Nitric Oxide Formation Contributes to β-Adrenergic Dilation of Resistance Coronary Vessels in Conscious Dogs

Robert Parent, Mohammed Al-Obaidi, Michel Lavallée

The contribution of the l-arginine/nitric oxide pathway to β-adrenergic dilation of resistance coronary vessels was examined in conscious dogs instrumented for measuring coronary blood flow (CBF), left ventricular (LV) wall thickening, and LV and aortic pressures and for intracoronary injections of acetylcholine (0.003 μg/kg), nitroglycerin (0.175 μg/kg), and graded doses of isoproterenol (0.0005 to 0.004 μg/kg). Peak increases in CBF with intracoronary isoproterenol (0.001 μg/kg) averaged 105±10% from baseline. With acetylcholine, CBF increased by 158±11%, and with nitroglycerin, CBF increased by 139±10%. After the administration of intracoronary Nω-nitro-l-arginine methyl ester (L-NAME, 10 μg/kg per minute for 12 minutes) to block nitric oxide synthesis from l-arginine, baseline CBF was not altered, and CBF increased by 49±7% with isoproterenol and by 94±6% with acetylcholine; both values were smaller (P<.01) than those before the arginine analogue. With nitroglycerin, CBF was increased by 145±11%, not significantly different from the value before L-NAME. Intracoronary l-arginine (1.0 mg/kg per minute for 12 minutes), the precursor of nitric oxide synthesis, partially reversed the inhibition of L-NAME on CBF responses to acetylcholine and isoproterenol. After β-adrenergic blockade, CBF responses to isoproterenol and acetylcholine were also reduced (P<.05) by the arginine analogue. When increases in CBF were prevented, peak changes in coronary vascular conductance with intracoronary bolus doses of acetylcholine and isoproterenol were attenuated (P<.01) by L-NAME. Thus, nitric oxide formation is an important intermediate in β-adrenergic dilation of resistance coronary vessels in conscious dogs. (Circulation Research 1993;73:241-251)

Key Words • β-adrenergic receptors • nitric oxide • endothelium-derived relaxing factor • coronary blood flow • l-arginine

Nitric oxide synthesized from l-arginine plays a pivotal role in the cascade of reactions leading to endothelium-dependent relaxation of conductance coronary vessels. In isolated coronary microvessels, Myers et al showed that hemoglobin reverses acetylcholine (ACH)-induced dilation, presumably because of inactivation of an endothelium-derived relaxing factor. Pharmacological or mechanical removal of endothelial cells in coronary microvessels blunts vascular relaxation to bradykinin. Thus, a relaxing factor formed by endothelial cells is ultimately responsible for the dilation of resistance coronary microvessels. Arginine analogues topically applied on or directly injected into coronary arteries to specifically block nitric oxide formation antagonized ACh-induced dilation of canine coronary resistance vessels. Therefore, nitric oxide formed from l-arginine is an important intermediate in endothelium-dependent relaxation of resistance coronary vessels.

The possibility that the l-arginine/nitric oxide pathway intervenes in β-adrenergic—mediated dilation of resistance coronary vessels has received limited attention. Rubanyi and Vanhoutte showed that isoproterenol (ISO)—induced relaxation of canine conductance coronary vessels precontracted with prostaglandin F2α was partially endothelium dependent, but the involvement of nitric oxide in this process was not considered. In contrast, Macdonald et al, using a similar approach, reported that β-adrenergic relaxation was endothelium independent. Consistent with these findings, Jackson and Busse found that endothelial removal did not influence ISO-induced relaxation of hamster thoracic aortas. However, in rat thoracic aortas, Gray and Marshall found that β-adrenergic dilation was completely endothelium dependent. Young and Vatner showed that iliac dilation to epinephrine was reversed to constriction after endothelial denudation. Therefore, an endothelium-derived substance could explain the dilation to this mixed α- and β-adrenergic agonist. In microvessels of feline hindquarters, an arginine analogue failed to block the vasodilation to ISO, whereas in hindquarters of rats, Nω-nitro-l-arginine reduced the vasodilation created by salbutamol, a β-adrenergic agonist. Thus, the role of nitric oxide in the dilation of resistance and conductance vessels to β-adrenergic stimulation remains debated.

The specific goal of the present study was to determine whether a powerful inhibitor of nitric oxide synthesis from l-arginine, modified the vasodilator responses of
resistance coronary vessels to ISO. Animals were studied in the conscious state and with intracoronary drug delivery to avoid the complicating influences of anesthesia and recent surgery on coronary regulation and the potentially confounding hemodynamic effects associated with the systemic administration of an arginine analogue.

Materials and Methods

Instrumentation

Under general anesthesia with sodium pentobarbital (30 mg/kg IV) and under sterile conditions, eight mongrel dogs (28±1 kg) underwent a left thoracotomy at the fifth intercostal space under artificial ventilation. The pericardium was widely incised parallel to the phrenic nerve. A Tygon (Norton Plastics and Synthetic Division, Akron, Ohio) catheter implanted in the thoracic aorta was used to measure arterial pressure (model 800, Bentley Trantec, Irvine, Calif). Mean arterial pressure (MAP) was obtained with an active filter with a time constant of 2 seconds. Through an apical stab wound, a solid-state pressure transducer (model P6.5, Konigsberg Instruments, Inc, Pasadena, Calif) was implanted in the left ventricular (LV) cavity to record LV systolic pressure (LVP) and to obtain the first derivative of LVP, LV dP/dt. A catheter was also implanted in the LV cavity to cross calibrate the miniature pressure gauge and to eliminate any drift of the instrument through repeated calibrations. A cardiotachometer (model 9857, Sensor Medics, Anaheim, Calif) triggered by the LV pressure pulse was used to monitor heart rate (HR). An ultrasonic Doppler blood flow transducer was implanted around the left circumflex coronary artery 2 to 3 cm from the bifurcation of the main left coronary artery. Coronary blood flow (CBF) was monitored using a 10-MHz pulsed Doppler flowmeter. Mean CBF was obtained with an active filter with a time constant of 2 seconds. The linearity of the relation between flow velocity and Doppler shift has been previously demonstrated for this instrument and was confirmed in our laboratory by in vitro testing. At necropsy, the internal circumference of the vessel under the probe was measured to obtain the vessel cross-sectional area and to calculate a calibration factor (in milliliters per minute per kilohertz). A Silastic (Dow Corning Co, Midland, Mich) catheter was implanted proximal to the flow probe, and its tip was advanced in the lumen of the circumflex coronary artery 1 to 2 cm from the bifurcation of the main left coronary artery using the approach described by Gwirtz.21 The portion of the catheter within the coronary vessel had an external diameter of 0.6 mm. A pair of 7-MHz crystals was implanted within the perfusion bed of the left circumflex coronary artery to measure regional wall thickening. One crystal was inserted in the endocardium of the LV wall, and the other was positioned at the epicardial surface. Regional wall motion was continuously monitored with an ultrasonic sonomicrometer (model 120, Triton Technology, San Diego, Calif). Adequate alignment of the transducers was confirmed during surgery by determining the position of the epicardial crystal where wall thickness was the smallest. The pericardium was left open, the chest was closed in layers, and the catheters and wires were exteriorized on the back of the animals. At necropsy, adequacy of the placement of endocardial crystals was verified.

Hemodynamic variables were recorded on an eight-channel tape recorder (model 3968A, Hewlett-Packard Co, San Diego, Calif) and monitored on a direct ink-writing strip-chart recorder (model 280Os, Gould, Cleveland, Ohio).

Protocols

Experiments were initiated 2 to 6 weeks after surgery in conscious healthy dogs lying quietly on their right sides in a dimly illuminated laboratory. While continuously monitoring HR, LVP, LV dP/dt, phasic arterial pressure and MAF, phasic and mean CBF, and regional LV wall thickness, bolus doses of 0.003 µg/kg ACh chloride (Sigma Chemical Co, St Louis, Mo), of 0.0005, 0.001, 0.002, and 0.004 µg/kg ISO hydrochloride (Sabex, Boucherville, Quebec, Canada), and of 0.175 µg/kg nitroglycerin (NTG, Parke-Davis, Scarborough, Ontario, Canada) were administered directly into the circumflex coronary artery. Drugs were freshly dissolved in 0.2 mL warm saline (38.9°C), slowly injected into the coronary catheter, and then flushed with 1.5 mL of saline delivered with a pump-driven syringe over a 12-second period. A continuous intracoronary infusion of warm saline (0.4 mL/min) was used to ensure the patency of the intracoronary catheter throughout these experiments. Bolus doses (0.2 mL) of saline were injected into the coronary artery to verify absence of any change in CBF related to the injection method. Our selection of doses for the various drugs was guided by preliminary experiments that demonstrated peak increases in CBF by nearly 150% above baseline with ACh and NTG and lack of other hemodynamic effects. Doses of ISO were selected to produce dose-dependent increases in CBF and minimal hemodynamic effects. The sequence of drug administration was randomly selected. At least 5 minutes was allowed between two injections for the return to a steady-state baseline. The same procedure was repeated after intracoronary administration of 10 µg/kg per minute L-NAME (Sigma) for 12 minutes, delivered in saline (0.5 mL/min). Five minutes was allowed after the completion of L-NAME delivery before any drug injection. An earlier study conducted in our laboratory showed that, under these conditions, the arginine analogue had a limited influence on the major determinants of coronary perfusion and provided a stable inhibition of ACh-induced coronary vasodilation for the duration of the experiments. The reversibility of the blockade of the coronary dilation to ACh and ISO (0.001 and 0.002 µg/kg) by L-NAME was then verified after intracoronary administration of L-arginine hydrochloride (Sigma) at 1.0 mg/kg per minute for a 12-minute period, delivered in saline (0.5 mL/min).

On a different day, 1.0 mg/kg IV atenolol (Stuart Pharmaceuticals, Wilmington, Del), a β1-adrenergic blocker, was administered to eliminate the inotropic effects of ISO and to consider β1-adrenergic coronary responses more specifically.22 This protocol was conducted in seven dogs. Adequacy of β1-adrenergic blockade was verified with intravenous injections of 0.1 µg/kg ISO before and after atenolol and at the completion of the experiments. Before atenolol, intravenous ISO (0.1 µg/kg) increased (P<.01) HR by 62±5 from 78±5 beats per minute and LV dP/dt by 2038±173 from 3165±109
mm Hg/s. MAP fell (P<.01) by 21±3 from 93±3 mm Hg, but LVP did not significantly change from baseline (115±2 mm Hg). After atenolol, increases in HR and LV dP/dt with intravenous ISO were limited (P<.01) to 19±3 from 74±4 beats per minute and 244±42 from 3150±132 mm Hg/s. MAP fell (P<.01) by 14±2 from 96±3 mm Hg and LVP by 11±2 from 120±3 mm Hg. In addition, the lack of increases in LV dP/dt and wall thickening with intracoronary ISO (0.002 μg/kg) confirmed the adequacy of β1-adrenergic blockade.

Under atenolol, intracoronary bolus doses of ACh (0.003 μg/kg), ISO (0.001, 0.002, and 0.004 μg/kg), and NTG (0.175 μg/kg) were administered before and after intracoronary L-NAME as previously described.

To examine the possibility that a flow-dependent mechanism influenced the coronary responses to ACh and ISO, six additional dogs (31±1 kg) were studied with and without controlling CBF before and after L-NAME. In these animals, the Doppler flow probe was placed close to the origin of the left circumflex coronary artery, and a hydraulic constrictor placed next to this flow probe was used for preventing drug-induced increases in CBF. An intracoronary catheter was implanted distal to the hydraulic constrictor to inject various drugs and to measure distal coronary pressure (DCP). Only those dogs in which the coronary anatomy allowed positioning the tip of the catheter before the first marginal branch of the circumflex coronary artery were included in this study. This ensured that no large arterial branches were located between the flow probe and the tip of the intracoronary catheter. This catheter was connected to a pressure transducer (model 800, Bentley Trantec) to monitor DCP. Mean DCP was obtained with an active filter with a time constant of 2 seconds.

After recording baseline hemodynamics and DCP, bolus doses of drugs (0.2 mL) were injected directly into the intracoronary catheter and flushed with 0.7 mL warm saline over 7 seconds. DCP recordings were immediately obtained thereafter. The same approach was used when CBF was controlled. In this situation, the hydraulic constrictor was inflated at the time CBF started to increase from baseline, and care was taken to limit its increases to less than 10% over baseline levels and to avoid even slight decreases of CBF under baseline levels. ACh (0.003 μg/kg) and ISO (0.001 and 0.002 μg/kg) were injected into the coronary artery under normal and controlled CBF conditions, before and after L-NAME, as described earlier.

Data Analysis

Hemodynamic data were read directly from the strip charts under baseline conditions and at peak increases of CBF for the various drugs. The excess of CBF created by the drugs, termed volume response (in milliliters), was determined by planimetry with a computer-assisted digitizing tablet. The duration of CBF increases was the interval between the initial rise and the return to baseline CBF. End-diastolic wall thickness was read immediately before the rapid rise of LVP, and end-systolic wall thickness was read 20 milliseconds before peak negative LV dP/dt. Wall thickening (WTh) is the difference between end-systolic and end-diastolic wall thickness. LV dP/dt and WTh were read at a time corresponding to their peak effects before β1-adrenergic blockade.

All values are reported as mean±SEM. Multiple simultaneous comparisons of baseline levels or responses for a given drug under control conditions, after L-NAME, and after L-arginine were made with analysis of variance for repeated measurements followed by Bonferroni’s t test to isolate specific contrasts.23,24 These analyses were performed for ACh and ISO (0.001 and 0.002 μg/kg). Comparisons of baseline levels or responses before and after L-NAME for ISO (0.0005 and 0.004 μg/kg) and NTG were performed with t tests for paired observations. Under atenolol, comparisons of baseline values or responses for a given drug, before and after L-NAME, were made with t tests for paired observations. Overall simultaneous comparisons of all baseline levels under control conditions, after L-NAME, and after L-arginine were made with analysis of variance for repeated measurements. After β1-adrenergic blockade, a similar procedure was used to simultaneously compare all baseline levels before and after L-NAME.

Data derived from experiments involving the control of CBF were obtained at peak changes in CBF and DCP. Coronary vascular conductance (CVC) was calculated as the ratio of CBF to DCP. Overall statistical comparisons of all baseline values were made as described above. Comparisons of baseline values or responses for a given drug, before and after L-NAME, were made with t tests for paired observations. Statistical significance was reached at P<.05.

All experimental procedures were approved by an ethical committee on animal care and performed in accordance with “Guide to the Care and Use of Experimental Animals” (Canadian Council on Animal Care publication No. [ISBN] 0-909187-10-8, Ottawa, 1980-1984).

Results

Normal CBF

Experiments were initiated in eight dogs. Hemodynamic variables are reported for all animals, except for WTh measurements that were not available for one dog. After atenolol, seven dogs were studied, and WTh measurements are reported in six animals. Intracoronary NTG injections were performed in five animals after atenolol. For reasons of clarity in the data presentation, intracoronary ACh, NTG, and ISO (0.001 μg/kg) responses are described thoroughly in “Results.” Responses to intracoronary ISO (0.0005, 0.002, and 0.004 μg/kg) are reported in the tables and figures only. Fig 1 illustrates hemodynamic and coronary responses to an intracoronary bolus of ISO (0.002 μg/kg) before and after L-NAME.

Hemodynamic baselines and responses to the intracoronary vasodilators are reported in Table 1 under control conditions, after L-NAME, and after L-arginine. CBF baseline values before the administration of various drugs are reported in Table 2.

Isolated differences between baseline hemodynamics were found and reported in Table 1. However, overall simultaneous comparisons made between baseline values (values before all drugs) did not reveal statistically significant differences before and after L-NAME for LVP, LV dP/dt, MAP, HR, and WTh. A similar situa-
FIG 1. Recording of left ventricular pressure (LVP), first derivative of LVP over time (LV dP/dt), phasic and mean arterial pressure (AP and AB respectively), regional wall thickness (WT), phasic and mean coronary blood flow (CBF and CBF respectively), and heart rate (HR) with intracoronary administration (arrow) of isoproterenol (ISO, 0.002 μg/kg) before and after intracoronary N^o-nitro- l-arginine methyl ester (L-NAME, 10 μg/kg per minute for 12 minutes). The increase in CBF with ISO was attenuated after administration of the arginine analogue.

The excess of CBF, averaged 9.1±0.5 mL and lasted 17.8±1.2 seconds, as reported in Fig 2.

After L-NAME, ACh resulted in peak increases in CBF by 94±6% and in volume responses of 3.9±0.3 mL lasting 13.7±0.7 seconds, all significantly (P<.01) less than before L-NAME.

L-Arginine partially reversed the inhibitory effects of L-NAME on ACh-induced CBF responses. Peak increases in CBF averaged 119±11%, and volume responses averaged 5.8±0.5 mL, both significantly (P<.01) greater than after L-NAME alone. The duration of CBF responses (15.2±0.6 seconds) was not significantly augmented.

After β-adrenergic blockade, L-NAME attenuated (P<.01) the effects of ACh on peak (from 160±8% to 103±9%), volume (from 8.8±0.6 to 3.9±0.5 mL), and duration (from 18.3±0.9 to 13.5±1.3 seconds) of CBF responses.
Other hemodynamic effects of intracoronary ACh were small and did not differ before L-NAME, after L-NAME, and after L-arginine (Table 1). Under β₁-adrenergic blockade, hemodynamic effects of ACh were trivial.

**Isoproterenol**

Intracoronary ISO (0.001 μg/kg) resulted in peak increases in CBF by 105±10%. Volume responses averaged 9.1±0.9 mL and lasted 31.7±3.8 seconds, as reported in Fig 2. After L-NAME, ISO resulted in peak increases in CBF by 49±7% and in volume responses of 2.9±0.5 mL lasting 18.8±2.6 seconds, all significantly (P<.01) less than before L-NAME.

L-arginine partially reversed the inhibitory effect of L-NAME on ISO-induced CBF responses. Peak in-
Nitroglycerin

Intracoronary NTG (0.175 μg/kg) resulted in peak increases in CBF by 139±10%. Volume responses averaged 8.0±0.8 mL and lasted 17.6±0.9 seconds.

After L-NAME, NTG resulted in peak increases in CBF by 145±11% and in volume responses of 7.0±0.5 mL lasting 17.0±1.1 seconds. These responses did not differ from those before L-NAME.

After β₁-adrenergic blockade, L-NAME failed to alter peak (155±12% and 158±4%), volume (7.0±0.6 and 7.2±0.5 mL), and duration (17.9±1.0 and 17.3±1.5 seconds) of CBF responses.

Hemodynamic responses to NTG were trivial and similar before and after L-NAME without (Table 1) and under β₁-adrenergic blockade.

Controlled CBF

In six additional dogs, responses to intracoronary ACh (0.003 μg/kg) and ISO (0.001 and 0.002 μg/kg)
were examined under normal CBF and also under controlled CBF conditions before and after intracoronary L-NAME. Fig 4 illustrates hemodynamic and coronary responses to an intracoronary bolus of ISO (0.002 μg/kg) under controlled CBF, before and after L-NAME. Data are reported under baseline conditions and at peak changes in CBF or DCP in Table 3. Overall simultaneous comparisons made between baselines did not reveal statistically significant differences before and after L-NAME. Only ACh and ISO (0.001 μg/kg) data are discussed thoroughly below. Data for ISO (0.002 μg/kg) are reported in Table 3 and Fig 5.

Acetylcholine

With intracoronary ACh (0.003 μg/kg), peak increases in CBF averaged 114±15%. When CBF was controlled, CBF increases were limited to 3±1%, while mean DCP fell by 47±2%.

After L-NAME, increases in CBF with ACh averaged 65±10%, significantly (P<.01) less than before L-NAME. Mean DCP fell by 33±3%, significantly (P<.01) less than before L-NAME, whereas CBF increases were limited to 3±1%.

After L-NAME, increases in CBF with ACh were significantly (P<.01) less than before L-NAME with and without controlling CBF (Fig 5).

Hemodynamic responses to ACh were trivial and similar under all conditions.

Isoproterenol

Intracoronary ISO (0.001 μg/kg) resulted in increases in CBF by 70±10%. When CBF was controlled, mean DCP fell by 45±4%, whereas CBF increased by only 3±1%.

After L-NAME, ISO increased CBF by 30±5%, significantly (P<.01) less than before L-NAME. When increases in CBF were limited to 4±1%, mean DCP fell by 27±4%, significantly (P<.01) less than before L-NAME.

After L-NAME, increases in CBF with ISO were significantly (P<.01) less than before L-NAME with and without controlling CBF (Fig 5).

Except for small increases in LVP and LV dP/dt, which were similar before and after L-NAME, other hemodynamic effects of ISO were trivial.

Discussion

The l-arginine/nitric oxide pathway is involved in β-adrenergic dilation of resistance coronary vessels in conscious dogs. This process is analogous to the endothelium-dependent dilation of conductance coronary vessels involving nitric oxide as the key intermediate for the relaxation of vascular smooth muscles.2,25,26 The present study extends previous findings showing that nitric oxide formation from l-arginine intervenes in the relaxation of coronary microvessels to ACh, adenosine, and transient ischemia.4 Endothelial cells are generally considered to be the site of nitric oxide formation under these conditions.27 In the present study, L-NAME, a powerful inhibitor of nitric oxide synthesis from l-arginine,17 not only reduced ACh-induced coronary vasodilator responses as expected but also antagonized ISO-induced coronary vasodilation. As for ACh, the inhibition of ISO-induced vasodilatation by the arginine analogue was partially reversed by L-arginine, the physiological precursor of nitric oxide synthesis.27 Blockade of the l-arginine/nitric oxide pathway by the arginine analogue was selective, because NTG-induced vasodilation, an endothelium-independent process, was not altered by the administration of L-NAME. Thus, the formation of nitric oxide from l-arginine is a common pathway involved in ISO and ACh vasodilator effects on canine coronary resistance vessels.

Nitric oxide formation and endothelium-dependent vasodilation have been initially described for conductance coronary vessels.28 These observations have been recently extended to the control of resistance coronary vessels. Similar to conductance coronary vessels, the control of resistance vessels involves a flow-dependent mechanism that requires an intact endothelium.5,29,30 Furthermore, vasodilator responses of coronary microvessels to ACh and bradykinin are abolished after endothelial denudation.5,5 Because arginine analogues antagonize ACh-induced dilation of resistance-sized coronary vessels, nitric oxide formed in endothelial cells is involved in the cascade of reactions leading to vascular relaxation.7,8,30 Direct measurements of nitric oxide content in the venous effluent of perfused hearts sup-
FIG 4. Recording of left ventricular pressure (LVP), first derivative of LVP over time (LV dP/dt), arterial pressure (AP), phasic and mean distal coronary pressure (DCP and DCP, respectively), phasic and mean coronary blood flow (CBF and CBF, respectively), and heart rate (HR) with intracoronary administration (arrow) of isoproterenol (ISO, 0.002 μg/kg) before and after intracoronary N*-nitro-L-arginine methyl ester (L-NAME, 10 μg/kg per minute for 12 minutes) under controlled CBF. The fall in DCP induced by ISO was decreased after L-NAME.

port this conclusion.31 L-Arginine is the physiological precursor of nitric oxide formation involved in endothelium-dependent relaxation.27 Therefore, endothelium-dependent relaxation and the synthesis of nitric oxide from L-arginine may play major roles in the vasomotor control of coronary microvessels.

Our selection of the dose and route of L-NAME administration were guided by preliminary experiments and an earlier study.8 With the present approach, the arginine analogue provided a stable blockade of CBF responses to intracoronary ACh for the duration of the experiments without the confounding effects of altered systemic hemodynamics. An incomplete blockade of nitric oxide synthesis may explain why ACh and ISO responses were not abolished. It is also possible that nitric oxide is not the sole intermediate involved in vasodilator responses to ACh.3,26 On the basis of our measurements of CBF responses, we can conclude that a major fraction of ACh-induced and ISO-induced coronary vasodilator responses is explained by nitric oxide formation.

The possibility that β-adrenergic dilation of resistance coronary vessels involves an endothelium-dependent mechanism linked to the l-arginine/nitric oxide pathway has not been directly considered earlier. β-Adrenergic vasodilation has been suggested to be endothelium dependent by studies in which endothelial denudation reduced responses to ISO in canine coronary arteries10 and in aortas and femoral arteries of normal13 and spontaneously hypertensive32 rats and in which norepinephrine-induced relaxation of rabbit aortic rings was blunted after removal of endothelial cells.33 Consistent with the possibility that an endothelium-dependent mechanism intervened in β-adrenergic relaxation of
conductance vessels, the dilation of the canine iliac artery to epinephrine was reversed to constriction after removal of the endothelium.\textsuperscript{14} In contrast, Jackson and Busse\textsuperscript{12} reported that endothelium removal did not prevent relaxation to ISO in hamster thoracic aortas. Macdonald et al\textsuperscript{11} also reported in canine coronary arteries that \(\beta\)-adrenergic dilation was endothelium independent. Opposite findings have been reported concerning a possible link between \(\beta\)-adrenergic dilation of resistance vessels and nitric oxide formation. In the feline hindquarters, L-NAME failed to block the vasodilation to ISO,\textsuperscript{15} whereas in the hindquarters of rats, \(N^G\)-nitro-l-arginine reduced the vasodilation created by salbutamol, a \(\beta_2\)-adrenergic agonist.\textsuperscript{16}

In the present study, L-NAME reduced the dilation to a mixed \(\beta_1\)- and \(\beta_2\)-adrenergic agonist, ISO, consistent with the involvement of the L-arginine/nitric oxide pathway in the vasorelaxation of resistance coronary vessels. Although ACh-induced vasodilation of resistance coronary vessels is clearly an endothelium-dependent process involving nitric oxide formation,\textsuperscript{5-8} we cannot directly conclude that endothelial cells involved in ISO-induced coronary dilation of resistance coronary vessels. Because of the similarities of coronary responses to ACh and ISO regarding their inhibition by L-NAME and their partial reversibility with \(L\)-arginine, \(\beta\)-adrenergic dilation of resistance coronary vessels most likely involves endothelium-derived nitric oxide. This hypothesis is further supported by the observation that \(L\)-arginine is the physiological precursor of nitric oxide formation, which mediates endothelium-dependent relaxation.\textsuperscript{27}

We considered that blockade of \(\beta\)-adrenergic receptors by L-NAME may explain the reduction of coronary

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**Table 3. Baseline Hemodynamics and Peak Changes With Intracoronary Acetylcholine and Isoproterenol Before and After Intracoronary \(N^G\)-Nitro-L-Arginine Methyl Ester With and Without Controlling Coronary Blood Flow**

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<th>Before L-NAME</th>
<th>After L-NAME</th>
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<tr>
<td></td>
<td>Baseline</td>
<td>Change from baseline</td>
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<tr>
<td>CBF (mL/min)</td>
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<td>ACh (0.003 μg/kg)</td>
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<tr>
<td>Normal CBF</td>
<td>43.9±4.0</td>
<td>+49.3±6.9*</td>
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<td>44.4±4.3</td>
<td>+1.5±0.4‡</td>
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<td>ISO (0.001 μg/kg)</td>
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<tr>
<td>Normal CBF</td>
<td>45.7±4.9</td>
<td>+30.2±2.6*</td>
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<td>Controlled CBF</td>
<td>46.4±5.3</td>
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<td>ISO (0.002 μg/kg)</td>
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<td>Normal CBF</td>
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<td>45.2±4.7</td>
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<td>DCP (mm Hg)</td>
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<td>ACh (0.003 μg/kg)</td>
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<tr>
<td>Normal CBF</td>
<td>89±2</td>
<td>−4±1‡</td>
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<tr>
<td>Normal CBF</td>
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<td>−3±1‡</td>
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<td>−48±3*</td>
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<td>MAP (mm Hg)</td>
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<tr>
<td>Normal CBF</td>
<td>91±2</td>
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<td>−1±1</td>
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<td>Normal CBF</td>
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L-NAME, \(N^G\)-nitro-L-arginine methyl ester; CBF, coronary blood flow; ACh, acetylcholine; ISO, isoproterenol; DCP, distal coronary pressure; MAP, mean arterial pressure. Values are mean±SEM (n=6).

*\(P<.01\) vs baseline.

†\(P<.01\) vs corresponding value before L-NAME.

‡\(P<.05\) vs baseline.
vasodilator responses to ISO. In this eventuality, the direct vasodilator effects of ISO on \( \beta_1 \) and \( \beta_2 \)-adrenergic receptors as well as the inotropic responses mediated by \( \beta_2 \)-adrenergic receptors should be compromised. Our analyses revealed similar changes in LV dP/dt and WTH with intracoronary ISO before and after L-NAME. Therefore, the arginine analogue did not directly interfere with \( \beta_2 \)-adrenergic receptors in general and with \( \beta_2 \)-adrenergic receptors in particular. However, the present study did not allow us to directly examine whether L-NAME interfered with \( \beta_2 \)-adrenergic vasodilation, which involves direct vasodilator effects as well as metabolically induced coronary dilation.

To specifically consider the role of nitric oxide formation in \( \beta_2 \)-adrenergic coronary vasodilation, experiments were carried out under atenolol. After \( \beta_2 \)-adrenergic blockade, increases in LV dP/dt and WTH with intracoronary ISO were prevented, and L-NAME strikingly reduced \( \beta_2 \)-adrenergic coronary vasodilator responses. In this situation, ACh-induced coronary vasodilation was also decreased by the arginine analogue, but NTG-induced responses were not. Thus, nitric oxide formation from L-arginine plays an important role in \( \beta_2 \)-adrenergic dilation of coronary microvessels.

A reduction of coronary responses to ACh and ISO after L-NAME could have resulted from the blockade of a flow-dependent process involving nitric oxide formation. This flow-dependent process could potentially magnify the direct effects of a vasodilator by promoting nitric oxide formation. Consequently, the reduction of coronary responses to ISO and ACh reported in the present study may be related to the blockade of this nonspecific flow-dependent component. Our finding that NTG responses were not altered by L-NAME does not completely rule out the possibility that with ACh and ISO a flow-dependent phenomenon intervened. It is conceivable that flow-dependent increases of nitric oxide formation cannot modulate cGMP levels already elevated through the direct effects of NTG.

The possibility that a flow-dependent process intervened in ACh and ISO responses was directly considered in experiments conducted under controlled CBF conditions in which DCP was used as an index of coronary vasomotion of resistance coronary vessels. The decreases in DCP and the increases in CVC created by the intracoronary injections of ACh and ISO were always greater before than after L-NAME. Therefore, the blockade of flow-dependent nitric oxide formation in resistance coronary vessels was not the primary mechanism by which L-NAME reduced the responses to ACh and ISO under the present experimental conditions. Nitric oxide formation is most likely directly coupled to \( \beta_2 \)-adrenergic and muscarinic receptors. However, our findings do not exclude the possibility that flow-dependent effects intervened when increases in CBF with ACh and ISO were allowed to occur. Even when CBF was controlled in epicardial coronary vessels after ACh and ISO, it is not certain that blood flow velocity in resistance coronary vessels remained at baseline. The dilation caused by ACh or ISO would be expected to lead to a decrease in blood flow velocity in resistance vessels that may result in a reduction of nitric oxide formation. In this eventuality, flow-dependent effects could have limited the fall in DCP that are reported in the present study before L-NAME administration under controlled CBF conditions.

In a recent report, Buxton et al.\(^{35} \) have indicated that methyl ester derivatives of arginine analogues may competitively block muscarinic receptors in addition to their inhibitory effects on nitric oxide synthesis. In contrast to the effects of L-NAME on nitric oxide synthase, muscarinic blockade could not be reversed by an excess of L-arginine. In the present study, the possibility that L-NAME reduced coronary responses to ACh by blocking muscarinic receptors cannot be completely excluded. However, the partial reversibility of the inhibitory effects of L-NAME on ACh responses argues against the possibility that the primary effect of L-NAME was the blockade of muscarinic receptors. Furthermore, L-arginine did not produce more reversal of ISO than ACh responses as expected if L-NAME acted as a muscarinic blocker. Consistent with the view that muscarinic blockade by L-NAME in vitro may not be as important as in experiments conducted in vivo, a recent study by Bellan et al.\(^{36} \) showed that, in the hindquarters vascular bed of the cat, L-NAME and \( N^\circ \)-nitro-L-arginine, a nonesterified derivative of L-arginine, produced similar maximal blockade of ACh-induced vasodilation. When the dose of L-NAME was further increased by 30-fold, additional blockade of ACh responses was not found. These findings indicate that blockade of muscarinic receptors was not a feature of L-NAME under these in vivo experiments. Thus, muscarinic blocking properties of L-NAME described.
in vitro may not be extended to experiments conducted in vivo.

L-NAME did not significantly influence baseline CBF in the present and in an earlier study. Because nitric oxide is spontaneously released under baseline conditions, a reduction of baseline CBF would be expected after the intracoronary administration of an arginine analogue. Chu et al failed to demonstrate significant changes in baseline CBF with intravenous N\textsuperscript{6} monomethyl-L-arginine but showed a reduction in the diameter of large epicardial coronary vessels in conscious dogs. Therefore, nitric oxide formation is apparently not as important for maintaining basal vasmotor tone in resistance and conductance coronary vessels in conscious animals. In contrast, nitric oxide formed from L-arginine plays a major role in the dilation of resistance coronary vessels to ISO and ACh, as demonstrated in the present study.

In conclusion, the L-arginine/nitric oxide pathway is involved in the cascade of reactions leading to the dilation of coronary resistance vessels induced by ISO and ACh. A receptor-operated mechanism rather than a flow-dependent phenomenon is involved. Endothelial cells are presumably the site of nitric oxide formation, which is ultimately responsible for an important fraction of ISO-induced coronary vasodilation. In addition, nitric oxide formation contributes to \( \beta \text{-adrenergic} \) dilation of coronary resistance vessels.

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