Adrenergic Coronary Tone during Submaximal Exercise in the Dog is Produced by Circulating Catecholamines
Evidence for Adrenergic Denervation Supersensitivity in the Myocardium but Not in Coronary Vessels

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SUMMARY. The goal of this study was to test the hypothesis that circulating catecholamines are primarily responsible for α-adrenergic coronary vasoconstriction during submaximal exercise. The experimental series consisted of chronic studies in which a regional left ventricular sympathectomy was performed with phenol. Myocardial perfusion to the innervated and sympathectomized left ventricular regions was measured in these animals during (1) a control period, (2) treadmill exercise, (3) exercise during β-adrenergic blockade, and (4) exercise during combined α- + β-adrenergic blockade. We found no differences in myocardial perfusion between the innervated and sympathectomized regions or the transmural distribution of perfusion during any of these interventions. Thus, there is no evidence for neurogenic α-adrenergic coronary vasoconstriction. However, during exercise in the presence of α- and β-blockade, coronary resistance (mmHg×min×100 g/ml) was significantly less in both the innervated (0.65 ± 0.07) and sympathectomized (0.68 ± 0.07) regions than during β-blockade, 0.90 ± 0.17 and 0.89 ± 0.16, respectively. This suggests that coronary α-adrenergic constriction was produced by circulating catecholamines. This concept of humorally mediated, α-adrenergic coronary vasoconstriction was strengthened by in vivo and in vitro studies that demonstrated that α-adrenergic supersensitivity of the coronary vasculature was not present. Myocardial β-adrenergic supersensitivity was observed in the phenol regional sympathectomy model; however, this effect was blocked by propranolol (1 mg/kg). This indicates that α-adrenergic vasoconstriction in both myocardial regions during submaximal exercise is produced by circulating catecholamines. The major conclusion of this study is that, during submaximal exercise in the canine, α-adrenergic coronary vasoconstrictor tone is predominately due to circulating catecholamines rather than direct neural effects. (Circ Res 58: 68–82, 1986)

ACTIVATION of the sympathetic nerves to the heart results in an α-adrenergic receptor coronary vasoconstrictor influence and β-adrenergic receptor-mediated positive inotropic and chronotropic effects on the myocardium which produce competing secondary local metabolic vasodilation (Feigl, 1983). This α-adrenergic coronary constriction competes with metabolic coronary vasodilation and reportedly limits coronary blood flow by approximately 30% under conditions of intracoronary infusion of nor-epinephrine or reflex sympathetic activation (Mohrman and Feigl, 1978), and during intense levels of exercise (Murray and Vatner, 1979). Heyndricks et al. (1982) have also reported that α-adrenergic blockade alters the relationship between myocardial oxygen consumption and myocardial perfusion during treadmill exercise. In addition, Gwirtz and Stone (1981) found that sympathetic coronary vasoconstriction during exercise limits coronary blood flow. An unresolved question is whether α-adrenergic coronary tone during exercise is due to activation of coronary α-adrenergic receptors by circulating catecholamines or to direct effects of the coronary sympathetic innervation.

The goal of this study was to employ an experimental model in which the direct neural and humoral effects of sympathetic coronary vasoconstriction during submaximal exercise could be assessed without confounding hemodynamic changes or nonspecific drug effects which may have influenced previous studies. Our experimental approach was to
Measure myocardial perfusion to an innervated and sympathectomized region of the left ventricle in conscious, exercising dogs. With this regional sympathectomy model, we are able to assess potential neurally or humorally mediated vasoconstriction during exercise, directly, because α-adrenergic receptor-mediated tone in the sympathetomized region should be due exclusively to circulating catecholamines.

Methods

Surgical Preparation: Conscious Studies

Mongrel dogs of either sex (22–36 kg) were acclimated to running on a motor-driven treadmill. After 1 week of this acclimation period, a regional left ventricular sympathectomy was performed. Dogs were anesthetized with sodium pentobarbital (25–30 mg/kg, iv), intubated and ventilated with room air using a mechanical respirator. By aseptic technique, a left thoracotomy was performed, the pericardium was incised, and the ventricle was exposed for the topical application of 85% phenol. Phenol was applied as previously described (Chilian et al., 1981). Briefly, the region of the posterior left ventricle to be sympathectomized was identified, and the arteries entering this region were carefully dissected. Umbilical tape, wetted with phenol, was passed under and wrapped around the vessels, and removed. The next step was the application of a 3- to 5-mm-wide line of phenol on the epicardial surface. Phenol was painted from the base to the apex in a series of interconnecting 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. Phenol was also applied to the anteroventricular groove above the sympathetomized region. A Konigsberg solid state transducer 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. The catheters and the transducer exited between the scapulae in the left atrial appendage, and a Konigsberg transducer was secured against aortic systolic pressure. The left atrial appendage was catheterized and the left circumflex artery was catheterized with a 22-gauge Teflon catheter.

Coronary blood flow velocity was measured simultaneously in the circumflex and left anterior descending arteries with a pulsed Doppler velocimeter modified from the description given by Hartley and Cole (1974). The Doppler probes consisted of a piezoelectric crystal (1 mm in diameter) housed in a Silastic suction cup. A 5–7 torr vacuum secured the probe to the vessel wall, and the crystal was oriented at 45° to the blood column.

Measurement of Myocardial Perfusion

Myocardial perfusion to the innervated and sympathetomized regions was measured with nuclide-labeled microspheres. The microspheres (4–15 μm) were labeled with 46Sc, 85Sr, 95Nb, 113Sn, 153Gd, or 141Ce. Approximately 1–5 x 10^6 microspheres were injected into the left atrial catheter over a 20-second period and flushed with 15 ml of 37°C saline. Starting 15 seconds before injection and continuing for 90 seconds after the microsphere injection, blood was withdrawn from the arterial catheter at a constant rate of 12.0 ml/min (conscious studies) or 3.8 ml/min from a femoral and brachial artery (anesthetized studies). Myocardial blood flow (MBF) was calculated by computer, correcting for isotope overlap, using the withdrawal rate of the pump (W), the total radioactivity of the arterial reference sample (Cr), and the radioactivity per unit weight of myocardium (Cm) according to the following expression:

\[ \text{MBF (ml/min per g)} = \frac{\text{Cm (counts/time per g)} W (ml/min)}{\text{Cr (counts/time)}} \]

Next, we killed the animals by anesthetizing them with sodium pentobarbital and injections of KCl. The heart then was removed, and tissue samples were taken for norepinephrine analysis and fixed in 6% formalin. After fixation, the left ventricle was systematically sectioned, using anatomic landmarks into the innervated and sympathetomized regions. (Analysis of tissue norepinephrine concentration verified the location of the two regions.) In the acute experiments, the circumflex region was stained with Evans blue and was sectioned from the other left ventricular regions.

The transmural tissue samples in the two regions were divided into equal thirds (subepicardial, mid-myocardial, and subendocardial) and weighed. Myocardial perfusion per unit weight was determined in each sample by measuring tissue radioactivity of each nuclide in a γ-counter and calculating flow according to formula. From these measurements of perfusion, we calculated the transmural distribution of blood flow (subendocardial:subepicardial flow ratio) and calculated coronary resistance (quotient of mean aortic pressure and myocardial blood flow) for the innervated and sympathetomized regions.

Measurement of Regional Myocardial Contractility

Left ventricular subendocardial segment shortening was measured in the innervated and denervated regions of the
left ventricle with ultrasonic transit-time dimension gauges (Pagani et al., 1978). The pairs of crystals were positioned and secured 6 mm beneath the epicardial surface. The instrument generates a voltage linearly proportional to the acoustic transit time (1.5 × 10^6 mm/sec) between two 7-MHz piezoelectric crystals. The voltage output was recorded on an oscillographic recorder and represents the actual phasic dimensions of the particular segment. The device was calibrated in 1.0-µsec steps, and the calibration and zero offset were checked frequently throughout the experiments.

These experiments were conducted in animals 2–3 weeks past the initial regional sympathectomy surgery. The animals were anesthetized with sodium pentobarbital (30–35 mg/kg), intubated, and ventilated with a positive end-expiratory pressure. A bilateral vagotomy was performed, the left carotid artery and jugular vein were catheterized for pressure measurements and supplemental fluids, respectively. Additional anesthesia was administered as required. Following a left thoracotomy, the heart was suspended in a pericardial cradle, and the ultrasonic crystals were secured in innervated and denervated regions of the myocardium. We measured two indices of left ventricular segment function: dL/dt and percent segment shortening calculated as: EDL-ESL/EDL × 100, where EDL and ESL are end-diastolic length and end-systolic length, respectively. Measurement of dL/dt were obtained with a differentiator which was calibrated with an internal sine wave generator. These measurements represent the maximum negative deflection of differentiated signal.

Isolated Vascular Ring Preparation

To examine the possibility of coronary α-adrenergic supersensitivity in the phenol-treated (sympathectomized) region, we removed 5–10 segments of epicardial coronary arteries from the innervated and sympathetomized regions in eight animals and examined their tension development to α-adrenergic agonists in organ chambers.

Each of these vascular ring segments was studied in organ chambers containing 25 ml of physiological salt solution of the following composition (mM) NaCl, 118.3; KCl, 4.7; CaCl₂, 2.5; MgSO₄, 1.2; KH₂PO₄, 1.2; NaHCO₃, 25; calcium-EDTA, 0.026; and propranolol (10⁻⁶ M). The baths were oxygenated with a mixture of 95% oxygen and 5% carbon dioxide. Each ring was suspended on two steel clips passed through the lumen. One clip was anchored inside the organ chamber and the other was connected with 4.0 surgical silk sutured to a force transducer (Grass FT03c). Changes in isometric force were recorded with a direct-writing recorder coupled to bridge amplifiers. The rings were placed at their optimum point in their length-tension relationship, as determined from repeated 3-minute exposures to 100 mM KCl. Basal length of the rings were increased gradually over approximately 1 hour until their contractions to 100 mM potassium chloride were optimized. This length was maintained throughout the experiment.

Tissue and Plasma Catecholamines

The regional sympathectomy was verified by analyses of tissue concentrations of norepinephrine in the anterior, normally innervated region and in the posterior phenol-treated sympathetomized region.

After sacrifice, the dog's heart was quickly removed, tissue samples from the anterior and posterior regions were excised, and these samples were immediately placed on dry ice. The tissue samples were transferred quickly and stored in a −135°C freezer for norepinephrine analyses.

Extraction of norepinephrine from tissue samples was carried out by the procedure of Anton and Sayer (1962). The high-performance liquid chromatograph consisted of a solvent delivery system (model 6000A) and U6K from Waters Associates, a C18 column (Biophase ODS, 5-µm particle size range, 250 × 4.6 mm), guard column (Biophase ODS, 5 µm), and an LC-17 electrochemical detector with LC-3 amperometric controller from Bioanalytical Systems.

The mobile phase was composed of three parts of 0.1 M citric acid and two parts 0.1 M sodium diasic phosphate and 0.1 M sodium octylsulfate. The detector potential was set at 0.75 V vs. the Ag/AgCl reference electrode. The mobile phase was pumped at a rate of 1.3 ml/min at an ambient temperature (Keller et al., 1976; Schmid et al., 1979). One-hundred microliters of the tissue extract were injected onto the analytical column. The retention time was 5.4 minutes for norepinephrine. Norepinephrine in the eluate was quantitated by comparing peak areas of the tissue extracts to that of known standards. Using this methodology, the relationship between peak area and increasing concentration of norepinephrine is linear. The minimum detectable amount of norepinephrine is 50 pg.

Blood samples for catecholamines were placed in prechilled tubes of ice, centrifuged, and the plasma was stored at −135°C. Plasma samples were prepared according to DeChamplain et al. (1976), and were assayed according to the method of Schmidt et al. (1982).

Experimental Protocols

Sensitivity of the Microsphere Method (n = 6)

The purpose of this series was to determine the sensitivity of the microsphere method by directly comparing myocardial blood flow measurements in the anterior and posterior regions of the left ventricle.

Myocardial perfusion (microspheres) and coronary blood flow velocity (Doppler) were measured simultaneously in six anesthetized, open-chest animals under control conditions and during infusion of the non-recirculating vasodilator (Roberts et al., 1976), prostaglandin E₁, into the circumflex artery to increase blood flow velocity by approximately 5%, 10%, and 25% without changing flow in the left anterior descending artery. Specifically, we wanted to ascertain if a small difference in perfusion (5% or 10%) between anterior and posterior regions could be determined with radioactive microspheres.

Exercise Protocol (n = 7)

To assess adrenergic coronary constriction during exercise, we sequentially measured myocardial perfusion in these animals during four experimental conditions: (1) control—standing on the treadmill, (2) exercise—light level of exercise indicated by heart rates of 180–200 beats/min, (3) exercise + β-adrenergic blockade [propranolol (1 mg/kg) was administered through the atrial catheter], and (4) exercise + (α+β)-blockade [propranolol (1 mg/kg) and phentolamine (1 mg/kg) were administered through the atrial catheter]. Between measurements of myocardial blood flow, the treadmill was slowed to a walking speed, and 3–5 minutes before the measurement of perfusion, the speed of the treadmill was increased to approximately the previous level. Despite the fact that adrenergic blockade could potentially affect exercise tolerance, the levels of exercise during adrenergic blockades were comparable.
to that during the unblocked condition. This was possible because, with our experimental regimen, submaximal exercise tolerance apparently was not affected by adrenergic blockade. To assess circulating levels of catecholamines, we obtained a 10-ml arterial blood sample immediately after the microsphere withdrawal for control, exercise, and exercise during combined α- and β-adrenergic blockade.

Assessment of α-Adrenergic Supersensitivity (n = 23)

To determine whether α-adrenergic receptors in the coronary vasculature in the sympathectomized are supersensitive to circulating catecholamines two experimental series were performed. First, myocardial perfusion was measured during intravenous norepinephrine infusion in 11 dogs that had been instrumented and prepared as described previously, except that they lacked solid state left ventricular transducers but had pacing leads. These studies were conducted in conscious, sedated (Innovar-Vet) dogs during β-blockage (propranolol, 1 mg/kg iv). Supplemental doses of propranolol (0.25 mg/kg) were administered hourly. The protocol involved the measurement of perfusion during: (1) control, with β-adrenergic blockade, (2) norepinephrine infusion, 0.1 μg/kg per min, (3) norepinephrine infusion, 0.5 μg/kg per min, (4) norepinephrine infusion, 1.0 μg/kg per min, and (5) norepinephrine infusion, 2.5 μg/kg per min. In four additional animals, the heart was paced at 180 beats/min (from pacing wires sutured to the right ventricular epicardium at the time of surgery), and the animals were administered propranolol (1 mg/kg) and hexamethonium (10.0 mg/kg) to prevent reflex changes in coronary tone associated with norepinephrine infusion. The measurements of myocardial blood flow were completed after a hemodynamic steady state of at least 5 minutes during each infusion period.

Isolated vascular ring preparations were also examined to verify sympathetic denervation of the coronary vasculature and to examine for α-adrenergic supersensitivity. The vascular ring preparations were allowed to equilibrate in the physiological saline solution in the presence of 10−6 M propranolol, and optimum tension was established. The contractile responses of the rings were tested with tyramine (10−4 M), to verify sympathetic denervation. Subsequently, cumulative dose response curves were obtained for each segment studied with the α1-adrenergic agonist phenylephrine and subsequently with the α1- and α2-adrenergic agonist norepinephrine. These drugs were initially administered in concentrations of 10−6 M, and the concentrations then were increased 3-fold in stepwise fashion to 10−2 M. Between these dose-response curves, the vessels were allowed to equilibrate for 45 minutes to 1 hour, and were washed repeatedly with oxygenated buffer.

In the isolated vascular ring studies, responses to each agonist are expressed as a percent of the constriction response to 100 mM KCl.

Assessment of β-Adrenergic Supersensitivity (n = 8)

Our intent in performing this series of experiments, was to examine the possibility of β-adrenergic supersensitivity in the sympathectomized region and to demonstrate the efficacy of the β-adrenergic blockade in both left ventricular regions. We measured dL/dt and percent segment shortening in innervated and sympathectomized region of the myocardium during infusion of β-adrenergic agonists. After stabilization of control conditions, isoproterenol was infused at rates of 0.1, 0.5, 1.0, and 2.5 μg/kg per min. Measurements were obtained during steady state conditions. After a return to stable control conditions, norepinephrine was administered at 0.1, 0.5, 1.0, 2.5 μg/kg per min. After the animal had been allowed to recover for several minutes, propranolol (1 mg/kg) was infused, slowly. To maintain arterial pressure at control levels and prevent large changes in cardiac contractility during propranolol administration, 6–12 mEq of CaCl2 was administered. Additional propranolol (0.25 mg/kg) was administered hourly. After stabilization, the infusion protocols of isoproterenol and norepinephrine was repeated.

Data Analysis

Myocardial oxygen consumption was calculated according to the method of Rooke and Feigl (1982). The formula for this calculation is:

\[ \text{MVO}_2 = 7.20 \times 10^{-4} (\text{SBP} \times \text{HR}) + 1.43 \]

where SBP = systolic blood pressure, and HR = heart rate.

Comparisons of myocardial blood flow, tissue norepinephrine, ratio of subendocardial to subepicardial blood flow, coronary resistance between innervated and sympathectomized regions, or vascular ring tension development were made with a paired t-test. Differences in myocardial blood flow, coronary resistance, hemodynamic variables, or the transmural ratio of blood flow in a given region during either the exercise protocol or supersensitivity protocol were tested with an analysis of variance. Specific intergroup differences were tested using the General Linear Model procedure of the SAS Institute which utilized the Ryan-Einot-Gabriel-Welsch multiple comparison test. Data comparing percent changes in blood flow velocity with percent changes in myocardial perfusion were analyzed with a least squares regression. A probability value of 0.05 or less was accepted as statistical significance. All values reported in this manuscript are mean ± SEM.

Results

Sensitivity of the Microsphere Methods

Mean arterial pressure and heart rate in this group under control conditions (before prostaglandin infusion) were 111 ± 5 mmHg and 159 ± 10 beats/min. Prostaglandin E1 infusion into the circumflex artery with doses from 0.05 to 0.20 μg/kg per min did not significantly change arterial pressure, 114 ± 4 mmHg, or heart rate, 154 ± 9 beats/min, from control. Figure 1 shows the percentage change in blood flow velocity detected with the Doppler on the abscissa and percent change in myocardial perfusion detected with microspheres on the ordinate. The correlation coefficient (r) is 0.88 (p < 0.05), and the slope is not different from the line of identity (slope = 0.96).

We also analyzed these data by comparing the average changes in Doppler measurements and microsphere measurements over ranges of 0–5%; the average changes in the microsphere and Doppler measurements were 3.0 ± 0.4% and 3.8 ± 1.7%, respectively. In the 5–10% range, the changes in the microsphere and Doppler measurements were 6.0 ± 1.1% and 9.5 ± 0.9%, respectively. Both of these
changes in the 5–10% range were significantly greater than zero ($P < 0.05$). In neither range were the differences in percent change in blood flow measured by the Doppler significantly different from that measured by microspheres.

**Exercise Protocol**

**Hemodynamics (Table 1)**

During exercise, heart rate, mean arterial pressure, left ventricular systolic pressure, and $dP/dt$ (% change from control) were significantly increased from control ($P < 0.05$). In five dogs, the basal value for $dP/dt$ averaged $3075 ± 420$ mm Hg/sec.

$\beta$-Adrenergic blockade during exercise significantly reduced mean arterial pressure, heart rate, and $dP/dt$ from that during exercise without adrenergic blockade ($P < 0.05$). Heart rate was still elevated from control during exercise and $\beta$-adrenergic blockade ($P < 0.05$). Left ventricular $dP/dt$ was not different from control.

During exercise and combined $\alpha$- and $\beta$-adrenergic blockade, heart rate was significantly greater than control ($P < 0.05$), although less than during exercise without pharmacological adrenergic blockade ($P < 0.05$). Mean arterial pressure during this intervention was less than that during control ($P < 0.05$) or during exercise ($P < 0.05$). During combined $\alpha$- and $\beta$-adrenergic blockade, left ventricular systolic pressure and $dP/dt$ were significantly less than these variables during exercise ($P < 0.05$), but were not changed from control.

Calculated myocardial oxygen consumption increased significantly from that of the controls during exercise ($P < 0.05$). During either adrenergic blockade, calculated myocardial oxygen consumption was greater than that of the control period ($P < 0.05$), but less than that during exercise alone ($P < 0.05$).

**Myocardial Blood Flow and Coronary Resistance (Table 2; and Fig. 2)**

During exercise, myocardial blood flow to the subepicardium and subendocardium increased significantly from control in both the innervated ($P < 0.05$) and sympathectomized ($P < 0.05$) regions.

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**Table 1**

<table>
<thead>
<tr>
<th>Hemodynamics during Control, Exercise, and Pharmacological Adrenergic Blockades during Exercise</th>
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<tbody>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
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<tr>
<td>Mean arterial pressure (mm Hg)</td>
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<tr>
<td>Left ventricular systolic pressure (mm Hg)</td>
</tr>
<tr>
<td>LV $dP/dt$ (% of control value)</td>
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<tr>
<td>MVO$_2$ index (ml/min per 100 g)</td>
</tr>
</tbody>
</table>

Data are mean ± SEM.

* $P < 0.05$ vs. control.
† $P < 0.05$ exercise + adrenergic blockade vs. exercise.
‡ Rooke-Feigl index.
TABLE 2

Myocardial Blood Flow to Subepicardial and Subendocardial Regions, Transmural Distribution of Blood Flow, and Transmural Coronary Resistance in the Sympathectomized and Innervated Regions*

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Exercise</th>
<th>Exercise + β-blockade</th>
<th>Exercise + (α + β)-blockade</th>
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<tr>
<td>Subendocardial blood</td>
<td></td>
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<tr>
<td>flow (ml/min per 100 g)</td>
<td></td>
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<tr>
<td>Innervated</td>
<td>131 ± 13</td>
<td>199 ± 21†</td>
<td>160 ± 38‡</td>
<td>157 ± 20‡</td>
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<tr>
<td>Sympathectomized</td>
<td>124 ± 13</td>
<td>185 ± 24†</td>
<td>145 ± 28‡</td>
<td>149 ± 23‡</td>
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<tr>
<td>Subepicardial blood</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>flow (ml/min per 100 g)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Innervated</td>
<td>106 ± 8</td>
<td>163 ± 12†</td>
<td>116 ± 18‡</td>
<td>124 ± 20‡</td>
</tr>
<tr>
<td>Sympathectomized</td>
<td>96 ± 10</td>
<td>158 ± 13†</td>
<td>110 ± 17‡</td>
<td>116 ± 15‡</td>
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<td>Subendocardial:subepicardial</td>
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<tr>
<td>Innervated</td>
<td>1.25 ± 0.05</td>
<td>1.24 ± 0.04</td>
<td>1.43 ± 0.14</td>
<td>1.37 ± 0.08</td>
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<tr>
<td>Sympathectomized</td>
<td>1.32 ± 0.06</td>
<td>1.22 ± 0.08</td>
<td>1.36 ± 0.08</td>
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<td>Transmural resistance</td>
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<td>(mm Hg × min × 100 g/ml)</td>
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<tr>
<td>Innervated</td>
<td>0.82 ± 0.05</td>
<td>0.65 ± 0.06†</td>
<td>0.90 ± 0.17‡</td>
<td>0.65 ± 0.07†‡</td>
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<tr>
<td>Sympathectomized</td>
<td>0.85 ± 0.06</td>
<td>0.65 ± 0.06†</td>
<td>0.89 ± 0.16‡</td>
<td>0.68 ± 0.07‡§</td>
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</table>

* During control, exercise, and pharmacological adrenergic blockade during exercise. n = 7. Data are mean ± SEM.
† P < 0.05 vs. control.
‡ P < 0.05 exercise + adrenergic blockade vs. exercise.
§ P < 0.05 exercise + β-blockade vs. exercise + (α + β)-blockade. There were no differences between the innervated and sympathectomized regions.

Subepicardial and subendocardial perfusion during exercise with β-adrenergic blockade and with combined α- and β-adrenergic blockade were decreased in both regions from that during exercise alone (P < 0.05), and was not changed from that of the control. There was no difference between perfusion to the innervated and sympathectomized regions during any of the experimental periods.

FIGURE 2. A plot of calculated myocardial oxygen consumption (MV02) vs. coronary resistance is shown for exercise, exercise with β-adrenergic blockade, and exercise with combined α- and β-adrenergic blockade. The horizontal standard error bars are for resistance. Inn = innervated region; Sx = sympathectomized region.
Coronary resistance decreased significantly during exercise and exercise with combined α- and β-adrenergic blockade from that of the control in both the innervated (P < 0.05) and sympathectomized regions (P < 0.05). Furthermore, coronary resistance during exercise alone and during exercise with combined α- and β-adrenergic blockade in both regions was less than that during exercise with β-adrenergic blockade (P < 0.05). Coronary resistance during exercise alone and during exercise with combined α- and β-adrenergic blockade in both regions was less than that during exercise with β-adrenergic blockade (P < 0.05). Coronary resistance levels in the innervated and sympathectomized regions were not significantly different during any of the experimental periods.

There were no differences in the subendocardial:subepicardial ratio of perfusion among any of the experimental periods or between the innervated and sympathectomized regions.

**Plasma and Tissue Catecholamines**

Before exercise, the plasma norepinephrine and epinephrine concentrations were 0.63 ± 0.10 and 0.57 ± 0.15 ng/ml (n = 5). During exercise, the plasma concentrations of norepinephrine and epinephrine increased to (P < 0.05), 1.51 ± 0.17 and 1.62 ± 0.15 ng/ml, respectively. Combined α- and β-adrenergic blockade increased plasma norepinephrine and epinephrine concentrations to 10.32 ± 1.56 and 3.10 ± 0.57 ng/ml, respectively. Tissue concentration of norepinephrine in the innervated region from six animals averaged 722 ± 100 ng/g and was significantly greater than that in the sympathectomized region 27 ± 8 ng/g (P < 0.05).

**Assessment of α-Adrenergic Supersensitivity: Conscious Animals**

Results from animals during norepinephrine infusion in the presence of β-adrenergic blockade are shown in Table 3. Neither of the two lower doses of norepinephrine (0.1 or 0.5 µg/kg per min) produced significant effects on the hemodynamic variables in the experimental group; the higher doses of

| Table 3: Hemodynamics, Myocardial Blood Flow, and Coronary Resistance in Different Layers of the Left Ventricle during β-Adrenergic Blockade with Norepinephrine |
|-------------------------------------------------|-----------|-----------|-----------|-----------|
| Norepinephrine (µg/kg per min)                   | Control   | 0.1       | 0.5       | 1.0       |
| Heart rate (beats/min)                           | 83 ± 6    | 86 ± 8    | 75 ± 4    | 69 ± 4*   |
| Mean pressure (mm Hg)                            | 87 ± 4    | 83 ± 5    | 87 ± 5    | 100 ± 6*  |
| Endo blood flow (ml/min per 100 g)               | 99 ± 9    | 101 ± 20  | 95 ± 10   | 86 ± 6    |
| Innervated                                       |           |           |           |           |
| Sympathectomized                                 | 97 ± 10   | 94 ± 26   | 93 ± 6    | 83 ± 9    |
| Mid blood flow (ml/min per 100 g)                | 93 ± 9    | 99 ± 23   | 97 ± 11   | 81 ± 8    |
| Innervated                                       |           |           |           |           |
| Sympathectomized                                 | 94 ± 11   | 99 ± 24   | 92 ± 7    | 71 ± 6    |
| Epi blood flow (ml/min per 100 g)                | 75 ± 8    | 74 ± 20   | 74 ± 9    | 61 ± 6    |
| Innervated                                       |           |           |           |           |
| Sympathectomized                                 | 72 ± 9    | 68 ± 18   | 66 ± 5    | 58 ± 6    |
| Endo resistance (mm Hg × min × 100 g/ml)         | 0.93 ± 0.10 | 0.96 ± 0.16 | 0.99 ± 0.16 | 1.15 ± 0.13 |
| Innervated                                       |           |           |           |           |
| Sympathectomized                                 | 0.94 ± 0.11 | 1.09 ± 0.17 | 0.96 ± 0.09 | 1.28 ± 0.14 |
| Mid resistance (mm Hg × min × 100 g/ml)          | 0.96 ± 0.12 | 0.98 ± 0.16 | 0.98 ± 0.16 | 1.27 ± 0.20 |
| Innervated                                       |           |           |           |           |
| Sympathectomized                                 | 1.02 ± 0.13 | 0.99 ± 0.15 | 0.99 ± 0.12 | 1.22 ± 0.12 |
| Epi resistance (mm Hg × min × 100 g/ml)          | 1.26 ± 0.13 | 1.38 ± 0.22 | 1.28 ± 0.21 | 1.64 ± 0.21 |
| Innervated                                       |           |           |           |           |
| Sympathectomized                                 | 1.38 ± 0.19 | 1.46 ± 0.21 | 1.37 ± 0.16 | 1.78 ± 0.16 |

Data are mean ± SEM. Mid = mid-myocardium, Epi = subepicardium, Endo = subendocardium.

* P < 0.05 vs. control. There were no differences between the innervated and sympathectomized regions.
TABLE 4

Hemodynamics, Myocardial Blood Flow, and Coronary Resistance in Different Layers of the Left Ventricle during Norepinephrine (NE) Infusion and Combined $\beta$-Adrenergic and Ganglionic Blockade

<table>
<thead>
<tr>
<th>Norepinephrine (µg/kg per min)</th>
<th>Control</th>
<th>0.1</th>
<th>0.5</th>
<th>1.0</th>
<th>2.5</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Systolic pressure (mm Hg)</strong></td>
<td>90 ± 8</td>
<td>102 ± 6*</td>
<td>131 ± 14*</td>
<td>159 ± 18*</td>
<td>209 ± 25*</td>
</tr>
<tr>
<td><strong>Diastolic pressure (mm Hg)</strong></td>
<td>65 ± 5</td>
<td>76 ± 5*</td>
<td>108 ± 13*</td>
<td>129 ± 16*</td>
<td>171 ± 20*</td>
</tr>
<tr>
<td><strong>Mean pressure (mm Hg)</strong></td>
<td>73 ± 6</td>
<td>86 ± 5*</td>
<td>117 ± 13*</td>
<td>139 ± 16*</td>
<td>184 ± 22*</td>
</tr>
<tr>
<td><strong>Endo blood flow (ml/min per 100 g)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Innervated</td>
<td>166 ± 52</td>
<td>165 ± 33</td>
<td>166 ± 9</td>
<td>172 ± 8</td>
<td>192 ± 10*</td>
</tr>
<tr>
<td>Sympathectomized</td>
<td>185 ± 51</td>
<td>188 ± 28</td>
<td>207 ± 41</td>
<td>202 ± 35</td>
<td>217 ± 30*</td>
</tr>
<tr>
<td><strong>Mid blood flow (ml/min per 100 g)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Innervated</td>
<td>159 ± 52</td>
<td>161 ± 28</td>
<td>177 ± 14</td>
<td>159 ± 3</td>
<td>189 ± 18</td>
</tr>
<tr>
<td>Sympathectomized</td>
<td>167 ± 44</td>
<td>176 ± 26</td>
<td>192 ± 30</td>
<td>168 ± 32</td>
<td>186 ± 36</td>
</tr>
<tr>
<td><strong>Epi blood flow (ml/min per 100 g)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Innervated</td>
<td>132 ± 46</td>
<td>124 ± 26</td>
<td>130 ± 11</td>
<td>134 ± 19</td>
<td>162 ± 20*</td>
</tr>
<tr>
<td>Sympathectomized</td>
<td>131 ± 33</td>
<td>136 ± 24</td>
<td>144 ± 27</td>
<td>137 ± 22</td>
<td>173 ± 18*</td>
</tr>
<tr>
<td><strong>Endo resistance (mm Hg × min × 100 g/ml)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Innervated</td>
<td>0.44 ± 0.13</td>
<td>0.52 ± 0.10</td>
<td>0.70 ± 0.08*</td>
<td>0.81 ± 0.07*</td>
<td>0.97 ± 0.08*</td>
</tr>
<tr>
<td>Sympathectomized</td>
<td>0.39 ± 0.12</td>
<td>0.46 ± 0.09</td>
<td>0.57 ± 0.14*</td>
<td>0.69 ± 0.22*</td>
<td>0.85 ± 0.19*</td>
</tr>
<tr>
<td><strong>Mid resistance (mm Hg × min × 100 g/ml)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Innervated</td>
<td>0.46 ± 0.13</td>
<td>0.53 ± 0.11</td>
<td>0.66 ± 0.05*</td>
<td>0.87 ± 0.04*</td>
<td>0.97 ± 0.09*</td>
</tr>
<tr>
<td>Sympathectomized</td>
<td>0.44 ± 0.14</td>
<td>0.49 ± 0.10</td>
<td>0.61 ± 0.10*</td>
<td>0.82 ± 0.15*</td>
<td>0.99 ± 0.16*</td>
</tr>
<tr>
<td><strong>Epi resistance (mm Hg × min × 100 g/ml)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Innervated</td>
<td>0.55 ± 0.13</td>
<td>0.69 ± 0.12</td>
<td>0.90 ± 0.06*</td>
<td>0.96 ± 0.16*</td>
<td>1.14 ± 0.12*</td>
</tr>
<tr>
<td>Sympathectomized</td>
<td>0.56 ± 0.10</td>
<td>0.63 ± 0.11</td>
<td>0.81 ± 0.15*</td>
<td>1.01 ± 0.18*</td>
<td>1.08 ± 0.09*</td>
</tr>
</tbody>
</table>

Data are mean ± SEM. Mid = mid-myocardium, Epi = subepicardium, Endo = subendocardium.

* $P < 0.05$ vs. control, $n = 4$.

norepinephrine (1.0 and 2.5 µg/kg per min) significantly increased systolic pressure in the animals ($P < 0.05$). Heart rate during administration of the higher doses of norepinephrine in animals was significantly less than that of the control ($P < 0.05$). The two lower doses of norepinephrine (0.1 and 0.5 µg/kg per min) did not produce any discernible changes in resistance from control in any of the layers in either the sympathectomized or innervated regions. Coronary resistance significantly increased in both the innervated and sympathectomized regions during norepinephrine infusion of 1.0 and 2.5 µg/kg per min ($P < 0.05$); however, myocardial perfusion was not significantly changed. Coronary resistance or myocardial perfusion in the innervated or sympathectomized regions during infusion of norepinephrine were not statistically different.

The results from four dogs with ganglionic and $\beta$-adrenergic blockade and pacing are shown in Table 4. In these animals with ganglionic blockade and pacing, arterial pressure increased ($P < 0.05$) during each of the different infusion rates of norepinephrine. Coronary resistance was increased from control at the higher infusion rates of norepinephrine ($P < 0.05$). Myocardial perfusion, however, increased only at the higher infusion rate of norepinephrine. During norepinephrine infusion, myocardial perfusion or coronary resistance were not different in the innervated and sympathectomized regions.

Assessment of $\alpha$-Adrenergic Supersensitivity: Isolated Blood Vessels

Figure 3 (top panel) illustrates the norepinephrine dose-response relationship in isolated vascular ring preparations from the innervated and sympathectomized regions. Figure 3 (bottom panel) shows the phenylephrine dose-response relationship from epicardial arteries in the two regions. There were no differences in the responses of the innervated and sympathectomized vessels at any dose of either
phehylephrine or norepinephrine. The calculated ED_{50} values for norepinephrine were \(4 \times 10^{-7} \text{M}\) and \(4 \times 10^{-7} \text{M}\) in the innervated and sympathectomized regions, respectively. The ED_{50} values for phelylephrine were \(1 \times 10^{-6} \text{M}\) and \(2 \times 10^{-6} \text{M}\) in the innervated and sympathectomized regions, respectively, and were not significantly different.

The responses to tyramine were markedly different in the vessels from the two regions. Tyramine (\(10^{-4} \text{M}\)) produced significant constriction in the ring preparations from the innervated region, 28.9 ± 7.8% of the maximum KCl response. In contrast, the response to tyramine was reduced to only 0.8 ± 0.6% in the sympathectomized vessels (\(P < 0.05\)). This value was not statistically different from zero. Moreover, only two of the nine sympathectomized vessels responded to tyramine: 1.6% and 5% of the KCl response, respectively.

**Assessment of \(\beta\)-Adrenergic Supersensitivity: Anesthetized Animals**

Results from this series are presented in Table 5, and representative phasic recording of hemodynamics, dL/dt, and segment shortening are shown in Figure 4. Isoproterenol produced a dose-related increase in heart rate (\(P < 0.05\)) and a dose-dependent decrease in mean arterial pressure (\(P < 0.05\)). Norepinephrine increased both arterial pressure and heart rate (\(P < 0.05\)). Propranolol markedly blunted the heart rate response. Only during the highest infusion rate of each agonist were significant increases in heart rate observed (\(P < 0.05\)). Segment shortening in the innervated and sympathectomized regions were not different from one another during infusion of isoproterenol; however, at the higher doses of isoproterenol, dL/dt was greater in the innervated than in the sympathectomized region. At the higher doses of norepinephrine, dL/dt and percent segment shortening were greater in the sympathetically denervated region than in the innervated region.

Propranolol blocked the contractile effects of both \(\beta\)-adrenergic agonists equally, except that, at the highest rates of norepinephrine infusion, the dL/dt was greater in the sympathectomized region than in the innervated region.

**Discussion**

The major findings of this study are: (1) direct sympathetic neural mechanisms via activation of the coronary sympathetic innervation do not produce coronary constriction during submaximal exercise; (2) circulating catecholamines are primarily responsible for \(\alpha\)-adrenergic tone during submaximal exercise; and (3) denervation supersensitivity occurs in the myocardium, but not in the coronary vasculature. These conclusions were based on the observations that there were no differences in either myocardial perfusion or coronary resistance between the sympathetically denervated and innervated regions of the left ventricle during exercise, but coronary resistance during exercise was less during combined \(\alpha\)- and \(\beta\)-blockade than during \(\beta\)-blockade alone. The vasculature in the sympathetically denervated region was not supersensitive to \(\alpha\)-adrenergic receptor stimulation by catecholamines, and the microsphere methodology was able to detect a 5–10% difference in blood flow. Thus, our failure to detect that coronary sympathetic innervation played a significant role in producing vasoconstriction cannot be explained by supersensitivity of the vasculature in the sympathetically denervated region to circulating catecholamines or to insensitivity of the microsphere methodology.

Our conclusions and interpretations depend on several important factors and observations. These include (1) efficacy of the regional sympathectomy model (2) sensitivity of the microsphere methodology, (3) question of adrenergic supersensitivity of the vasculature and myocardium in the denervated region to circulating catecholamines, (4) nonspecific...
neuronal uptake of labeled norepinephrine is normal. During stellate nerve stimulation, the extraction of oxygen and lactate is augmented in the innervated region, but is not changed in the sympathectomized region. Also, during stellate nerve stimulation, there is norepinephrine overflow from the innervated region, but, under the same conditions, the sympathectomized region extracts norepinephrine. These data provide substantial evidence that the sympathetic innervation to the posterior wall of the left ventricle has been ablated and that the innervation to the anterior left ventricle is intact. We also have shown that the tissue damage produced by phenol is minor, since tissue concentrations of high energy phosphates and lactate are similar in the phenol-treated and control regions (Chilian et al., 1981). Also, Randall et al. (1968) reported that the coagulation necrosis produced by phenol is very superficial, being confined to 0.25 mm of the epicardial surface.

Our results from the isolated vascular ring studies...
also show that innervation to the anterior vasculature is intact (significant response to tyramine), whereas the vessels in the phenol-treated vasculature are sympathectomized (insignificant or zero response to tyramine).

Application of epicardial phenol to the myocardium reportedly does not interrupt efferent vagal influences on effective refractory periods (Martins and Zipes, 1980). However, application of phenol to the atrioventricular groove between the origin of the right coronary artery and the posterior descending artery eliminates vagal influences on effective refractory periods in the left ventricular free wall (Takahashi et al., 1985). These results, along with those of Kent (1974) and Denn and Stone (1975), have promulgated the concept that the efferent parasympathetic fibers to the left ventricle penetrate the myocardium within a few centimeters of the atrioventricular groove and innervate the left ventricular free wall via a subendocardial or intramural nerve plexus. We do not know precisely whether or not the parasympathetic innervation to the coronary vasculature has been affected by the phenol treatment, since part of the phenol application is to the posterior atrioventricular groove. Even if the vagal innervation was partially interrupted in the sympathectomized region because of phenol treatment, this would not change our conclusions, since, during exercise, there is withdrawal of vagal tone (Vatner and Pagani, 1976). Thus, possible vagal cholinergic vasodilator influences would be minimal during exercise.

Another potential problem of the regional sympathectomy model would be interruption of afferent nerves coursing in the epicardium and posterior atrioventricular groove. Barber et al. (1984) have found that sympathetic afferent fibers course in the subepicardium, whereas parasympathetic fibers course in the subendocardium, or mid-myocardium and ascend to the subepicardium as they approach the atrioventricular groove. We do not think possible deafferentation of such cardiac nerves would alter our interpretations, because the hemodynamic changes we observed during exercise were indicative of a classical autonomic response, i.e., sympathetic activation and parasympathetic withdrawal. Thus, the general autonomic neural response appeared to be unaffected by the disruption of sympathetic and parasympathetic afferents from the posterior wall of the left ventricle.

**Sensitivity of the Microsphere Methodology**

A possible reason for not finding a difference in blood flow between the innervated and sympathectomized regions could be the insensitivity of microsphere methodology. Our results show that when we utilized a paired experimental design in which a region of the left ventricle served as the experimental region and another region served as the control, microspheres detected a small difference (5–10%) in perfusion. This information is supported by a recent study (Dole et al., 1981) in which a comparable error of only 8–10% is inherent in microsphere-measured perfusion.
α-Adrenergic and β-Adrenergic Supersensitivity

Another reason that we did not observe a difference in perfusion to the innervated and sympathectomized regions would be supersensitivity of α-adrenergic receptors in the sympathectomized region to circulating catecholamines. However, myocardial perfusion and coronary resistance, to the innervated and sympathectomized regions were not different during norepinephrine infusion (Table 3 and Table 4). These observations are also supported by the results from the in vitro isolated vascular ring studies, in which sympathectomized blood vessels were not supersensitive to the α₁-adrenergic agonist, phenylephrine, or the α₁- and α₂-adrenergic agonist, norepinephrine. Thus, in our experimental model, the coronary vasculature in the sympathectomized region was not supersensitive to α-adrenergic receptor agonists, and we conclude that supersensitivity of α-adrenergic receptors in the sympathectomized region cannot explain our results. Another important result shown in Table 3 and Table 4 is that the coronary vasculature in both the innervated and sympathectomized regions was responsive to α-adrenergic constrictor stimuli because coronary resistance increased substantially during norepinephrine infusion.

The present results also indicate that there was no supersensitivity of β-adrenergic receptors to isoproterenol in the sympathectomized region. This was based on our observations that isoproterenol did not increase regional contractility (% shortening, dL/dt) more in the sympathectomized region than in the innervated region (Table 5). Since isoproterenol is not transported into sympathetic nerves, any supersensitivity to this β-adrenergic agonist would be mediated exclusively by a postjunctional mechanism (Dempsey and Cooper, 1968; Trendelenburg, 1976). Norepinephrine, however, increased regional contractility (% shortening, dL/dt) more in the sympathectomized region (P < 0.05) (Table 5). This would indicate that the absence of sympathetic neuronal uptake is responsible for the supersensitivity. This belief is based on the observation that the primary disposition mechanism of norepinephrine in the myocardium is via active uptake into the sympathetic nerves, and blockade of such uptake results in acute β-adrenergic supersensitivity (Trendelenburg, 1976). Our present results are also supported by several reports in which β-adrenergic supersensitivity in the surgically or chemically denervated heart was mediated by the absence of norepinephrine uptake into sympathetic nerves (Dempsey and Cooper, 1968; Nadeau, 1971). These studies generally reported that the denervated heart is supersensitive to norepinephrine, but not isoproterenol.

In contrast, several investigators have found an increase in the sensitivity of the myocardium to isoproterenol, which, presumably, is due to an increased number of β-adrenergic receptors. Tenner et al. (1982) found an increased number of β-receptors in the left ventricular myocardium following reserpine treatment, which resulted in an increased sensitivity of papillary muscles to isoproterenol. Bannister et al. (1981) and Korczyñ et al. (1982) found denervation sensitivity of heart rate responses to isoproterenol. In addition, Vatner et al. (1985) reported supersensitivity of heart rate and contractility responses to isoproterenol in conscious dogs. These latter investigators also reported that there was an increased number of β-adrenergic receptors in the surgically denervated heart which correlated well with the amount of chronotropic and inotropic supersensitivity to isoproterenol. However, the up-regulation of β-adrenergic receptors accounted for only a minor amount of denervation supersensitivity to norepinephrine. Thus, these authors concluded that the major mechanism of denervation supersensitivity to norepinephrine was the lack of reuptake of norepinephrine in the denervated heart. It is possible that, in our experimental model, there is an interaction between the innervated and denervated regions during isoproterenol or norepinephrine infusion, which could possibly mask the full effect of denervation supersensitivity. If so, this could account for the rather modest β-adrenergic supersensitivity we observed in our studies.

Since we did not observe coronary vascular α-adrenergic supersensitivity despite the lack of neuronal reuptake of norepinephrine (Fig. 3), we can only speculate that the coronary sympathetic nerves are not primarily responsible for catecholamine disposition in the coronary blood vessels. This hypothesis is supported by Kalsner and Nickerson (1969), who found epinephrine and norepinephrine reuptake by sympathetic nerves in vascular tissue to be far less important than extraneuronal enzymatic pathways (catechol-O-methyl transferase, monoamine oxidase) for the inactivation of these catecholamines. Within this context, studies of sympathetic innervation of the coronary vasculature demonstrate that the nerves penetrate only to the media-adventitial junction, and specialized neuromuscular junctions are not observed (Lever et al., 1965; Dolezel et al., 1978). Thus, the sympathetic nerves may not be primarily responsible for the metabolism (or reuptake) of norepinephrine from coronary vascular smooth muscle because of their distant anatomical location; rather, norepinephrine may be enzymatically inactivated directly by vascular smooth muscle.

Nonspecific Drug Effects

Nonspecific drug effects should also be considered in our data interpretations. Our experimental models and those employed by Murray and Vatner (1979), Heyndrickx et al. (1982), and Gwirtz and Stone (1981) entail measurement of blood flow before and after α-adrenergic blockade with phentolamine. Since the α-adrenergic receptor antagonist, phentolamine, is a muscarinic and histaminergic agonist
(Weiner, 1980), and a direct vasodilator (Taylor, 1965), it could be potentially difficult to discern if coronary vasodilation produced by the drug was due to α-adrenergic antagonism, direct vasodilation, or muscarinic and/or histaminergic receptor stimulation. The way we reconcile these potentially confounding effects of phentolamine is that, in a previous study under resting conditions, phentolamine did not produce significant coronary dilation (Chilian et al., 1981). Thus, its nonspecific effects on the coronary system appear to be minimal.

Phentolamine also can block presynaptic α-adrenergic receptors (Langer, 1977). This can lead to facilitated norepinephrine release which can stimulate myocardial metabolism that indirectly decreases coronary resistance. In our experimental protocol, propranolol administration prior to phentolamine would prevent this stimulation of myocardial metabolism, and such a facilitation or norepinephrine release should not affect our results.

**Calculations of Myocardial Oxygen Consumption**

Our conclusions about the role of circulating catecholamines in the control of coronary vascular resistance during exercise are based on the relationship between coronary resistance and calculated myocardial oxygen consumption. Our interpretations and calculations of myocardial oxygen consumption, according to the method of Rook and Feigl (1982), assume that myocardial oxygen demands were similar in both innervated and denervated regions during exercise. Although oxygen consumption in the two regions may differ during exercise because of possible differences in contractility, β-adrenergic blockade with propranolol should normalize the metabolic consequences of sympathetic activation in the two regions. Our results from the anesthetized studies support this contention. Propranolol blocked the β-receptor-mediated effects at all but the highest infusion rates of isoproterenol and norepinephrine. An infusion rate of this magnitude would certainly result in an unphysiological plasma concentration of β-adrenergic agonists. Based on the recent study by Young et al. (1985), we calculated plasma norepinephrine during an infusion of 2.5 μg/kg per min as 50 ng/ml. This concentration was 10-fold greater than the plasma level of catecholamines during exercise; thus, metabolic demands in the innervated and sympathetomized regions should be equivalent during exercise, after β-adrenergic blockade with propranolol.

Figure 2 further illustrates these points concerning the relationship between coronary resistance and myocardial oxygen consumption during exercise in our study. If the control of coronary resistance was mediated exclusively by vasoactive metabolites, coronary resistance should be inversely related to metabolic rate. This, however, was not our experimental observation, since calculated myocardial oxygen consumption was greater during exercise than during combined α- and β-blockade, yet coronary resistance was the same during these periods. This directly suggests the presence of α-adrenergic constrictor tone. Another way of analyzing these data is to compare coronary resistance during exercise with β-adrenergic blockade and with combined α- and β-adrenergic blockade. For the same myocardial oxygen consumption (15.1 vs. 14.1 ml/min per 100 g, P < 0.3), coronary resistance was significantly less during combined adrenergic blockade than during β-adrenergic blockade (P < 0.05). Since the resistance values in both the innervated and sympathetomized regions were equivalent during the experimental interventions, and changed to a similar extent during combined α- and β-blockade, we concluded that the α-adrenergic tone in the coronary circulation during exercise was produced by circulating catecholamines.

**Implications of the Present Study**

Several laboratories have reported that during exercise there is coronary vasoconstriction mediated by α-adrenergic receptors (Murray and Vatner, 1979; Schwartz and Stone, 1979; Heyndrickx et al., 1980, 1982; Gwirtz and Stone, 1981). Our results not only support their primary conclusions, but also add the important conclusion that circulating catecholamines during certain experimental conditions, such as submaximal exercise, can be responsible for coronary α-adrenergic tone. Peronnet et al. (1981) have reported that during submaximal exercise in the canine, coronary sinus levels of catecholamines are less than arterial levels. The interpretation of this result was that sympathetic activity to the heart was less than that to the adrenomedullary region under these experimental conditions. Also, during their submaximal exercise protocol, plasma catecholamines concentrations were similar to those in our study. Under conditions of severe short-duration exercise, coronary sinus catecholamines exceeded arterial levels, indicating greater activation of sympathetic nerves to the heart than to the adrenomedullary region. Thus, α-adrenergic tone in the coronary system during exercise may be due to circulating catecholamines and/or coronary sympathetic nerves, depending on the level and duration of the exercise period which dictates the degree of activation of sympathetic nerves to the heart and to the periphery.

An important implication of our results is that the plasma concentrations of catecholamines during submaximal exercise are sufficient to produce coronary vasoconstriction, and that neurally mediated sympathetic tone in the coronary system is insignificant. However, Young et al. (1985) reported that concentrations of circulating catecholamines do not accurately predict the inotropic state of the myocardium. They concluded that circulating catecholamines during exercise are insufficient to maintain
ventricular inotropic activation, and that cardiac sympathetic nerves must be activated in order to maintain the increases in myocardial contractility. To reconcile our data of humoral vs. neuronal adrenoceptor activation with those of Young et al. (1985), we have postulated three mechanisms which may cause differential cardiac vs. coronary neuronal effects. First, during submaximal exercise, there may be differential activation of cardiac sympathetic nerves and coronary sympathetic nerves. This idea is appealing, because, recently, Segal et al. (1984) have suggested a central neural area which, when activated with picrotoxin, produces selective coronary vasoconstriction before the onset of hemodynamic alterations; thus, cardiac and coronary sympathetic efferents may stem from different brain centers. Second, the release of norepinephrine from coronary sympathetic nerves may be inhibited to a greater extent during exercise than that from cardiac nerves by norepinephrine, especially with high circulating levels as in the present study. Our results support this latter hypothesis, because circulation levels of catecholamines were markedly increased during phentolamine administration. Phentolamine, an $\alpha_1$- and $\alpha_2$-antagonist, will augment norepinephrine release from sympathetic nerves by blockade of the inhibitor presynaptic $\alpha_2$-adrenergic receptor (Langer, 1977). Heyndrickx et al. (1984) have recently reported that, under physiological conditions (treadmill exercise), norepinephrine release from sympathetic nerves in the heart during exercise was actually inhibited by $\alpha_2$-adrenergic activation on the sympathetic nerves. Blockade of this negative feedback mechanism augmented norepinephrine release into the coronary sinus by 100%. Third, Williams et al. (1985) have found that adenosine inhibited norepinephrine overflow in coronary veins in conscious dogs. Also, other putative coronary metabolic vaso- dilators (H$^+$ osmolarity, ATP, ADP, AMP, K$^+$) have been reported to inhibit the release of norepinephrine from sympathetic nerves (Vanhoutte et al., 1981). Thus, an increase of interstitial levels of a putative metabolic vasodilator(s) during exercise may inhibit release of norepinephrine from coronary sympathetic nerves. The relative importance of these inhibitory mechanisms in coronary blood vessels vs. cardiac muscle, however, is not known.

In conclusion, our study indicates that there is significant $\alpha$-adrenergic coronary constrictor tone during submaximal exercise in the dog. Our data strongly suggest that $\alpha$-adrenergic tone during submaximal exercise is due to the vasoconstrictor effects of circulating catecholamines. Our results also indicate that denervation supersensitivity occurs in the myocardium, but not in the coronary vasculature.

References


INDEX TERMS: Neural control • Sympathetic nerves • Coronary blood flow • Exercise • Supersensitivity • α-Adrenergic receptors • β-Adrenergic receptors
Adrenergic coronary tone during submaximal exercise in the dog is produced by circulating catecholamines. Evidence for adrenergic denervation supersensitivity in the myocardium but not in coronary vessels.

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