Endothelium-Dependent Relaxation Competes With α₁- and α₂-Adrenergic Constriction in the Canine Epicardial Coronary Microcirculation

Christopher J.H. Jones, MBBS; David V. DeFily, PhD; Jan L. Patterson, MS; and William M. Chilian, PhD

Background. The purpose of this study was to determine whether endothelium-dependent relaxation competes with α₁- and α₂-adrenergic coronary microvascular constriction in the beating heart in vivo.

Methods and Results. Coronary microvascular diameters were measured using stroboscopic epillumination and intravitral microscopy during fluorescein microangiography in open-chested dogs (n=20). Both α₁- and α₂-adrenergic receptors were selectively activated by intracoronary infusions of norepinephrine (0.05 and 0.2 μg · kg⁻¹ · min⁻¹) in the presence of the α₁-adrenergic antagonist rauwolscine (0.2 mg/kg) or the α₂-adrenergic antagonist prazosin (0.75 mg/kg) during β-adrenergic blockade (1 mg/kg propranolol). Microvascular diameters during selective α₂-adrenergic receptor activation were measured under baseline conditions and after inhibition of endogenous nitric oxide synthesis by an analogue of L-arginine, either N⁴-nitro-L-arginine (L-NA, 30 mg/kg) or N⁴-nitro-L-arginine methyl ester (L-NAME, 30 mg/kg). Under baseline conditions, α₂-adrenergic activation constricted small arteries (vessels with diameters between 100 and 300 μm) (4±1% and 5±1% decrease in diameter for the low and high doses of norepinephrine, respectively, both p<0.05) but did not change the diameter of arterioles (vessels with diameters <100 μm). In contrast, α₁-adrenergic activation by the lower but not the higher dose of norepinephrine induced constriction of arterioles (6±2% and 3±4% decrease in diameter, p<0.05 and NS, respectively) but not small arteries. Inhibition of nitric oxide synthase activity by either L-NA or L-NAME produced constriction of small coronary arteries (9±2% decrease in diameter, p<0.01) and arterioles (6±1% decrease in diameter, p<0.05). The dilatation of small arteries and arterioles by acetylcholine (0.05 μg · kg⁻¹ · min⁻¹ intracoronary infusion; 10±1% increase in diameter under baseline conditions, p<0.05) was abolished by either analogue. Both α₁- and α₂-adrenergic coronary microvascular constriction were markedly potentiated after L-NA or L-NAME. α₁-Adrenergic constriction was unmasked in arterioles (7±3% and 10±4% decrease in diameter, p<0.05), although it was not significantly increased in small arteries. Conversely, α₂-adrenergic constriction was unmasked in small arteries (8±1% and 6±2% decrease in diameter, both p<0.05) and potentiated in arterioles (12±1% and 8±4% decrease in diameter, both p<0.05). After L-NA or L-NAME, microvessels retained the ability to dilate to sodium nitroprusside (0.1 μg · kg⁻¹ · min⁻¹ intracoronary infusion; 10±2% increase in diameter, p<0.05). α₂-Adrenergic constriction was not accentuated by increased tone alone, since it was either attenuated or converted to dilatation during a similar degree of preconstriction by the endothelium-independent vasoconstrictor angiotensin II (p<0.05 for both α₁- and α₂-adrenergic activation).

Conclusions: These data confirm that α₁-adrenergic receptors are widespread in the coronary microcirculation, with the baseline functional responses to α₁-adrenergic activation predominating in small arteries and those to α₂-adrenergic activation predominating in arterioles. Furthermore, coronary microvascular constriction caused by both α₁- and α₂-adrenergic receptor activation is significantly modulated by endothelium-dependent relaxation, being markedly potentiated by inhibition of nitric oxide synthase activity. The data imply that α₂-adrenergic activation will assume considerable importance as a determinant of coronary microvascular resistance in pathophysiological situations associated with coronary endothelial impairment. (Circulation 1993;87:1264–1274)

KEY WORDS • coronary circulation • coronary microcirculation • norepinephrine • nitric oxide

Coronary blood flow is regulated through changes in microvascular resistance caused by various dilator and constrictor control mechanisms. Coronary microvascular dilation may be caused by metabolites accumulated during anaerobic myocardial metabolism,¹ by autoregulatory adjustments to reduc-
tions in local transmural pressure,2 or by endothelial release of locally acting vasodilators both basally and in response to circulating agonists or changes in shear stress.3 Conversely, coronary microvascular constriction may be invoked in the autoregulatory myogenic response to an increase in transmural pressure2 or by circulating hormones including the β-adrenergic receptor agonist norepinephrine.4-6 Modulation of coronary β-adrenergic constriction by one or more dilator mechanisms would confer an advantage by protecting the myocardium against inappropriate reductions in coronary blood flow during exercise, hemorrhage, or stress.

Both α1- and α2-adrenergic receptor subtypes have been shown to cause coronary constriction in studies using selective agonists, antagonists, and different preparations.7-9 α1-Adrenergic constriction occurs throughout the coronary microcirculation, whereas α2-adrenergic constriction predominates in arterioles <100 μm in diameter when the opposing influence of autoregulatory dilatation is avoided by controlled hypoperfusion.7 Although α-adrenergic coronary constriction may increase coronary vascular resistance, the constriction is of lesser degree than that in systemic microvascular networks.10 This may be due to differences in α-adrenergic receptor density or function or to the tonic action of a regulatory dilator mechanism. In the absence of altered metabolic demand or coronary perfusion pressure, this modulating mechanism may be endothelium-dependent relaxation.

Endothelium-dependent relaxation in response to flow is mediated by endothelium-derived relaxing factor (EDRF) in the coronary microcirculation.3 It is inhibited by stereo-specific L-arginine analogues, which inhibit the activity of nitric oxide synthase.11,12 Inhibition of nitric oxide synthesis constricts epicardial canine coronary arteries in vivo13 and may increase coronary vascular resistance in some animal species,14,15 suggesting that the endothelium may help to maintain a relatively low basal coronary vascular resistance.

β-Adrenergic constriction and endothelium-dependent relaxation are known to interact in coronary arteries. Endothelium-dependent relaxation is induced by β2-adrenergic activation by norepinephrine in isolated canine epicardial coronary arteries,16 and inhibition of nitric oxide synthesis potentiates the constriction by norepinephrine of large coronary arteries from dogs and humans.17 Ohyanagi et al18 have shown that adrenergic and endothelial control mechanisms also interact in the systemic microcirculation, as β2-adrenergic constriction is potentiated by inhibition of nitric oxide synthesis in a rat cremaster preparation. Whether similar competitive interaction occurs in the coronary microcirculation is unknown. Accordingly, we hypothesized that β-adrenergic constriction is tonically modulated by endothelium-dependent relaxation in the coronary microcirculation. Furthermore, we proposed that this interaction may not be specific for activation of β2-adrenergic receptors as in other vascular beds but also that the endothelium may transduce and respond to an increase in blood velocity and shear stress through coronary microvessels constricted by β2-adrenergic activation. To investigate these hypotheses, we measured changes in epicardial coronary microvascular diameters in the beating canine heart during selective α1- and α2-adrenergic activation by norepinephrine before and after inhibition of the synthesis of nitric oxide by administration of L-arginine analogues.

Methods

Animal Preparation

Adult mongrel dogs of either sex weighing between 4 and 11 kg were sedated with droperidol (0.5 mg/kg) and anesthetized with pentobarbital (30 mg/kg i.v.). They were placed on a homeothermic blanket to maintain body temperature at 37°C. The right femoral artery and vein were cannulated for measurement of aortic pressure and administration of fluids and drugs. A 5F fluid-filled catheter was inserted into the right carotid artery and advanced to a stable position within the left ventricular cavity so that left ventricular pressure could be recorded. The rate of change of left ventricular pressure (LV dp/dt) was determined from the left ventricular pressure signal by an on-line differentiator.

To minimize the periodic movement of the heart caused by inflation of the lungs, the dogs were ventilated by a jet ventilator synchronized to the cardiac cycle. An 18-gauge cannula was inserted into the trachea and advanced to the level of the carina, and an expiratory tracheal tube was positioned under 1–3 cm H2O. Using the peak rate of rise of left ventricular pressure (LV dp/dtmax) as a timing reference, a solenoid connected to a pressure source (60% N2, 40% O2) at 6–12 psi was triggered to open for 20–35 msec at the same time in each cardiac cycle. Respiratory movements of the heart were thus minimized by the small tidal volume and occurred at the same frequency as the heartbeat. Arterial blood gases and pH were analyzed at approximately 30-minute intervals and were maintained in the following ranges by adjustment of the position of the tracheal cannula or by administration of sodium bicarbonate: PCO2, 25–40 mm Hg; PO2, 100–200 mm Hg; pH 7.34–7.44.

The heart was exposed by a left thoracotomy in the fifth left intercostal space and was partially stabilized in a pericardial cradle. The proximal circumflex coronary artery was exposed, and a 24-gauge cannula was inserted for measurements of coronary artery pressure and intracoronary administration of norepinephrine and fluorochromes. As reported previously, coronary pressure and flow are not affected significantly by this procedure because of the small size of the cannula.7 An inferior vena cava tie was applied that could be tightened or released to allow the accurate control of left ventricular preload and ultimately, systemic aortic pressure after the systemic administration of inhibitors of nitric oxide synthase activity.

Microvascular Preparation

The diameters of epicardial coronary microvessels were measured using an intravital microscope (Leitz Ploemopak, Wild Leitz, USA, Inc.) and a Dage silicon-intensified tube video camera (model 66, Dage-MTI Inc., Michigan City, Ind.). The surface of the heart was illuminated by a stroboscopic light source (Chadwick-Helmuth, 100-W xenon arc, El Monte, Calif.). The stroboscope flashed once during each cardiac cycle at the same time during late diastole. A PDP 11/73 computer (Digital Equipment Corp., Nashua, N.H.) received the LV dp/dt signal and triggered the stroboscope to flash.
(20–30 μsec) after a preset delay between successive heartbeats. A polarizing filter reduced the glare from the epicardial surface. The combined use of low tidal volume jet ventilation and brief epicardial illumination, both synchronized to the cardiac cycle, caused the surface coronary microvessels to appear virtually motionless when viewed through the microscope. The microscope objectives used were the Leitz EF4 (4×; numerical aperture, 0.22) and the Leitz L10 (10×; numerical aperture, 0.22). Used with 10× magnification eyepieces, the resulting magnification was either 40× or 100×. The resolution obtained by this system was 6 μm for the 4× objective and 2.6 μm for the 10× objective.

After an area of epicardium with easily visible microvessels was selected, the heart was restrained by the passage of four 22-gauge pins through the left ventricle. The pins were attached to a rod fixed externally in such a position that vertical cardiac motion was eliminated while vigorous myocardial contraction in the plane of the region selected for microvascular measurements continued. As described previously, neither resting blood flow nor vasodilator reserve are affected by this use of myocardial restraint to improve the quality of microvascular images.

**Microvascular Diameter Measurements**

To enhance visualization of the inner diameters of the epicardial microvessels and to allow small arteries and arterioles to be distinguished from venous vessels, fluorescein isothiocyanate-dextran (MW, 2,000,000) was injected in short pulses through the circumflex coronary cannula. The volume of each injected aliquot was 50–100 μl of a 25 mg/ml solution. A Leitz H2 excitation barrier filter was used to activate the fluorescein and receive the emitted light. Arterial and venous vessels fluoresced sequentially for 5–15 seconds after each injection.

The anatomic landmarks of a particular vessel were identified, and between five and eight images were obtained during late diastole at the same point on the vessel over a period <30 seconds. Typically, microvascular measurements over this period varied by less than ±3% from the average value. Control images were obtained at least 15 minutes after each intervention, and vessels in which the microvascular diameters varied from the prior control diameters by >10% were excluded. The fluorescent images were digitized directly from the camera by a frame digitizer (Imaging Technology Inc., Woburn, Mass.) and transferred to a Macintosh IIfx computer (Apple Computer Inc., Cupertino, Calif.) for diameter measurements using appropriate software (*IMAGE* 2.18, National Institutes of Health Research Services Branch). Diameters were measured by aligning cursors at the vessel edges, the measurements in pixels being converted to micrometers using a conversion factor determined in previously described calibration experiments using microspheres of different sizes.

**Experimental Protocol**

Coronary microvascular diameters and hemodynamic variables were measured at the following intervals during two experimental protocols (the methodological details are given below).

**Protocol 1.** 1) Control under baseline conditions; 2) α1-adrenergic activation (n=9) or α2-adrenergic activation (n=6) under baseline conditions; 3) repeat control under baseline conditions 30 minutes later; 4) acetylcholine administration under baseline conditions; 5) control after administration of L-arginine analogue to inhibit nitric oxide synthesis; 6) α1- or α2-adrenergic activation during blockade of nitric oxide synthesis; 7) repeat control during nitric oxide blockade; 8) acetylcholine administration during nitric oxide blockade; 9) repeat control during nitric oxide blockade; 10) sodium nitroprusside administration during nitric oxide blockade.

**Protocol 2.** 1) Control under baseline conditions; 2) α1-adrenergic activation (n=3) or α2-adrenergic activation (n=2) under baseline conditions; 3) repeat control under baseline conditions; 4) angiotensin II administration to cause coronary microvascular constriction to establish a new baseline; 5) repeated α1- or α2-adrenergic activation during angiotensin II administration; 6) repeat baseline measurement during angiotensin II infusion. This protocol was undertaken because inhibition of nitric oxide synthesis (protocol 1) was found to lead to coronary microvascular constriction; therefore, we wished to determine whether increased microvascular tone alone accounted for altered α-adrenergic responses.

α1-Adrenergic activation. Selective activation of α1-adrenergic receptors was achieved by intracoronary infusion of norepinephrine (0.05 μg·kg⁻¹·min⁻¹ and 0.2 μg·kg⁻¹·min⁻¹, infusion commenced 5 minutes before measurements were obtained) in the presence of the selective α2-adrenergic antagonist rauwolscine (0.2 mg/kg intravenous bolus) and the nonselective β-adrenergic antagonist propranolol (1 mg/kg intravenous bolus). Supplementary propranolol (0.25 mg/kg) was administered 15 minutes before the repeat infusions of norepinephrine.

α2-Adrenergic activation. Intracoronary norepinephrine (as described above) was infused in the presence of the selective α1-adrenergic antagonist prazosin (0.75 mg/kg intravenous bolus) and propranolol (as described above).

**Inhibition of nitric oxide synthase activity.** Nitric oxide synthesis was inhibited using L-arginine analogues. These inhibitors were administered as follows: L-NA, 30 mg/kg intravenous injection given over 10 minutes (n=6; α1-activation, n=1; α2-activation, n=5) or L-NAME, 30 mg/kg intravenous injection given over 10 minutes (n=9; α1-activation, n=8; α2-activation, n=1). L-NAME was used in more studies simply because it was easier to handle than L-NA, which tended to precipitate rapidly at a physiological pH. The rise in systemic arterial pressure that was observed after the administration of both agents was reversed by applying tension to the inferior vena cava. Microvascular responses to acetylcholine (0.05 μg·kg⁻¹·min⁻¹ intracoronary infusion commenced 5 minutes before measurements were obtained), an endothelium-dependent vasodilator in the dog, were determined before and 60 minutes after administration of L-NA or L-NAME to confirm that endothelium-dependent relaxation had been attenuated or abolished. Measurements that showed <50% attenuation of acetylcholine-induced vasodilatation after L-NA or L-NAME were excluded.
from further analysis. The ability of smooth muscle to relax after L-NA or L-NAME was evaluated using sodium nitroprusside (0.1 μg·kg⁻¹·min⁻¹ intracoronary infusion for 3 minutes), an endothelium-independent vasodilator.

**Coronary microvascular constriction induced by angiotensin II.** To determine whether the effects of L-NA or L-NAME were due purely to an increase in vascular tone, angiotensin II (0.1 μg·kg⁻¹·min⁻¹ intracoronary infusion) was administered in separate control experiments as a vasoconstrictor. Selective α₁- or α₂-adrenergic activation was accomplished before and during the administration of angiotensin II. The rise in systemic arterial pressure caused by angiotensin II was reversed by increasing the tension on the inferior vena caval tie.

**Data Analysis**

Animals were randomly assigned to the various groups. Microvascular diameters during α-adrenergic activation are expressed as a percent change from the appropriate control diameters obtained immediately preceding the experimental measurement ("+" percent change indicates dilatation; "−" percent change indicates constriction). The significance of the percent diameter changes induced by pharmacological interventions was assessed by multivariate repeated-measures ANOVA using a MANOVA model (Complete Statistical System version 2.1, Statsoft Inc., Tulsa, Okla.) with planned comparisons between the percent changes of diameters using linear contrasts. Although the microvascular responses were generally graded with respect to vessel size, data for small arteries (>100-μm diameter) and arterioles (<100-μm diameter) were analyzed separately in view of the well-recognized differences in physiological behavior between vessels of these size classes.5-7,19,20 Because of these well-accepted differences between arterioles and arteries, vessels within the same animal were considered independent units of observation. The significance of the differences in hemodynamics was assessed using repeated-measures ANOVA followed by Scheffe’s tests. All data are presented as mean±SEM, and a probability value of 5% was used as the criterion of statistical significance.

**Results**

**Systemic Hemodynamics**

Table 1 shows the coronary pressure, aortic pressure, and heart rate during the experiments performed with norepinephrine for α₁- and α₂-adrenergic activation and L-arginine analogues for inhibition of nitric oxide synthesis. These interventions did not significantly change systemic hemodynamics, although we emphasize that, without the use of an inferior vena caval tie, pressure would have been significantly increased after adminis-

**Table 1. Systemic Hemodynamics During Experiments Performed With Norepinephrine and l-Arginine Analogues**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>0.05 NE</th>
<th>0.2 NE</th>
<th>ACH</th>
<th>Control</th>
<th>0.05 NE</th>
<th>0.2 NE</th>
<th>ACH</th>
<th>SNP</th>
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</thead>
<tbody>
<tr>
<td>a1-Adrenergic activation</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Mean coronary pressure</td>
<td>74±7</td>
<td>74±4</td>
<td>77±4</td>
<td>77±3</td>
<td>90±4</td>
<td>82±6</td>
<td>83±5</td>
<td>91±2</td>
<td>66±3*</td>
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<tr>
<td>Mean aortic pressure</td>
<td>76±4</td>
<td>77±5</td>
<td>79±5</td>
<td>79±4</td>
<td>87±6</td>
<td>85±5</td>
<td>85±6</td>
<td>86±7</td>
<td>67±8*</td>
</tr>
<tr>
<td>Mean heart rate</td>
<td>123±9</td>
<td>126±9</td>
<td>128±9</td>
<td>131±10</td>
<td>130±10</td>
<td>138±13</td>
<td>137±12</td>
<td>131±12</td>
<td>138±15</td>
</tr>
<tr>
<td>a2-Adrenergic activation</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Mean coronary pressure</td>
<td>72±7</td>
<td>73±6</td>
<td>75±6</td>
<td>71±6</td>
<td>79±5</td>
<td>79±6</td>
<td>79±6</td>
<td>83±6</td>
<td>73±5*</td>
</tr>
<tr>
<td>Mean aortic pressure</td>
<td>81±2</td>
<td>79±3</td>
<td>80±2</td>
<td>77±3</td>
<td>83±3</td>
<td>79±4</td>
<td>81±6</td>
<td>87±6</td>
<td>76±5</td>
</tr>
<tr>
<td>Mean heart rate</td>
<td>94±4</td>
<td>92±5</td>
<td>91±5</td>
<td>96±3</td>
<td>85±5</td>
<td>95±2</td>
<td>88±5</td>
<td>93±3</td>
<td>97±5</td>
</tr>
</tbody>
</table>

NOx, data during inhibition of nitric oxide synthase (*p<0.05 vs. control); bpm, beats per minute; NE, norepinephrine; ACH, acetylcholine; SNP, sodium nitroprusside.

**Table 2. Hemodynamic Variables During Angiotensin II–Induced Preconstriction**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>0.05 NE</th>
<th>0.2 NE</th>
<th>Control</th>
<th>0.05 NE</th>
<th>0.2 NE</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>a1-Adrenergic activation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean coronary pressure</td>
<td>71±5</td>
<td>72±7</td>
<td>74±5</td>
<td>75±8</td>
<td>79±6</td>
<td>79±4</td>
<td>80±4</td>
</tr>
<tr>
<td>Mean aortic pressure</td>
<td>68±5</td>
<td>74±11</td>
<td>75±7</td>
<td>75±7</td>
<td>81±7</td>
<td>76±5</td>
<td>77±7</td>
</tr>
<tr>
<td>Mean heart rate</td>
<td>122±2</td>
<td>124±2</td>
<td>125±3</td>
<td>127±4</td>
<td>127±3</td>
<td>128±2</td>
<td>127±2</td>
</tr>
</tbody>
</table>

NE, norepinephrine; ang II, angiotensin II; bpm, beats per minute.
tation of the L-arginine analogues. Table 2 shows the same hemodynamic variables measured during the experiments using angiotensin II-induced preconstriction. Systemic hemodynamics were stable in these experiments.

Microvascular Responses to \( \alpha_1 \)- and \( \alpha_2 \)-Adrenergic Activation

\( \alpha_1 \)-Adrenergic activation. Microvascular diameters were not changed by administration of rauwolscine (+1±1%, mean±SEM percent change in diameter, NS). Figure 1 shows the percent changes in microvascular diameters during \( \alpha_1 \)-adrenergic activation under baseline conditions. Small coronary arteries were constricted significantly by both doses of norepinephrine (0.05 \( \mu \)g \cdot kg\(^{-1} \) \cdot min\(^{-1} \), −4±1%; 0.2 \( \mu \)g \cdot kg\(^{-1} \) \cdot min\(^{-1} \), −5±1%; both \( p<0.05 \)), whereas mean arteriolar diameter did not change significantly at either dose (0.05 \( \mu \)g \cdot kg\(^{-1} \) \cdot min\(^{-1} \), 0±2%; 0.2 \( \mu \)g \cdot kg\(^{-1} \) \cdot min\(^{-1} \), −3±2%; both NS).

\( \alpha_2 \)-Adrenergic activation. Microvascular diameters were not changed by administration of prazosin (+4±2%, NS). Percent changes in microvascular diameter during \( \alpha_2 \)-adrenergic activation under baseline conditions are shown in Figure 2. In contrast to the changes observed during \( \alpha_1 \)-adrenergic activation, \( \alpha_2 \)-adrenergic constriction occurred in arterioles (0.05 \( \mu \)g \cdot kg\(^{-1} \) \cdot min\(^{-1} \), −6±2%, \( p<0.05 \); 0.2 \( \mu \)g \cdot kg\(^{-1} \) \cdot min\(^{-1} \), −3±4%; NS) but not in small arteries (0.05 \( \mu \)g \cdot kg\(^{-1} \) \cdot min\(^{-1} \), −2±1%; 0.2 \( \mu \)g \cdot kg\(^{-1} \) \cdot min\(^{-1} \), −1±3%; both NS).

Microvascular Responses to Inhibition of Nitric Oxide Synthase Activity

Percent changes in microvascular diameters 30 minutes after the administration of either L-NA or

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**FIGURE 1.** Scatterplots show percent changes in diameter of individual coronary small arteries (>100-\( \mu \)m diameter) and arterioles (<100-\( \mu \)m diameter) to \( \alpha_1 \)-adrenergic activation by norepinephrine (NE, 0.05 and 0.2 \( \mu \)g \cdot kg\(^{-1} \) \cdot min\(^{-1} \)) in the presence of rauwolscine (0.2 mg/kg) and propranolol (1 mg/kg).

**FIGURE 2.** Scatterplots show percent changes in diameter of individual coronary small arteries (>100-\( \mu \)m diameter) and arterioles (<100-\( \mu \)m diameter) to \( \alpha_2 \)-adrenergic activation by norepinephrine (NE, 0.05 and 0.2 \( \mu \)g \cdot kg\(^{-1} \) \cdot min\(^{-1} \)) in the presence of prazosin (0.75 mg/kg) and propranolol (1 mg/kg).
L-NAME are shown in Figure 3. Significant microvascular constriction was seen (−8±1%, *p*<0.05). No difference was apparent between the magnitude of the constrictor responses to L-NA (−9±1%) and L-NAME (−5±1%). Furthermore, small arteries (−9±2%, *p*<0.05) and arterioles (−6±1%, *p*<0.05) constricted to a similar degree, as did the vessels in the groups of animals pretreated with prazosin (−7±2%, *p*<0.01) or rauwolscine (−9±1%, *p*<0.05).

Acetylcholine caused coronary microvascular dilation under baseline conditions (+10±1%, *p*<0.05) and was abolished 60 minutes after L-NA or L-NAME was administered (−3±2%, NS), indicating effective inhibition of agonist-induced nitric oxide synthase activity in the vessels studied. Acetylcholine given after L-NA or L-NAME constricted microvessels in animals pretreated with rauwolscine (−6±2%, *p*<0.05) but caused no change in diameter in those pretreated with prazosin (−2±2%, NS). Sodium nitroprusside, given after L-NA or L-NAME, caused significant dilatation (+10±2%, *p*<0.05).

**Microvascular Responses to α₁- and α₂-Adrenergic Activation During Inhibition of Nitric Oxide Synthase Activity**

Coronary microvascular constriction by either α₁- or α₂-adrenergic activation was markedly potentiated after the synthesis of nitric oxide was inhibited.

**α₁-Adrenergic activation.** Figure 4 shows the changes in diameter of individual vessels during α₁-adrenergic activation before and after the administration of L-NA or L-NAME. The magnitude of the constriction of the small arteries (0.05 *μg·kg⁻¹·min⁻¹, −6±2%; 0.2 *μg·kg⁻¹·min⁻¹, −8±2%; both *p*<0.05) was unchanged from that observed before administration of the l-arginine analogues. However, arterioles, which previously did not change in diameter, constricted significantly (0.05 *μg·kg⁻¹·min⁻¹, −7±3%; 0.2 *μg·kg⁻¹·min⁻¹, −10±4%; both *p*<0.05).

**α₂-Adrenergic activation.** Figure 5 shows the changes in individual microvascular diameters induced by α₂-adrenergic activation before and after the administration of L-NA or L-NAME. The magnitude of α₂-adrenergic constriction was potentiated after administration of the l-arginine analogues in small arteries, which previously did not change in diameter during α₂-adrenergic activation (0.05 *μg·kg⁻¹·min⁻¹, −8±2%; 0.2 *μg·kg⁻¹·min⁻¹, −6±2%; both *p*<0.05 versus baseline and prior α₂-adrenergic response), and also in arterioles (0.05 *μg·kg⁻¹·min⁻¹, −12±1%; 0.2 *μg·kg⁻¹·min⁻¹, −8±4%; both *p*<0.05 versus baseline and prior α₂-adrenergic activation).
**FIGURE 5.** Line plots show individual coronary microvascular responses to α2-adrenergic activation by norepinephrine (NE) before (baseline, data also shown in Figure 2) and after inhibition of nitric oxide (NOx) synthesis. Probability values indicate the significance of the differences between mean changes in diameter.

α1- and α2-Adrenergic Activation After Constriction by Angiotensin II

**α1-Adrenergic activation.** The influence of increasing microvascular tone by angiotensin II on the microvascular responses to norepinephrine in the presence of propranolol and rauwolscine are shown in Figure 6. Significant α1-adrenergic constriction was observed under baseline conditions (0.05 μg · kg⁻¹ · min⁻¹, -7±2%; 0.2 μg · kg⁻¹ · min⁻¹, -11±2%; both p<0.05). Angiotensin II (0.1 μg · kg⁻¹ · min⁻¹) constricted microvessels (-8±2%, p<0.05) to a comparable degree to the L-arginine analogues. However, in contrast to the responses after the L-arginine analogues, α1-adrenergic activation predominantly caused dilatation after constriction by angiotensin II (0.05 μg · kg⁻¹ · min⁻¹, +6±3%; 0.2 μg · kg⁻¹ · min⁻¹, +11±2%; both p<0.05 versus baseline and prior α1-adrenergic response).

**α2-Adrenergic activation.** Figure 6 also shows the influence of angiotensin II on the microvascular responses to norepinephrine in the presence of propranolol and prazosin. α2-Adrenergic activation caused significant constriction only during the lower dose of norepinephrine under baseline conditions (0.05 μg · kg⁻¹ · min⁻¹, -4±1%, p<0.05; 0.2 μg · kg⁻¹ · min⁻¹, 0±1%, NS). Angiotensin II again caused significant constriction (-5±1%, p<0.05). Angiotensin II significantly modified the response to the lower dose of norepinephrine in favor of dilatation (0.05 μg · kg⁻¹ · min⁻¹, +2±1%; p<0.05 versus prior α2-adrenergic response), although the response to the higher dose was unchanged (0.2 μg · kg⁻¹ · min⁻¹, +6±2%).

**FIGURE 6.** Line plots show individual microvascular responses to α1- and α2-adrenergic activation before (baseline) and during coronary constriction by angiotensin II (ANG II) 0.1 μg · kg⁻¹ · min⁻¹. Results are shown for both doses of norepinephrine (NE) in each case. Note that α-adrenergic constriction was attenuated, abolished, or converted to dilatation by angiotensin II. Probability values refer to the significance of the differences between mean responses without and with angiotensin II.
Discussion

The major finding of this study is that coronary microvascular constriction by both α₁- and α₂-adrenergic activation is unmasked or accentuated by inhibition of nitric oxide synthesis. Our results also show that nitric oxide influences the basal diameter of small epicardial coronary arteries and arterioles. These findings imply that nitric oxide release modulates resting coronary microvascular tone and tonically opposes the constrictor action of catecholamines. We have also confirmed the previous findings from this laboratory that the functional responses to α₁-adrenergic receptor activation occur predominantly in small coronary arteries under baseline conditions when autoregulatory mechanisms are intact and that α₂-adrenergic effects predominate in arterioles. We will address the limitations of our study with particular reference to the methods we used to measure microvascular diameters, inhibit endogenous nitric oxide synthesis, and activate α-adrenergic receptors and will discuss the interpretation and implications of our results.

Measurement of Coronary Microvascular Diameters

The major advantage of our experimental system is that it allows individual coronary microvascular diameters down to 30–40 μm to be measured in the intact beating heart in vivo. Our preparations were stable, since control measurements before and after all the reported interventions varied by <10%, blood gases were maintained at physiological levels, and changes in systemic arterial pressure that may have induced metabolic and autoregulatory adjustments in coronary microvascular diameters were prevented by an inferior vena cava tie. Vessels with diameters <100 μm (arterioles) were imaged using a 10× objective to maximize the spatial resolution of our measurements to <3 μm, and each data point presented represents the average of at least five measurements obtained from images obtained over a 1–2-minute period. The use of fluorescein microangiography ensured that we measured the inner diameters of the microvessels. Although our preparation is anesthetized and open-chested, no technology exists that would allow us to measure coronary microvascular diameters under more physiological conditions.

Some methodological limitations remain, however. Pressure measurements are ideally required for the interpretation of changes in diameters, particularly of arterioles, in actively autoregulating microvascular networks. Although pressure measurements are possible, they are technically difficult and would have been impractical in the context of the current experimental protocols. A limitation of stroboscopic epi-illumination is that microvascular diameters are measured only on the epicardial surface. Data from this laboratory have recently confirmed that the distributions of microvascular resistances differ in subepicardium and subendocardium, so that subepicardial microvascular diameter changes cannot be considered representative of changes in subendocardial microvessels. Furthermore, α-adrenergic constriction is not uniformly distributed across the ventricular wall. Myocardial blood flow studies using radiolabeled microspheres have demonstrated that α-adrenergic constriction predominates in the subepicardium during exercise, maintaining subendocardial flow to advantage in the presence of a coronary stenosis. The transmural distribution of myocardial perfusion was not evaluated in this study, so our results are valid only for the epicardial canine coronary microcirculation.

Inhibition of Nitric Oxide Synthase Activity

The activity of the nitric oxide synthase enzyme was inhibited in the present study by the analogues of L-arginine, L-NA, or L-NAME. L-NA was chosen initially in view of its relatively high potency, but it caused problems through its instability in solution. L-NAME was found to be a more stable compound in solution than L-NA. These agents similarly reduced resting coronary microvascular diameters, presumably by unmasking myogenic constriction, and similarly attenuated coronary microvascular dilatation by acetylcholine. Before the use of the inferior vena cava tie, both agents caused an increase in systemic blood pressure, as has been reported previously.

As the nitric oxide production by coronary microvascular endothelium may have been nonuniformly inhibited by L-NA or L-NAME, we included only vessels in which acetylcholine-induced dilatation was attenuated by >50%. However, it is worth noting that acetylcholine-induced dilatation of microvessels of all sizes was either abolished completely or converted to constriction by the L-arginine analogues in most of the vessels studied (=70%), whereas endothelium-independent relaxation in response to sodium nitroprusside was intact. This result differs from that of Komaru et al., who found that topically applied Nω-monomethyl-L-arginine (L-NMMA) inhibited acetylcholine-induced dilatation only in vessels >120 μm in diameter. This difference may be due to the different doses and efficacies of the L-arginine analogues in the two studies, but topical administration of a drug may be expected to produce different local effects on an intact microvascular network than the same drug given by the intravascular route because of differences in its diffusion, circulation, and site of action in the vascular wall and in the autoregulatory behavior of the network. In agreement with Komaru et al, however, we found that microvessels of all sizes were dilated by acetylcholine under control conditions. Our results indicate that inhibition of nitric oxide synthesis affects resting smooth muscle tone and agonist-induced endothelial responses throughout the coronary microcirculation.

α₁- and α₂-Adrenergic Activation

The present study is the first to show the effects on the coronary microcirculation of the beating heart of selective α₁- and α₂-adrenergic activation by norepinephrine. This approach was chosen in view of the high affinity for their specific receptor subtype of the α₁- and α₂-adrenergic antagonists rauwolscine and prazosin (PA1 values in isolated canine coronary arteries of 8.5 and 8.8, respectively). Furthermore, rauwolscine exhibits >50-fold selectivity for the α₁-adrenergic receptor and prazosin >100-fold selectivity for the α₁-adrenergic receptor. We did not denervate the coronary microcirculation in our animals, introducing the possibility that norepinephrine release from sympathetic nerve endings may have contributed to our results. Both rauwolscine and prazosin may antagonize postsynaptic α-adrenergic receptors at sympathetic nerve endings.
and augment norepinephrine release. This possibility cannot be excluded, but the lack of any change in microvascular diameter with either antagonist implies that neurotransmitter norepinephrine release was not a significant factor in our study.

Norepinephrine was given as an intracoronary infusion to minimize systemic effects. β-Adrenergic receptor blockade by propranolol given as an intravenous bolus before each norepinephrine infusion should have prevented major changes in cardiac contractility. Although cardiac muscle contains predominantly β-adrenergic receptors, our experimental protocol did not exclude changes in myocardial work and metabolism mediated by functional myocardial α-receptors, which are mainly of the α1-receptor subtype. However, metabolic vasodilatation secondary to myocardial α1-adrenergic activation appears unlikely in our study because constriction was the predominant response to α1-adrenergic activation and was greater at the higher dose of norepinephrine. Although α1-adrenergic activation did not constrict arterioles <100 μm in diameter under baseline conditions, these vessels only show α1-adrenergic constriction when the coronary perfusion pressure is reduced beyond the autoregulatory range. Thus, metabolic effects are unlikely to have influenced our findings during α1-adrenergic activation.

**Interpretation of Experimental Results**

Coronary vascular resistance is increased after α-adrenergic activation in vivo. Whereas some studies have indicated that α1-adrenergic vasoconstriction predominates, others have indicated that α2-adrenergic constriction predominates during coronary hyperperfusion, exercise, and hypoxia. These studies consider the coronary microcirculation as a lumped resistance bed and have consequently provided little information about the microvascular mechanisms of α1-adrenergic constriction. Our results confirm that α1-adrenergic activation predominantly constricts small arteries, although arterioles “escape,” presumably by autoregulation. Conversely, α1-adrenergic activation predominantly constricts arterioles under baseline conditions in the present study, a different result from that obtained previously when α1-adrenergic receptors were activated by BHT-933, a preferential α2-adrenergic agonist. BHT-933 produced no change in coronary microvascular diameters under baseline conditions, but it constricted arterioles when perfusion pressure was reduced. The different findings of these two studies are consistent with findings in systemic microvascular networks in which arterioles constrict more during α1-adrenergic activation by norepinephrine than by BHT-933 because of a greater apparent sensitivity to norepinephrine (PD2 of 7.4) than to BHT-933 (PD2 of 5.1). Importantly, both of our studies indicate that the principle coronary site for α1-adrenergic vasoconstriction is in arterioles.

Both α1- and α2-adrenergic constriction were markedly potentiated when endogenous nitric oxide synthesis was inhibited. This result implies that α1-adrenergic activation will increase coronary vascular resistance considerably when endothelium-dependent relaxation is impaired, since the vascular resistance to flow varies with the fourth power of the diameter. α1-Adrenergic constriction was unmasked in arterioles, whereas α2-adrenergic constriction was unmasked in small arteries. Endothelium-derived nitric oxide thus modulates α1-adrenergic constriction mainly in arterioles and α2-adrenergic constriction mainly in small arteries. The disparity between these sites further implies a widespread role of nitric oxide in the control of coronary microvascular tone and also that functional α1- and α2-adrenergic receptors are widely distributed in the coronary microcirculation.

We considered the possibility that α-adrenergic responses were modified nonspecifically by the increase in resting coronary vascular tone induced by L-NA and L-NAME. To address this, we evaluated the effects of α1- and α2-adrenergic activation before and during endothelium-independent constriction by angiotensin II in separate groups of animals. We achieved comparable degrees of constriction with angiotensin II as with L-NA and L-NAME, and the baseline microvascular responses to norepinephrine were similar to those in the larger groups of animals. However, α-adrenergic responses were affected differently by preconstriction caused by angiotensin II rather than by L-NA or L-NAME. Both α1- and α2-adrenergic constriction were either abolished or converted to dilatation during preconstriction by angiotensin II. α1-Adrenergic dilatation in preconstricted microvessels may be due to autoregulatory “escape” or to EDRF release in response either to increased shear stress or activation of endothelial α1-adrenergic receptors. We can conclude from these experiments that inhibition of endothelial nitric oxide synthesis specifically potentiated α1-adrenergic constriction in our experiments.

EDRF may modulate α-adrenergic constriction by various mechanisms. Basal EDRF release in the absence of flow depresses contractile responses to α1- and α2-adrenergic agonists in rat aorta, probably because of functional antagonism. EDRF inhibits noradrenaline release from adrenergic nerves in rabbit carotid arteries and dog mesenteric arteries. Furthermore, there is evidence that, in preconstricted canine epicardial coronary arteries, norepinephrine causes endothelium-dependent relaxation blocked by rauwolscine and L-arginine analogues. The results obtained in the present study with norepinephrine and prazosin are compatible with α1-adrenergic receptor–mediated release of nitric oxide. The finding that α2-adrenergic constriction was potentiated in arterioles by L-NA and L-NAME suggests that α1-adrenergic activation also may mediate EDRF release. This has not been demonstrated in arteries but it may account for the potentiation of α1- but not α2-adrenergic constriction after endothelial removal in rabbit veins. Inhibition of EDRF synthesis in the present study would have uncovered α1-adrenergic constriction if α-adrenergic receptor subtypes that release EDRF when activated were present on endothelial cells. α1-Adrenergic receptors take the form of several different subtypes, α1A, α1B, α1C, α1D, etc., which have differing molecular structure, agonist and antagonist binding characteristics, and intracellular effector mechanisms. It is noteworthy that the nature and function of α-adrenergic receptor subtypes on the endothelium and smooth muscle of coronary microvessels remain unknown.

Another mechanism by which endothelium-dependent relaxation may modify α1-adrenergic constriction is
nitric oxide release in response to increased shear stress during microvascular constriction. Endothelium may thus exert a “braking” dilator action on constriction caused by different stimuli. Changes in shear stress are involved in endothelial modulation of arterial constriction in isolated rat resistance arteries. Changes in shear stress may also account for increased nitric oxide release in the isolated rabbit heart during vasconstriction by endothelin-1 and during pulsatile cardiac contraction. Unfortunately, our results do not allow us to say with certainty how EDRF modulates both $\alpha_1$- and $\alpha_2$-adrenergic constriction in the coronary microcirculation.

**Physiological and Pathophysiological Significance of the Results**

Competition between $\alpha$-adrenergic coronary microvascular constriction and endothelium-dependent relaxation may limit the reduction in myocardial perfusion caused by $\alpha$-adrenergic vasconstriction during augmented sympathoadrenal drive. $\alpha$-Adrenergic vasconstriction may be a desirable consequence of catecholamine release during stress or exercise in the systemic microcirculation, but it would not appear to be beneficial in the coronary microcirculation. A shear stress-related dilator mechanism, activated by constriction itself and possibly varying with the degree of constriction, would be particularly advantageous in “braking” the effects of catecholamines. If this modulating mechanism is disturbed in disease, accentuated $\alpha$-adrenergic coronary microvascular constriction may increase vascular resistance and threaten myocardial perfusion.

Our results may explain the relation between coronary endothelial impairment and sensitivity to sympathetic stimulation in atherosclerosis. Microvascular endothelium-dependent relaxation is attenuated in the presence of atherosclerosis in animal models and humans. Coronary vascular resistance increases more than normal during cold pressor testing in atherosclerotic patients with endothelial dysfunction, suggesting that the dilator actions of endothelium and the constrictor actions of catecholamines normally oppose each other. Endothelial impairment may thus account for enhanced $\alpha$-adrenergic constriction during cigarette smoking and cold pressor testing in atherosclerotic patients. We speculate that this may occur in other conditions associated with loss of EDRF activity, notably hypercholesterolemia, diabetes, hypertension, heart failure, and microvascular angina (syndrome X).

In summary, the present study demonstrates that endothelium-dependent relaxation by nitric oxide contributes to the control of tone in coronary microvessels with diameters between 40 and 250 $\mu$m. Furthermore, widespread loss of EDRF activity in the coronary microcirculation markedly accentuates $\alpha$-adrenergic constriction in the beating heart by actions at different microvascular levels during $\alpha_1$- and $\alpha_2$-adrenergic receptor activation, respectively. These findings could explain the apparent increase in coronary vascular sensitivity to sympathetic stimulation in patients with impaired coronary endothelial function.

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**References**


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