Absence of Functioning α-Adrenergic Receptors in Mature Canine Coronary Collaterals

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with the technical assistance of Douglas H. Barnes

To determine if mature coronary collateral vascular smooth muscle contains functioning α-adrenergic receptors, we studied 13 dogs, 6–10 months after circumflex ameroid occlusion. Regional myocardial blood flow was measured with radioactive microspheres in a blood-perfused heart preparation at constant aortic pressure (80 mm Hg). Normal zone resistance was calculated as aortic pressure divided by normal zone flow, and transcollateral resistance was calculated as aortic pressure minus circumflex pressure distal to the ameroid constrictor divided by coronary collateral flow. Flow and resistance were measured during adenosine vasodilatation before and during graded doses of a constant infusion of the α-adrenergic agonist methoxamine (n = 6) or the α2-adrenergic agonist clonidine (n = 7). In the hearts that received methoxamine, normal zone resistance increased from a control of 0.29 ± 0.06 to 0.39 ± 0.06 mm Hg × min/ml per 100 g (resistance units) during infusion of 10−4 M methoxamine (p < 0.05). In contrast transcollateral resistance averaged 0.24 ± 0.02 resistance units under control conditions and did not change during methoxamine infusion. In the hearts that received clonidine, normal zone resistance averaged 0.24 ± 0.03 resistance units and increased to 0.39 ± 0.07 resistance units (p < 0.05) with the highest dose of clonidine administered (10−6 M). Transcollateral resistance averaged 0.17 ± 0.03 resistance units during control conditions and did not change with clonidine infusion. In separate studies isometric tension development by the left anterior descending and coronary collateral vessels was examined in organ baths. The left anterior descending coronary artery demonstrated dose-dependent constriction to phenylephrine (peak response 22 ± 5% of the response to 100 mM KCl). Clonidine produced weak constrictr responses in the left anterior descending coronary artery (5 ± 2.5% maximal KCl response). In contrast, neither phenylephrine nor clonidine produced responses in mature collaterals. We also examined responses of mature collateral vessels to nonadrenergic agonists. In the vascular ring preparation the mature collaterals developed tension in the presence of KCl (2.3 ± 0.9 g), prostaglandin F2α (16 ± 8% of the KCl responses), and vasopressin (90 ± 30% of the KCl response). In adenosine-vasodilated hearts, pharmacologic doses of vasopressin caused a two-fold increase in transcollateral resistance. Thus, these studies performed on intact hearts and isolated vascular rings demonstrate that mature coronary collaterals do not contain functioning α-adrenergic receptors. Consequently, mature coronary collateral resistance is not regulated by α-adrenergic neurohumoral mechanisms. (Circulation Research 1986;59:133–142)

In several species, including man, gradual occlusion of a coronary artery leads to the development of large intracoronary collateral vessels.1–7 In dog and man, this process is thought to be principally the result of enlargement of preexisting native coronary collaterals. Among other morphologic changes, a prominent feature of this process is proliferation of the internal elastic lamina is not continuous, and 2) in contrast to the more proximal epicardial vessels the adrenergic nerves are sparse.4 This paucity of adrenergic nerves suggests that α-receptor-mediated control of mature collaterals may differ from other portions of the coronary circulation. The presence of adrenergic nerves, however, is not a prerequisite for the presence of functioning α-adrenergic receptors as several vascular tissues have been shown to have such receptors not in proximity to neurovascular junctions or in the absence of sympathetic innervation.6–10 In the coronary circulation, this issue is further complicated because the distribution of α-adrenergic receptors, and α-adrenergic receptor subtypes, appear to vary from one arterial segment of the coronary circulation to another.11–13 Thus, it is not possible to predict whether α receptors may be present and functioning in mature collaterals based on studies of other portions of the coronary circulation.

To gain further insight into the role of α-adrenergic control in the coronary collateral circulation, we assessed the functional capacity of α-adrenergic receptors to modulate vascular smooth muscle tone in mature collaterals. We studied mongrel dogs 6–10
months after placement of ameroid constrictors and used two separate methods to examine the responses of mature collaterals to a variety of agonists. An isolated blood-perfused heart preparation was used to examine coronary collateral resistance before and during infusion of α-adrenergic agonist or vasopressin. In addition, segments of coronary collaterals were studied in isolated tissue baths and isometric tension development recorded during agonist administration.

**Materials and Methods**

**Production of Mature Coronary Collaterals**

Thirteen mongrel dogs (weight 19–30 kg) were anesthetized with sodium pentobarbital (30 mg/kg) IV, the trachea intubated, and ventilation maintained by a mechanical respirator. After sterile preparation a left thoracotomy was performed. The pericardium was opened and the circumflex coronary artery was dissected free near its origin. An ameroid occluder either 2.77 or 3 mm in diameter (Three Point Products, Montreal, Canada) was placed around the vessel at this site. The pericardium was closed and the thoracotomy repaired. The animals were given 600,000 units of EM penicillin and returned to the animal care quarters.

**Isolated Blood Perfused Heart Preparation**

The studies were performed 6–10 months following placement of the ameroid constrictor. The animals were again anesthetized with sodium pentobarbital (30 mg/kg) IV. The trachea was intubated and respiration maintained using a mechanical ventilator. The chest was opened by a midsternal incision. The left brachiocephalic trunk was ligated and the right brachiocephalic artery was cannulated with a large-bore metal cannula that was advanced to the ascending aorta. Pressure was monitored at the cannula tip. The dogs were then given 5,000 units of heparin IV. Approximately 800–1,000 ml of the study dog's blood was removed and used to prime the isolated heart perfusion apparatus. During exsanguination, the aorta distal to the left brachiocephalic was gradually occluded to maintain coronary perfusion pressure above 50 mm Hg. Following exsanguination the heart was rapidly excised and attached to the perfusion apparatus (Figure 1). The aorta was perfused retrogradely through the previously placed cannula. The left ventricle was vented with a cannula through the mitral valve. Blood from the coronary sinus was drained from the right atrium and returned by a roller pump to the jugular vein of a support dog. Blood was pumped from cannulas in the carotid arteries of the support dog to a pressurized reservoir and from there returned to the heart and the coronary circulation. The reservoir was constructed so that pressure could be controlled with compressed air and an adjustable gauge. The aortic perfusion tubing contained a mixing chamber in which a small magnetic stir bar assured adequate dispersion of microspheres. Beyond the mixing chamber was a lucite loop with a withdrawal port on each side used for obtaining reference samples during microsphere injections.

**Figure 1.** Schematic representation of the isolated blood-perfused heart preparation used in these experiments. The coronary arteries are perfused from the aorta. The circumflex is cannulated distal to the site of the ameroid constrictor and is perfused independently of the remainder of the circulation. Following occlusion of the tubing leading to the cannula in the circumflex, peripheral coronary pressure is monitored. The left and right ventricles are vented and do not generate pressure. Regional myocardial blood flow is measured using radioactive microspheres injected into the aortic perfusion line. Other details of this preparation are provided in the text.
Determination of Regional Myocardial Perfusion With Microspheres

Regional myocardial perfusion was measured using 15-μ radioactively labeled microspheres. For each flow measurement, 5–8 × 10^3 microspheres were injected directly into the aortic perfusion cannula. Reference samples were obtained using a withdrawal rate of 4.94 ml/min beginning 10 seconds prior to and continuing for 1.5 minutes following microsphere injection.

After completion of the study the hearts were fixed in formaldehyde for at least 2 days. After fixation, the right ventricle and both atria were trimmed from the left ventricle, and the left ventricle was cut into 1-cm slices from the base to the apex parallel to the A-V groove. The base and apex pieces were discarded. Each slice was cut into endocardial, midwall, and epicardial rings. Each ring was subsequently cut radially into 14–25 pieces beginning at the junction of the anterior free wall and the septum (near the site of the left anterior descending). Each segment was weighed to the nearest 0.01 g, placed in a scintillation tube, and counted in a well-type gamma counter for 5 minutes. Separation of the energy spectra for the various microsphere labels was accomplished using standard techniques. Regional myocardial perfusion for each segment of myocardium was determined using the formula

\[ \text{myocardial blood flow} = \frac{C_m \times \text{RBF} \times 100}{C_e} \]

where \( C_m \) represents counts per gram of myocardial tissue in each sample, \( \text{RBF} \) represents reference blood flow in milliliters per minute (rate of reference sample withdrawal), and \( C_e \) equals counts in reference sample.

Isolated Vascular Ring Studies

Following 6 of the isolated heart experiments we removed 5–7 mm segments of epicardial coronary vessels for study in organ baths. The segments studied were as follows: 1) the proximal left anterior descending coronary artery, 2) portions of the left anterior descending diagonal branches giving rise to the collaterals and segments of the circumflex marginal branch immediately distal to the collaterals, 3) segments taken from the midportion of the collateral. Collateral vessels were easily identified on the surface of the left ventricle. These appeared as tortuous vessels extending from the diagonal branch of the left anterior descending to the marginal branches of the circumflex and from the left anterior descending around the apex of the left ventricle to the posterior descending branch of the circumflex. These vessels ranged from approximately 0.5 to 1 mm in diameter. While it was difficult to discern the exact origin and termination of the collaterals, the midportion of the collaterals was easily identified. The adjacent segments were rings taken from the transition between the collaterals and native vascularure.

Each of these vascular ring segments was studied in organ chambers containing 25 ml of a physiologic solution of the following composition (millimolar): NaCl 118.3, KCl 4.7, MgSO_4_2, KH_2PO_4_1.2, CaCO_3_25, Ca EDTA 0.026. The baths were oxygenated with a mixture of 95% oxygen and 5% CO_2. Each ring was suspended on two steel clips passed surgical silk sutured to a force transducer (Grass FT03c). Changes in isometric force were recorded using a direct writing recorder coupled to bridge amplifiers. The rings were placed at the optimal point in their length–tension relationship, as determined from repeated 3-minute exposures to 100 mM KCl. Basal length of the rings was increased gradually over approximately 1 hour until their contractions to 100 mM KCl were optimized. This length was maintained throughout the experiment.

Methoxamine, phenylephrine, clonidine, angiotensin II, and prostaglandin F_2alpha were purchased from Sigma Chemical Company (St. Louis, Mo.). Vasopressin was obtained as the commercially available 8-arginine vasopressin compound from Parke-Davis (Pitressin). Each agent was dissolved in water in concentrations such that no more than 0.1 ml was added to the organ chambers for each dose administered.

Protocols

Isolated Blood-Perfused Heart Preparation

Following the preparatory measures described above, the circumflex coronary artery was dissected free and cannulated with a short metal cannula constructed so that pressure could be measured immediately adjacent to its orifice. This cannula was attached to a second length of perfusion tubing, and the circumflex was perfused independently of the remainder of the coronary circulation. The hearts were studied while in ventricular fibrillation. Maximal vasodilation was achieved with adenosine, 12 mg/min, given into the perfusion reservoir. In preliminary studies we had shown that this dose of adenosine abolishes the reactive hyperemic response and further vasodilation is not achieved with additional doses of adenosine.

Use of the Shadow Technique to Identify the Collateral Dependent Region in Hearts After Chronic Coronary Occlusion

During an initial microsphere injection in each study, aortic and circumflex pressure were adjusted to equal 60 mm Hg. One label of radioactive microspheres was then injected into the aortic perfusion line while the circumflex was being perfused at an identical pressure with microsphere-free blood. This injection served to label the circumflex perfusion field (the field dependent on collateral blood flow). When the hearts were subsequently sectioned, data from this microsphere injection was used to identify the collateral dependent region. Each transmural ring was cut in a radial fashion into 14–25 segments, and flows into each segment from this microsphere injection were...
plotted against the segment number. An example of such an analysis is shown in Figure 2.

When the shadow technique is used in studies of hearts with native collaterals, there are invariably several segments of tissue in each heart slice in which microsphere-measured flow is less than 1 ml/min·100 g. In the present studies, 8 of the 13 hearts studied had segments with flows of less than 1 ml/min·100 g. In the other hearts some of the microspheres injected in the aortic perfusion line were present in all segments despite the circumflex being perfused with microsphere-free blood at aortic pressure. However, in all hearts a sharp transition was present between the normally perfused segments and collateral dependent segments. We included segments in the collateral dependent region if they contained less than 30% of the microsphere concentration in the normally perfused region on the shadow injection. In all studies, a total of 238 segments of tissue were included in the collateral dependent zone, 123 of which had less than 5% normal zone flow and 115 of which had 5–30% normal zone flow. In 9 hearts, segments of tissue containing 0–5% normal zone flow and 5–30% normal zone flow were both present. We are confident that the tissues with 5–30% normal zone flow identified from the shadow technique injection were truly in the collateral dependent regions for two reasons: 1) Following circumflex cannula occlusion, the resultant collateral flow in the 5–30% segments was 222 ± 37 and in the segments with 0–5% normal zone flow it was 216 ± 22. If the 5–30% segments contained substantial overlap flow, flow to these segments after occlusion of the circumflex cannula would have been higher than flow to the 0–5% segments. 2) The responses of the 5–30% normal zone flow segments to α-adrenergic agonist administration were identical to that of the 0–5% segments. Thus, our results and conclusions are not altered by inclusion of the segments.

Collateral Flow Measurements During Control Conditions and α-Adrenergic Agonist Administration

Following the initial shadow microsphere injection, the tubing to the circumflex perfusion cannula was occluded while we continued to monitor peripheral coronary pressure. Aortic pressure was adjusted to 80 mm Hg, and a control measurement of regional myocardial blood flow was obtained using radioactive microspheres. The α-adrenergic agonist methoxamine (6 hearts) or the α2-adrenergic agonist clonidine (7 hearts) was then administered into the aortic perfusion line. The adrenergic agonist administered to each heart was randomly assigned. These drugs were dissolved in saline, and the concentration of the agonist and the infusion rate were adjusted so that the final concentration...
of the drug when infused was 10^{-9} and 10^{-4}M for clonidine and 10^{-7} and 10^{-3}M for methoxamine for the two successive microsphere injections.

Collateral Flow Measurements During Vasopressin Administration

In 4 of the 13 hearts (2 in the clonidine group and 2 in the methoxamine group), we performed two additional microsphere injections while continuing adenosine infusion. Ten minutes after stopping the \( \alpha \)-adrenergic agonists, when coronary flow had returned toward control, a fifth injection of microspheres was performed. Vasopressin was then infused into the aortic perfusion line at a rate and concentration to bring the final concentration in the perfusate to 10^{-7}M. A final measurement of regional perfusion was made 1 minute after starting vasopressin infusion.

Isolated Vascular Ring Studies

Response to \( \alpha \)-Adrenergic Agonists. Following equilibration and establishing optimal tension, cumulative concentration response curves were recorded during the administration of the \( \alpha \_2 \)-agonist phenylephrine and subsequently during administration of the \( \alpha \_1 \)-agonist clonidine. These drugs were initially administered in a concentration of 10^{-9}M and the concentration increased three fold in a stepwise fashion to a concentration of 10^{-4}M. Between these dose-response curves, the vessels were allowed to equilibrate for 45 minutes to 1 hour and were repeatedly washed with oxygenated buffer.

Responses to Non-\( \alpha \)-Adrenergic Constrictor Agents. To determine if the vessels studied were responsive to nonadrenergic agonists we examined responses to 100 mM KCl, 3 \( \times \) 10^{-4} M PGF_{2\alpha}, 3 \( \times \) 10^{-7} M angiotensin II, and 2 \( \times \) 10^{-3}M vasopressin.

Data and Statistical Analysis

In the isolated heart studies three resistances were calculated. Normal zone resistance was calculated by determining the quotient of aortic pressure divided by flow into the normally perfused region of the left ventricle (the left anterior descending perfusion field). Total collateral resistance was calculated as flow into the collateral dependent region divided into aortic pressure. Transcollateral resistance was calculated as the pressure gradient across the collateral vessels divided by flow into the collateral dependent region. In the isolated vascular ring studies, responses to each agonist are expressed as a percent of the constriction response to 100 mM KCl.

The data are expressed as the mean \( \pm \) the standard error of the mean. Paired t tests using a Bonferroni correction factor were used to compare resistances before and after agonist administration. A \( p \) value of less than 0.05 was considered significant.

Results

Ameroid constrictor placement produced large visible collaterals in all dogs studied. No infarction was observed on gross examination of individual heart slices. The transcollateral resistance for the entire group averaged 0.18 \( \pm \) 0.02 mm Hg/ml/min \( \cdot \) 100 g (Resistance Units = RU) during control conditions. Using this preparation, native transcollateral resistance averaged approximately 4.0 RU. Thus, all of the dogs studied had developed mature collaterals as a result of chronic coronary occlusion.

Intact Heart Studies

Responses to Methoxamine Infusion (Tables 1 and 2 and Figure 3). Normal zone flow decreased during methoxamine infusion from a control value 321 \( \pm \) 51 ml/min \( \cdot \) 100 g to 234 \( \pm \) 36 ml/min \( \cdot \) 100 g at the 10^{-5} M concentration of methoxamine. Resistance to normal zone perfusion increased significantly from 0.29 \( \pm \) 0.06 RU to 0.39 \( \pm \) 0.06 RU (\( p < 0.05 \)) during 10^{-3} M methoxamine. Changes in flow and resistance were similar in the subepicardium, midwall, and subendocardium. Thus, methoxamine caused constriction of the vasculature perfusing the left anterior descending region of myocardium.

Peripheral coronary pressure increased during methoxamine infusion from a baseline value of 41 \( \pm \) 2 mm Hg to 49 \( \pm \) 3 mm Hg. Before methoxamine collateral flow averaged 174 \( \pm \) 27 ml/min \( \cdot \) 100 g. During methoxamine infusion collateral flow decreased to 140 \( \pm \) 25 ml/min \( \cdot \) 100 g. Total collateral resistance averaged 0.53 \( \pm \) 0.08 RU of myocardium. This value did not change significantly during infusion of 10^{-3} M methoxamine.

Transcollateral resistance averaged 0.24 \( \pm \) 0.02 RU under control conditions and did not change with either dose of methoxamine administered.

Response to Clonidine (Tables 1 and 2 and Figure 4). Under control conditions normal zone flow averaged 363 \( \pm \) 45 ml/min \( \cdot \) 100 g. During 10^{-8} M clonidine infusion flow decreased 266 \( \pm \) 64 ml/min \( \cdot \) 100 g. Normal zone resistance averaged 0.24 \( \pm \) 0.03 RU under control conditions and increased in a dose-depend-

![Figure 3. Normal zone and transcollateral resistance during control conditions and during infusion of the selective \( \alpha \_1 \)-agonist, methoxamine. Normal zone resistance increased significantly from 0.29 \( \pm \) 0.04 resistance units to 0.39 \( \pm \) 0.05 during the highest dose of methoxamine. Transcollateral resistance did not change during methoxamine.](http://circres.ahajournals.org/Downloaded from http://circres.ahajournals.org/)

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ent fashion to a value of .39 ± .08 RU at the 10^{-5} M concentration. Equivalent degrees of constriction occurred in subepicardial, midwall, and subendocardium. Thus, clonidine produced vasoconstriction in the normally perfused region of myocardium.

Under control conditions, peripheral coronary pressure was 49 ± 3 mm Hg. During clonidine infusion peripheral coronary pressure increased in a dose-dependent fashion. During infusion of 10^{-5} clonidine peripheral coronary pressure averaged 56 ± 3 mm Hg. Total collateral resistance averaged .35 ± .03 RU under control conditions and increased to .48 ± .06 RU at the highest dose of clonidine. This was accompanied by a decrease in collateral flow from 227 ± 27 ml/min/100 g under control conditions to 178 ± 41 ml/min/100 g at the highest dose of clonidine.

Transcollateral resistance averaged .17 ± .03 RU under control conditions and did not change during either dose of clonidine administered.

Response to Vasopressin (Table 3). Vasopressin produced intense constriction of the vasculature in the normally perfused myocardium, reducing flow by 59%. Collateral flow per 100 g of myocardium decreased from 186 to 88 ml/min. Peripheral coronary pressure during vasopressin infusion increased from 39 ± 3 to 45 ± 4 mm Hg. Both total collateral resistance and transcollateral resistance increased by more than two fold.

Isolated Vascular Ring Studies

Optimal resting tension averaged 12.7 ± 1.2 g for the left anterior descending, 2.2 ± 0.7 g for the collaterals, and 2.8 ± 0.8 g for the vessels immediately adjacent to the collaterals (Table 4).

Responses to Non-α-Adrenergic Agonists (Table 4). Responses to 100 mm KCl averaged 8.7 ± 1.0, 3.3 ± 0.7, and 2.0 ± 1.0 g for the LAD, vessels adjacent to the collaterals, and collateral vessels respectively. Prostaglandin F_2α (3·10^{-6} M) and angiotensin II (3·10^{-7} M) also caused constriction of the LAD and collaterals. Vasopressin (2·10^{-7} M) caused a significantly greater constrictor response in the collateral vessels than in either the left anterior descending or the vessels immediately adjacent to the collaterals.

Responses to α-Adrenergic Agonists. Responses to α-adrenergic agonists are shown in Table 4 and Figure 5. Phenylephrine caused dose-dependent constrictions in the left anterior descending. The peak LAD response to phenylephrine was 21.3 ± 4.2% of the response to 100 mM KCl (ED_50 = 2.0·10^{-6} M). The peak left anterior descending response to clonidine averaged 5.6 ± 2.4% of the response to 100 mM KCl (ED_50 = 3·10^{-6} M). In contrast, neither clonidine nor phenylephrine caused constriction in either the collateral segments or the segments immediately adjacent to the collaterals.

Discussion

In these studies we used two different experimental preparations to examine the effect of α-adrenergic agonists on coronary collateral vascular tone. In isolated heart studies, α-adrenergic agonists produced constriction of noncollateral vessels but did not change transcollateral resistance. In isolated vascular ring studies, coronary collaterals were unresponsive to either α₁- or α₂-adrenergic agonists. In both preparations, nonadrenergic agonists produced coronary collateral constriction. In the isolated heart preparation vasopressin produced greater than a two-fold increase in transcollateral resistance. Isolated rings of collateral vessels responded to vasopressin, prostaglandin F_2α, angiotensin II, and KCl. Thus, nonadrenergic agonists in both preparations constrict mature coronary collaterals. Taken together these two groups of studies demonstrate that mature canine coronary collaterals do not contain functioning α-adrenergic receptors but may be responsive to other constrictor agents. It may be inferred from these studies that resistance of mature collaterals is not regulated by α-adrenergic mechanisms.

During exercise or other physiologic stress, there is an increase in sympathetic efferent activity to the heart and an increase in circulating catecholamines. These factors, in addition to peripheral adaptive mechanisms, increase myocardial contractility and may worsen myocardial ischemia in the setting of chronic coronary occlusion by increasing myocardial oxygen demand. In addition, if mature collateral vessels contained post-junctional α-adrenergic receptors, of either the α₁ or the α₂ subtype, activation of these receptors might produce collateral constriction and increase collateral resistance. This would further worsen myocardial ischemia by limiting oxygen supply. Our studies
produced significant increases in normal zone resistance while collateral resistance; however, phenylephrine also sig-
ificant increases in normal zone resistance while collateral resistance remained unchanged.

**Relationship to Prior Work**

Previous work examining responses of mature coronary collaterals to adrenergic stimuli has been scant. Sink et al performed studies on dogs 4 weeks after ameroid placement. During cardiopulmonary bypass and ventricular fibrillation collateral resistance was measured with radioactive microspheres. Phenylephrine infusion was observed to increase coronary collateral resistance; however, phenylephrine also significantly increased flow to both the normally perfused region of myocardium and the collateral dependent region. The mechanism for this increase remains unclear as coronary driving pressure remained constant (80 mm Hg). In contrast to the commonly held notion that phenylephrine possesses only α-agonistic properties, there are at least two studies that suggest phenylephrine in high doses activates β receptors and may increase myocardial contractility. It is conceivable that in Sink et al's study phenylephrine activated β receptors and thus produced coronary vasodilation by stimulation of vascular β receptor and by increasing myocardial metabolic demand. Thus, the resultant in-
crease in collateral resistance may have been related to changes in extravascular compressive forces associated with increasing the myocardial inotropic state. Extravascular compressive forces have been shown to play an important role in regulating collateral resistance and may have produced the observed increase in total collateral resistance.

Schaper administered pharmacologic doses of the α₁-adrenergic agonist methoxamine to isolated hearts with mature collaterals stimulated by 6 months of ameroid constrictor placement. In these studies, methoxamine did not decrease peripheral coronary pressure. It was assumed that peripheral coronary pressure would decrease if methoxamine decreased collateral flow. Collateral flow was not measured, and it is conceivable that subtle changes in collateral resistance not detectable by alterations in peripheral coronary pressure may have occurred.

**Advantages of the Methods Used**

We studied animals at least 6 months after placement of ameroid constrictors. Schaper has shown that collateral growth is an active process and that while collateral resistance declines significantly 6 weeks following placement of an ameroid constrictor, continued maturation of the vessel wall occurs for approximately 6 months. At this time, the appearance of the vessel is similar to that of other epicardial coronary arteries. Thus, the vascular smooth muscle present has undergone considerable hyperplasia by 6 months. While it is conceivable that longer periods of maturation may be necessary for α-adrenergic receptor function to develop, the duration of ameroid constriction in our study ranged from 6 to 10 months and the findings were similar in all animals studied.

We used an isolated heart preparation to measure collateral and normal zone resistance. This preparation is useful because aortic pressure can be controlled, peripheral coronary pressure is easily measured, and extravascular compressive forces are minimized. Because extravascular forces and aortic pressure are major determinants of collateral flow, it is critical that these factors be controlled during pharmacologic interventions.

<table>
<thead>
<tr>
<th>Methoxamine group</th>
<th>Aortic pressure (mm Hg)</th>
<th>Peripheral coronary pressure (mm Hg)</th>
<th>Collateral flow (ml/min-100 g)</th>
<th>Total collateral resistance (mm Hg/ml/min-100 g)</th>
<th>Transcollateral resistance (mm Hg/ml/min-100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>82 ± 1</td>
<td>41 ± 2</td>
<td>174 ± 27</td>
<td>0.53 ± .08</td>
<td>0.24 ± .02</td>
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<td>10⁻⁷ M</td>
<td>81 ± 1</td>
<td>46 ± 2*</td>
<td>172 ± 29</td>
<td>0.51 ± .10</td>
<td>0.22 ± .03</td>
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<tr>
<td>10⁻⁵ M</td>
<td>80 ± 1</td>
<td>49 ± 3*</td>
<td>140 ± 25*</td>
<td>0.58 ± .05</td>
<td>0.26 ± .05</td>
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<tr>
<td>Clonidine group</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>81 ± 1</td>
<td>49 ± 3</td>
<td>227 ± 27</td>
<td>0.35 ± .03</td>
<td>0.17 ± .03</td>
</tr>
<tr>
<td>10⁻⁹ M</td>
<td>83 ± 1</td>
<td>53 ± 3*</td>
<td>181 ± 23*</td>
<td>0.39 ± .04*</td>
<td>0.18 ± .03</td>
</tr>
<tr>
<td>10⁻⁵ M</td>
<td>82 ± 1</td>
<td>56 ± 3*</td>
<td>178 ± 41*</td>
<td>0.48 ± .06*</td>
<td>0.16 ± .05</td>
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* p < .05 from control.
The rationale for the use of adenosine in these studies was based on two concepts: 1) Under basal flow conditions, there is only a minimal pressure gradient (usually less than 10 mm Hg) across mature collaterals and there are no differences in regional perfusion between the normal zone and collateral dependent regions of myocardium. These are the two parameters used to calculate collateral resistance. Thus, subtle changes in collateral diameter might alter the pressure gradient only minimally and produce minimal or no change in flow to the collateral dependent zone. During adenosine vasodilatation, the transcollateral pressure gradient is magnified and flow to the collateral dependent zone is significantly less than that to the normally perfused region of myocardium. Under these circumstances, subtle decreases in collateral diameter due to changes in collateral smooth muscle tone are more likely to be detected. 2) In contrast to the commonly held notion that intense vasodilation may abolish vasoconstrictor responses to α agonists, recent work suggests that α-mediated vasoconstriction may be observed and in fact enhanced in the presence of vasodilation. Johannsen et al showed that intracoronary phenylephrine and sympathetic nerve stimulation caused coronary constriction in the presence of adenosine vasodilatation. In a recent study by Heusch and Deussen, sympathetic nerve stimulation produced vasoconstriction only in the presence of metabolic vasodilation secondary to a severe coronary stenosis.

It might be postulated that other α-adrenergic agonists might produce constriction of coronary collaterals. In our opinion, this is unlikely. First, the α-adrenergic agonist used in the isolated heart experiments was different than that used in the isolated ring studies (methoxamine vs. phenylephrine). Neither produced collateral constriction. Second, in one preliminary isolated heart experiment, we examined responses to phenylephrine infusion. During adenosine infusion only, normal zone and transcollateral resistance were 0.34 and 0.16 RU respectively. Phenylephrine infusion (200 μg/minute) increased normal zone resistance to 0.67 but did not increase transcollateral resistance. In addition, in one isolated vessel experiment, noradrenaline (10^-9 to 10^-8 M) after propranolol (10^-6 M) administration failed to constrict segments of mature collaterals. Thus, we believe the lack of adrenergic responses in collateral vessels was not due to the agonist selected but due to diminished responsiveness of collateral vascular smooth muscle.

We used a variation of the shadow technique initially employed by Patterson and Kirk to identify the collateral dependent region of myocardium. When this technique is used in hearts with unstimulated native collaterals, it is generally possible to identify numerous segments of tissue with virtually no microspheres present in the collateral dependent region. In this study, only 8 of 13 hearts had segments with flows calculated to be less than 1 ml/min·100 g of tissue obtained during the shadow injection. Thus, in the presence of mature collaterals with low resistances, there is a tendency for microspheres to enter the collateral dependent region even when the collateral dependent region is perfused with microsphere-free blood at a pressure equal to aortic pressure. We attributed this to shunting of microspheres across the low resistance collaterals even when no or a minimal pressure gradient was present. Another possible explanation relates to work of Downey et al. These investigators showed that 2 weeks after ameroid constrictor placement, during diversion of retrograde flow from the recipient vessel, microspheres injected into the left atrium reached the collateral dependent region. It was concluded that a portion of the stimulated collaterals entered the recipient vessels beyond a major site of resistance and that the pressure at the origin of the stimulated collaterals was greater than the pressure at the site of their insertion. If this phenomenon had occurred in our studies, it would have explained why some microspheres entered the collateral dependent region during the shadow microsphere injection when

### Table 3. Responses to Vasopressin (n = 4)

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<tr>
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<th>Aortic pressure (mm Hg)</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>80 ± 1</td>
<td>39 ± 3</td>
<td>273 ± 82</td>
<td>186 ± 48</td>
<td>0.52 ± 0.13</td>
<td>0.24 ± 0.05</td>
</tr>
<tr>
<td>Vasopressin</td>
<td>80 ± 1</td>
<td>45 ± 4*</td>
<td>111 ± 29*</td>
<td>88 ± 26*</td>
<td>1.24 ± 0.59*</td>
<td>0.53 ± 0.28*</td>
</tr>
</tbody>
</table>

*p < .05 from control.

### Table 4. Optimal Resting Tension and Responses to α- and Non-α-Adrenergic Agonists

<table>
<thead>
<tr>
<th></th>
<th>Optimal tension (g)</th>
<th>KCL (g)</th>
<th>PGF2α (3 × 10^-7 M) (% KCl)</th>
<th>Angiotension II (3 × 10^-7 M) (% KCl)</th>
<th>Vasopressin (2 × 10^-7 M) (% KCl)</th>
<th>Phenylephrine peak response (% KCl)</th>
<th>Clonidine peak response (% KCl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left anterior descending</td>
<td>12.4 ± 1.0</td>
<td>8.0 ± 1.1</td>
<td>37 ± 6</td>
<td>70 ± 16</td>
<td>5.0 ± 1</td>
<td>21.3 ± 4.2</td>
<td>5.6 ± 2.4</td>
</tr>
<tr>
<td>Adjacent vessel</td>
<td>3.0 ± 0.7</td>
<td>3.4 ± 0.6</td>
<td>19 ± 8</td>
<td>61 ± 11</td>
<td>19 ± 5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Collateral vessels</td>
<td>2.4 ± 10.6</td>
<td>2.3 ± 0.9</td>
<td>16 ± 8</td>
<td>30 ± 6</td>
<td>90 ± 30</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
the circumflex was perfused with microsphere-free blood at a pressure equal to aortic pressure. It was still possible for us clearly to demarcate the collateral dependent zone. As shown in Figure 2, a sharp transition was present between the high concentration of microspheres in the normally perfused region and the lower concentration of microspheres in the collateral dependent region.

In the isolated vessel studies, the collaterals demonstrated marked constrictor responses to vasopressin while the left anterior descending and adjacent vessels constricted only minimally. While vasopressin is known to produce marked increases in arteriolar resistance, its effect on large vessels has been less well known to produce marked increases in arteriolar resistance,28 its effect on large vessels has been less well studied. Recently, Katusic et al have shown that vasopressin produces relaxation rather than constriction in large coronary arteries and that this response is in large part dependent on the presence of endothelium.29 These data would suggest that there is substantial variability in the response to vasopressin between large and smaller coronary arteries. It is of interest that the collaterals (and to a lesser extent the adjacent vessels) in our study demonstrated a constrictor response to vasopressin similar to that observed in resistance vessels reported by others.28 Despite these observations, we do not believe that our work conclusively demonstrates an important role for vasopressin in regulating collateral resistance. The concentration of vasopressin administered in these studies was several fold greater than that encountered under physiological conditions. Smaller doses of vasopressin did not consistently produce constrictor responses in any of the isolated vessels we studied.

Recently, Yoshida et al have shown that diffusible indicators may diffuse into or be removed from an ischemic region of myocardium when microsphere-measured collateral flow has been diverted by opening a cannula placed in the occluded vessel.30 These investigators have concluded that there is an occult component of ischemic zone perfusion that is not measured by radioactive microspheres. The exact source of this perfusion remains undefined. Based on our studies using radioactive microspheres, we cannot exclude an effect of α-adrenergic agonists on this component of perfusion to ischemic myocardium.

In our isolated heart studies, we found the α-adrenergic agonist methoxamine and the α2-adrenergic agonist clonidine produced similar amounts of vasconstriction in the normally perfused myocardium. In contrast, in the vascular ring studies clonidine produced only weak constrictor responses in the left anterior descending while phenylephrine, a selective α1-adrenergic agonist produced substantial constriction. This apparent discrepancy relates to the portion of the coronary circulation affected by each of the agonists studied and the methodology employed. In the intact heart studies, the changes in normal zone resistance were most likely due to the agonist activity on small resistance vessels. Several recent studies have suggested that the predominant α receptor in the coronary microvasculature is of the α1 subtype.11,28 In contrast, isolated vascular ring studies have shown that the predominant α receptor in the proximal circumflex and left anterior descending is of the α2 subtype.11,12 Our isolated vessel studies are compatible with these findings.

In summary: These studies have demonstrated a lack of functioning α-adrenergic receptors in mature collateral vascular smooth muscle. Based on these observations it seems unlikely that mature collateral resistance can be modulated by α-adrenergic influences. These observations do not exclude the possibility that α-adrenergic receptors are present but are either uncoupled or weakly coupled to underlying effector mechanisms.

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References

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