Transmural Differences in Sympathetic Coronary Constriction During Exercise in the Presence of Coronary Stenosis

William M. Chilian and Peter H. Ackell

The goal of this study was to determine the effect of sympathetic neural activation on the transmural distribution of myocardial perfusion distal to a flow-limiting coronary artery stenosis. Treadmill exercise in conscious dogs was used as a physiological stimulus to activate the sympathetic nervous system. In the experimental model, the anterior region of the circumflex artery was innervated, but the posterior circumflex region was treated with phenol to produce regional sympathectomy within the stenotic territory. Myocardial perfusion to innervated and sympathectomized left ventricular regions was measured before and after inflation of the occluder to reduce distal coronary pressure to 45 mm Hg. Measurements were obtained during control conditions with the animal standing on the treadmill, during inflation of the occluder with the animal standing, during exercise alone, during exercise with β-adrenergic blockade, and during exercise with combined α- and β-adrenergic blockade. Exercise (6 km/hr) resulted in a marked increase in heart rate from 128 ± 9 (standing) to 218 ± 7 beats/min. β-Adrenergic blockade blunted the tachycardia during exercise (146 ± 6 beats/min). Under control conditions (while standing), there were no differences in myocardial perfusion between the innervated and sympathectomized regions, 187 ± 26 and 181 ± 24 ml/min/g, respectively. During exercise or in combination with β-adrenergic blockade, subepicardial perfusion was significantly less (18-25%) in the innervated stenotic region than that in the sympathectomized stenotic region. In contrast, subendocardial perfusion was significantly greater in the innervated stenotic region (17-26%) than that in the sympathectomized stenotic region. The subendocardial-to-subepicardial blood flow ratio during exercise was 0.60 ± 0.08 in the innervated stenotic region and 0.42 ± 0.07 in the sympathectomized stenotic region (p<0.05). During exercise with β-adrenergic blockade, the endocardial-to-subepicardial blood flow ratios in the innervated and sympathectomized stenotic regions were 0.47 ± 0.09 and 0.37 ± 0.07, respectively (p<0.05). These differences were abolished during α- and β-adrenergic blockade. These data indicate that α-adrenergic coronary constriction distal to a flow-limiting stenosis facilitates redistribution of blood flow toward the subendocardium. This redistribution was produced by α-adrenergic constriction in the outer layers of the left ventricle.

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Many investigators have found that infusion of catecholamines produces α-adrenergic receptor-mediated coronary constriction.1-6 Electrical stimulation of the stellate ganglion has been shown to cause neurogenic sympathetic coronary constriction.7-10 Physiological interventions that have been used to activate the sympathetic nervous system and induce α-adrenergic coronary constriction have included carotid sinus hypotension,11-13 hemorrhage,14 and exercise.15-20 Despite accumulation of evidence describing α-adrenergic coronary constriction in the coronary circulation, few studies have addressed a physiological role or purpose for such effects.21 Some laboratories have assessed a physiological role of coronary α-adrenergic constriction by measuring transmural myocardial perfusion during infusion of catecholamines or stimulation of sympathetic nerves. Johannsen et al22 found that sympathetic nerve stimulation during maximal coronary dilation produced selective subepicardial coronary constriction. Under these conditions, sympathetic nerve stimulation increased the endocardial-to-epicardial distribution of flow. Buffington and Feigl23 observed that phenoxybenzamine decreased the endocardial-to-epicardial distribution of myocardial blood flow during noradrenaline infusion in hypoperfused hearts. Nathan and Feigl24 reported that activation of α-adrenergic receptors with norepinephrine lessened transmural steal in an experimental model designed to mimic a stenosis. These results suggest α-adrenergic constriction in the coronary circulation is not uniform in different transmural layers of the left ventricular wall. The impression from these studies is that a possible role of coronary adrenergic constriction would be to enhance subendocardial perfusion via subepicardial constriction. Also, such a mechanism may be operative under physiological conditions because Huang and Feigl25 observed that coronary α-adrenergic activation during exercise resulted in preferential subepicardial constriction.

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Despite these results, which suggest that coronary α-adrenergic constriction may be beneficial, some investigators report that coronary α-adrenergic constriction may be detrimental. Heusch and Deussen found that in anesthetized dogs sympathetic neural stimulation produced myocardial ischemia (lactate production) when coronary reserve was markedly compromised. Also, recently, α-adrenergic blockade was reported to improve function during exercise in a stenotic left ventricular region.

The goal of this study was to determine the effects of sympathetic neural activation on the transmural distribution of myocardial perfusion distal to a stenosis in conscious animals. Specifically, we tested the hypothesis that in the presence of a severe flow-limiting stenosis, physiological activation of sympathetic nerves would result in preferential subepicardial and subendocardial coronary constriction and enhance subendocardial perfusion. The experimental approach was to measure myocardial perfusion to innervated and sympathectomized stenotic regions of the left ventricle in conscious, exercising dogs.

**Materials and Methods**

**Surgical Preparation**

Mongrel dogs of either sex (22–28 kg) were acclimated to running on a motor driven treadmill. After acclimation to the treadmill, the animals were instrumented in the following manner. Dogs were anesthetized with sodium pentobarbital (25–30 mg/kg i.v.), intubated, and ventilated with room air using a mechanical respirator. Using aseptic technique, a left thoracotomy was performed, the pericardium was incised, and the heart was exposed for topical application of 85% phenol and for instrumentation (Figure 1). Phenol was applied to the posterior portion (distal circumflex region) of the left ventricular free wall, always distal to the first marginal branch. The region of the posterior left ventricle to be sympathectomized was identified, and arteries entering this region were carefully dissected. Umbilical tape wetted with phenol was passed under and wrapped around the vessels and removed. Phenol was then applied to the epicardial surface using the wooden end of a cotton-tip applicator stick. Phenol was painted from the base to the apex in an interconnecting series of lines along the anterior boundary to the apical dimple of the left ventricle. The posterior boundary was painted from the apical dimple to the base, and a connecting line was painted across the base of the heart. Following this procedure, a solid-state transducer (Konigsburg, Pasadena, California) was secured in the left ventricle via a stab wound in the apex. A Tygon catheter was placed in the left atrium via the left atrial appendage, and a Teflon catheter was secured in the thoracic aorta. A hydraulic occluder was placed around the proximal circumflex artery, and a catheter was placed distal to the occluder according to the method of Gwirtz and Stone. The catheters, transducer, and occluder were exteriorized between the scapulae through a subcutaneous channel. The thoracotomy was repaired, the chest evacuated of air, and the animal was given antibiotics (Bicillin) for 1 week. The catheters were flushed once every 2 days with heparinized saline (500 U/ml). Following a 7–10-day recovery period, the animals were reacclimated to the treadmill. The speed of the treadmill and duration of the exercise period were progressively increased so that the animals could exercise without discomfort within 2–3 weeks after the initial surgery.

**Hemodynamic Measurements**

In all animals, the following hemodynamic variables were measured during the different interventions: heart rate, left ventricular systolic and end-diastolic pressures, left ventricular dP/dt, mean arterial pressure, and coronary pressure distal to the occluder. Aortic and coronary pressures were measured with strain gauges (Statham, Hato Rey, Puerto Rico) zeroed at heart level. Left ventricular pressure was measured with a Konigsberg transducer. The Konigsberg transducer was calibrated in vitro against a mercury manometer, and in vivo left ventricular systolic and end-diastolic pressures were calibrated against aortic systolic and left atrial pressures, respectively. Peak left ventricular dP/dt was measured from the left ventricular pressure signal with a differentiator that was calibrated with an internal sine wave oscillator. All hemodynamic variables were recorded on an oscillographic recorder.

**Measurement of Myocardial Perfusion**

Myocardial perfusion to the innervated and sympathectomized regions was measured with nuclide-labeled microspheres (15 μm) labeled with ⁴⁶Sc, ⁴⁵Sr, ¹¹⁵Sn, ¹⁴¹Ce, ⁹⁹Nb, ⁹⁰Cd, ⁵⁷Co, ⁵¹Cr, or ¹⁰⁸Ru. Microspheres (2.8–4.5 × 10⁹) were injected into the left atrial catheter over a 20-second period and flushed with 15–20 ml of 37° C saline. Starting 15 seconds before injection of the microspheres and continuing for 90 seconds after the injection, blood was withdrawn from the arterial catheter at a constant rate of 12.0 ml/min.

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**Figure 1.** A schematic of the instrumentation procedures. Note that in the circumflex perfusion field distal to the occluder, only the posterior portion of this region was treated with phenol (sympathectomized). The anterior portion of this stenotic region has intact coronary innervation.
Blood reference samples were placed in counting vials to determine nuclide activity. After killing the animal with an overdose of sodium pentobarbital, the heart was excised, and tissue samples from the innervated and sympathectomized regions were removed immediately. (See “Tissue and Plasma Catecholamines” for details regarding sample procurement and analyses.) The phenol-treated “stenotic” region (sympathectomized) was distinguished from the innervated “stenotic” region by marking the two different regions with sodium fluorescein (yellow) or Evans blue. The dyes were injected simultaneously into the proximal circumflex artery and the posterior circumflex artery. The heart was then fixed in 4% formalin. After fixation, the left ventricle was sectioned and divided into three regions: the anterior portion of the left ventricle that was supplied by the left anterior descending artery (unstained), the innervated region within the stenotic territory of the circumflex artery (sodium fluorescein), and the sympathectomized region contained within the stenotic territory (Evans blue). Analysis of tissue norepinephrine concentration verified the location of the different regions.

Following fixation, transmural tissue samples from the three regions were divided into equal thirds (subepicardial, midmyocardial, and subendocardial) and weighed. The tissue weights ranged from 0.30 to 0.49 g. We calculated that each tissue sample would contain at least 400 microspheres for each nuclide, which should ensure an adequate number for blood flow measurement. This calculated value was derived from the original specific activity of the microspheres, the efficiency of the detector, the decay constant of the nuclide, and the time elapsed from the original assay date to when the activity was measured, yielding an estimate of counts per minute per microsphere. Tissues with less than the predicted number of counts for 400 microspheres were omitted from analysis. Myocardial perfusion per unit weight was determined in each tissue sample by measuring the tissue activity of each nuclide in a germanium well-type detector, the decay constant of the nuclide, and the time elapsed from the original essay date to when the activity was measured, yielding an estimate of counts per minute per microsphere. Tissues with less than the predicted number of counts for 400 microspheres were omitted from analysis.

Myocardial blood flow (MBF) was calculated from the withdrawal rate of the pump (W), the activity of a nuclide in the arterial reference sample (Ra), and the activity per unit weight of tissue for a given nuclide (Ta); according to the following expression:

$$\text{MBF (ml/min/100 g)} = \frac{\text{Ta (activity/g) } W \text{ (ml/min)} \times 100}{\text{Ra (activity)}}$$

**Tissue and Plasma Catecholamines**

The regional sympathectomy was verified by analyses of tissue concentrations of norepinephrine in the anterior, normally innervated region (left anterior descending region), the innervated, anterior portion of the circumflex perfusion field (within the stenotic territory), and the posterior phenol-treated sympathectomized region of the circumflex artery (within the stenotic territory). After killing the animal, the heart was quickly removed, and tissue samples from these three regions were excised and immediately placed on dry ice. The samples were transferred quickly to a −70° C freezer.

Extraction of norepinephrine from tissue samples was carried out on alumina by the procedure of Anton and Sayer. Analysis of norepinephrine was performed via high performance liquid chromatography and electrochemical detection. This system consists of a solvent delivery system (model 6000 A, Waters Associates, Milford, Massachusetts) and U6K (Waters), a C-18 column (Biophysical ODS 5-μm particle size range, 250×4.6 mm), a guard column (Biophysical ODS, 5-μm), and an LC-17 electrochemical detector with LC-3 amperometric controller (Bioanalytical Systems, West Lafayette, Indiana). The mobile phase was composed of 3 parts of 0.1 M citric acid and 2 parts 0.1 M sodium dibasic phosphate and 0.1 M sodium octysulfate. The detector potential was set at 0.75 V versus the Ag/AgCl reference electrode. The mobile phase was pumped at a rate of 1.3 ml/min at an ambient temperature. The tissue extract (1–100 μl) was injected onto the analytical column. The retention time was 5.4 minutes for norepinephrine.

Norepinephrine in the eluate was quantified by comparing peak areas of the tissue extracts to those of known standards. Using this methodology, the relation between the peak area and increasing concentration of norepinephrine is linear. The minimal detectable amount of norepinephrine is 50 pg.

Blood plasma samples were obtained during control conditions and during exercise. Plasma concentrations of catecholamines were assayed according to the method of Peuler and Johnson. Briefly, 5 ml blood was added to a tube that contained 20 μl of a solution consisting of 95 mg EGTA and 60 mg reduced glutathione per milliliter at pH 6–7 (pH adjusted with 6 N sodium hydroxide). Immediately upon collection, the blood was mixed with the preservatives by gentle mixing, and the tubes were placed in an ice bath and then centrifuged to separate plasma from the red blood cells. The plasma was decanted and stored in tubes at −70° C. For the assay, the samples were thawed and analyses of epinephrine and norepinephrine were made using a radioenzymatic method. This method involves methylation of norepinephrine to normetanephrine and epinephrine to metanephrine using COMT (catechol-O-methyl-transferase) and [3H]methyl donor. [3H]Metanephrine is distinguished from [3H]normetanephrine using thin-layer chromatography, and the amounts are quantitated using liquid scintillation. The sensitivity of this assay is 3 pg, and the coefficient of variation averages between 0.5% and 4%.

**Stenosis Protocol**

To test the hypothesis that neural adrenergic coronary constriction during exercise will prevent transmural steal of blood in a stenotic region, myocardial perfusion was sequentially measured during five experimental conditions in 9 dogs: 1) control conditions — when the animal was standing on the treadmill; 2) control conditions with stenosis — the circumflex occluder was...
inflated to reduce distal pressure to 45 mm Hg; 3) exercise with circumflex stenosis (distal pressure maintained at 45 mm Hg); 4) exercise during β-adrenergic blockade (propranolol, 1 mg/kg) with the circumflex stenosis maintained (distal pressure maintained at 45 mm Hg); and 5) exercise plus α- and β-adrenergic blockade (propranolol, 1 mg/kg; phentolamine, 1 mg/kg) with the circumflex stenosis (distal pressure maintained at 45 mm Hg). In these experiments, exercise was used as a physiological stimulus to activate the sympathetic nervous system. Propranolol (β-adrenergic blockade) was used to normalize possible differences in metabolic demands in the innervated and sympathectomized regions during exercise. α-Adrenergic blockade (phentolamine) was used in combination with β-adrenergic blockade to eliminate α-adrenergic constrictor effects. Phentolamine and propranolol were administered through the left atrial catheter. During all interventions that required the presence of the stenosis, the distal pressure was maintained relatively constant (44–46 mm Hg) with adjustments of the pressure within the occluder. Measurements of myocardial perfusion were completed after 1.5–2 minutes following the initiation of exercise. This was the time required to make adjustments of the pressure within the occluder. Measurements of myocardial perfusion were completed after 1.5–2 minutes following the initiation of exercise. The levels of exercise during the three experimental periods were identical for each animal (6 km/hr). This was possible because with our experimental protocol, submaximal exercise tolerance was not affected by adrenergic blockade or the production of the stenosis. Between measurements of myocardial blood flow during exercise (6 km/hr), the treadmill was slowed to a walking speed and the stenosis released. To assess circulating blood plasma levels of catecholamines, we obtained an arterial blood sample from 4 animals immediately after the microsphere withdrawal for the control and exercise periods.

Data Analysis

All regional differences (left anterior descending, innervated stenotic region, sympathectomized stenotic region) in perfusion, endocardial-to-epicardial flow ratio, and norepinephrine concentration were analyzed using paired experimental comparisons in conjunction with the Bonferroni Inequality. The blood flow data were compared only to respective paired values within a single intervention, e.g., innervated stenotic subendocardium versus sympathectomized stenotic subendocardium versus normally perfused subendocardium. Blood flow comparisons between interventions were not performed. Differences in hemodynamics or blood flow among the different interventions (standing, standing with stenosis, etc.) were examined with an analysis of variance. Specific differences among the interventions were examined using the Bonferroni Inequality. Statistical significance was determined as p<0.05. All reported values are mean ± SEM.

Results

Systemic Hemodynamics

The hemodynamic results for the stenosis experiments are presented in Table 1. The stenosis was maintained during all interventions with the exception of the first control measurement. Heart rate increased significantly from control during all exercise periods (p<0.05). Mean arterial pressure increased during exercise (p<0.05) but was significantly reduced during combined α- and β-adrenergic blockade during exercise (p<0.05). Left ventricular end-diastolic pressure increased significantly during the production of the stenosis under control conditions (p<0.05) and increased substantially during exercise and exercise plus β-adrenergic blockade (p<0.05). Peak left ventricular dP/dt increased during exercise (p<0.05), but β-adrenergic blockade reduced this experimental variable to control levels during the exercise periods.

Myocardial Perfusion

Table 2 shows the regional myocardial perfusion data during the stenosis experiments. Under control conditions, there were no differences in perfusion or the endocardial-to-epicardial ratio of blood flow in either the normally perfused innervated region, the innervated stenotic region, or the sympathectomized stenotic region. Production of the stenosis at rest signifi-

Table 1. Hemodynamics During Stenosis Experiments

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Control + stenosis</th>
<th>Exercise + stenosis</th>
<th>Exercise + βα + stenosis</th>
<th>Exercise + (αα + ββ) + stenosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td>116 ± 7</td>
<td>132 ± 11</td>
<td>218 ± 7*†</td>
<td>146 ± 6*†</td>
<td>148 ± 7*†</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>99 ± 5</td>
<td>108 ± 7</td>
<td>119 ± 8*†</td>
<td>110 ± 11*</td>
<td>89 ± 6*†</td>
</tr>
<tr>
<td>LV systolic pressure (mm Hg)</td>
<td>128 ± 8</td>
<td>121 ± 7</td>
<td>152 ± 11*†</td>
<td>129 ± 9§</td>
<td>111 ± 7*§</td>
</tr>
<tr>
<td>LV end-diastolic pressure (mm Hg)</td>
<td>5 ± 1</td>
<td>9 ± 2*</td>
<td>17 ± 3*†</td>
<td>22 ± 2*†</td>
<td>14 ± 2*†</td>
</tr>
<tr>
<td>LV dP/dt (mm Hg/sec)</td>
<td>3,316 ± 259</td>
<td>3,401 ± 229</td>
<td>6,549 ± 230*</td>
<td>3,318 ± 254§</td>
<td>3,244 ± 169§</td>
</tr>
<tr>
<td>Circumflex pressure (mm Hg)</td>
<td>99 ± 5</td>
<td>46 ± 2</td>
<td>45 ± 2</td>
<td>45 ± 2</td>
<td>45 ± 1</td>
</tr>
<tr>
<td>n</td>
<td>9</td>
<td>8</td>
<td>9</td>
<td>9</td>
<td>8</td>
</tr>
</tbody>
</table>

All values are mean ± SEM. n, number of dogs.

 αα, α-adrenergic blockade; ββ, β-adrenergic blockade.
* p<0.05 vs. Control.
† p<0.05 vs. Control + stenosis.
‡ p<0.05. Exercise + ββ + stenosis vs. Exercise + stenosis or Exercise + stenosis + (αα + ββ).
§ p<0.05. Exercise + stenosis vs. Exercise + stenosis + adrenergic blockade.
Table 2. Regional Myocardial Perfusion During Stenosis Experiments

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Control + stenosis</th>
<th>Exercise + stenosis</th>
<th>Exercise + (\alpha_x + \beta_x) + stenosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subendocardial Blood Flow (ml·min(^{-1})·100 g(^{-1}))</td>
<td>N-I</td>
<td>175 ± 18</td>
<td>221 ± 25†</td>
<td>388 ± 51†</td>
</tr>
<tr>
<td></td>
<td>St-I</td>
<td>186 ± 25</td>
<td>120 ± 28</td>
<td>91 ± 17*</td>
</tr>
<tr>
<td></td>
<td>St-Sx</td>
<td>181 ± 23</td>
<td>115 ± 26</td>
<td>71 ± 14</td>
</tr>
<tr>
<td>Midmyocardial Blood Flow (ml·min(^{-1})·100 g(^{-1}))</td>
<td>N-I</td>
<td>186 ± 19</td>
<td>219 ± 29†</td>
<td>418 ± 68†</td>
</tr>
<tr>
<td></td>
<td>St-I</td>
<td>163 ± 16</td>
<td>126 ± 30</td>
<td>116 ± 15</td>
</tr>
<tr>
<td></td>
<td>St-Sx</td>
<td>165 ± 21</td>
<td>132 ± 28</td>
<td>124 ± 20</td>
</tr>
<tr>
<td>Subepicardial Blood Flow (ml·min(^{-1})·100 g(^{-1}))</td>
<td>N-I</td>
<td>136 ± 20</td>
<td>190 ± 21†</td>
<td>375 ± 42†</td>
</tr>
<tr>
<td></td>
<td>St-I</td>
<td>126 ± 12</td>
<td>133 ± 21</td>
<td>177 ± 35*</td>
</tr>
<tr>
<td></td>
<td>St-Sx</td>
<td>120 ± 15</td>
<td>134 ± 23</td>
<td>193 ± 38</td>
</tr>
<tr>
<td>Endo/epi Ratio</td>
<td>N-I</td>
<td>1.39 ± 0.06</td>
<td>1.22 ± 0.07†</td>
<td>1.04 ± 0.09†</td>
</tr>
<tr>
<td></td>
<td>St-I</td>
<td>1.48 ± 0.08</td>
<td>0.89 ± 0.07</td>
<td>0.58 ± 0.08*</td>
</tr>
<tr>
<td></td>
<td>St-Sx</td>
<td>1.50 ± 0.05</td>
<td>0.84 ± 0.17</td>
<td>0.38 ± 0.07</td>
</tr>
</tbody>
</table>

All values are mean ± SEM. \(n\), number of dogs.

\(\alpha_x\), \(\alpha\)-adrenergic blockade; \(\beta_x\), \(\beta\)-adrenergic blockade.

N-I, normally perfused, innervated region; St-I, stenotic innervated region; St-Sx, stenotic sympathectomized region.

*\(p<0.05\), innervated stenotic vs. sympathectomized stenotic.

†\(p<0.05\), normal region vs. stenotic regions.

Table 3 summarizes the differences between the innervated and sympathectomized regions during the experimental interventions. This table is constructed to show the mean differences between these two regions and the standard error of the differences. The major differences shown are in the presence of the stenosis during exercise and exercise plus \(\beta\)-adrenergic blockade. The subendocardial blood flow during these two periods was significantly higher in the innervated region than in the sympathetomized region, as indicated by the positive values. Midmyocardial blood flow and subepicardial blood flow were less in the innervated regions than in the sympathetomized regions, as indicated by the negative values. The subendocardial-to-subepicardial flow ratio also reflected the redistribution of flow: the ratio was significantly greater in the innervated region than that in the sympathetomized region.

Table 3. Differences Between Innervated and Sympathectomized Regions During Stenosis Experiments

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Control + stenosis</th>
<th>Exercise + stenosis</th>
<th>Exercise + (\alpha_x + \beta_x) + stenosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Innervated-sympathectomized subendocardial blood flow (ml·min(^{-1})·100 g(^{-1}))</td>
<td>5 ± 6</td>
<td>5 ± 6</td>
<td>20 ± 7*</td>
<td>10 ± 5*</td>
</tr>
<tr>
<td>Innervated-sympathectomized midmyocardial blood flow (ml·min(^{-1})·100 g(^{-1}))</td>
<td>2 ± 5</td>
<td>-6 ± 9</td>
<td>-8 ± 6</td>
<td>-17 ± 5*</td>
</tr>
<tr>
<td>Innervated-sympathectomized subepicardial blood flow (ml·min(^{-1})·100 g(^{-1}))</td>
<td>1 ± 4</td>
<td>-16 ± 6*</td>
<td>-10 ± 6</td>
<td>-4 ± 6</td>
</tr>
<tr>
<td>Innervated-sympathectomized endo/epi ratio</td>
<td>-0.02 ± 0.05</td>
<td>0.05 ± 0.06</td>
<td>0.20 ± 0.06*</td>
<td>0.09 ± 0.03*</td>
</tr>
</tbody>
</table>

All values are mean ± SEM. \(n\), number of dogs.

\(\alpha_x\), \(\alpha\)-adrenergic blockade; \(\beta_x\), \(\beta\)-adrenergic blockade.

*\(p<0.05\), innervated vs. sympathetomized.
region. These differences in blood flow were abolished following α-adrenergic blockade.

**Tissue and Plasma Catecholamines**

Left ventricular concentrations of norepinephrine were 761 ± 58 ng/g in the innervated left anterior descending region and 693 ± 63 ng/g in the innervated stenotic circumflex region. These values were not significantly different. The norepinephrine concentration was reduced significantly to 76 ± 22 ng/g (p < 0.05) in the phenol-treated, sympathectomized circumflex region.

During control conditions, blood plasma norepinephrine and epinephrine concentrations were 160 ± 46 and 259 ± 22 pg/ml, respectively. Both norepinephrine and epinephrine were significantly increased (p < 0.05) during exercise to 630 ± 71 and 570 ± 50 pg/ml, respectively.

**Discussion**

The major finding of this study was that in the presence of a flow-limiting stenosis, activation of coronary sympathetic nerves during exercise redistributes blood flow toward the subendocardium. This redistribution of flow resulted from neuronally mediated α-adrenergic vasoconstriction in the subepicardial and midmyocardial left ventricular regions because α-adrenergic blockade with phentolamine equalized the transmural distribution of perfusion in the innervated and sympathectomized stenotic regions. Also, the differences in the distribution of blood flow in the two stenotic regions were not dependent on differential myocardial metabolism or contractility in the innervated and sympathectomized stenotic regions because redistribution of perfusion was observed during β-adrenergic blockade. Thus, α-adrenergic coronary constriction should be considered as a regulatory factor involved in the transmural distribution of myocardial perfusion.

Our interpretations and conclusions depend upon important considerations regarding the experimental methodology, the experimental model, results that support nonuniform coronary α-adrenergic constriction, and suitable hemodynamic and physiologic mechanisms that support this concept.

**Methodological Considerations**

Microsphere determination of myocardial perfusion was the most critical experimental method on which our conclusions were based. We have previously reported that paired measurements of myocardial perfusion were accurate to within a 5–10% difference. The differences in perfusion between the innervated and sympathectomized stenotic regions ranged between 8% and 21%; thus, our blood flow measurement technique had the necessary precision to identify such a difference. Determination of blood flow in the present study could not have been made with an electromagnetic flow probe or a Doppler flowmeter because such devices cannot detect transmural variations in coronary blood flow. The use of nuclide-labeled microspheres was essential for regional blood flow measurements.

Another important methodological consideration regards our experimental protocol. Previously, we have found that during sustained, submaximal exercise, a significant portion of α-adrenergic coronary constrictor tone was due to circulating catecholamines. In the present study, we have found that sympathetic neurogenic α-adrenergic tone was the important factor for decreasing transmural steal in a stenotic region. Our present protocol involved measurement of myocardial perfusion during the early stage of exercise (1.5–2 minutes following initiation). This is in contrast to our previous study in which measurements were obtained 5–10 minutes following initiation of exercise. As a consequence of these different experimental protocols, the circulating levels of plasma catecholamines were also very different. In our previous report, the total concentration of plasma epinephrine and norepinephrine averaged 3,136 pg/ml during sustained submaximal exercise. In contrast, the plasma catecholamine level in the present study (1.5–2 minutes after initiation of exercise) was 1,266 pg/ml. Thus, using the present exercise protocol, i.e., measurements obtained shortly after initiation of exercise, neurogenic adrenergic influences predominated.

**Experimental Model Considerations**

The efficiency of the phenol-treated regional sympathectomy model is well established. Previously, in a regional sympathectomy model we found 90–99% reduction in tissue norepinephrine in the phenol-treated region compared with that of the anterior innervated region. This was also confirmed by the present results. Neuronal uptake of norepinephrine was also absent in the sympathectomized region but normal in the anterior innervated region. Stellate nerve stimulation also resulted in marked norepinephrine overflow from the anterior innervated region, whereas the sympathectomized region extracted norepinephrine. We have also reported functional evidence for denervation. Stellate nerve stimulation was associated with an increased lactate and oxygen extraction in the anterior innervated region but not in the posterior sympathectomized region. Phenol application did not produce extensive damage to the myocardium because Randall et al found that necrosis produced by phenol treatment is restricted to the superficial 0.25-mm epicardial surface. Moreover, tissue concentrations of high energy phosphate are similar in the treated and untreated regions.

Application of phenol also produced coronary vascular denervation as indicated by a marked reduction in labeled-norepinephrine uptake in vessels from the phenol-treated region. Also, we recently reported that large epicardial vessels from the phenol-treated region do not show constrictor responses to tyramine, whereas normally innervated vessels show substantial constriction to tyramine.

Although Martins and Zipes found that application of epicardial phenol did not interrupt efferent vagal influences on effective refractory periods in the left ventricle, we cannot directly assess the status of the
coronary parasympathetic innervation. We emphasize, however, that even if vagal efferent innervation was partially interrupted in the sympathectomized region, there is withdrawal of vagal tone during exercise \textsuperscript{28}; thus, cholinergic influences on the heart and coronary circulation would be minimal.

Another important characteristic of the regional sympathectomy model is that coronary $\alpha$-adrenergic supersensitivity does not occur.\textsuperscript{29} This conclusion was based on the observations that myocardial perfusion or coronary resistance in the innervated and sympathectomized regions of the left ventricle of conscious, $\beta$-adrenergically blocked dogs was not different during norepinephrine infusion. Also, results from in vitro isolated vascular ring studies indicated that sympathectomized epicardial coronaries were not supersensitive to phenylephrine ($\alpha_1$-adrenergic agonist) or norepinephrine ($\alpha_2$-adrenergic agonist). Thus, potential $\alpha$-adrenergic coronary vascular supersensitivity in the sympathectomized region does not complicate interpretation of our results.

In the aggregate, these data provide substantial evidence that the regional sympathectomy model is efficacious. An advantage offered by the regional sympathectomy model is that it enables a paired comparison of myocardial perfusion in two left ventricular regions (innervated versus sympathectomized) that have identical preload, afterload, and driving pressure and are influenced equally by drugs and humoral substances.

**Nonuniform Transmural $\alpha$-Adrenergic Coronary Constriction**

Our results are consistent with the hypothesis that sympathetic neural activation during exercise lessened the transmural steal of blood flow within the stenotic region. This interpretation was based on two important results. First, during exercise, there was greater subendocardial perfusion in the innervated stenotic region than in the sympathectomized stenotic region. Second, there was less subepicardial and midmyocardial perfusion in the innervated stenotic regions than in the sympathetomized stenotic region. Thus, these data indicate that physiological activation of coronary sympathetic innervation redistributed myocardial blood flow away from the subepicardium and midmyocardium toward the subendocardium in a stenotic region of the left ventricle. Our conclusion is strengthened by the fact that the perfusion pressures were equal in the stenotic sympathetomized and innervated regions. This is an important controlled variable since the transmural distribution of flow is critically dependent on perfusion pressure.\textsuperscript{30}

Since $\alpha$-adrenergic coronary constriction appears to result in a beneficial redistribution of myocardial blood flow in the stenotic region, we might have expected $\beta$-adrenergic blockade to unmask and augment this beneficial neurogenic $\alpha$-adrenergic tone. This, however, was not the experimental observation since during exercise with $\beta$-adrenergic blockade, the endocardial-to-epicardial flow ratio was 0.47 in the innervated stenotic region but was 0.60 in the same region during exercise alone. The decrease in the endocardial-to-epicardial ratio during exercise of $\beta$-adrenergic blockade was coincidental with an increase in left ventricular end-diastolic pressure from 17 to 22 mm Hg in the absence and presence of $\beta$-adrenergic blockade, respectively. Since left ventricular end-diastolic pressure is related inversely to left ventricular subendocardial perfusion,\textsuperscript{30} the observed increase in this variable could account for the decrease in the endocardial-to-epicardial flow ratio observed in the stenotic regions during $\beta$-adrenergic blockade.

**Hemodynamic Mechanism For Nonuniform Transmural $\alpha$-Adrenergic Coronary Constriction**

Steal of blood normally occurs when vasodilation in one vascular bed lowers the perfusion pressure to a vascular bed in which flow is pressure-dependent. Chiariello et al\textsuperscript{40} reported that $\alpha$-adrenergic coronary constriction could "reverse" coronary steal between the circumflex and left anterior descending vascular beds. In their preparation, the left anterior descending artery was ligated, and collateral flow was measured during infusion of methoxamine (an $\alpha_1$-adrenergic agonist) during constant arterial pressure. Methoxamine produced constriction of blood vessels in the nonoccluded zone. Blood flow fell from 90 to 77 ml/min/100 g, but blood flow increased in the ischemic zone from 21 to 41 ml/min/100 g. This suggested that $\alpha$-adrenergic constriction in the circumflex bed promoted collateral flow by increasing the driving pressure across the left anterior descending bed. Favorable redistribution of blood flow to the ischemic zone was interpreted as a mechanism that caused "reverse" coronary steal. Giudicelli et al\textsuperscript{41} also found that left stellate stimulation reversed coronary steal in the presence of $\beta$-adrenergic blockade.

Transmural steal of myocardial blood flow occurs by a mechanism similar to steal between adjacent vascular beds in which dilation of a vascular bed (subepicardial) lowers the perfusion pressure of a parallel bed (subendocardial) in which flow is pressure-dependent. A reverse transmural steal would occur if vasoconstriction in subepicardial and midmyocardial regions shunted blood toward the subendocardium. Our data are consistent with this explanation: in the presence of a stenosis, subepicardial and midmyocardial $\alpha$-adrenergic constriction redistributed blood flow toward the subendocardium. In the sympathetomized stenotic region, such a mechanism was not operative, and blood flow was distributed away from the subendocardium.

There are reports in the literature consistent with an $\alpha$-adrenergic antisteal mechanism. Buffington and Feigl\textsuperscript{42} reported that $\alpha$-adrenergic coronary constriction in transmural layers of the myocardium was dependent on perfusion pressure. These investigators found uniform transmural coronary constriction with norepinephrine at coronary perfusion pressures of 100 and 70 mm Hg. No constriction was observed during norepinephrine infusion in any transmural region at a perfusion pressure of 38 mm Hg. At a pressure of 50...
Nonuniform Transmural Coronary Constriction

mm Hg, norepinephrine produced constriction in the subepicardial and midmyocardial regions but not in the subendocardial region. Recently, Nathan and Feigl48 used a constant flow preparation and demonstrated that \( \alpha \)-adrenergic coronary constriction in the outer layers of the myocardium exerted an antisteal effect. This conclusion was based on the fact that during graded reductions in coronary blood flow, \( \alpha \)-adrenergic receptor activation with norepinephrine increased endocardial-to-epicardial blood flow ratios. Our experimental results importantly extend these observations from anesthetized animals into a conscious animal model of coronary artery stenosis during physiological activation of sympathetic nerves. An important implication of our results and those from previous reports23,24 is that sympathetic coronary constriction should not always be considered detrimental. In fact, adrenergic coronary vasoconstriction may be beneficial in that transmural steal of blood flow is decreased.

An important question provoked by our results relates to the functional consequences of the enhanced subendocardial flow in the innervated stenotic region compared with the sympathectomized stenotic region. Since subendocardial blood flow and wall thickening are intimately related,41 it is possible to estimate wall thickening in the innervated and sympathectomized stenotic regions. Using the equations reported by Gallagher et al (see their Figure 5),27 the calculated percent wall thickening in the innervated stenotic region and sympathectomized stenotic region would be 20–25% and 10–15%, respectively. Thus, it is conceivable that the redistribution of flow toward the subendocardium observed in the innervated stenotic region could improve function. Whether or not this actually occurs, however, is yet unknown.

Physiological Mechanisms Responsible For Nonuniform Transmural \( \alpha \)-Adrenergic Coronary Constriction

There are several explanations for adrenergic activation producing nonuniform coronary constriction in different transmural layers of the left ventricular wall. First, during coronary hypoperfusion, the transmural gradient of ischemia is most intense in the subendocardium.37,43,44 Thus, a buildup of ischemic metabolites or hypoxia in this region could impair adrenergic vasoconstriction to a greater extent than in the outer layers. Within this context, Heistad et al49 found that hypoxia inhibited coronary constriction to norepinephrine. Also, Curro and Greenburg60 observed that acidosis impaired \( \alpha \)-adrenergic constriction in the skeletal muscle microcirculation. A buildup of ischemic metabolites could also impair adrenergic neurotransmission via presynaptic mechanisms58 and limit endocardial adrenergic constriction. A second possibility for nonuniform transmural adrenergic coronary constriction could be related to a gradient in the density of coronary \( \alpha \)-adrenergic receptors across the left ventricular wall. This explanation seems remote because Chilian et al20 found that infusion of norepinephrine produced uniform constriction in different transmural layers in conscious animals with \( \beta \)-adrenergic and ganglionic blockade. Also, Johannsen et al22 found that phenylephrine and norepinephrine produced uniform coronary constriction across the wall of the left ventricle in dogs with adenosine-induced maximal coronary vasodilation. Another explanation for heterogeneous adrenergic coronary constriction across the wall of the left ventricle could be related to the distribution of the sympathetic nerves. This is speculative because there has been no systemic study of the density of adrenergic innervation of the coronary vasculature across the wall of the left ventricle. Johannsen et al22 found that sympathetic nerve stimulation produced primarily subepicardial vasoconstriction in dogs with maximal coronary vasodilation (produced by adenosine infusion). However, tyramine infusion produced uniform coronary constriction. These authors argued that nonuniform transmural coronary constriction was related not to the density of the sympathetic nerves but to prejunctional effects of adenosine modulating norepinephrine release. Our results do not allow definitive conclusions regarding the physiological mechanism for nonuniform transmural effects of adrenergic coronary constriction in our experimental model. However, because of the evidence provided from the literature, we favor the first hypothesis that ischemic vasodilator metabolites or local hypoxia in the subendocardium impaired vascular reactivity to \( \alpha \)-adrenergic constriction or sympathetic neural transmission.

Several investigators have reported that sympathetic activation can produce coronary constriction of sufficient magnitude to impair function or produce myocardial ischemia. Recently, Gwirtz et al19 found that \( \alpha \)-adrenergic blockade during exercise improved subendocardial contractile function. These investigators proposed that sympathetic coronary constriction during exercise limited subendocardial perfusion and oxygen delivery to a sufficient extent to impair function. These authors, however, did not determine whether subendocardial perfusion was enhanced by \( \alpha \)-adrenergic blockade; thus, their experimental evidence favoring their conclusion was equivocal. Furthermore, other indices of subendocardial function (percent segment shortening) were not improved by \( \alpha \)-adrenergic blockade. In a preliminary report, \( \alpha \)-adrenergic blockade was found to improve coronary blood flow and function in a stenotic left ventricular region in exercising dogs.37 Heusch and Deussen26 found that sympathetic nerve stimulation produced myocardial ischemia in dogs with a severe coronary stenosis. Sympathetic nerve stimulation caused a significant increase in coronary resistance and resulted in myocardial lactate production. In humans with coronary artery disease, reflex sympathetic coronary constriction was found to be sufficient to produce myocardial ischemia43 and left ventricular dysfunction.40 In these studies of human coronary pathophysiology, sympathetic coronary constriction would probably influence the resistance of the stenosis. If this were to occur, modest adrenergic coronary
constriction could markedly influence the stenotic resistance and promote myocardial ischemia. In our experimental model, stenotic resistance was varied during the experimental interventions to maintain pressure distal to the stenosis at 45 mm Hg; thus, possible α-adrenergic constriction at the stenosis was controlled. This difference in experimental design is a plausible reason to explain why we found a beneficial effect of α-adrenergic constriction, whereas other laboratories have found a detrimental effect.

In conclusion, in the conscious animal, sympathetic activation during exercise will produce nonuniform transmural coronary constriction in a stenotic region. The nonuniform constriction promotes subendocardial perfusion by α-adrenergic constriction in midmyocardial and subepicardial regions. The net effect is to decrease a transmural steal of blood in the stenotic region.

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