Adrenergic Coronary Vasoconstriction Helps Maintain Uniform Transmural Blood Flow Distribution During Exercise

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The hypothesis that α-adrenergic coronary vasoconstriction helps maintain a uniform transmural distribution of myocardial blood flow during exercise was tested in dogs. Carotid artery loops were surgically constructed and a splenectomy performed three weeks prior to study. On the day of study, the dog was anesthetized briefly (fentanyl and nitrous oxide) for percutaneous catheterization, and α-receptors in one myocardial region were blocked with phenoxybenzamine (0.25 mg/kg) infused selectively into the left circumflex coronary artery. Recirculation of phenoxybenzamine was minimized by drainage of coronary sinus outflow during the infusion. After the dog recovered from the anesthesia, regional blood flow was measured at rest and during graded treadmill exercise with the microsphere technique calibrated by reference blood samples. Average transmural flow was limited by α-vasoconstriction and was less in the region where α-receptors were intact than in the region where they were blocked, as has been described by others. The ratio of inner layer myocardial blood flow to outer layer flow was better maintained in the region with α-receptors intact than in the region with α-receptors blocked when myocardial oxygen consumption was 150 μl/min/g or greater (p<0.001). Even though average transmural flow was limited by α-receptor activation, inner layer myocardial blood flow was greater in the region with α-receptors intact than in the region with α-receptors blocked when myocardial oxygen consumption was 500 μl/min/g or more (p<0.05). In conclusion, adrenergic coronary vasoconstriction mediated by α-receptors helps to maintain a uniform transmural distribution of myocardial blood flow during exercise in spite of limiting average transmural flow.

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When the coronary vessels are dilated pharmacologically with adenosine and the heart is electrically paced from 100 to 250 beats/min, the ratio of inner myocardial blood flow to outer myocardial blood flow (inner/outer flow ratio) falls dramatically from 1.0 to 0.4." This is consistent with the concept that myocardial compression impedes blood flow to the inner layers of the myocardium more than it does to the outer layers. Because a larger proportion of each cardiac cycle is spent in systole as heart rate increases, tachycardia tends to exaggerate the effects of this transmural gradient of compression.

In contrast to the situation with adenosine and pacing, when coronary vasodilation and tachycardia develop in response to exercise, the transmural distribution of coronary blood flow changes little, and the inner/outer flow ratio remains at or above 1.0.14 This suggests that some component of the physiological response to exercise helps maintain the uniform transmural distribution of flow.

Several laboratories have demonstrated that adrenergic coronary vasoconstriction mediated by α-receptors is part of the physiological response to exercise. The resulting restriction of functional hyperemia seems paradoxical because the metabolic demand for myocardial blood flow is greatly elevated during exercise. However, the adrenergic vasoconstriction during exercise might improve the match between myocardial metabolism and blood flow across the ventricular wall by altering the transmural distribution of blood flow.

Radioactive microspheres were used to make paired comparisons of flow between α-intact and α-blocked regions of the left ventricle during graded treadmill exercise. Coronary blood flow and its transmural distribution were analyzed as functions of myocardial oxygen consumption, an index of exercise intensity directly relevant to the myocardium. The transmural distribution of blood flow was more uniform, and flow in the inner layer of the left ventricle was better maintained with α-receptors intact than with α-receptors blocked. This indicates that coronary vasoconstriction mediated by adrenergic α-receptors may be beneficial during exercise.

Materials and Methods

The general experimental strategy was to compare the transmural distribution of myocardial blood flow in α-receptor blocked and α-receptor intact regions of the same left ventricle during rest and graded treadmill exercise. The α-blocked region was selectively marked with radioactive microspheres injected together with the α-blocking agent so that this area could be identified postmortem when sections of the left ventricle were analyzed for radioactivity. Transmural coronary blood

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flow in both regions was determined with other radioactive microspheres injected into the left atrium (Figure 1).

Experimental Groups

Mongrel dogs, 14.5–26.8 kg, were selected on the bases of overall health, tractability, and enthusiasm for treadmill exercise. These were divided into three experimental groups. 1) α-blocked group (20 dogs). Phenoxybenzamine infused selectively into the left circumflex coronary artery blocked α-receptors in the region perfused by that vessel. 2) α- and β-blocked group (15 dogs). In addition to phenoxybenzamine selectively infused into the circumflex coronary artery, the dogs in this group also received intravenous propranolol. Because prejunctional α-receptors, involved in feedback inhibition of norepinephrine release, are blocked along with postjunctional α-receptors by phenoxybenzamine, norepinephrine release may be enhanced in the α-blocked region. The additional blockade of β-receptors prevents the increase in myocardial metabolism and the associated metabolic vasodilation likely to result from this enhanced release of norepinephrine and thereby helps to distinguish the direct effects of blocking postjunctional coronary α-receptors from the indirect prejunctional α-receptor-mediated effects (see “Discussion”). 3) Vehicle group (17 dogs). Instead of phenoxybenzamine, only the vehicle for phenoxybenzamine was infused into the left circumflex coronary artery. Differences between the myocardial regions, independent of the effects of α-blockade, were measured in this group.

Preliminary Training and Surgery

After each dog became familiar with the treadmill, preliminary sterile surgery was performed under anesthesia induced with sodium thiopental (Surital, 18 mg/kg i.v.) and maintained by spontaneous inhalation of halothane. The common carotid arteries were exteriorized in protective skin tubes to form arterial loops. The spleen was removed. Postoperative discomfort was controlled with morphine (0.25 mg/kg s.c.). At least three weeks were allowed for recovery from surgery and resumption of treadmill training before study. The training protocol was not designed for physical conditioning.

Experimental Preparation

On the day of study, the dog was anesthetized for about 2 hours with fentanyl citrate (0.25 mg/kg i.v. for induction, plus 1.0-mg supplements every 30 minutes or as needed) and nitrous oxide (80% in oxygen, ventilation with Harvard respirator pump, model 607). Prophylactic heparin sodium (100 U/kg s.c.) was given at this time, complementing the effect of aspirin (324 mg p.o.) given 12–18 hours earlier. End-expired CO₂ was monitored (Beckman LB-2 Medical Gas Analyzer) and maintained at about 5% by adjustments in ventilatory rate, and standard base excess in arterial blood was maintained above −4.0 meq/l with NaHCO₃ (8.4% solution i.v.). A catheter introducer sheath (USCI Hemaquet, F8) was inserted percutaneously into each carotid artery loop with the Seldinger technique, then the left circumflex region of the myocardium was selectively treated, and the dog was instrumented as described below. The skin and subcutaneous tissues of the neck were infiltrated with a long-acting local anesthetic (bupivicaine hydrochloride, 0.0625%), and general anesthesia was reversed with a combination of narcotic antagonists: naloxone hydrochloride (0.2 mg/kg i.v.), which was very rapid and short-acting, and naltrexone hydrochloride (0.1 mg/kg i.m.), which was slow and long-acting. During recovery, the dog was given water to drink and was allowed to roam about the laboratory at will. A supplement of naloxone (0.2 mg/kg i.v.) was given before exercise began, following 2½ hours of recovery.
Regional α-Receptor Blockade

Phenoxybenzamine hydrochloride (Dibenzyline, 50 mg/ml) was diluted to 4 mg/ml with normal saline and mixed with radionuclide-labeled microspheres (1 × 10^5 beads) for marking the distribution of phenoxybenzamine. This was suspended in 24 ml of arterial blood and infused at 11.5 ml/min (Harvard syringe pump, model 945) via a 7.5F Sones catheter (USCI 007561) passed down the right carotid artery and, under fluoroscopic guidance, into the left circumflex coronary artery (Figure 1A). The intracoronary dose of phenoxybenzamine was 0.25 mg/kg, which has been shown to effectively block adrenergic coronary vasoconstriction during a carotid sinus reflex or intracoronary norepinephrine infusion.14

Recirculation of phenoxybenzamine was minimized by draining coronary sinus effluent, during and for 1 minute after the selective coronary infusion, via a modified 14F Foley catheter with its tip in the coronary sinus. The Foley catheter was introduced via the right jugular vein under fluoroscopic guidance and sealed in the coronary sinus ostium with its balloon. The drained blood was discarded and the volume (about 200 ml) replaced with 10% dextran (10 dogs) or with blood drawn from the dog about 3 weeks beforehand (42 dogs) and stored with CPDA-1 (anticoagulant citrate phosphate dextrose adenine solution) at 4.5°C. The coronary arterial and sinus catheters were then removed. The vehicle group was treated identically except that the vehicle in which Dibenzyline was supplied (48.5% ethanol and 0.5% HCl in propylene glycol) was diluted and infused instead of Dibenzyline.

Instrumentation

The left atrium was catheterized transseptally via the left jugular vein, with a modified version of the technique described by Phillips et al.15 A Swan-Ganz catheter (American Edwards 93-111-7F) was secured in the left atrium by inflating its balloon (Figure 2). A 7.5F Sones catheter (USCI 007561) was passed down the right jugular vein and fluoroscopically guided into the coronary sinus. Postmortem, the catheter tip was found to lie 22–60 mm beyond the coronary sinus ostium where it selectively sampled coronary venous blood.16

A catheter-tipped manometer (Millar Instruments PC-470, Houston, Texas) inserted via the introducer sheath in the right carotid artery was positioned at the level of the heart in the descending aorta for continuous recording of arterial pressure and heart rate during exercise.

An 8F polyurethane catheter was positioned with its tip 1 cm beyond the end of the introducer sheath in the left carotid artery for withdrawal of reference blood samples.17

Experimental Protocol

Five predetermined conditions were obtained by adjusting the speed and grade of the treadmill. Succesive target heart rates were 1) resting, 2) moderate warm-up, 3) near-maximal (judged from previous exercise training sessions), 4) intermediate between warm-up and near-maximal, and 5) intermediate between resting and warm-up. Rest periods after each run allowed heart rate and arterial pressure to return to near the resting levels. After the protocol was completed, the dog was sacrificed with intravenous sodium pentobarbital.

When the dog had a steady heart rate and blood pressure, arterial and coronary sinus blood samples were drawn, and radionuclide-labeled microspheres were infused into the left atrium over 15–30 seconds. A reference blood sample was drawn (7.47 ml/min, Harvard syringe pump, model 968) starting at least 15 seconds before, and continuing for 90 seconds after, the infusion began.

In the α- and β-blocked group, systemic β-blockade was imposed with propranolol hydrochloride (2.0 mg/kg i.v.) at least 10 minutes prior to the first infusion of microspheres. Heart rate responses to isoproterenol hydrochloride (bolus doses, 0.003–10.0 μg/kg i.v. in half-log increments) were recorded before propranolol and again after completion of the exercise protocol. After β-blockade, the isoproterenol dose-response curve was shifted 1.6 orders of magnitude to the right. Thus, β-receptors were effectively blocked.

Blood Samples

Arterial and coronary sinus blood samples were heparinized for blood gas analysis (Instrumentation Laboratories 1302 pH/blood gas analyzer) and for measurement of oxygen content (Lex O2 Con, Lexington Instruments). The microsphere reference blood
samples were mixed with EDTA and sodium bisulfite and centrifuged at 1,500 rpm for 10 minutes at 4° C. Then, the plasma was collected for analysis of catecholamine content by high-performance liquid chromatography with electrochemical detection (performed by the clinical laboratory of University Hospital, University of Washington, Seattle, with a modified version of the technique of Hallman et al)."

**Tissue Samples**

The left ventricular free wall was cut into 30–45 transmural sections (Figure 1B); each was trimmed of epicardium, epicardial fat, large vessels, and papillary muscles, and each was divided into three layers, inner, middle, and outer myocardium, of equal thickness (Figure 1C). These, plus a sample of the right ventricular free wall (1–1.5 g) and 4 cortical samples from each kidney (about 0.4 g each, 44 dogs), were fixed in 37% formaldehyde solution.

**Analysis of Radioactivity**

The microspheres used (10 μm diameter in 15 dogs, 15 μm diameter in 37 dogs) were labeled with 51Cr, 103Ru, 111In, 141Ce, and 153Sm and were supplied by New England Nuclear, Boston, Massachusetts, in 10% dextran with 0.01% Tween 80. One isotope (111In or 141Ce) was used to mark the treated region (described above), and the other five isotopes, in random order, were used to measure blood flow. The microsphere dose (usually 2.24 × 10^6 beads) was calculated, with adjustments for radioactive decay, so that at least 400 beads, emitting at least 10,000 counts in 1,000 seconds, were trapped in each layer of each myocardial region. Each dose was diluted to 2.5 ml with normal saline and subjected to ultrasoundation and intermittent vortex-mixing, to disperse the microspheres, for 2 hours before infusion.

The gamma activity of each tissue and blood sample was analyzed in a Packard Auto-Gamma Scintillation Spectrometer, and the counts from the six different nuclide labels were separated by the simultaneous equation method described by Baer et al. The separated counts were calibrated in units of flow according to the activities of the reference samples (counts/minute/milliliter). The number of microspheres present was also calculated from the separated counts according to the activities of samples containing known numbers of microspheres counted under a microscope.

**Regional Analysis**

Myocardial regions were defined on the basis of the density of marker microspheres in each transmural section (number of marker microspheres present per unit mass of tissue) relative to the mean marker density (total number of marker microspheres present in the sampled left ventricular myocardium divided by its total mass). The treated region was defined by the following prospective criteria: 1) all contiguous transmural sections with at least twice the mean marker density were included; and 2) successive less densely marked contiguous transmural sections were included only if they had marker densities at least 1.75 times the mean, and until the treated region contained at least three sections with at least 400 microspheres and 10,000 counts of each label in each layer. Similarly, the untreated region included all contiguous transmural sections with no more than one tenth the mean marker density, provided they were not contiguous with any section having a marker density greater than or equal to the mean. These criteria separated the two regions and minimized inclusion of partially treated tissue in either region (Figure 1).

Experiments were rejected when the regional selectivity of the treatment was poor according to the following criteria: 1) if fewer than three transmural sections were included in each myocardial region (six dogs), because such small regions suggest that the circumflex region was poorly perfused with the treatment mixture and/or that the rest of the myocardium was contaminated by it; and 2) if the density of marker microspheres in peripheral tissues was high, because this suggests that part of the treatment mixture infused into the left circumflex coronary artery refloxed into the aorta and was distributed systemically. Ten dogs were rejected because the right ventricular density was greater than one tenth the mean left ventricular density. One dog was rejected because the marker density of the renal cortex was greater than the mean left ventricular density.

The counts from each section in a region were added together, and the region was analyzed as a single sample divided into inner, middle, and outer layers. For each nuclide, transmural flow (ml/min/g) was calculated for each region from the total counts summed over all three layers of all sections and divided by the total mass of the region corrected for dehydration in formaldehyde. Count densities and flows were also calculated for each layer. The inner/outter flow ratio was calculated from the layer count densities (rather than layer flows), for computational precision: inner/outter ratio = count density of inner layer/count density of outer layer.

Total flow to the left ventricular free wall (ml blood/min/g) was calculated for each nuclide (total counts in all layers of all sections divided by total mass calibrated with the reference samples), and multiplied by the arteriovenous difference in oxygen content across the left ventricular myocardium, yielding average myocardial oxygen consumption (μl O2/min/g), according to the Fick equation.

**Analysis of Error in Microsphere Method**

In a flow measurement made with microspheres, the relative error due to the stochastic nature of microsphere distribution is defined as the deviation of the measurement from the true flow, relative to the magnitude of the true flow. According to Dole et al., the 95% confidence limits for the relative error are then ± 1.96(1/Nt + 1/Nr)0.5, where Nt and Nr refer to the number of microspheres (with a given label) trapped in the tissue and in the reference sample, respectively. These confidence limits were calculated for each layer.
of each region; they were within ±10% for 94.5% of the observations and were never greater than ±15%.

In 42 dogs, microsphere distributions to the two kidneys differed by less than 10% in 94.2% of the observations, and never by more than 16.9%, indicating that the microspheres were adequately mixed with the cardiac output. Two dogs from which renal cortical samples were taken were excluded from this analysis because of evidence of errors in the tissue sampling procedure.

Statistical Analyses

The general statistical approach was to fit a separate least-squares regression line to the observations from each dog versus myocardial oxygen consumption (as in Figure 5). These individual regression lines were then summarized by multiple linear regression computed with the SPSS program.21 The difference in response between the two myocardial regions in each dog was analyzed as the dependent variable to preserve the paired nature of the observations. The differences between regions within each group were tested with paired t tests, and the differences between groups were tested with unpaired t tests. Differences in regression slopes reflected the overall relation between dependent variables and myocardial oxygen consumption. Differences in the magnitude of the dependent variables varied with the level of myocardial oxygen consumption. Accordingly, tests of differences in magnitude were performed at intervals of 50 μl O₂/min/g to estimate the level of myocardial oxygen consumption at which the responses became significant.

The multiple regression calculation required symmetry in the data sets, so for each missing observation, the mean of the other four flow measurements in the same dog was substituted. This maneuver restored symmetry without changing the average value, the slope, or the intercept of the individual regression line that the multiple linear regression analysis is based on. There were four missing data points, one from each of the three dogs in the vehicle group (three observations out of 85) and one missing point in the α-blocked group (one observation out of 100).

Because the logarithmic transformation of a ratio variable tends to be more normally distributed than the ratio itself is, analyses were performed with ln (inner/outer ratios) in addition to the usual inner/outer flow ratio. The results were not meaningfully different, so the data and analyses are presented here in terms of the conceptually more direct, untransformed variables.

The variability of the data is shown in the figures. Figure 5 shows the scatter of individual observations around the simple linear regression lines for each region in one dog (within-dog variability). Figures 6, 7, and 8 (Panels B and C) show the variability of the simple regression lines calculated for each dog (among-dog variability). Figures 9 and 10 show the residual (within-dog) variability of the observations, scattered around the mean lines calculated by multiple linear regression, which accounts for the differences among dogs. Tests of the effect of α-blockade were based on the paired differences (treated, left circumflex region versus untreated, left anterior descending region) and the residual variability, as plotted in Figures 9 and 10.

Results

General Response to Exercise

The dogs ran on the treadmill at speeds up to 9.6 km/hr in combination with grades from 0.0% to 20%. The average resting myocardial oxygen consumption in the vehicle group was 122 ± 12 (SEM) μl/min/g and increased to an average maximum of 454 ± 25 μl/min/g. In the α-receptor blocked group, the average myocardial oxygen consumption values at rest and maximum exercise were 156 ± 12 and 559 ± 42 μl/min/g, respectively. β-Receptor blockade markedly attenuated the cardiac exercise response. The average resting value was 112 ± 7 μl O₂/min/g and increased to an average maximum value of only 289 ± 14 μl O₂/min/g in the α- and β-receptor blocked group.

Heart rate increased from as low as 52 beats/min at rest to as high as 291 beats/min during exercise (Figure 3). The average resting heart rate was 79.4 ± 4.7 beats/min (SEM) in the vehicle group, 100.4 ± 5.9 beats/min in the α-receptor blocked group, and 81.4 ± 5.2 beats/min in the α- and β-blocked group. The average maximum heart rate was 238.3 ± 4.8 beats/min in the vehicle group, 245.2 ± 6.5 beats/min in the α-blocked group, and 189.0 ± 3.1 beats/min in the α- and β-blocked group. Systolic blood pressure increased significantly (p<0.0001) during exercise in all experimental groups, while diastolic blood pressure changed little with exercise (Figure 3). The dogs hyperventilated during exercise, with a significant (p<0.0001) progressive decline in Paco₂ from approximately 31–32 mm Hg at rest to approximately 24–26 mm Hg during maximal exercise. This was accompanied by a small increase in Pao₂ from approximately 87–91 mm Hg at rest to approximately 94–96 mm Hg during maximal exercise, which was statistically significant (p<0.05) in the two groups with α-blockade. Arterial plasma epinephrine and norepinephrine concentrations, indicative of the degree of sympathetic activation, increased dramatically in all groups from less than 300 pg/ml at rest to 500–1,400 pg/ml during maximal exercise. Interestingly, the catecholamine response was more marked in the two groups with α-blockade than in the vehicle group.

Transmural Coronary Flow

Coronary blood flow increased consistently with exercise intensity in all three experimental groups (Figure 4). Flow increased approximately in proportion with myocardial oxygen consumption in the α-blocked and vehicle groups, so there were no significant changes in coronary sinus oxygen tension (approximately 16–17 mm Hg) or in the coronary arteriovenous difference in oxygen content (approximately 12–13 ml O₂/100 ml blood). In contrast, in the group with both α- and β-blockade, coronary sinus oxygen tension fell significantly (from approximately 16 to 11 mm Hg,
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α-Receptors and Transmural Flow in Exercise

**FIGURE 3.** Hemodynamic responses to exercise plotted as functions of myocardial oxygen consumption. Individual points illustrate variability within and among dogs. Lines were calculated by multiple linear regression.

*p*<0.0001), and the arteriovenous difference in oxygen content widened (from approximately 13 to 15 ml O_2/100 ml blood, *p*<0.0001) with exercise.

**Regional Transmural Coronary Flow**

The magnitude of the regional α-receptor blockade may be estimated as the difference in flow between the paired regions, relative to the flow in the circumflex region. In the α-blocked group, the flow in the α-intact (anterior descending) region averaged 12.2% less than in the α-blocked (circumflex) region. In the vehicle group, the flow in the anterior descending region averaged 6.3% less than in the circumflex region. Thus, the differential effect of α-receptor blockade was approximately 6% (12.2% minus 6.3%), and this difference was significant (*p*<0.01) when myocardial oxygen consumption was 200 μl/min/g or more. This demonstrates that α-receptors in the circumflex region were effectively blocked.

The left ventricular inner/outer blood flow ratio decreased with increasing levels of exercise in all experimental groups (Figures 5–8). The α-intact left anterior descending region had a more favorable inner/outer blood flow ratio than the α-blocked circumflex region in the same heart (Figure 6). In contrast, the inner/outer flow relation between anterior descending and circumflex regions was reversed in the vehicle group (Figure 7). The difference in inner/outer ratio between the α-blocked and α-intact regions was significant (*p*<0.01) when myocardial oxygen consumption was 150 μl/min/g or greater. A similar trend in the inner/outer flow ratio was observed between the α-intact and α-blocked regions in the group with prior β-blockade; however, myocardial oxygen consumption was very limited, and a significant difference could be predicted only by extrapolation (Figure 8).

The paired differences in inner/outer flow ratio between the circumflex and anterior descending regions for the α-blocked and vehicle groups are compared in Figure 9. The inner/outer flow ratio was greater in the circumflex region than in the anterior descending region in the vehicle (α-receptors intact) group, and this did not change with exercise. In contrast, when the circumflex region was selectively α-blocked, the inner/outer ratio in this region was less than in the paired anterior descending region, and this difference widened with increasing levels of exercise. These results indicate that α-receptor activation during exercise helps maintain a more uniform transmural blood flow in the left ventricle.

Because the inner/outer ratio may be affected by changes in both subepicardial and subendocardial blood flow, a further comparison of blood flows to just the inner myocardial layer was made. The paired differences in inner layer blood flow between the circumflex and anterior descending regions for the α-blocked and vehicle groups are compared in Figure 10. Flow to the inner layer was greater in the circumflex region than in the anterior descending region in the vehicle (α-receptors intact) group, and this difference increased with exercise. In contrast, when the circumflex region was selectively α-blocked, this effect of exercise was not observed (Figure 10). The paired differences in cir-
ALPHA BLOCKED

10

8

6

4

2

0

20 dogs
99 observations
P < 0.001 above
500 μl O₂/min/g
• intact
• anterior descending

ALPHA-BETA BLOCKED

7.5

6

4

2

0

15 dogs
75 observations
P < 0.001 above
200 μl O₂/min/g
• intact
• anterior descending

VEHICLE

8

6

4

2

0

17 dogs
82 observations
P < 0.001 above
200 μl O₂/min/g
• intact
• anterior descending

MYOCARDIAL OXYGEN CONSUMPTION (μl O₂/min/g)

FIGURE 4. Paired observations of regional transmural flow in the treated (circumflex) and the untreated (anterior descending) regions, plotted against total left ventricular myocardial oxygen consumption, illustrate the variability of the data within and among dogs. Lines were calculated by multiple linear regression. The slopes of the two lines in each panel were significantly different (p<0.001, 0.025, and 0.005 for Panels A, B, and C, respectively), so the differences in regional transmural flow between paired regions became statistically significant when exercise elicited high rates of myocardial oxygen consumption, as shown.

circumflex minus anterior descending inner layer flow for the α-blocked group and vehicle group became significantly different (p<0.05) when myocardial oxygen consumption was 500 μl/min/g or greater. This indicates that, despite the vasodilation caused by both the prejunctional and postjunctional α-receptor blocking actions of phenoxybenzamine, flow to the inner layer of the circumflex region (relative to the paired reference flow to the inner layer of the anterior descending region) was greater when α-receptors were intact than when they were blocked (see "Discussion").

Discussion

The results presented here indicate that coronary vasoconstriction mediated by α-receptors, during exercise, helps maintain a uniform distribution of coronary blood flow across the left ventricular wall when myocardial oxygen consumption exceeds approximately 150 μl/min/g.

General Response to Exercise

The hemodynamic response to exercise, with increases in heart rate and blood pressure, observed in the

Discussion

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General Response to Exercise

The hemodynamic response to exercise, with increases in heart rate and blood pressure, observed in the
**ALPHA BLOCKED GROUP**

**FIGURE 6.** Inner/outer flow ratio vs. myocardial oxygen consumption in the α-blocked group. A: Lines summarize observations in the α-receptor intact (anterior descending) region and in the α-receptor blocked (circumflex) region according to multiple linear regression analysis. The slopes of the two lines were different (p<0.05). The inner/outer flow ratio of the α-blocked region was below the paired α-intact region when myocardial oxygen consumption was 150 μl O₂/min/g or more (p<0.01). B and C: Simple regression lines calculated for the α-intact (anterior descending) and α-blocked (circumflex) regions of each dog illustrate the variability among dogs.

**VEHICLE GROUP**

**FIGURE 7.** Inner/outer flow ratio vs. myocardial oxygen consumption in the vehicle group. A: Lines summarize observations in the control (anterior descending) region and in the vehicle-treated (circumflex) region according to multiple linear regression analysis. The slopes of the two lines were not significantly different (0.8<p<0.9). The inner/outer ratio in the circumflex region was significantly higher than in the paired anterior descending region when myocardial oxygen consumption was 100 μl/min/g or more (p<0.001). B and C: Simple regression lines calculated for the control (anterior descending) and vehicle-treated (circumflex) regions of each dog illustrate the variability among dogs.
The present experiments was similar to that observed by others. The resting values of heart rate and myocardial oxygen consumption reported here are not truly basal because the animals were waiting to run on a treadmill in a laboratory where they had been trained to exercise. The dogs hyperventilated during exercise and developed arterial hypocapnia and hyperoxia. As described by Bainton and Mitchell, hyperventilation occurs in response to both increased heat load and exercise per se. Rectal temperature was measured in 19 dogs in the present study and increased significantly (p<0.001) from 38.9°C at rest to 39.4°C after the maximum exercise run, and all dogs panted. Clifford et al recently reported that arterial hypocapnia developed in dogs even during mild exercise in a cool ambient temperature. Unfortunately, neither body temperatures nor the occurrence of panting were reported. When exercise is severe enough to result in net lactate production, respiratory compensation for the metabolic acidosis also results in arterial hypocapnia and hyperoxia, as has been described in humans.

The increase in coronary blood flow during exercise has been documented many times. A widening of the arteriovenous oxygen content difference across the coronary circulation and/or a fall in coronary venous oxygen tension during exercise has been observed in some studies but not others. In the present study, the coronary arteriovenous oxygen content difference widened, and the coronary sinus oxygen tension fell during exercise in the α- and β-blocked group, but not in the vehicle or α-blocked groups. The present experimental design does not elucidate a mechanism for this difference.

**Regional Transmural Coronary Flow**

The paired experimental design provided simultaneous measurements of blood flow in the circumflex and left anterior descending regions at rest and during four graded levels of exercise in each dog. In the vehicle group, the circumflex region was treated with only the phenoxybenzamine vehicle and marker microspheres. Under these conditions, the inner/outer flow ratio was greater in the circumflex region than in the paired anterior descending region (Figure 7), and this difference is similar to that previously reported by Wüsten et al. Because Wüsten’s experiments did not involve phenoxybenzamine vehicle, the difference is probably due to an inherent difference between the circumflex and anterior descending regions. However, the present

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**Figure 8.** Inner/outer flow ratio vs. myocardial oxygen consumption in the α- and β-blocked group. A: Lines summarize observations in the α-intact (anterior descending) region and α-blocked (circumflex) region according to multiple linear regression analysis. Dotted lines illustrate values predicted at high rates of myocardial oxygen consumption by extrapolation of regression lines. The slopes of the two lines were significantly different (p<0.005). The inner/outer ratio of the α-blocked region became significantly less than in the α-intact region when myocardial oxygen consumption is extrapolated beyond 400 μl/min/g (p<0.05). B and C: Simple regression lines calculated for the α-intact (anterior descending) and α-blocked (circumflex) regions of each dog illustrate the variability among dogs.

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The arterial concentrations of epinephrine and norepinephrine increased during exercise, as previously described for the dog by Péronnet et al and Chilian et al. Splenic contraction is a normal component of the exercise response in dogs and results in an increased hematocrit. Because increases in hematocrit make the inner/outer flow ratio more sensitive to stress, splenectomy was performed in each dog in the present study, and hematocrit never increased by more than 1% during the experimental protocol.

The increase in coronary blood flow during exercise has been documented many times. A widening of the arteriovenous oxygen content difference across the coronary circulation and/or a fall in coronary venous oxygen tension during exercise has been observed in some studies but not others. In the present study, the coronary arteriovenous oxygen content difference widened, and the coronary sinus oxygen tension fell during exercise in the α- and β-blocked group, but not in the vehicle or α-blocked groups. The present experimental design does not elucidate a mechanism for this difference.

The present experiments was similar to that observed by others. The resting values of heart rate and myocardial oxygen consumption reported here are not truly basal because the animals were waiting to run on a treadmill in a laboratory where they had been trained to exercise. The dogs hyperventilated during exercise and developed arterial hypocapnia and hyperoxia. As described by Bainton and Mitchell, hyperventilation occurs in response to both increased heat load and exercise per se. Rectal temperature was measured in 19 dogs in the present study and increased significantly (p<0.001) from 38.9°C at rest to 39.4°C after the maximum exercise run, and all dogs panted. Clifford et al recently reported that arterial hypocapnia developed in dogs even during mild exercise in a cool ambient temperature. Unfortunately, neither body temperatures nor the occurrence of panting were reported. When exercise is severe enough to result in net lactate production, respiratory compensation for the metabolic acidosis also results in arterial hypocapnia and hyperoxia, as has been described in humans.

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Regional Transmural Coronary Flow

The paired experimental design provided simultaneous measurements of blood flow in the circumflex and left anterior descending regions at rest and during four graded levels of exercise in each dog. In the vehicle group, the circumflex region was treated with only the phenoxybenzamine vehicle and marker microspheres. Under these conditions, the inner/outer flow ratio was greater in the circumflex region than in the paired anterior descending region (Figure 7), and this difference is similar to that previously reported by Wüsten et al. Because Wüsten’s experiments did not involve phenoxybenzamine vehicle, the difference is probably due to an inherent difference between the circumflex and anterior descending regions. However, the present
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**INNER/OUTER FLOW RATIO DIFFERENCE**

![Graph showing inner/outer flow ratio difference](image)

**MYOCARDIAL OXYGEN CONSUMPTION (μl O2/min/g)**

**INNER MYOCARDIAL FLOW**

![Graph showing inner myocardial flow difference](image)

**MYOCARDIAL OXYGEN CONSUMPTION (μl O2/min/g)**

Experimental design would not separate effects due to region from those due to vehicle. When the α-receptors were blocked selectively in the circumflex region, the inner/outer flow ratio was less in that region than in the simultaneously paired anterior descending region (Figure 6). Thus, the inner/outer flow ratio is normally greater in the circumflex region than in the anterior descending region, but selective α-blockade in the circumflex region reverses this relation. The comparison of the inner/outer ratio differences between the circumflex and anterior descending areas for the vehicle and α-blocked groups is given in Figure 9, which indicates that the inner/outer flow ratio was greater with α-receptors intact. These results demonstrate that the transmural distribution of flow during exercise is more uniform when α-receptors are intact than when they are blocked.

Coronary adrenergic vasoconstriction is mediated postjunctionally by both α1- and α2-receptors. Thus, a combined α1- and α2-receptor antagonist was chosen for these experiments. However, there are also prejunctional α2-receptors involved in feedback inhibition of norepinephrine release from sympathetic nerves. Therefore, blockade of prejunctional α2-receptors results in an augmented norepinephrine release during exercise. The augmented norepinephrine concentration stimulates myocardial β-receptors, thereby magnifying inotropic effects that increase myocardial oxygen consumption and local metabolic vasodilation. The present experiments combining α- and β-blockade were designed to examine the hypothesis free of the effects mediated by β-receptors, secondary to prejunctional α-receptor blockade. The results in the α- and β-blocked group are consistent with the hypothesis that vasoconstriction mediated by postjunctional α-receptors helps maintain a uniform transmural blood flow during exercise. However, the cardiac exercise response was so blunted by β-blockade that a significant difference could be predicted (Figure 8) only when the exercise response was extrapolated to values observed in the other groups without β-blockade (Figures 6 and 7).

Because the inner/outer flow ratio may be altered by changes in flow in either the inner or outer layer, an additional comparison of only the inner layer flows was made. The paired differences in inner layer blood flow between the circumflex and anterior descending regions are presented in Figure 10. The inner layer blood flow in the untreated anterior descending region was used as a paired reference to avoid the variability associated with differences in left ventricular diastolic pressure, aortic pressure, cardiac output, hematocrit, and other variables that combine to influence myocardial oxygen consumption among different dogs. In the vehicle group, the inner layer flow in the circumflex region was
greater than the inner layer flow in the anterior descending region, and this difference widened with increasing levels of exercise. This indicates an intrinsic regional difference between the two areas. In contrast, when the circumflex region was selectively α-blocked, the inner layer flow in this region differed from the vehicle group. This difference between groups was significant (p < 0.05) when myocardial oxygen consumption was 500 μl/min/g or greater (Figure 10). Thus, when myocardial oxygen consumption exceeded 500 μl/min/g, the inner layer blood flow in the circumflex region was greater with α-receptors intact (vehicle) than with α-receptors blocked (the inner layer anterior descending region serving as a simultaneous paired reference). The inner layer blood flow was greater with α-receptors intact than blocked, despite the coronary vasodilatory effects of both prejunctural and postjunctural α-blockade, as discussed above.

The present results may be an underestimate of the true effect of α-receptor–mediated coronary vasoconstriction on transmural flow distribution for several reasons: 1) phenoxybenzamine was used because it binds noncompetitively and irreversibly by alkylation of α-receptors,29 properties that were necessary for the paired region experimental design. However, the 2.5-minute exposure time of the drug may have been too short for optimal blockade. 2) Because some 35% of left circumflex coronary artery blood flow leaves the mycardium via Thebesian veins40 and, therefore, could not be captured by the coronary sinus drain, some phenoxybenzamine recirculated and partially blocked the anterior descending region. 3) Although phenoxybenzamine was infused into the circumflex region via a catheter with its tip beyond the bifurcation between the anterior descending and circumflex coronary arteries, the distribution of marker microspheres indicated some reflux into the anterior descending artery and even into the right coronary artery in some cases (see "Regional Analysis" in "Materials and Methods").

4) The findings of Constantine and Lebel41 that phenoxybenzamine does not completely block α2-receptors in the dog and the observation by Holtz et al17 that canine coronary adrenergic vasoconstriction is mediated by α2-receptors, as well as α-receptors, suggest that adrenergic coronary vasosconstriction may not have been completely blocked.

All of these shortcomings (brief exposure time, recirculation, reflux, and incomplete α-blockade) would limit the differences between the two regions and make it more difficult to demonstrate an α-receptor–mediated effect. In the present study, the coronary vasoconstrictor effect mediated by α-receptors during exercise was approximately 6%, which is smaller than reported in other studies. Using a sequential experimental design, Mohrman and Feigl14 observed a 30% α-receptor–mediated constrictor effect on flow in a carotid sinus baroreceptor reflex in anesthetized dogs. The 6% vasoconstrictor effect observed in the present study may be compared to a 14% α-vasoconstrictor effect observed by Heyndrickx et al3 or a 30% effect found by Gwirtz et al6 when exercise was studied sequentially before and after α-blockade. If the true α-receptor–mediated coronary vasoconstrictor effect during exercise is 14–30%, rather than the approximate 6% observed in the present study, then the true adrenergic effect on transmural flow distribution may be several times larger than the 0.1–0.2 effect on the inner/outer flow ratio observed here (Figure 9).

The disadvantage of the simultaneously paired experimental design used in the present study is that only a small differential regional α-blockade was achieved. The advantage is that heart rate, diastolic left ventricular pressure, aortic pressure, catecholamine levels, etc., are the same for both regions, obviating the attempt to match these variables at different exercise levels before and after α-blockade that a sequential design would require.

Possible Mechanisms

There are several possible mechanisms that could be involved in the maintenance of more uniform transmural distribution of blood flow when α-receptors remain intact.

1) The simplest mechanism is that either sympathetic α-receptor density or coronary innervation is greater in the outer layers of the left ventricle than in the inner layers. A transmural gradient of coronary postsynaptic α-receptors has not been observed in four studies that used norepinephrine infusions to examine the question842–44; thus, it seems an unlikely mechanism at this time. Johannsen et al42 did not find evidence for a transmural gradient of sympathetic coronary innervation under normal conditions but did find an appropriate gradient during pharmacological hyperemia produced with adenosine. They postulate a differential prejunctural interaction between adenosine and sympathetic norepinephrine release across the left ventricular wall. To the extent that adenosine is involved in the cardiac response during exercise, this is an interesting possibility.

2) Nathan and Feigl44 observed a beneficial effect of adrenergic coronary vasoconstriction on transmural flow distribution during hypoperfusion. The mechanism for this effect was an anti-transmural steal whereby α-receptor–mediated vasoconstriction in the outer layer of the left ventricle helped maintain the perfusion pressure for the inner layer. Chilian and Ackell45 also observed an adrenergic antitransmural steal mechanism distal to a coronary stenosis in exercising dogs. The basis of a vascular steal is that the arterial pressure distal to a flow restriction is below the autoregulatory range so that a change in vascular resistance in one parallel vascular bed alters the perfusion pressure for a second parallel vascular bed. An anti-transmural steal mechanism is unlikely to explain the present results because there was no coronary artery stenosis, and even unintended stenosis secondary to surgery was avoided with the closed-chest technique.

3) An additional mechanism for transmural redistribution of coronary flow by adrenergic vasoconstriction is based on changes in vascular capacitance and...
vessel wall stiffness associated with vasoconstriction, as suggested by Hoffman et al.4 Myocardial vessels are narrowed by systolic compression; thus, some diastolic time is spent reexpanding narrowed vessels and filling their capacitance before flow through the vessels resumes. Stiffening of vessel walls, as by vasoconstriction, may help the vessels resist narrowing during systole and also facilitate reexpansion during diastole. Furthermore, vascular capacitance tends to be reduced by vasoconstriction so that less blood is required for filling the capacitance and more of the diastolic inflow is available for tissue perfusion. Thus, vasoconstriction may facilitate flow through inner myocardial vessels, which are particularly subject to narrowing by systolic compression. Flow through vessels in the outer myocardium, subject to lower myocardial compression than those in the inner myocardium, would be affected more by the reduction of vessel diameter (increased resistance to flow) than by the changes in wall stiffness and vessel capacitance with vasoconstriction, so the net effect of vasoconstriction in outer myocardial vessels would be to reduce flow.

Although the results reported here demonstrate a beneficial effect of sympathetic coronary vasoconstriction during exercise, other investigators have observed that coronary α-adrenergic vasoconstriction can be detrimental under some circumstances. Heusch and Deussen6 observed that α-receptor–mediated coronary vasoconstriction was augmented in the presence of coronary stenosis and was powerful enough to induce ischemia. In a preliminary report, Seiltelberger et al.44 indicate that αα-adrenergic vasoconstriction during exercise depresses the inner myocardial blood flow, inner/outer flow ratio, and systolic wall thickening in myocardium distal to a coronary stenosis. In both these studies, it is possible that slight vasoconstriction of the epicardial artery at the site of the stenosis increased its severity enough for the effect of transmural hypoperfusion to dominate over the beneficial transmural effects observed in the present and other studies.44,45 Gwirtz and coworkers report that the rate of shortening of myocardial segment length (but not percent segment shortening) was enhanced by αα-blockade during exercise.9 However, this does not necessarily indicate that normal adrenergic coronary vasoconstriction during exercise interferes with myocardial function by making the myocardium ischemic, because increasing coronary flow in nonischemic myocardium augments cardiac function by the Gregg effect.32

In conclusion, the present results indicate that adrenergic coronary vasoconstriction has a beneficial effect on left ventricular transmural blood flow distribution during exercise in normal dogs without coronary artery stenosis. This observation may answer the paradox as to why there is sympathetic coronary vasoconstriction during exercise, when myocardial oxygen consumption is so great.

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