulation and suggests that either myogenic and/or metabolic mechanisms are dominant. In contrast to our findings in conscious animals, Ueeda et al\(^1\) found that the ability of the nonworking buffer-perfused guinea pig heart to autoregulate flow over the autoregulatory plateau was improved after inhibiting nitric oxide synthesis with NG\(^\text{\textsuperscript{-}}\text{nitro-L-arginine (NNLA).}\) After administering NNLA, autoregulatory gain significantly increased at coronary pressures between 25 and 85 mm Hg and was associated with a reduction in resting flow and venous PO\(_2\). Similar effects of L-NMMA were observed in the rabbit ear, in which inhibition of nitric oxide production enhanced autoregulation in a vascular bed where autoregulation is usually poor.\(^1\) The major difference between these results and our findings in conscious dogs appears to be related to differences in the intrinsic ability to autoregulate flow under basal conditions. Under resting conditions, autoregulatory gain averaged 0.16 over a pressure range of 85 to 45 mm Hg in the study of Ueeda et al,\(^1\) whereas it was 0.84 in our study. This lower intrinsic autoregulatory gain may have reflected a relatively vasodilated circulation due to elevated release of nitric oxide in the buffer-perfused isolated heart. Alternatively, it could represent a species difference or be secondary to the effects of acute surgical instrumentation in the isolated guinea pig preparation. Since perfusion pressures greater than arterial pressure cannot be obtained in intact preparations such as ours, we cannot exclude the possibility that autoregulatory relations might be affected by inhibiting nitric oxide production when coronary pressure exceeds left ventricular pressure. Nevertheless, we conclude that, at coronary pressures below aortic pressure, the characteristics of autoregulation over the autoregulatory plateau are not affected by inhibiting nitric oxide production, provided that the intrinsic ability of the heart to autoregulate flow is high.

In contrast to the failure of L-NAME to affect the control of coronary flow over the autoregulatory plateau in conscious dogs, L-NAME had a profound effect on shifting the lower autoregulatory break point as well as the relation between coronary pressure and flow during ischemia. Under control conditions, coronary flow began to fall when pressure decreased below 45 mm Hg. This is similar to the lower autoregulatory pressure limit that we found for subendocardial flow using microspheres in conscious dogs.\(^7\) After L-NAME, the lower autoregulatory pressure limit increased to 61
mm Hg (Fig 4). The shift in the lower autoregulatory break point and increase in coronary vascular resistance during ischemia are compatible with the notion that inhibition of flow-mediated vasodilation in resistance vessels increases the vulnerability of the myocardium to ischemia. Alternatively, L-NAME may have attenuated the response of locally released vasodilator substances acting through endothelium-dependent mechanisms that required nitric oxide production. The magnitude of the shift in the autoregulatory relation produced by inhibiting nitric oxide production was comparable to that which we previously found during tachycardia. When heart rate was increased from 100 to 200 beats per minute, the lower limit of autoregulation was increased to 59 mm Hg in conscious dogs. Since the shifts in the latter study were entirely related to increases in metabolic demand and the increases in compressive determinants of subendocardial flow during tachycardia, it is important to consider the potential role of such effects as well as collateral flow on our observations.

Despite the fact that L-NAME caused an increase in systolic and mean arterial pressure, we prevented the reflex bradycardia by pacing the animals at their control rate. Thus, the constancy of heart rate as well as the failure of left ventricular end-diastolic pressure to increase after L-NAME indicates that the compressive determinants of diastolic coronary perfusion remained unchanged. Effects of L-NAME on metabolic demand in our preparations are more difficult to address directly, since we were unable to measure myocardial oxygen consumption. Several reports have indicated that inhibiting nitric oxide production decreases resting coronary flow or, in constant flow preparations, increases perfusion pressure. We, as others, have found that flow remains unchanged when heart rate is constant. Nevertheless, we cannot exclude the possibility that the increase in systolic pressure may have increased myocardial oxygen consumption through an unmeasured increase in oxygen extraction. Such an increase could have affected the lower autoregulatory break points but would have caused our results based on flow to underestimate the actual difference in the lower autoregulatory break point. Differences in oxygen consumption and oxygen extraction could not explain the fact that the slope of the relation between coronary pressure and flow below the break point was decreased. Since the compressive determinants of diastolic perfusion were constant, the reduction in the slope of the pressure-flow relation during ischemia implies that L-NAME reduced oxygen delivery by limiting coronary vasodilation in the setting of ischemia. Such an effect could presumably be reversed by administering an exogenous vasodilator but remains to be established.

Although the driving pressure for collateral flow increased because of the increase in arterial pressure after L-NAME, its quantitative importance is unlikely to have affected our results. We found that peripheral coronary pressure, an index of collateral flow into a totally occluded vascular bed, averaged 15 mm Hg under control conditions and 17 mm Hg after L-NAME (Fig 3). Although this difference did not reach statistical significance, it could have represented a small increase in unmeasured collateral flow into the circumflex region after L-NAME. Nevertheless, this small difference could not have accounted for the large shift we observed in the autoregulatory break point and the pressure-flow relation during ischemia after L-NAME.

Effects of Inhibiting Nitric Oxide Production on Reactive Hyperemia and Flow Recruitment to Increases in Metabolic Demand

Several studies have demonstrated that inhibiting nitric oxide production blunts the reactive hyperemic response following a transient coronary occlusion. Most studies have demonstrated a reduction in the duration of reactive hyperemia as reflected by flow repayment and/or the percentage of flow repayment to flow debt. The effect of L-arginine analogues on peak reactive hyperemic flow has been variable. In conscious dogs, Chu et al. found that L-NMMA did not affect peak reactive hyperemic flow to a 20-second occlusion. Nevertheless, the minimal coronary vascular resistance obtainable was probably increased, since arterial pressure increased after L-NMMA. In isolated buffer-perfused hearts, Kostic and Schrader found that L-NAME reduced the flow repayment following a 60-second occlusion but did not affect peak flow. Similarly, Yamabe et al. found that intracoronary L-NMMA blunted the percentage of flow repayment to flow debt but that it did not affect peak reactive hyperemic flow in open-chest anesthetized dogs.

Our results differ from these studies in that, after L-NAME, both peak reactive hyperemic flow and the percentage of flow repayment to flow debt were reduced. Similar responses have been reported after NNLA in a recent study by Parent et al. Thus, like the effects of L-NAME on autoregulatory flow adjustments during ischemia, the ability of ischemic stimuli to reduce coronary vascular resistance to a transient occlusion was attenuated by inhibiting nitric oxide production in a setting where the compressive determinants of coronary flow also remained constant.

In contrast to the significant effects of L-NAME on ischemic vasodilation during autoregulation and reactive hyperemia, it had no effect on flow recruitment to metabolic stimuli. We found that the increase in coronary flow produced by pacing to approximately 200 beats per minute was similar before and after L-NAME. Several factors may explain these apparently disparate results. First, the increase in flow required to meet metabolic needs during pacing was modest (approximately a twofold increase in flow). Because of this, metabolic vasodilation alone or in conjunction with myogenic mechanisms may have been able to overcome the inhibition of flow-dependent vasodilation. Second, during increases in myocardial demand, the metabolic stimuli responsible for vasodilation are present in both the endocardium and epicardium, and competition among mechanisms regulating perfusion occurs across the entire myocardial wall. In contrast, during autoregulatory adjustments in tone, reductions in coronary pressure cause a selective impairment of subendocardial perfusion at a time when flow to the outer layers is maintained.

In this setting, the metabolic stimuli reflecting an imbalance between oxygen supply and demand will only modulate vascular tone in subendocardial resistance vessels. Resistance vessels carrying blood to the subendocardium, which are located in the outer
layers, may not be subjected to these metabolic mediators (see below). Finally, the mechanisms involved in controlling vascular resistance may be distributed in series such that metabolic, flow-mediated, and myogenic control mechanisms predominate in different classes of arteriolar vessels. With this type of arrangement, appropriate metabolic recruitment could simply reflect the ability of local resistance adjustments in arterioles where metabolic or myogenic vasodilation predominates to compensate for the inhibition of nitric oxide–mediated flow-dependent vasodilation in other classes of resistance arterioles. Such an effect has been suggested by Kostic and Schrader,24 who found adenosine release to increase after inhibiting nitric oxide production in isolated guinea pig hearts.

Physiological and Pathophysiological Importance of Nitric Oxide–Dependent Resistance Adjustments in the Control of Coronary Blood Flow In Vivo

Our findings suggest that, in vivo, nitric oxide–dependent coronary resistance adjustments are necessary for regulating myocardial perfusion during ischemia. Since isolated coronary arterioles are almost completely vasodilated at flows that are only slightly higher than their estimated resting values,23 inhibition of nitric oxide synthesis appears to increase the minimal resistance attainable in vessels in which this mechanism is operative. The extent to which this will affect the overall regulation of coronary flow in vivo will depend on whether competing metabolic and/or myogenic mechanisms can overcome these abnormalities. One possible explanation for our findings is that the control of a significant portion of the vascular network supplying blood to the subendocardium is physically disassociated from local metabolic stimuli. Chilian25 has demonstrated that penetrating arterioles that span the myocardial wall contribute a substantial pressure drop to perfusion of the subendocardial microcirculation. Because of their anatomic orientation, these vessels will be subjected to metabolic stimuli that can vary along their length in the setting of nontransmural ischemia. Thus, the portion of these vessels that lies in the subendocardium will be subjected by increased concentrations of metabolic mediators for vasodilation. In the nonischemic outer layers, however, the metabolic stimuli for vasodilation may be absent until coronary pressure is sufficiently low. Under normal conditions, endothelium-dependent nitric oxide released in response to changes in local flow will be able to adjust the tone of these resistance vessels, which are physically removed from the ischemic subendocardium. In contrast, after inhibiting nitric oxide, they will constrict and contribute an additional “fixed” resistance to flow that will increase the minimal coronary vascular resistance obtainable for any given coronary driving pressure. As a result, the impairment of flow-dependent resistance adjustments will increase the vulnerability of the myocardium to nontransmural ischemia. As coronary pressure is lowered and ischemia propagates from the endocardium to the epicardium, the inhibition of flow-dependent mechanisms may eventually be overcome by local metabolic stimuli for vasodilation. Although the pathophysiological importance of this hypothesis remains to be defined, both epicardial conduit arteries in humans with atherosclerosis26 and isolated coronary resistance arterioles from animals with hypercholesterolemia27 have been shown to have impaired flow-mediated vasodilatory responses. Thus, the physiological significance of any given stenosis may reflect both hydraulic effects of the epicardial stenosis as well as an impairment of flow-mediated resistance adjustments in the distal vascular bed. Further studies will be required to address this hypothesis directly.

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References


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