Heterogeneous Microvascular Coronary α-Adrenergic Vasoconstriction

William M. Chilian, Susan M. Layne, Charles L. Eastham, and Melvin L. Marcus

We tested the hypothesis that humoral or neurogenic α-adrenergic activation in the coronary circulation would produce heterogeneous vascular reactions. To accomplish this, the epicardial coronary microcirculation was viewed through an intravital microscope using stroboscopic epi-illumination. Microvascular diameters were measured under control conditions during β-adrenergic blockade (propranolol 1 mg/kg) and β-adrenergic blockade with pacing; during coronary α-adrenergic activation in the presence of β-adrenergic blockade with three doses of norepinephrine infusion (0.1, 0.5, and 1.0–2.0 μg/kg/min) or three frequencies of bilateral stellate nerve stimulation (2, 10, and 20 Hz); and during combined α- and β-adrenergic blockade (phentolamine 2 mg/kg and propranolol 1 mg/kg).

Diameters of both arterial and venous vessels were reduced during β-adrenergic blockade but returned back to baseline with pacing. At the lowest level of norepinephrine infusion or frequency of bilateral stellate stimulation, microvessel constriction was not observed. At the higher doses of norepinephrine (5.1±0.9% (1.0–2.0 μg/kg/min) and 4.0±1.1% (0.5 μg/kg/min) decrease in diameter of arterial vessels greater than 100 μm in diameter were observed (p<0.05). At 10 Hz and 20 Hz of stellate stimulation, diameter decreased by 4.8±1.9% and 4.4±2.1%, respectively, in these relatively large vessels. Small coronary arterioles (<100 μm diameter) dilated significantly during the highest levels of nerve stimulation (9.2±2.5% increase in diameter) or infusion rate of norepinephrine (13.6±2.7% increase in diameter) (p<0.05). These constrictor and dilator responses were abolished following combined α- and β-adrenergic blockade.

norepinephrine infusion resulted in a decrease in diameter of coronary veins and venules (7.2±1.3%) (p<0.05), whereas stellate stimulation did not significantly reduce venous and venules in diameter. In summary, the coronary venous and venular vasculature responds to α-adrenergic activation from circulating norepinephrine but is not affected by stellate stimulation. In contrast, stellate stimulation and norepinephrine infusion elicit similar responses in the coronary arterial and arteriolar microvasculature.

Constriction occurs in vessels greater than 100 μm in diameter, whereas dilation predominates in vessels less than 100 μm in diameter. Such heterogeneous arterial responses would undoubtedly result in a redistribution of coronary vascular resistance toward larger coronary arteries and arterioles. (Circulation Research 1989;64:376–388)

The heart is characterized by extensive innervation to the myocardium and the coronary blood vessels. Activation of sympathetic innervation to the heart results in β-adrenergic receptor–mediated increases in heart rate and contractility along with simultaneous coronary vasoconstriction, secondary to activation of α-adrenergic receptors. Infusion of catecholamines also has been shown to produce α-adrenergic coronary constriction. Other physiological interventions that have been used to activate the sympathetic nervous system and produce consequent α-adrenergic coronary constriction include exercise, hemorrhage, and carotid sinus hypotension. Despite the substantial information regarding α-adrenergic modulation of coronary resistance, there is relatively little information regarding the coronary microvascular locations of such constrictor mechanisms.

The classic approach used to study segmental α-adrenergic coronary constriction has involved calculations of large epicardial coronary artery resis-
tance and total coronary vascular resistance. Using such an approach, Kelley and Feigl found that the increase in large epicardial coronary artery resistance was similar in magnitude to the increase observed in total coronary vascular resistance during norepinephrine infusion or sympathetic nerve stimulation. Vatner developed a sonomicrometric technique to measure coronary artery diameters and found that large coronary arteries were receptive to α-adrenergic stimulation in conscious animals. The results from these studies, however, cannot be extended to provide insight into possible segmental α-adrenergic control in the coronary microcirculation.

α-Adrenergic vasoconstriction in response to sympathetic nerve stimulation has been extensively documented in the microcirculation of other organ systems. In skeletal muscle, sympathetic nerve stimulation produces greater constriction in large arterioles and small arteries than in small arteriolar vessels. Also, in the intestinal wall, sympathetic nerve stimulation produces preferential constriction of small arteries and large arterioles. In the cerebral circulation, sympathetic stimulation produces constriction of large arteries but not of small blood vessels. The net result of such heterogeneous constriction in these different vascular beds is redistribution of vascular resistance.

The purpose of this investigation was to document coronary microvascular responses to α-adrenergic receptor activation by humoral (norepinephrine infusion) or neuronal (bilateral stellate nerve stimulation) mechanisms. We hypothesized that, similar to other vascular beds, the coronary microcirculation would exhibit heterogeneous responses to α-adrenergic receptor stimulation. This hypothesis was based in part on our preliminary report, which indicated that epinephrine produced non-uniform coronary constriction. Also, we assessed possible differences between α-adrenergic activation mediated via humoral or neuronal stimulation. The latter goal was related to the fact that circulating catecholamines can significantly influence coronary vascular resistance, and there may be possible differences between the distributions of α-adrenergic receptors and sympathetic nerves. Our experimental approach was to measure coronary microvascular diameters in the epicardial coronary microcirculation during norepinephrine infusion or bilateral stellate nerve stimulation in the presence of β-adrenergic blockade. These measurements allow precise documentation of microvascular sites in the coronary circulation that are receptive to α-adrenergic stimuli.

Materials and Methods

General Preparation

Mongrel cats (n=81) of either sex were sedated with ketamine (2 mg/kg i.m.) and anesthetized with sodium pentobarbital (30 mg/kg i.m.). Each animal was placed on a homeothermic blanket system to maintain body temperature at 37–38°C. The femoral artery and vein were catheterized for hemodynamic measurements, fluid and drug administration, and arterial blood gas analyses. The femoral arterial catheter was advanced retrograde to the aortic arch. A solid-state transducer (5F, Millar, Houston, Texas) was introduced into the left ventricle via the right carotid artery for measurements of left ventricular pressure and left ventricular dP/dt (LV dP/dt). Both left and right stellate ganglia were isolated, decentralized, and placed in stimulating electrodes.

High-frequency jet ventilation was used to eliminate cardiac movement caused by pulmonary inflation. This procedure consisted of introduction of an 18-gauge cannula into the trachea and advancement of the cannula to the carina. An expiratory tracheal tube was positioned under 1–3 cm of water. A solenoid valve, triggered from LV dP/dt, was connected to a pressure source (compressed air) and a pressure regulator. The pressure regulator was connected to the tracheal cannula. The solenoid was open for only 10–20 msec during a respiratory cycle, and pressure in the tracheal cannula was regulated to 3–7 psi. With this jet ventilation system, pulmonary inflation produced no discernible effects on cardiac motion because of the small tidal volume. Arterial blood gases and pH were analyzed approximately every 60 minutes and were maintained within physiological limits by varying the duration that the solenoid valve was open, the position of the cannula in the trachea, and/or the regulated pressure.

After this ventilation procedure was performed, the heart was exposed by a midsternal split and partially stabilized with a pericardial cradle. The left atrium was catheterized, and snares were placed around the inferior vena cava and descending thoracic aorta for control of arterial pressures during the experimental maneuvers.

Microvascular Preparation

Measurements of microvascular diameter in the beating heart were accomplished with an intravital microscope (Leitz Ploemopak) that was optically coupled to a silicon-intensified tube video camera (General Electric). The preparation was illuminated by a fiberoptic connected to a stroboscopic light source (Chadwick-Helmuth, 150 W xenon arc, El Monte, California). The strobe was flashed once per cardiac cycle at the same point (late diastole) during successive cardiac cycles. A PDP 11/73 computer (Digital Equipment Corp., Nashua, New Hampshire) received the LV dP/dt signal and was adjusted to maintain the trigger point for the strobe light. The epicardial microvasculature appeared to be motionless when viewed through the microscope because the heart was illuminated for a short time (15–25 μsec) at the same point during successive cardiac cycles. The fiberoptic prevented heat damage to the epicardial microcirculation from the light source, and a polarizing filter was used to reduce the glare.
from the epicardial surface. The microscope objectives used for this preparation were the Leitz A6 (×6) and Leitz L10 (×10) with numerical apertures 0.18 and 0.22, respectively. These objectives were used in conjunction with ×10 magnification eyepieces, and the resulting magnification was either ×60 or ×100. The resolution of our measurement system was 2.6 and 4.7 μm for the ×10 and ×6 objectives, respectively.

Arterial and arteriolar diameter measurements were made during late diastole. The image acquisition and analysis system consisted of a frame digitizer (Imaging Technology Incorporated, Woburn, Massachusetts) that digitized the image directly from the camera. These digitized images were displayed on a high-resolution video monitor (Panasonic) and stored on a hard disk or transferred to magnetic tape for permanent data storage and subsequent analysis. Diameter measurements were accomplished with digitized images that were displayed on the high-resolution monitor. A digitizing tablet (Summa Graphics, Fairfield, Connecticut) was used to align the cursors with the vessel edges, and a computer program was used to calculate the vessel diameter in microns. Each vessel was measured four to eight times, using different images of a particular vessel obtained over a 10–15-second period.

To distinguish small coronary arterioles from small coronary venules and enhance visualization of these small coronary microvessels, fluorescein isothiocyanate dextran (MW= 149,700) was administered as a 5–10-mg bolus (50 mg/ml solution) into the left atrium. Following injection of the labeled dextran, the arteries and veins would illuminate sequentially (i.e., arteries would illuminate first and, because of the transit time for plasma flow, veins would illuminate a few seconds later). The fluorescein molecule was activated and visualized with fluorescence techniques and filters (Leitz H2 filter) in conjunction with a Ploem system.

**Experimental Protocols**

Measurements of coronary microvascular diameters were completed during three experimental protocols: 1) β-adrenergic blockade, 2) α-adrenergic activation, and 3) α-adrenergic blockade.

**β-Adrenergic blockade protocol.** Diameter measurements were made under three experimental conditions: 1) baseline conditions, 2) β-adrenergic blockade (propranolol 1 mg/kg), and 3) pacing (return heart rate to baseline levels) during β-adrenergic blockade. The purpose of this experimental series was to assess the effects of β-adrenergic blockade on coronary microvascular diameters and determine whether the effects were due to changes in oxygen demands associated with β-adrenergic blockade or nonspecific effects of propranolol.

**α-Adrenergic activation protocol.** Measurements of microvascular diameters in the different classes of arterial and venous vessels were completed under the following conditions: 1) control measurement in the presence of β-adrenergic blockade (propranolol 1 mg/kg), 2) α-adrenergic activation in the presence of β-adrenergic blockade, and 3) β-adrenergic blockade. Any measurement that did not return to a diameter within 10% of that before α-adrenergic activation (comparison of 1 vs. 3) was excluded from analysis. α-Adrenergic activation was accomplished with bilateral stellate stimulation (2, 10, and 20 Hz) or norepinephrine infusion (0.1, 0.5, and 1.0–2.0 μg/kg/min). The purpose of this series was to assess the effects of α-adrenergic activation by humoral (norepinephrine infusion) or neurogenic (stellate stimulation) activation on coronary microvascular diameters with the metabolic consequences of such stimuli eliminated (or greatly attenuated) with β-adrenergic blockade. We observed a pressor response associated with nerve stimulation or norepinephrine infusion. Following this increase, pressure was returned to baseline values with adjustment of the inferior vena caval snare. Diameter measurements were obtained usually 2–5 minutes after initiation of α-adrenergic activation. This delay was necessary to obtain adequate adjustment of pressure. Following successful measurement of diameter and other variables (e.g., perfusion), the snare was released, and the increase in arterial pressure was compared with that initially obtained before snaring. The pressor responses had to be within 10 mm Hg of each other for the stimulation period to be considered in a steady state and for the measurement to be included in the final analysis.

**α-Adrenergic blockade protocol.** Coronary microvascular diameters were measured during the following conditions: 1) β-adrenergic blockade (propranolol, 1 mg/kg); 2) α-adrenergic activation (norepinephrine infusion, 2 μg/kg/min, or bilateral stellate stimulation, 20 Hz); 3) combined α- and β-adrenergic blockade (phentolamine, 2 mg/kg; propranolol, 1 mg/kg); and 4) α-adrenergic activation with combined α- and β-adrenergic blockade. The purpose of this experimental protocol was to examine whether the effects of norepinephrine or bilateral stellate stimulation on coronary microvascular diameters were mediated through α-adrenergic receptors (i.e., to determine whether phentolamine would block the effects). The dose of phentolamine was chosen because it completely blocked the pressor effects of stellate stimulation and norepinephrine infusion.

**Measurement of Myocardial Perfusion**

After successful measurement of coronary microvascular diameters, myocardial perfusion was measured with nuclide-labeled microspheres (15 μm diameter labeled with 45Sc, 87Sr, 113Sn, 141Ce, and 59Nb). Microspheres (1×10⁶) were agitated and injected into the left atrium and flushed with 2 ml warm saline. Before (10–15 seconds) and for 1.5 minutes after the microsphere injection, arterial blood was collected with a constant withdrawal pump from the femoral arterial catheter at a rate of
0.97 ml/min. Blood reference samples were placed in counting vials to determine nuclide activity. After killing of the animal, the heart was removed and tissue samples from the left ventricle were obtained, divided into subepicardial and subendocardial portions, and weighed. Since microvascular diameter measurements were confined to the subepicardium, only measurements of subepicardial perfusion are reported. Myocardial blood flow (MBF) per gram was calculated from the following expression:

\[
MBF = \frac{Cm \times Wr}{Cr}
\]

where \(Cm\) is nuclide activity per gram weight of tissue, \(Wr\) is withdrawal rate of the pump, and \(Cr\) is total nuclide activity in the blood reference sample. Nuclide activity was determined with a Ge detector.26

**Data Analysis**

All hemodynamic variables (systolic, diastolic, and mean arterial pressures and heart rate) were recorded on an oscillographic recorder. These variables, along with myocardial perfusion measurement, were compared through the use of \(t\) tests in conjunction with the Bonferroni inequality for any multiple comparisons. Microvascular diameter data following \(\beta\)-adrenergic blockade or \(\alpha\)-adrenergic activation were plotted as the percent change in diameter versus the initial diameter. These data were "best fit" with multiple regression analyses.27 Comparisons of the coefficients of determination of the regression lines were performed with analysis of variance ANOVA.28 The percent changes in vessel diameters were also grouped together for a particular intervention and compared with zero using a paired \(t\) test. All data are presented as mean±SEM and \(p<0.05\) was used as the probability level for statistical significance.

Microvessel hindrance for the arterial vessels during stellate stimulation and norepinephrine infusion was calculated according to Firrell et al.29 and was expressed as the percent change that occurred during norepinephrine infusion or nerve stimulation from that during \(\beta\)-adrenergic blockade. Although hindrance calculations are likely not a quantitative representation of resistance because factors such as vessel length or microvascular blood viscosity are not included, such a measurement does normalize changes in diameter to the original diameters. This is obviously important because a 7-\(\mu\)m decrease in diameter would be more physiologically significant for a 25-\(\mu\)m arteriole than for a 250-\(\mu\)m artery.

**Results**

**Systemic Hemodynamics and Myocardial Perfusion**

\(\beta\)-Adrenergic blockade protocol. Table 1 shows systemic hemodynamics and myocardial perfusion during the \(\beta\)-adrenergic blockade protocol. \(\beta\)-Adrenergic blockade decreased heart rate and myocardial perfusion from that during unblocked, baseline conditions (\(p<0.05\)). Arterial pressure did not change during \(\beta\)-adrenergic blockade. Pacing increased heart rate and myocardial perfusion to baseline (preblockade) levels.

**TABLE 1. Systemic Hemodynamics and Myocardial Blood Flow During \(\beta\)-Adrenergic Blockade Protocol**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>(\beta)</th>
<th>(\beta)+pacing</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hemodynamics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>207±3</td>
<td>151±4*</td>
<td>206±3</td>
</tr>
<tr>
<td>Systolic pressure (mm Hg)</td>
<td>107±3</td>
<td>112±4</td>
<td>111±3</td>
</tr>
<tr>
<td>Diastolic pressure (mm Hg)</td>
<td>81±2</td>
<td>82±2</td>
<td>85±3</td>
</tr>
<tr>
<td>Mean pressure (mm Hg)</td>
<td>94±3</td>
<td>95±2</td>
<td>96±3</td>
</tr>
<tr>
<td>Myocardial blood flow (ml/min·g)</td>
<td>1.47±0.03</td>
<td>1.10±0.04*</td>
<td>1.39±0.08</td>
</tr>
</tbody>
</table>

Values are mean±SEM (n=7). \(\beta\), \(\beta\)-adrenergic blockade. *\(p<0.05\) vs. control.

**FIGURE 1.** Plot of effects of \(\beta\)-adrenergic blockade on coronary arterial and arteriolar microvascular diameters. There was a significant homogeneous decrease of microvascular diameter throughout the microcirculation following \(\beta\)-adrenergic blockade.
The effects of β-adrenergic blockade on diameters in the coronary arterial system and coronary venous system are shown in Figures 1, 2, and 3. The equations describing the regression lines are shown in Table 4. Although there was not a linear relation between the percent change in vessel diameter after β-adrenergic blockade and the initial diameter in the coronary arterial system (Figure 1), the average percent change in diameter (−10.8±1.0, p<0.05) was uniform in the different sizes of coronary arteries and arterioles. Pacing returned arterial diameters back to baseline values, that is, produced uniform vasodilation of coronary arteries and arterioles (Figure 2). Also, the coronary venous system was not characterized by a significant relation between percent change in diameter following β-adrenergic blockade and initial diameter (Figure 3, Table 4). There was, however, a uniform −4.9±0.9% decrease in venous diameter (p<0.05). Pacing increased venous diameters to control baseline values, only −0.8±0.7% less than control (p=NS).

α-Adrenergic activation. During all doses of norepinephrine or stellate stimulation, heart rate or myocardial perfusion did not change from that during control conditions with β-adrenergic blockade (Table 2). Arterial pressure was controlled with adjustments of the vena caval snare and did not significantly change.

Figures 4 and 5 show the microvascular effects of the different doses of norepinephrine or various levels of bilateral stellate ganglion stimulation on coronary arterial and arteriolar diameters (Table 4 presents the regression equations). Figure 4 illustrates the effects of norepinephrine on coronary venous system to β-adrenergic blockade. β-Adrenergic blockade produced a significant uniform decrease in diameters in the coronary venous system.
Table 2. Systemic Hemodynamics and Myocardial Blood Flow During α-Adrenergic Activation Protocol

<table>
<thead>
<tr>
<th>Hemodynamics</th>
<th>α (48)</th>
<th>2 Hz+α (18)</th>
<th>10 Hz+α (25)</th>
<th>20 Hz+α (33)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td>146±8</td>
<td>148±9</td>
<td>150±6</td>
<td>156±7</td>
</tr>
<tr>
<td>Systolic pressure (mm Hg)</td>
<td>115±8</td>
<td>116±7</td>
<td>120±8</td>
<td>115±10</td>
</tr>
<tr>
<td>Diastolic pressure (mm Hg)</td>
<td>84±4</td>
<td>84±8</td>
<td>83±5</td>
<td>88±9</td>
</tr>
<tr>
<td>Mean pressure (mm Hg)</td>
<td>99±5</td>
<td>100±7</td>
<td>101±4</td>
<td>101±9</td>
</tr>
<tr>
<td>Myocardial blood flow (ml/min/g)</td>
<td>1.52±0.14</td>
<td>1.59±0.11</td>
<td>1.63±0.19</td>
<td>1.75±0.11</td>
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</tbody>
</table>

Norepinephrine infusion

<table>
<thead>
<tr>
<th>Hemodynamics</th>
<th>α (63)</th>
<th>0.1 NE+α (22)</th>
<th>0.5 NE+α (7)</th>
<th>1-2 NE+α (38)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td>134±5</td>
<td>131±5</td>
<td>134±8</td>
<td>141±5</td>
</tr>
<tr>
<td>Systolic pressure (mm Hg)</td>
<td>114±7</td>
<td>119±8</td>
<td>117±6</td>
<td>104±6</td>
</tr>
<tr>
<td>Diastolic pressure (mm Hg)</td>
<td>81±5</td>
<td>83±5</td>
<td>80±6</td>
<td>79±5</td>
</tr>
<tr>
<td>Mean pressure (mm Hg)</td>
<td>98±5</td>
<td>100±6</td>
<td>93±7</td>
<td>92±5</td>
</tr>
<tr>
<td>Myocardial blood flow (ml/min/g)</td>
<td>1.58±0.11</td>
<td>1.66±0.13</td>
<td>1.64±0.10</td>
<td>1.67±0.15</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Number in parentheses is the number of observations. α, α-adrenergic blockade; NE, norepinephrine infusion rate in units of μg/kg/min.

Table 3. Systemic Hemodynamics During α-Adrenergic Blockade Protocol

<table>
<thead>
<tr>
<th>Hemodynamics</th>
<th>β + BSS</th>
<th>α + NE</th>
<th>α + β + BSS</th>
<th>α + β + NE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td>138±7</td>
<td>148±10</td>
<td>141±8</td>
<td>135±6</td>
</tr>
<tr>
<td>Systolic pressure (mm Hg)</td>
<td>121±6</td>
<td>127±6</td>
<td>117±6</td>
<td>118±9</td>
</tr>
<tr>
<td>Diastolic pressure (mm Hg)</td>
<td>84±8</td>
<td>78±9</td>
<td>83±7</td>
<td>86±7</td>
</tr>
<tr>
<td>Mean pressure (mm Hg)</td>
<td>100±6</td>
<td>106±9</td>
<td>97±5</td>
<td>103±9</td>
</tr>
</tbody>
</table>

Values are mean±SEM (n=7). β, β-adrenergic blockade; α, α-adrenergic blockade; BSS, 20-Hz bilateral stellate stimulation; NE, norepinephrine infusion at 2 μg/kg/min.
FIGURE 4. Plots of responses of coronary artery and arteriolar vessels to different rates of norepinephrine infusion. Note that at the higher doses most vessels >100 µm in diameter constricted, whereas the smaller arterioles dilated. These data were best fit by linear and third-order polynomial expressions. %ΔD, percent change in diameter; Di, diameter.

heart rate or arterial pressure did not significantly change (Table 3). Heart rate and arterial pressure did not change during combined α- and β-adrenergic blockade (Table 3).

The effects of α- and β-adrenergic blockade on coronary microvascular diameters are shown in Figure 8. Both stellate stimulation (20 Hz) and norepinephrine infusion (2 µg/kg/min) produced constriction of the arterioles and arteries greater than 100 µm diameter (−6.1±2.0% and 5.9±2.1%, respectively). During both interventions, dilation was observed in the one arteriole less than 100 µm in diameter. α-Adrenergic blockade did not significantly change coronary microvascular diameters from control (β-adrenergic blockade) and abolished the responses during either stellate stimulation or norepinephrine infusion.

Hindrance changes during α-adrenergic stimulation. Figure 9 shows the calculated percent change in hindrance in arterial vessels greater and less than 100 µm during the highest levels of stellate nerve stimulation (20 Hz) or norepinephrine infusion (1–2 µg/kg/min). This division of vessels was based on the data shown in Figures 4 and 5 in which during α-adrenergic activation coronary arterioles less than 100 µm dilated, whereas larger vessels constricted. In vessels greater than 100 µm, hindrance increased by 17.0±7.1% and 14.5±6.9% during norepinephrine infusion and stellate stimulation, respectively. In the smaller arterioles, hindrance decreased by 30.4±7.3% during norepinephrine infusion and by 28.1±8.7% during stellate stimulation.

Discussion

There are three new observations in this study. First, β-adrenergic blockade and the associated reduction in oxygen demand result in decreases in diameter of coronary arteries, arterioles, venules, and veins. Second, both neuronal and humoral α-adrenergic stimulation during β-adrenergic blockade produce nonuniform segmental vascular reactions in the coronary microcirculation. Specifically, constriction was observed in coronary arteries and large coronary arterioles (greater than 100 µm in
Bilateral Stellate Stimulation

FIGURE 5. Plots of responses of coronary artery and arteriolar vessels to different levels of bilateral stellate stimulation in the presence of β-adrenergic blockade. Note the heterogeneous vascular reactions, small vessel dilation (<100 μm diameter), and large vessel constriction at the higher rates of stimulation. %ΔD, percent change in diameter; Di, diameter.

Our data interpretations and conclusions depend on several factors, including methodology, local metabolic and myogenic control of the coronary circulation, and α-adrenergic control of the coronary circulation.

Experimental Methodology

The accuracy of our experimental measurements and the viability of our preparation are critical to the conclusions. We are able to resolve 4.7 μm with the ×6 objective and 2.6 μm for the ×10 objective. Thus, small changes in microvascular caliber could be detected.

Each experimental preparation was characterized by systemic hemodynamics and arterial blood gases in the physiological range. Also, our experimental design ensured that the preparation was not deteriorating during the course of the experiment; for example, before and after α-adrenergic activation, measurements of microvascular diameter had to be within 10% of each other. If this experimental criterion was not satisfied, the measurement was
TABLE 4. Regression Equations for Coronary Arterial and Venous Systems During Experimental Interventions

<table>
<thead>
<tr>
<th>System</th>
<th>Equation</th>
<th>r²</th>
</tr>
</thead>
</table>
| Arterioles and arteries
| β-adrenergic blockade: | y = -10.57 + (1.52 × 10⁻⁴)x; r² = 0.01 |         |
| NE 1–2 μg/kg/min*†: | y = 43.14 – 0.63x + (2.49 × 10⁻³)x² – (2.99 × 10⁻⁴)x³; r² = 0.61 |         |
| NE 0.5 μg/kg/min*: | y = 12.71 – 0.27x + (1.24 × 10⁻³)x² – (1.74 × 10⁻⁴)x³; r² = 0.17 |         |
| NE 0.1 μg/kg/min: | y = 3.08 – (1.79 × 10⁻²)x; r² = 0.03 |         |
| BSS 20 Hz*:      | y = 3.61 ± 0.30x – (4.02 × 10⁻³)x² + (1.05 × 10⁻³)x³; r² = 0.49 |         |
| BSS 10 Hz*:      | y = 39.33 – 0.74x + (3.76 × 10⁻³)x² + (5.89 × 10⁻⁶)x³; r² = 0.26 |         |
| BSS 2 Hz:        | y = 5.59 – (3.38 × 10⁻²)x; r² = 0.07 |         |

Venules and veins

<table>
<thead>
<tr>
<th>System</th>
<th>Equation</th>
<th>r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-adrenergic blockade:</td>
<td>y = -9.17 + (2.22 × 10⁻²)x; r² = 0.05</td>
<td></td>
</tr>
<tr>
<td>NE 1–2 μg/kg/min:</td>
<td>y = -8.79 + (8.04 × 10⁻⁴)x; r² = 0.01</td>
<td></td>
</tr>
<tr>
<td>BSS 20 Hz:</td>
<td>y = 1.29 – (1.15 × 10⁻³)x; r² = 0.02</td>
<td></td>
</tr>
</tbody>
</table>

y, %Δ in diameter; x, initial diameter; NE, norepinephrine infusion; BSS, bilateral stellate stimulation.

*p<0.05 vs. β-adrenergic blockade.

†p<0.05 vs. NE 1–2 or BSS 20 Hz.

not used. Approximately 20% of all experimental measurements failed to meet this criterion. Another important consideration regarded the viability of the epicardial microcirculation. Previously we reported that our experimental method maintains the epicardial environment similar to that when the pericardium is closed. Thus, our experimental techniques had adequate resolution and maintained the viability of the subepicardial vasculature.

A limitation of our experimental design was that pressure measurements were not obtained in the microvessels during α-adrenergic activation. Therefore, a decrease in microvascular diameter may, in part, be related to a decrease in intraluminal pressure (i.e., passive decrease in diameter). Such a mechanism would have been related to the reduction of diameters in coronary venules less than 50 μm in diameter because these vessels are devoid of vascular smooth muscle. However, the degree of arterial constriction and dilation were similar during both stellate stimulation and norepinephrine infusion, yet only norepinephrine infusion caused venous constriction. Thus, in our opinion, this decrease in diameter of veins larger than 50 μm in diameter was an active response to α-adrenergic activation. Despite arterial constriction, venular diameter did not change significantly during stellate stimulation. We speculate that the observed arteriolar dilation must have decreased the resistance in this segment, lessening the pressure drop, and maintaining capillary and venular pressures relatively constant. We must emphasize, however, that the dilation we observed in the small coronary arterioles (<100 μm diameter) during either norepinephrine infusion or sympathetic nerve stimulation must have been due to an active mechanism. A passive mechanism should have only reduced the diameter of these vessels. In our opinion, this observation of downstream dilation during upstream constriction is an important finding because we may have identified the site for coronary autoregulation.

Local Control of Coronary Blood Flow

A close relation exists between myocardial oxygen demands and coronary blood flow. Increases or decreases in myocardial oxygen demands are
Bilateral Stellate Stimulation: Coronary Veins and Venules

FIGURE 7. Plot of responses of coronary venous and venular vessels to bilateral stellate stimulation (20 Hz) in the presence of β-adrenergic blockade. The changes in venous diameter were insignificant.

FIGURE 8. Plot of effects of α-adrenergic blockade on coronary microvascular diameters during norepinephrine infusion (2 μg/kg/min) and bilateral stellate stimulation (20 Hz). Constriction was observed in the absence of α-adrenergic blockade, but no changes in diameter were observed following α-adrenergic antagonism. βα, β-adrenergic blockade; C, control; NE, norepinephrine infusion; αα, α-adrenergic blockade.

associated with increases or decreases, respectively, in coronary blood flow to match oxygen delivery with oxygen demands. Only a few studies have addressed specific coronary vascular locations of these metabolic adjustments.

Vatner and Hintze reported that a reduction of myocardial oxygen consumption due to β-adrenergic blockade resulted in a reduction of coronary blood flow and a decrease in the diameter of large coronary arteries. The same laboratory also found that stimulation of myocardial metabolism with β-adrenergic agonists produced metabolic coronary vasodilation and dilation of large epicardial coronary arteries. These results suggested that the caliber of large epicardial coronary arteries was regulated by a metabolite or mediated through changes in vascular shear stress associated with alterations in coronary blood flow. Our results (Figures 1, 2, and 3) have significantly extended these observations into the coronary microcirculation. We found that diameter decreased throughout the coronary microvessels when myocardial oxygen demands were decreased after β-adrenergic blockade. The decrease in vessel diameter was likely mediated by a reduction in the production of a metabolic vasodilator and/or lower vascular shear stresses associated with the reduced coronary blood flow. We must emphasize that the results are not due to a nonspecific effect of propranolol, because when heart rate was returned to control levels (preblockade) with pacing (Figure 2), diameters returned to baseline levels. Moreover, we emphasize that if the metabolic consequences of catecholamine stimulation are not controlled, metabolic dilator factors override α-adrenergic constriction, and uniform dilation of coronary blood vessels is observed.

Another possible explanation for constriction following β-adrenergic blockade could be related to the existence of a myogenic mechanism in the coronary circulation; however, recent data remain controversial concerning the importance of this putative control mechanism. If the smallest arterioles are under predominant metabolic control, a decrease in oxygen demands would produce constriction. Such downstream constriction would increase pressure upstream, which could potentially result in myogenic constriction in these upstream vascular segments. Such integrated metabolic and myogenic control mechanisms in various vascular segments have been suggested in other vascular systems. Metabolic control is thought to be confined primarily to the smallest arterioles. These vessels, however, are not believed to participate in myogenic responses. Davis and Gore found that larger arterioles were responsible for myogenic autoregulatory adjustments. It is conceivable that in the coronary vasculature, shear, myo-
genic, and metabolic mechanisms are integrated for the control of oxygen delivery.

\textbf{\textit{\alpha-Adrenergic Vasoconstriction}}

The coronary microcirculation is characterized by heterogeneous responses to \textit{\alpha}-adrenergic receptor stimulation. This conclusion was based on the observations that either norepinephrine infusion or sympathetic nerve stimulation produced constriction in coronary arterial vessels greater than 100 \mu m in diameter simultaneously with dilation in the smaller arteriolar vessels. These constrictor and dilator responses were abolished with \textit{\alpha}-adrenergic blockade. Such disparate vascular reactions to a common stimulus would undoubtedly cause redistribution of resistance, as suggested by Figure 9.

We have three explanations for heterogeneous arterial and arteriolar \textit{\alpha}-adrenergic constriction. First, there could be uneven distribution of \textit{\alpha}-adrenergic receptors throughout the coronary circulation. Related to this hypothesis, Muntz et al reported that the distribution of coronary \beta-adrenergic receptors was heterogeneous. Coronary arterioles of dogs had five times the number of \beta-adrenergic receptors than were found in small arteries. If there were a reciprocal distribution of \textit{\alpha}- and \beta-adrenergic receptors, as suggested by some laboratories, \textit{\alpha}-adrenergic responses would be expected to predominate in larger coronary vessels. Second, it is possible that endothelial-dependent \textit{\alpha}-adrenergic vasodilation could occur in coronary microvessels. Cocks and Angus have reported that such a mechanism can induce relaxation in isolated coronary arteries; however, the importance of such a microvascular mechanism in vivo is undocumented. A third explanation for heterogeneous \textit{\alpha}-adrenergic coronary constriction could be downstream autoregulatory adjustments in vasomotor tone during sustained \textit{\alpha}-adrenergic constriction. Related to this explanation is the possibility that all coronary arteries and arterioles constricted initially during \textit{\alpha}-adrenergic activation. However, since the measurements of diameter were completed 2–5 minutes after commencement of stimulation, we may have missed an initial transient constriction and observed only the autoregulatory "escape" from the \textit{\alpha}-adrenergic constriction.

Previously, we reported that a single dose of epinephrine (1–2 \mu g/kg/min) produced nonuniform coronary constriction. However, in that report, coronary arterial and arteriolar constriction (>100 \mu m diameter) were less than that observed in the present study. Moreover, arteriolar dilation was not observed. Thus, it is possible that a critical level of upstream constriction is necessary to cause the concomitant downstream dilation. These observations regarding simultaneous upstream constriction with downstream dilation have substantial implications for mechanisms that control myocardial oxygen delivery. Escape from \textit{\alpha}-adrenergic coronary constriction may be related to autoregulatory adjustments in small coronary arterioles. One such adjustment could be due to an increase in the concentration of a vasodilator metabolite associated with the upstream vasoconstriction. Another possibility could relate to a myogenic mechanism in which a decrease in pressure in the smaller arterioles, produced by constriction in the upstream vessels, resulted in myogenic vasodilation. We postulate that different regulatory mechanisms dominate the control of the upstream and downstream resistances in the coronary circulation. Coronary arteries, large arterioles,
and the venous vasculature may be regulated by neurohumoral mechanisms, whereas smaller microvessels (in which about 50–60% of total coronary resistance occurs) may be under preferential local autoregulatory control mechanisms.

It is tempting to speculate about the physiological importance of such heterogeneous vascular reactions during α-adrenergic activation. Dilatation of arterioles less than 100 μm in diameter would increase the cross-sectional area of these vessels. This would tend to decrease blood flow velocity and red cell transit time in this vascular segment and possibly facilitate oxygen exchange extraction because of the increased "contact time" of the red cells in the vessels. Although such a mechanism is entirely speculative, several laboratories have found that coronary α-adrenergic activation increases myocardial oxygen extraction. Moreover, in skeletal muscle a significant component of oxygen exchange occurs in precapillary vessels. Thus, the redistribution of coronary vascular resistance during α-adrenergic activation and the possible changes in blood flow velocities in certain vascular segments may aid in oxygen delivery to the myocardium.

We observed that the quantitative effects of humoral and neuronal α-adrenergic activation in the coronary microcirculation differ only in the coronary venous system (Figures 7 and 8). Although the vascular endothelium imposes a barrier to the transvascular flux of catecholamines, our results indicated that sufficient amounts of blood-borne norepinephrine can produce steady-state coronary constriction in coronary arterial vessels equal to that attained with stellate nerve stimulation (Figures 4 and 5). We must emphasize that our results were obtained a few minutes after the initiation of norepinephrine infusion or stellate nerve stimulation; thus, transient differences between neuronal versus humoral α-adrenergic stimulation remain unknown.

Conclusions

Two important implications from this study are that all classes of coronary arterial vessels are responsive to changes in myocardial oxygen demands produced by β-adrenergic blockade, and that there may be different regulatory mechanisms that predominate in large and small coronary arterial resistances. Neurohumoral control mechanisms may dominate in coronary arteries and large coronary arterioles, whereas local myogenic and/or metabolic mechanisms may regulate smaller coronary arterioles.

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