Progressive Coronary Vasoconstriction during Relative Ischemia in Canine Myocardium

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SUMMARY. Under certain conditions, a progressive increase in vascular resistance occurs within ischemic myocardium during the first 3 hours after coronary artery stenosis. We tested the hypothesis that the increased resistance is due to local release of a vasoconstrictor substance in the ischemic region. Relative ischemia was produced in anesthetized dogs by a combination of acute coronary arterial stenosis and increased myocardial metabolism. A hydraulic occluder on the left anterior descending coronary artery was adjusted to maintain distal left anterior descending coronary artery pressure at 50 mm Hg during atrial pacing at 180 beats/min. Myocardial blood flows were measured with 15 fL microspheres after 30 and 180 minutes of relative ischemia. During this time, transmural left anterior descending coronary artery flow decreased by an average of 30%. Infusion of adenosine into the ischemic region after 180 minutes produced a 74 ± 17% increase in flow, indicating the presence of substantial vasodilator reserve. Stimulation of metabolism in the ischemic area by norepinephrine infusion increased flow by 54 ± 17%. Indomethacin treatment after 180 minutes of ischemia, however, caused left anterior descending coronary artery flow to decrease by 22 ± 8%. α-Receptor blockade (phenoxybenzamine) in the left anterior descending coronary artery bed prior to ischemia prevented the flow decrease between 30 and 180 minutes. Phentolamine administration after 180 minutes also resulted in increased left anterior descending coronary artery flow. However, the addition of propranolol to phenoxybenzamine-treated hearts reversed the vasodilatory influence of phenoxybenzamine, resulting in a flow decrease within the ischemic region similar to that seen without any pharmacological intervention. We conclude that during 3 hours of relative ischemia in this preparation, blood vessels in the ischemic area (1) are not maximally dilated, and (2) undergo a progressive vasoconstriction via an unknown mechanism. Phenoxybenzamine prevents the progressive vasoconstriction, apparently by increasing norepinephrine release. (Circ Res 51: 411–420, 1982)
Arterial pH was regulated by the rate of an intravenous infusion of isotonic sodium bicarbonate. Hematocrit was kept within normal limits by administration of a 6% dextran (mol. wt. 60,000) solution. We measured temperature in the esophagus, and used heating pads if the temperature fell below 38°C.

The right femoral vein was cannulated for the administration of intravenous fluids and anesthetic supplements. Mean arterial pressure (MAP) was measured in the thoracic aorta by means of a catheter advanced through a femoral or brachial artery. In radioactive microsphere experiments, a cannula for the collection of reference flow blood samples was placed in the right femoral artery. In some experiments, a Teflon catheter for the measurement of left ventricular end-diastolic pressure was placed in the left ventricle by way of a carotid or a femoral artery.

We exposed the heart through a left lateral thoracotomy in the 5th intercostal space and constructed a pericardial cradle. In microsphere experiments, a polyethylene catheter for administration of the spheres was placed in the left atrial appendage. We isolated a segment of the left anterior descending coronary artery (LAD) 1-3 cm from its origin, and placed a hydraulic occluder around the vessel. The occluder was connected to a syringe pump to facilitate fine control of the degree of inflation. Just distal to the occluder, we inserted a thin catheter constructed according to the method of Herd and Barger (1964). This catheter had a side hole within the artery and two ends emerging from the artery. We measured pressure through one end of this catheter (we will refer to this as distal LAD pressure), and we used the other end of the catheter to make drug infusions directly into the LAD vascular bed. Platinum pacing electrodes were inserted into the left atrial appendage, and the heart was paced at 180 beats/min when spontaneous heart rate was below this value. Pacing stimuli from a Grass stimulator consisted of square wave pulses of 1.5 V and 6-10 msec duration. Pacing assured an elevated myocardial metabolism in all preparations.

In some experiments, we collected coronary venous blood samples from the LAD bed for analysis of plasma lactate and potassium ion concentrations. A 20-gauge Teflon catheter (Becton-Dickinson I.V. Cath) was inserted into a branch of the great cardiac vein and advanced well into the area distal to the occluder. This catheter did not occlude the vein. Blood was allowed to drip continuously from the catheter (2-4 ml/min) through tubing near heart level. These animals were heparinized (750 units/kg), and blood not used for sampling was returned to the dog. The hemodynamic results from heparinized dogs did not differ from those of unheparinized dogs. Blood samples were collected into tubes containing sodium fluoride to arrest lactate metabolism. They were then centrifuged and the plasma was separated for later analysis. Potassium ion concentration was measured via flame photometry or a K+-sensitive electrode (Orion Biomedical). Lactate was measured using an enzymatic assay (Sigma).

Arterial pressure and distal LAD pressure were recorded continuously by means of Statham pressure transducers and a Grass model 7 Polygraph. Heart rate was obtained from a tachograph connected to the arterial pressure channel.

**Experimental Protocols**

Relative myocardial ischemia was produced by inflating the LAD cuff until mean distal LAD pressure fell to approximately 50 mm Hg. We maintained this pressure constant throughout the ischemic period by making frequent adjustments of cuff inflation, using the syringe pump. Myocardial blood flows were measured with radioactive microspheres after 30 minutes of ischemia, and again after 180 minutes of ischemia. We chose 30 minutes for the first flow measurement because Guyton et al. (1977) showed, in a very similar preparation, that flow in the ischemic region increases slightly during the first 30 minutes. Comparison of the flows at 30 and 180 minutes allowed us to determine whether flow in the ischemic region fell during this period.

Several series of experiments were performed in an attempt to determine the mechanism of this progressive flow decrease. In most of these series, we made a pharmacological intervention after 180 minutes of ischemia and observed the effect on blood flow in the LAD bed. Drugs were administered either intracoronarily or intravenously. Unless otherwise stated, all intracoronary infusions were at a rate of 3.5 ml/min. In preliminary experiments using dye infusion, this infusion rate resulted in uniform staining of the distal LAD bed and did not raise distal LAD pressure by more than 1 mm Hg.

**Adenosine Series (n = 6)**

In order to determine whether the ischemic area was maximally dilated after 180 minutes of relative ischemia, we infused adenosine (2-3 μg/kg per min) into the distal LAD bed. A final flow measurement was made after 15 minutes of adenosine infusion.

**Norepinephrine Series (n = 5)**

After 180 minutes of ischemia, norepinephrine (1-2 μg/min) was infused into the distal LAD for 5-15 minutes, followed by a final flow measurement. In a separate series of three dogs, we assessed the effect of 180 minutes of relative ischemia and subsequent norepinephrine infusion on contractile force development in the LAD bed. We sewed a Walton-Brodie strain gauge arch to the subepicardial surface within the distal LAD bed before initiating ischemia. Contractile force development during the ischemic period was expressed as a percentage of the force developed just before beginning ischemia.

**Indomethacin Series (n = 7)**

Following 180 minutes of ischemia, indomethacin (5 mg/kg) was given intravenously. A final flow measurement was made 30 min later.

**Phenoxy benzamine Series (n = 7)**

Before beginning the ischemic period, we infused phenoxy benzamine into the LAD bed. A total dose of 0.25 mg/kg was infused over a 30-minute period at a flow rate of 1 ml/min. After discontinuing the phenoxy benzamine infusion, a microsphere blood flow measurement was made. Relative ischemia then was induced and flows were measured as usual after 30 and 180 minutes. A second dose of phenoxy benzamine, identical to the first, was administered between the 90- and 120-minute points of the ischemic period in order to maintain α-blockade. In order to produce a sufficient reduction in blood flow (see below for acceptance criteria) in this series of dogs, we found it necessary to reduce distal LAD pressure to a lower level (35 ± 1 mm Hg) than in the other series. Preliminary experiments were performed in five anesthetized dogs to establish the effectiveness of coronary α-blockade, using this regimen and dosage of phenoxy benzamine. An electromagnetic flow probe (Zepeda Instruments) and hydraulic occluder were placed around the LAD, and a coronary catheter was in-
serted just distal to the occluder. β-Adrenergic blockade was achieved with intravenous propranolol (3 mg/kg, plus infusion of 1 mg/kg per hr). Bolus intracoronary injections of norepinephrine (1–4 μg) resulted in coronary vasoconstriction (peak flow decreases of 20–40%). Intracoronary phenoxybenzamine infusion as described above completely abolished this vasoconstrictor response to injected norepinephrine during a subsequent 3-hour period.

Phenoxybenzamine Plus Propranolol Series (n = 6)

Phenoxybenzamine in the dose mentioned above was infused into the LAD bed prior to beginning the ischemic period. Ischemia then was begun (P_LAD = 35 mm Hg) and flows were measured after 30 and 180 minutes. β-Adrenergic blockade then was produced in the LAD bed by an intracoronary infusion of propranolol (60 μg/ml at 1.0 ml/min) for 15 minutes. Next, propranolol infusion was discontinued and a final flow measurement made. β-Adrenergic blockade then was checked by observing the response of distal LAD pressure to bolus intracoronary injections of isoproterenol (1 μg) and adenosine (400 μg). Both of these agents normally produced a decrease in LAD pressure indicative of vasodilation under restricted flow conditions. Propranolol treatment blocked the response to isoproterenol but not to adenosine.

Phentolamine Series (n = 9)

Phentolamine (0.25–0.5 mg/kg) was given intravenously after 180 minutes of ischemia. We chose intravenous rather than intracoronary administration because we found that, after 180 minutes of ischemia, intracoronary phentolamine usually caused ventricular fibrillation. In two dogs, phentolamine caused a significant drop in systemic pressure. In these dogs, we tightened a snare around the descending thoracic aorta in order to return mean proximal aortic pressure to the value observed just before giving phentolamine. Flow was measured 5 minutes after phentolamine administration. α-Blockade was tested after the final flow measurement by an iv injection of 1 mg methoxamine, which produced little or no response.

Time Control Series (n = 4)

The purpose of this series was to establish the stability of myocardial blood flow after 180 minutes of relative ischemia in this preparation with no pharmacological intervention. After the flow measurement at 180 minutes, we waited 15 additional minutes and measured flow again. Isotonic saline was infused into the coronary catheter during all three flow measurements.

Short-Term Adenosine Series (n = 5)

The purpose of this series was to determine the effect of adenosine on ischemic bed flow after a much shorter period of ischemia. We made the first microsphere flow measurement under conditions of resting flow (no stenosis). The occluder then was inflated to reduce distal LAD pressure to approximately 50 mm Hg, and after 10 minutes, a second microsphere flow measurement was made. Without releasing the stenosis, we then began an infusion of adenosine into the LAD at 2–3 μg/kg per min. During adenosine infusion, the stenosis was adjusted to maintain the same distal LAD pressure as existed just before adenosine infusion. Flow was measured again after 10 minutes of adenosine infusion.

All drugs except indomethacin were dissolved in isotonic saline within 1 hour of administration. Indomethacin was dissolved in 50 ml of saline containing 0.2% sodium bicarbonate. When a microsphere flow measurement was made during an intracoronary drug infusion, isotonic saline was infused during the other two flow measurements as a control. At the conclusion of the experiment, we stained the ischemic area by infusing a crystal violet dye solution through the coronary catheter. The outlines of this stain were followed when sectioning the heart for microsphere flow determinations.

Acceptance Criteria

Because we used radioactive microspheres to measure coronary blood flow, we could not know the degree of ischemia created by the LAD stenosis until well after each experiment was completed. In some preparations, the reduction in distal LAD pressure did not reduce LAD bed flow. We therefore needed a criterion for an acceptable degree of relative ischemia. Using flow in the untouched circumflex bed as an index of flow demand, we accepted preparations in which the ratio of flows (circumflex/transmural bed) decreased by 20% or less when 75% of circumflex transmural flow after 30 minutes of stenosis. This criterion resulted in the rejection of 10 of 56 dogs.

In 85% of the preparations meeting this criterion of ischemia, flow in the LAD bed decreased between the 30- and 180-minute measurements. This confirms the experience of Guyton et al. (1977) who used a very similar preparation. The flow decrease was the phenomenon we wished to study, and so we have not included the results of the 15% of experiments in which it did not occur. However, inclusion of these experiments changes only the magnitude, not the direction or statistical significance of the changes we observed. In the discussion, we will deal with possible reasons for the variability of the time-dependent flow decrease. Of course, this acceptance criterion was not applied when we attempted to block the time-dependent flow decrease in the phenoxybenzamine series.

Radioactive Microsphere Techniques

We used 15-μm radioactive microspheres to measure regional myocardial blood flows. The nuclides chosen were 85Sr, 141Ce, and 46Sc or 51Cr. The stock microsphere suspension as obtained from the 3M Company had an initial activity of 0.05 mCi/ml, and the suspending agent was 10% dextran. The stock suspension was agitated mechanically with a Vortex mixer, then sonicated in an ultrasonic cleaner with further intermittent agitation for at least 15 minutes prior to use. Approximately 1.5 ml of the stock solution (2–4 × 10⁶ spheres) was drawn into a syringe and diluted to 10 ml with warm (40°C) saline. We periodically observed this suspension under a microscope to assure ourselves that this mixing protocol resulted in adequate dispersion of the microspheres. This suspension was then infused into the left atrium at 12.1 ml/min with a Harvard syringe pump. The infusion tubing was flushed with 5 ml of warm saline, which also was infused with the syringe pump.

We used an arterial reference sample in order to calculate flows in ml/min per 100 g. The reference blood sample withdrawal pump was turned on just before microsphere infusion was started. Blood was withdrawn from the right femoral artery at a known rate (6.3 or 7.0 ml/min) until 2 minutes after completing the flush of the microsphere infusion line. Thus, blood was withdrawn for a total of approximately 4 minutes.

The reference blood sample was transferred from the collecting syringe into plastic scintillation vials. The
blood in these vials was hemolyzed and the protein precipitated by the addition of approximately 5 ml of 2 N KOH in methanol. We then placed the vials in a 70°C oven and evaporated as much fluid as possible. The purpose of this procedure was to get the microspheres to the bottom of the scintillation vials, thereby minimizing potential errors due to differences in sample geometry within the counting well.

The left ventricle was sectioned into several pieces for determination of regional flows. Epicardial fat and large vessels were removed. We then divided the ventricle into sections from the ischemic LAD bed and from the circumflex bed. The borders of the ischemic LAD bed were defined by the dye which had been infused prior to sacrifice. We sliced the circumflex region into three equal layers along lines perpendicular to the base-apex axis. The ischemic area was divided into a "core" region and two "border" regions. Each layer then was subdivided into subepicardial and subendocardial halves. Pieces of myocardium were placed in tared plastic scintillation vials and weighed. Samples then were digested in methanolic KOH, as described earlier.

The myocardial samples and reference blood samples were counted in a Packard Auto-Gamma Scintillation Spectrometer. Samples were counted until at least 9000 counts had been recorded for each nuclide in the sample. A computer program was used to correct the counts for background and overlap from other nuclides, and to calculate flows in ml/min per 100 g. Although flow in the LAD "core" region often was lower than in "border" regions, the directional changes in flow were identical. We therefore made no distinction between these areas and have reported the combined flow measurement.

Although we measured blood flow at three different times in each experimental series, the critical flow measurements in each case were the final pair, which measured the effect of the intervention. Changes in flow between the final pair of measurements were analyzed using the paired t-statistic. Changes in flow between the first two flow measurements in each series were not analyzed statistically because we selected only those dogs which showed a flow increase in the LAD bed during that period. Changes in the endo/epi flow ratio in the LAD bed, in left ventricular end-diastolic pressure, and in potassium and lactate flux were determined in tared plastic scintillation vials and weighed. Samples then were digested in methanolic KOH, as described earlier.

Results

The results of adenosine infusion after prolonged reduction in perfusion pressure are presented in Figure 1. Between 30 and 180 minutes transmural LAD bed flow decreased by 29%. Subsequent adenosine infusion increased this flow dramatically. Adenosine increased subepicardial flow from 64 ± 3 to 119 ± 15 (P < 0.05) and subendocardial flow from 26 ± 5 to 37 ± 9 ml/min/100g (P = 0.05). This dose of adenosine had no effect on systemic arterial pressure. Flow in nonischemic areas did not show the time-dependent decrease, nor was it affected by the adenosine infusion (Table 1). Adenosine infused after only 20 minutes of relative ischemia (short-term adenosine series, Fig. 2) did not significantly increase transmural LAD bed flow. To the extent that flow did rise in this case, the increase was entirely subepicardial. Comparison of the short-term vs. the long-term adenosine results indicates that the increase in flow due to adenosine (an indication of vasodilator reserve) was at least as large after 180 minutes as after 20 minutes of relative ischemia.

Norepinephrine infusion into the LAD bed after 180 minutes of relative ischemia resulted in a significant increase in LAD flow (Fig. 3). There was a tendency for norepinephrine to increase flow in nonischemic areas, as well (Table 1), due to effects on systemic pressure and/or recirculation of the drug. In the three dogs equipped with Walton-Brodie strain gauge arches, contractile force development in the ischemic region fell to 56 ± 12% of control within 5 minutes after imposition of the stenosis. After 180 minutes, this value was 38 ± 14% of control. Subsequent norepinephrine infusion into the ischemic region increased flow to 108 ± 11% of control.

The effect of indomethacin on flow in the ischemic region is shown in Figure 4. Indomethacin definitely did not reverse the decline in ischemic bed flow; instead, it reduced the flow even further. In nonischemic areas, indomethacin increased flow, perhaps as a result of increased systemic pressure (Table 1).

When hearts were treated with phenoxybenzamine before we caused ischemia, we found that distal LAD pressure reduction to 50 mm Hg seldom produced flows low enough to meet our criterion of initial ischemia. In three such animals, the LAD flow after 30 minutes of stenosis was 92 ± 18, vs. 99 ± 14 ml/min/100 g in the nonischemic circumflex bed. We therefore reduced distal LAD pressure to lower values (35 ± 1 mm Hg) in subsequent animals. In both series in which phenoxybenzamine was administered, the flow decrease between 30 and 180 minutes in the ischemic area was completely abolished (Table 2). When propranolol was added to the ischemic region of these hearts after 180 minutes, flow decreased.
Time control
n = 4
180 113 ± 4 49 ± 1 144 ± 18 46 ± 5 0.30 ± 0.06 17 ± 2*
195 115 ± 6 51 ± 1 151 ± 19 48 ± 6 0.33 ± 0.11 17 ± 2

Time = duration of relative ischemia, P<sub>LAD</sub> = distal LAD pressure, ENDO/EPI = subendocardial/subepicardial flow ratio in the LAD bed, LVEDP = left ventricular end-diastolic pressure. A drug abbreviation beside a time value indicates that the drug was present during the flow measurement. ADO = adenosine, NE = norepinephrine, INDO = indomethacin, PT = phenolamine.

* = P < 0.05 compared to the previous time point. All values are means ± standard errors. No statistical comparison has been made between the LAD flows at 30 minutes vs. 180 minutes because we selected only preparations in which LAD flow declined during this time. If such a comparison had been made, the difference would of course be statistically significant.

The purpose of this study was to investigate the cause of the progressive decrease in blood flow which often occurs during the first few hours of maintained myocardial ischemia. The first report of such a decline in flow was made by Grayson and co-workers (Grayson and Lapin, 1966; Grayson et al., 1968). Using implanted thermocouples to measure changes in myocardial thermoconductivity (an index of blood flow), they reported that, following coronary occlusion, flow in the ischemic region fell sharply over a 4- to 5-hour period, and that this decline in flow could be prevented by adrenergic blockade. This observation of a decline in flow following coronary occlusion could not be repeated, however, using different techniques of measuring flow (Redding and Rees, 1968; Pasyk et al., 1971; Bishop et al., 1971; Marshall et al., 1974). However, three groups, including our own, have found that if a coronary artery is only partially occluded, a progressive decline in flow within the ischemic region usually is observed. Guyton et al. (1977) reported that blood flow declined over a 3-hour period in a region distal to a fixed coronary stenosis. The decline in flow may have resulted from vasodilation within nonischemic regions, which initiated a “steal” of collateral flow away from the ischemic area. The decline in flow also occurred when the stenosis was varied to maintain a constant peripheral coronary arterial pressure, however, indicating that increased vascular resistance within the ischemic region had occurred. Frame and Powell (1976) also

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TABLE 1
Hemodynamic Data

| Series      | Time (min) | MAP (mm Hg) | P<sub>LAD</sub> (mm Hg) | Circumflex flow
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<tr>
<td></td>
<td></td>
<td>LVEDP (mm Hg)</td>
<td>LAD flow (ml/min per 100 g)</td>
<td>LAD flow (ml/min per 100 g)</td>
</tr>
<tr>
<td>Short-term</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>n = 5</td>
<td>180</td>
<td>120 ± 5</td>
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<td>141 ± 15</td>
</tr>
<tr>
<td>n = 8</td>
<td>195</td>
<td>118 ± 5</td>
<td>52 ± 1</td>
<td>141 ± 15</td>
</tr>
<tr>
<td>n = 9</td>
<td>180</td>
<td>124 ± 2</td>
<td>48 ± 1</td>
<td>121 ± 7</td>
</tr>
<tr>
<td>Time control</td>
<td>180</td>
<td>113 ± 4</td>
<td>49 ± 1</td>
<td>148 ± 6*</td>
</tr>
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</table>

Intravenous phenolamine administration after 180 minutes of ischemia caused flow to increase in the ischemic region (Table 1). In the time control series, we found that flow in the ischemic zone did not change significantly between the flow measurements at 180 and 200 minutes. The additional time required to perform pharmacological interventions and measure flow after 180 minutes of ischemia therefore is not responsible for any of the flow changes. These data are presented in Table 1.

The data on potassium and lactate flux in the LAD bed are presented in Table 3. Lactate was neither released from the ischemic area. Potassium and lactate release occurred at all times during ischemia. The first report of such a decline but net lactate production occurred at all times during ischemia. The decline in flow also occurred when the stenosis was varied to maintain a constant peripheral coronary arterial pressure, however, indicating that increased vascular resistance within the ischemic region had occurred. Frame and Powell (1976) also...
have studied this phenomenon, using a preparation in which the cannulated LAD was perfused at constant pressure. They were able to prevent the fall in ischemic bed flow by administration of hyperosmotic mannitol solutions, suggesting that cell swelling in the ischemic area caused a passive increase in vascular resistance. However, hyperosmotic mannitol relaxes coronary vascular smooth muscle (Krishnamurty et al., 1978), and so its ability to increase flow could be due to reduced vascular tone rather than reduced cell swelling. Our results suggest that, in this preparation, the decrease in flow is due, at least in part, to increased vascular smooth muscle tone.

The preparation we have used employs a coronary artery stenosis rather than total occlusion, resulting in relative ischemia rather than the severe ischemia found after occlusion. This preparation can best be viewed as a model of situations where myocardial O₂ supply is relatively high but is insufficient to meet an even higher myocardial O₂ demand. We have documented the presence of relative ischemia by (1) the lowered ratio of ischemic bed flow to normal zone flow, (2) the switch to lactate production in the ischemic areas, and (3) the rapid reduction in contractile force which follows imposition of the stenosis.

Because of the variability in the flow reduction caused by the stenosis, we found it necessary to impose the previously mentioned acceptance criterion for initial ischemia. At least two factors may have contributed to the variability of flow reduction. First, the amount of collateral flow into the ischemic area is highly variable in the dog. Second, even though we attempted to standardize metabolism by assuring an elevated heart rate in all preparations, there may have been a variable amount of vasodilator reserve in the LAD bed depending on the prestenosis level of O₂ consumption. A distal LAD pressure of 50 mm Hg would therefore be below the autoregulatory range in most preparations. Alternatively, we might have chosen a lower distal LAD pressure. However, preliminary experiments indicated that a lower pressure increases the relative influence of ventricular compression forces on the vessels, and makes vascular smooth muscle tone a less important determinant of vascular resistance. This was undesirable, because we wanted to know...
the potential for changes in vascular smooth muscle tone in the ischemic area. With our acceptance criterion of a LAD transmural flow less than 75% of circumflex transmural flow after 30 minutes, only the subendocardium became ischemic in some preparations. Therefore, we also analyzed the data using the acceptance criterion that LAD subepicardial flow must be less than 75% of circumflex supracardial flow at 30 minutes. Although this more stringent criterion of ischemia caused the rejection of more experiments, the results do not differ from those already reported.

We have made the assumption that changes in ischemic bed flow reflect changes in vascular caliber within the bed. This assumption is valid only if changes in the other determinants of flow are insignificant. The determinants of flow are the pressure difference across the bed, and the resistance to flow determined by the physical properties of blood and by vessel caliber. The pressure difference is distal LAD pressure minus outflow pressure. We have held distal LAD pressure constant by adjusting the inflation of the periarterial cuff. The physical location of outflow pressure in the coronary circulation is not agreed upon, but its absolute value is probably well above right atrial pressure, and may be determined by intramyocardial pressure or, in the subendocardium, by intraventricular cavitary pressure (Downey and Kirk, 1975; Munch and Downey, 1980). This probably is the reason for the relatively high zero flow intercept in coronary pressure-flow diagrams (Bellamy, 1978). We have measured left ventricular end-diastolic pressure as an index of outflow pressure in three of the groups of experiments reported here (Table 1). It often increased during a 180-minute period of relative ischemia, but the drug interventions which increased flow caused no systematic decrease in end-diastolic pressure. Thus, we doubt that changes in outflow pressure are the major factor responsible for the changes in flow we have observed. Changes in extravascular compression within the ischemic region may, however, be poorly reflected by changes in

<table>
<thead>
<tr>
<th>Series</th>
<th>Time (min)</th>
<th>MAP (mm Hg)</th>
<th>PLAD (mm Hg)</th>
<th>Circumflex flow (ml/min per 100 g)</th>
<th>LAD flow (ml/min per 100 g)</th>
<th>LAD ENDO/EP1</th>
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<tbody>
<tr>
<td>Phenoxybenzamine</td>
<td>0</td>
<td>108 ± 4</td>
<td>101 ± 4</td>
<td>92 ± 6</td>
<td>109 ± 9</td>
<td>0.91 ± 0.04</td>
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<tr>
<td>n = 7</td>
<td>30</td>
<td>104 ± 5</td>
<td>35 ± 1</td>
<td>120 ± 18</td>
<td>57 ± 5</td>
<td>0.57 ± 0.05*</td>
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<tr>
<td></td>
<td>180</td>
<td>93 ± 3</td>
<td>35 ± 1</td>
<td>122 ± 27</td>
<td>72 ± 11*</td>
<td>0.58 ± 0.08</td>
</tr>
<tr>
<td>Phenoxybenzamine + propranolol</td>
<td>30</td>
<td>101 ± 4</td>
<td>35 ± 1</td>
<td>85 ± 9</td>
<td>49 ± 5</td>
<td>0.60 ± 0.08</td>
</tr>
<tr>
<td>n = 6</td>
<td>180</td>
<td>93 ± 4</td>
<td>35 ± 1</td>
<td>100 ± 12</td>
<td>59 ± 7</td>
<td>0.58 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>195 (P)</td>
<td>92 ± 4</td>
<td>36 ± 1</td>
<td>76 ± 8</td>
<td>36 ± 5*</td>
<td>0.65 ± 0.10</td>
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</table>

All hearts in both series were pretreated with phenoxybenzamine. (P) indicates propranolol treatment. * = $P < 0.05$ compared to value at the previous time point. As before, no statistical test was done on LAD pressure or flow changes due to imposition of the stenosis.

![Figure 5. Combined results of phenoxybenzamine series and phenoxybenzamine plus propranolol series. Numbers within bars indicate number of animals at each time point. Phenoxybenzamine was present at all times; propranolol was added for flow measurement at 195 minutes. * = $P < 0.05$ compared to 180-minute flow.](http://circres.ahajournals.org/)

![Figure 6. Phentolamine series. Flows in the ischemic region after 30 minutes, 180 minutes, and in the presence of phentolamine after 190 minutes of relative ischemia. Intravenous phentolamine resulted in increased flow.](http://circres.ahajournals.org/)
coronary vasoconstriction (Bohr and Goulet, 1961; Murray et al., 1979). We inhibited the synthesis of these cyclooxygenase-dependent metabolites by giving indomethacin after 180 minutes of ischemia. We have shown previously that the use of this dose of indomethacin blocks the vasodilator effects of intracoronary arachidonate in normal myocardium (Harlan et al., 1978). Indomethacin decreased flow even further in the ischemic bed. This suggests that although there may be TXA2 and PGF2α release in the ischemic area, the net effect of cyclooxygenase activity is vasodilation, perhaps through synthesis of PGE2 and/or PGI2 (Berger et al., 1976; Needleman et al., 1978). Whereas this indomethacin result rules out TXA2 and PGF2α as the source of the time-dependent vasocconstriction, it does not rule out other vasoconstrictor arachidonic acid metabolites, such as SRS-A, whose production is not dependent on cyclooxygenase activity.

Norepinephrine is released from sympathetic fibers within ischemic myocardium, either as a direct result of ischemia (Wollenberger and Shahab, 1965) or due to an increased sympathetic discharge (Malliani et al., 1969; Brown and Malliani, 1971). Whereas norepinephrine causes a metabolically induced vasodilation in nonischemic myocardium, it has become clear in recent years that it simultaneously exerts a direct α-adrenergic vasoconstrictor influence on the coronary vasculature (Feigl, 1967; Mohrmann and Feigl, 1978; Murray and Vatner, 1979). During ischemia, this balance between metabolic vasodilator production and α-adrenergic vasoconstriction may tip in favor of vasoconstriction (Mudge et al., 1976). We performed four series of experiments in an attempt to determine whether norepinephrine is responsible for the time-dependent vasocconstriction seen under the conditions of our experiments.

Infusion of norepinephrine into the ischemic region after 180 minutes of ischemia resulted in vasodilation. This result suggests that exogenous norepinephrine is still capable of raising metabolism and causing vaso- dilation within the ischemic bed. However, this does not necessarily mean that endogenous norepinephrine would have the same effects, because the dose employed (1–2 μg/min) and the route of administration might not mimic the effect of endogenously released norepinephrine. We did further experiments employing α-blockade to address this question. Phentolamine administration after 180 minutes increased flow in the ischemic region. Whereas this suggests that α-adrenergic tone does exist after 180 minutes of ischemia, it does not prove that the vasocconstriction between 30 and 180 minutes is caused by increased α-adrenergic

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Lactate (mg/dl)</th>
<th>Potassium (mEq/l)</th>
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<tr>
<td>A</td>
<td>V</td>
<td>n</td>
</tr>
<tr>
<td>Control</td>
<td>23.8 ± 5.0</td>
<td>23.2 ± 5.9</td>
</tr>
<tr>
<td>30 min</td>
<td>22.4 ± 6.9</td>
<td>39.1 ± 8.5</td>
</tr>
<tr>
<td>60 min</td>
<td>23.7 ± 8.4</td>
<td>38.9 ± 10</td>
</tr>
<tr>
<td>120 min</td>
<td>22.0 ± 7.7</td>
<td>37.7 ± 8.7</td>
</tr>
<tr>
<td>180 min</td>
<td>20.3 ± 6.5</td>
<td>35.6 ± 7.5</td>
</tr>
</tbody>
</table>

Lactate and potassium concentrations in LAD bed plasma during 180 minutes of relative ischemia. Ischemia resulted in net lactate efflux (V-A difference significantly greater than control at each time point, P < 0.05), but no release of K+. A = arterial, V = venous, n = number of animals.
amount of a-adrenergic tone that was also present early in the ischemic period (Buffington and Feigl, 1981). This objection was circumvented in the phenoxycbenzamine series, in which we blocked the a-receptors in the LAD bed before the 180-minute ischemic period. Under these conditions, there was no flow decrease between 30 and 180 minutes. This indicated that increasing a-adrenergic tone may have been responsible for the progressive vasoconstriction observed in this preparation. However, phenoxycbenzamine blocks both a1-receptors of vascular smooth muscle and a2-receptors of sympathetic nerve endings (Starke, 1971; Farah and Langer, 1974). This means that it is possible that phenoxycbenzamine treatment not only prevented a-adrenergic vasoconstriction, but also simultaneously increased norepinephrine release in the ischemic area. Such increased norepinephrine release could then have caused metabolic vasodilation, and this could account for the lack of vasoconstriction in the ischemic area. This possibility was tested by administering propranolol to phenoxycbenzamine-treated hearts after 180 minutes of ischemia. If phenoxycbenzamine causes elevated norepinephrine release and subsequent metabolic vasodilation, propranolol should prevent the metabolic vasodilation and reverse the vasodilatory effect of phenoxycbenzamine. This is exactly the result we obtained. Given this series and the norepinephrine series, we conclude that the net effect of norepinephrine release in the ischemic region is vasodilation, and that norepinephrine is not the cause of the increasing vascular resistance in this preparation between 30 and 180 minutes.

In order to produce ischemia in phenoxycbenzamine-treated hearts, we had to employ a lower distal LAD pressure than in other series (35 vs. 50 mm Hg). It might be argued that the resulting abolition of the flow decrease between 30 and 180 minutes was related to the lower perfusion pressure (e.g., decreased filtration and decreased tissue pressure), rather than to the phenoxycbenzamine. This is not a likely explanation, however, because the addition of propranolol causes the expected flow decrease to reappear. Furthermore, Frame and Powell (1976) have observed similar flow decreases at a perfusion pressure of ~25 mm Hg.

In summary, we have provided evidence that, under very constrained experimental conditions, coronary resistance vessels within an ischemic region are not fully dilated, and undergo progressive vasoconstriction in the face of continued ischemia. We have been unable to determine the cause of this progressive vasoconstriction, but it does not appear to be caused by the release of potassium ion, PGF2α, TXA2, or norepinephrine. It may be caused by the release of a vasoconstrictor substance which we have not tested. Alternatively, this vasoconstriction may result from a decreasing release of vasodilator metabolites in the ischemic region. Our results do not rule out cell swelling as an additional source of increased vascular resistance in ischemic myocardium. Cell swelling seems particularly likely to play a role in preparations in which the ischemia is more intense than it is in ours, or in preparations in which prolonged coronary occlusion is followed by reflow (Willerson et al., 1975; Kloner et al., 1976; Powell et al., 1976).

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References


INDEX TERMS: Myocardial ischemia • Coronary artery stenosis • a-Adrenergic receptors • Radioactive microspheres • Myocardial blood flow
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