Nonuniform Loss of Regional Flow Reserve During Myocardial Ischemia in Dogs


To determine whether coronary vasodilator reserve that persists during myocardial ischemia is present in all left ventricular regions, we measured regional blood flow in 192 left ventricular pieces (mean weight, 201 mg) in each of eight dogs by using radioactive microspheres while perfusing the left main coronary artery at 70, 50, 40, and 30 mm Hg. Flows were measured before and during adenosine infusion to determine flow reserve. Perfusion at 40 and 30 mm Hg produced ischemia in all dogs. At 70 mm Hg, 100% of left ventricular regions had significant flow reserve, compared with 92%, 55%, and 8% during perfusion at 50, 40, and 30 mm Hg, respectively. A greater amount of flow reserve and a greater number of regions responded to adenosine in the subepicardium than in the subendocardium at 50, 40, and 30 mm Hg. We conclude that coronary flow reserve persists in only a subset of left ventricular regions during ischemia and that the number of regions with persistent flow reserve decreases with perfusion pressure. These findings may best be explained by a model in which regional ischemia is a maximal coronary vasodilator and persistent pharmacological vasodilator reserve seen when global markers indicate ischemia simply reflects persistent endogenous flow reserve in myocardial regions not yet ischemic. (Circulation Research 1990;67:253–264)

Maximal dilatation of coronary resistance vessels has generally been accepted to occur when myocardial ischemia is produced by decreasing perfusion pressure below the effective range for autoregulation.1-5 If this tenet is true, then ischemia should be a maximal stimulus for coronary vasodilatation. A number of studies, however, have noted that pharmacological agents such as adenosine or carbochromen can augment coronary blood flow at perfusion pressures well below the autoregulatory range.6-9 Warltier et al10 observed greater coronary flow during pharmacological vasodilatation than during peak reactive hyperemia after a transient coronary occlusion. Still others have demonstrated persistent vasodilator reserve distal to a critical coronary stenosis.11-15 These studies imply that myocardial ischemia is not a maximal stimulus for coronary vasodilatation and that endogenous flow reserve may be exhausted while pharmacological vasodilator reserve persists.

The mechanisms responsible for the persistence of pharmacological vasodilator reserve during ischemia are unclear. Downey et al16 demonstrated that the hyperemic response to transient coronary occlusion was not synchronous across the left ventricular wall; the subendocardial layer reached peak reactive hyperemia earlier than did the subepicardial layer. They suggest that this difference in time to peak reactive hyperemia would cause transmural flow measurements at any given point in time to underestimate true steady-state maximal flow. These findings, however, do not explain why pharmacological vasodilator reserve is observed when ischemia is produced by constant low-pressure perfusion.

Recently, Austin et al,17 working in our laboratory, demonstrated a marked heterogeneity in the amount of pharmacological flow reserve present in small regions of the left ventricle at normal perfusion pressures. This observation led us to postulate that small myocardial regions may exahust their pharmacological flow reserve over a range of perfusion pressures. In the current experiment, therefore, we evaluated the variability of regional flow reserve during ischemia. Specifically, our study was designed to show that during ischemia 1) only some left ventricular regions maintain the capacity to vasodilate in response to adenosine and 2) the number of...
regions with pharmacological vasodilator capacity decreases as perfusion pressure decreases.

Materials and Methods

Experimental Preparation

Thirteen adult mongrel dogs of either sex weighing 22–33 kg were premedicated with an intramuscular injection of 2–3 ml fentanyl citrate/droperidol (Innovar-Vet; Pitman-Moore, Washington Crossing, N.J.). General anesthesia was induced with intravenous sodium pentobarbital (25 mg/kg) and maintained with a mixture of 1% halothane in O₂-enriched room air delivered through an endotracheal tube by a Harvard respirator (South Natick, Mass.). Arterial blood gases were measured frequently, and ventilator settings were adjusted to maintain pH, Pₐₒ₂, and PCO₂ within the normal range.

A vinyl catheter was placed in the descending aorta via the left femoral artery to monitor aortic pressure and obtain blood gases. Bilateral thoracotomies were performed in the fourth intercostal space. In three dogs, the proximal right coronary artery was exposed through a small right-sided pericardiotomy and ligated to prevent possible collateral blood flow into the left coronary artery perfusion field. In the same dogs, a 16F vinyl catheter with side holes was passed from the right atrium deep into the coronary sinus to drain coronary venous blood for measurements of myocardial lactate extraction and oxygen consumption. In all dogs, the heart was suspended in a pericardial cradle via a left-sided pericardiotomy. The left main coronary artery (LMCA) was isolated, and a silk ligature was placed loosely around it. Heparin sodium (5,000 units) was given intravenously before the perfusion circuit was primed with blood; maintenance doses (2,000 units) were given each half hour thereafter. A perfusion circuit was then placed from the femoral artery to the LMCA. Blood from the left femoral artery was pumped through a filter into a pressurized reservoir, then passed through a modified Gregg cannula (o.d., 4 mm; i.d., 3 mm), which was inserted via a subclavian arteriotomy into the LMCA and secured with the external ligature. A fluid-filled catheter located just inside the tip of the Gregg cannula was used to measure LMCA pressure and provided input to the servo-controlled air-pressure reservoir. Thus, mean coronary perfusion pressure could be set at any level and held constant, independent of aortic pressure. Side ports in the perfusion cannula provided for the injection of radionuclide-labeled microspheres and for continuous infusion of intracoronary adenosine. Mean LMCA flow was measured with an in-line electromagnetic flow transducer (Howell Instruments, Camarillo, Calif.) connected to a flowmeter (model RT500, Narco Bio-Systems, Inc., Houston). Mechanical zero was checked, and offsets were recorded after each flow measurement. The accuracy of flow measurements was checked against a timed collection of blood into a graduated cylinder. Heart rate was controlled by pacing the left atrial appendage (model 5880A, Medtronic, Inc., Minneapolis). Aortic pressure was kept constant by constriciting the descending aorta as needed. The aortic and LMCA catheters were connected to Statham P23Db pressure transducers (Gould, Cleveland, Ohio), and zero pressure was set at midchest level. All pressure and flow data were recorded on a strip chart (model R612, Beckman Instruments, Inc., Fullerton, Calif.).

During low-pressure perfusion, ischemia was demonstrated in several ways. In two dogs, intramyocardial pH was measured in the subendocardium of the left ventricular free wall with a BGA 1000-CM pH meter (Orange Medical Instruments, Costa Mesa, Calif.). In three dogs, left ventricular subendocardial segment shortening was measured with piezoelectric crystals and a Triton sonomicrometer (Triton Technology, Inc., San Diego). In the remaining three dogs, ischemia was confirmed by a change from net myocardial lactate extraction to net myocardial lactate production.

Experimental Protocol

Coronary blood flow was measured at four different perfusion pressures, first with autoregulation intact and then during maximal vasodilatation with adenosine. Coronary pressure was set at 70 mm Hg, and the heart was allowed to achieve a steady state for at least 15 minutes. Radioactive microspheres (1–1.5 million) labeled with a single radionuclide were injected into the LMCA while LMCA flow, mean aortic pressure, and mean LMCA pressure were recorded simultaneously. After allowing 60 seconds for the establishment of a steady state at each perfusion pressure, this procedure was repeated (using different radionuclides) at pressures of 40 and 30 mm Hg. The total duration of ischemia did not exceed 5 minutes. Maximal coronary vasodilatation was then produced by intracoronary administration of adenosine (20 μg/kg/min) (Sigma Chemical Co., St. Louis). Vasodilatation was considered maximal when doubling the adenosine infusion rate failed to increase LMCA flow. After allowing 60 seconds for the establishment of a steady state at each perfusion pressure, microspheres were then injected at 40, 30, and 70 mm Hg by using the methods described above. In four dogs, LMCA and regional flow measurements were also made at 50 mm Hg.

Early results demonstrated that adenosine infusion at 70 mm Hg resulted in very high LMCA flows that did not return to control levels more than 2 hours after adenosine had been discontinued. For this reason, measurements made with autoregulation intact were completed before adenosine infusion. Also, during adenosine infusion, measurements at low pressures were completed before those at 70 mm Hg.

Regional Blood Flow Measurements

Myocardial blood flow to small regions of the left ventricle was measured with radionuclide-labeled microspheres 15±1 μm in diameter (mean±SD) (3M...
Co., New England Nuclear, Boston). Up to eight measurements were made in each experiment with \( ^{85} \text{Sr}, ^{57} \text{Co}, ^{103} \text{Cd}, ^{51} \text{Cr}, ^{58} \text{Co}, \) and \( ^{111} \text{In} \). Microspheres were suspended in saline containing 0.01% Tween 80 to help prevent aggregation. After vigorous vortex mixing, microspheres were injected directly into the perfusion circuit 35 cm proximal to the orifice of the Gregg cannula according to methods previously described.\(^{18,19} \) However, in lieu of taking a reference sample, the in-line flow transducer was used to determine reference flows. Each set of microspheres was injected over 60 seconds to average any cyclical changes in metabolic flow regulation. Injections had no discernible effect on coronary or systemic hemodynamics.

At the end of each experiment, while the dog was still under anesthesia, cardiac arrest was induced by an intracoronary injection of 10% potassium chloride. The heart was excised, and all fat and vessels were trimmed. All hearts were free of any evidence of preexisting myocardial disease. In the three dogs whose right coronary artery was tied off, a probe was inserted into the right coronary ostium to ensure that it had been completely ligated. In all dogs, the papillary muscles were removed, and the subendocardial and subepicardial surfaces were examined for evidence of hemorrhage. Hearts from two dogs showed visible evidence of myocardial hemorrhage and were excluded from analysis.

The left ventricle was opened, flattened, and fixed in 4% formalin for 72 hours. A \( 6 \times 6 \) cm square was cut from the center of the left ventricular free wall. This square was cut into three layers, and each layer was then cut into 64 pieces for a total of 192 left ventricular regions, each having a mean weight of 201±46 mg (mean±SD). The precise anatomic location of each myocardial piece was recorded. The remainder of the heart was cut less rigorously and used to quantitate total radioactivity of the heart.

Radionuclide emissions were counted as previously described by Baer et al.,\(^{19} \) using a NaI(Tl) detector (Tracor Analytic; TM Analytic, Brandon, Fla.), multichannel pulse-height analyzer (Norland Corp., Fort Atkinson, Wisc.), and a NOVA-3 minicomputer (Data General, Boston). All samples were counted for 3 minutes. Blood flow to each tissue sample was calculated with radionuclide counts referenced to mean LMCA inflow (electromagnetic flowmeter) as follows:\(^{18,19,21} \)

\[
Q_{\text{sample}} = Q_{\text{LMCA}} \times \left( \frac{A_{\text{sample}}}{A_{\text{total}}} \right) \times \left( \frac{1}{W_{\text{sample}}} \right)
\]

where \( Q_{\text{sample}} \) is the blood flow to tissue sample (milliliters per minute per gram), \( Q_{\text{LMCA}} \) is the mean LMCA inflow (milliliters per minute), \( A_{\text{sample}} \) is the activity of tissue sample, \( A_{\text{total}} \) is the summated activity of whole heart, and \( W_{\text{sample}} \) is the weight of tissue sample (grams). Only dogs with adequate numbers of spheres in all myocardial regions (>400 spheres/region) with each radionuclide were considered acceptable for analysis. Three dogs were rejected for analysis because more than 1% of regions in the left ventricle had fewer than 400 microspheres of a given radionuclide.

Flow reserve was calculated by subtracting autoregulatory flow from maximal flow during adenosine infusion. Throughout this manuscript, the term flow reserve refers to pharmacological flow reserve and is technically distinct from endogenous or autoregulatory flow reserve, a term properly used when endogenous vasodilators cause the maximal flow response (e.g., during reactive hyperemia).\(^{22} \)

**Oxygen Consumption**

During microsphere injections in three dogs, arterial and coronary sinus blood were withdrawn to determine hemoglobin concentration, \( P_{\text{O}} \), and percent oxygen saturation. Using these measurements and LMCA flow, we calculated oxygen consumption as

\[
\text{Oxygen consumption (ml \( P_{\text{O}} \)/min) = } (O_{\text{2}} \text{sat}_{\text{art}} - O_{\text{2}} \text{sat}_{\text{cs}}) \times Hb \times 1.36 \times Q_{\text{LMCA}}
\]

where \( O_{\text{2}} \text{sat}_{\text{art}} \) is the oxygen saturation of arterial blood, \( O_{\text{2}} \text{sat}_{\text{cs}} \) is the oxygen saturation of coronary sinus blood, \( Hb \) is the hemoglobin concentration (gram per liter of blood), * denotes a constant (milliliters of \( P_{\text{O}} \) per gram of \( Hb \)), and \( Q_{\text{LMCA}} \) is the blood flow to the LMCA (liters of blood per minute).

**Myocardial Lactate Production**

Blood lactate was measured in arterial and coronary sinus blood samples according to lactic acid procedure No. 826-UV from Sigma.\(^{23} \) Net myocardial lactate extraction was calculated from the arteriovenous difference in measured blood lactate. A negative lactate extraction (lactate production) was taken as evidence of left ventricular ischemia.\(^{24} \)

**Myocardial Segment Shortening**

Left ventricular free wall segment shortening was measured according to previously described methods\(^{25,26} \) in eight dogs from a pilot study and in three dogs used for regional flow analysis in the current study. Subendocardial piezoelectric crystals were placed 1–2 cm apart in the myocardium and aligned so that the intercrystal axis was parallel to the expected direction of fiber shortening.\(^{27} \) Percent segment shortening was calculated as

\[
\text{Percent segment shortening} = \left( \frac{DD - SD}{DD} \right) \times 100 \]

where \( DD \) is the maximum diastolic length (millimeters) and \( SD \) is the minimum systolic length (millimeters).

**Intramycocardial pH**

Intramycocardial pH was measured with fiberoptic probes, which were inserted into the myocardium at approximately the level of the subendocardium and secured in place with a ligature.
### Statistical Methods

For each flow measurement based on microsphere radionuclide activity, the estimated error was calculated according to published methods in which

$$\text{EE}_{\text{total}} = Q_{\text{sample}} \times \sqrt{1+b/(SD_{\text{sample}}/C_{\text{sample}})^2}$$

where $\text{EE}_{\text{total}}$ is the total estimated error of flow to the tissue sample (milliliters per minute per gram), $Q_{\text{sample}}$ is the blood flow to the tissue sample (milliliters per minute per gram), $b$ is the number of microspheres in the tissue sample, $C_{\text{sample}}$ is the radioactive counts in the tissue sample, and $SD_{\text{sample}}$ is the standard deviation of radioactive counts in the tissue sample.

Calculations of flow reserve included the subtraction of two numbers, each of which was associated with an error. Thus, a propagation of errors occurred, and the total error for flow reserve was determined as

$$\text{Flow reserve error}_{\text{total}} = \sqrt{(1/\text{EE}_{\text{autoreg}})^2 + (1/\text{EE}_{\text{vasodil}})^2}$$

where flow reserve error$_{\text{total}}$ is the total error for each flow reserve calculation, EE$_{\text{autoreg}}$ is the total estimated error of flow to the tissue sample during autoregulation, and EE$_{\text{vasodil}}$ is the total estimated error of flow to the tissue sample during maximal vasodilatation.

Left ventricular function and chemical analyses were compared by a one-way analysis of variance (ANOVA). Baseline coronary blood flows in different layers of the left ventricle were compared by a one-way ANOVA; individual comparisons were made with Scheffe’s test. Coronary flows before and during maximal vasodilatation at each pressure for the whole left ventricle and for different myocardial layers were compared by a paired t-test. LMCA flows observed at 30 mm Hg before and during adenosine infusion were not normally distributed and were therefore compared with the Wilcoxon signed rank test. All statistical differences were considered significant if $p<0.05$. Flow reserve in each individual region was considered significant if it exceeded twice the total estimated flow reserve error, giving a 95% confidence level. Data are presented as mean±SD unless otherwise stated.

### Results

In a pilot study ($n=8$), when LMCA perfusion pressure was reduced from 70 to 40 mm Hg, subendocardial segment shortening, as measured by piezoelectric crystals, decreased significantly from 17±5% to 7±3% ($p<0.05$, paired t test), indicating myocardial ischemia. Segment shortening decreased by at least 8% in all dogs at a coronary pressure of 40 mm Hg. For this reason, perfusion pressures of 40 and 30 mm Hg were used to produce ischemia in the current study.

Of the 13 dogs used in the experimental protocol, a total of five dogs were excluded from analysis because of myocardial hemorrhage (two dogs) or inadequate numbers of radioactive microspheres (three dogs). Thus, the following results are based on data obtained from eight dogs.

#### Hemodynamics and Assessment of Myocardial Ischemia

Table 1 gives mean values for heart rate, aortic pressure, percent segment shortening, intramyocardial pH, and myocardial lactate extraction; values are given at each perfusion pressure both before and during adenosine infusion. Mean aortic blood pressure remained constant as LMCA pressure was lowered from 70 to 50, fell slightly at 40 mm Hg, but decreased significantly when coronary pressure was reduced further to 30 mm Hg. Mean aortic blood pressure did not change significantly with adenosine infusion at any LMCA pressure. In all dogs, when LMCA pressure was reduced to 40 or 30 mm Hg, there was evidence of myocardial ischemia.

#### Transmural and Layer Flow Reserve

Flow reserve in the whole left ventricle was calculated from LMCA inflow before and during maximal vasodilatation. Results are shown in Table 2. Flow reserve was significantly different from zero at 70, 50, 40, and 30 mm Hg in all dogs ($p<0.05$). The response to adenosine at 30 mm Hg was variable, with some dogs showing no increase in flow and others a rather dramatic increase (e.g., dogs 1 and 7 versus dog 8, etc.).
Table 2). The amount of pharmacological vasodilator reserve in the left ventricle decreased with decreasing perfusion pressure and was exhausted in some dogs at 30 mm Hg. The variability of flow reserve from dog to dog was related to the aortic pressure at the time of the measurement (Table 2).

Microsphere data were used to determine flow reserve within the subendocardial, middle, and subepicardial layers of the left ventricle. As shown in Table 3, significant pharmacological flow reserve persisted in all layers at 70, 50, and 40 mm Hg in all dogs. At an LMCA pressure of 30 mm Hg significant flow reserve persisted in only the subepicardium. Table 3 also demonstrates that the subendocardial-to-subepicardial flow reserve ratio fell significantly when perfusion pressure was decreased from 70 to 50, 40, or 30 mm Hg.

**Flow Reserve in Small Myocardial Regions**

Blood flow to small myocardial regions under control conditions (LMCA pressure, 70 mm Hg, no adenosine) averaged 0.80±0.16 ml/min/g. Figure 1 shows the frequency distributions of flow reserve at different perfusion pressures for two representative dogs. In both dog 3 and dog 5, all regions demonstrated vasodilator capacity when perfused at 70 mm Hg. Each frequency histogram describes a relatively normal distribution with a mean of 3.5 and 2.1 ml/min/g, respectively. The histograms of flow reserve at 40 mm Hg shown in Figures 1a and 1b are skewed to the right. The majority of regions in both graphs have no apparent flow reserve at 40 mm Hg and are distributed about zero. Flow reserve was exhausted in these regions even though LMCA flow more than doubled with maximal vasodilation in both dogs. A number of other regions at this same pressure, however, did vasodilate in response to adenosine, with the maximum being 2.2 and 1.7 ml/min/g in Figures 1a and 1b, respectively. Because a negative flow reserve most likely represents error rather than a true decrease in flow during pharmacological vasodilation, this area of the histograms gives a rough measure of methodological error. Figure 1b shows that even at an LMCA pressure of 50 mm Hg a small number of regions had exhausted their flow reserve despite the large degree of flow reserve present in other regions (also note the large increase in LMCA flow from 112 to 385 ml/min with pharmacological vasodilation). Regions both with and without flow reserve were consistently observed in the frequency

<table>
<thead>
<tr>
<th>Dog</th>
<th>70 mm Hg</th>
<th>50 mm Hg</th>
<th>40 mm Hg</th>
<th>30 mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>−Ad +Ad Reserve</td>
<td>Mean P&lt;sub&gt;Ao&lt;/sub&gt; (mm Hg)</td>
<td>−Ad +Ad Reserve</td>
<td>−Ad +Ad Reserve</td>
</tr>
<tr>
<td>1</td>
<td>120</td>
<td>892</td>
<td>772</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>94</td>
<td>865</td>
<td>771</td>
<td>60</td>
</tr>
<tr>
<td>3</td>
<td>63</td>
<td>422</td>
<td>359</td>
<td>74</td>
</tr>
<tr>
<td>4</td>
<td>75</td>
<td>513</td>
<td>438</td>
<td>70</td>
</tr>
<tr>
<td>5</td>
<td>125</td>
<td>537</td>
<td>412</td>
<td>77</td>
</tr>
<tr>
<td>6</td>
<td>62</td>
<td>200</td>
<td>138</td>
<td>95</td>
</tr>
<tr>
<td>7</td>
<td>104</td>
<td>370</td>
<td>266</td>
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</tr>
<tr>
<td>8</td>
<td>68</td>
<td>600</td>
<td>532</td>
<td>62</td>
</tr>
<tr>
<td>Mean</td>
<td>461†</td>
<td>164†</td>
<td>69†</td>
<td>16‡</td>
</tr>
<tr>
<td>SD</td>
<td>225</td>
<td>97</td>
<td>42</td>
<td>20</td>
</tr>
</tbody>
</table>

LMCA, left main coronary artery; −Ad, before adenosine infusion; +Ad, during adenosine infusion; reserve, pharmacological flow reserve in the left ventricle.

*Mean aortic pressure was 4 mm Hg higher during adenosine infusion than before adenosine infusion at 30 mm Hg in this dog.

†p<0.05 by paired t test (−Ad vs. +Ad).

‡p<0.05 by Wilcoxon signed rank test.

**Table 3.** Mean Flow Reserve (ml/g/min) in Different Myocardial Layers and Mean Flow Reserve Ratio at Different Perfusion Pressures

<table>
<thead>
<tr>
<th></th>
<th>70 mm Hg</th>
<th>50 mm Hg</th>
<th>40 mm Hg</th>
<th>30 mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subendocardium</td>
<td>3.86±0.11*</td>
<td>0.51±0.03*</td>
<td>0.14±0.02*</td>
<td>0.02±0.01</td>
</tr>
<tr>
<td>Midwall</td>
<td>4.45±0.11*</td>
<td>1.29±0.06*</td>
<td>0.41±0.02*</td>
<td>0.06±0.01</td>
</tr>
<tr>
<td>Subepicardium</td>
<td>3.19±0.08*</td>
<td>1.37±0.06*</td>
<td>0.73±0.03*</td>
<td>0.12±0.02*</td>
</tr>
<tr>
<td>Endo/epi reserve ratio</td>
<td>1.2</td>
<td>0.37†</td>
<td>0.19†</td>
<td>0.17†</td>
</tr>
</tbody>
</table>

Values are mean±SEM, n=8 for all except 50 mm Hg, where n=4. LMCA, left main coronary artery; endo/epi reserve ratio, subendocardial flow reserve/subepicardial flow reserve.

*Significant flow reserve, p<0.01, paired t test, comparing flow before and during adenosine infusion.

†Significant decrease compared with endo/epi ratio at 70 mm Hg, p<0.05, paired t test.
distributions of flow reserve at 50 and 40 mm Hg in all dogs. At 30 mm Hg, the histogram in Figure 1a is slightly skewed to the right with a mean flow reserve of 0.1 ml/min/g, indicating that a small amount of flow reserve was present in some myocardial regions in this dog. Consistent with this result, adenosine increased LMCA flow from 35 to 45 ml/min. In general, when coronary reserve decreased below 0.25 ml/min/g, no predictable patterns could be found; some dogs had a mean flow between -0.25 and 0 ml/min/g, and other dogs demonstrated mean flows between 0 and +0.25 ml/min/g (e.g., compare histograms in the bottom right-hand corner of Figure 2a for dog 3 and Figure 2b for dog 5). For this reason, we considered ±0.25 ml/min/g our limit of resolution of regional flow reserve, and all regions with a flow reserve in this range were considered not different from zero. Figure 1b demonstrates that in dog 5 a large majority of regions had no flow reserve at 30 mm Hg; however, the distribution tails off to the right slightly, showing that flow reserve was maintained in some regions. This result is consistent with the observed increase in LMCA flow during adenosine infusion in dog 5 at 30 mm Hg (Table 2). Six of eight dogs demonstrated flow reserve in some myocardial regions at 30 mm Hg. The remaining two dogs showed no regional flow response to adenosine at this coronary pressure. If all myocardial regions had lost flow reserve in a uniform fashion, the frequency histograms should have remained normally distributed at all perfusion pressures.

The distribution of flow reserve within each myocardial layer was also examined, and the different layers were compared. Figures 2a and 2b show the frequency histograms of flow reserve within the subendocardium and subepicardium at each perfusion pressure for two representative dogs. The amount of flow reserve and the shape of the frequency histograms in different layers at 70 mm Hg are similar in both dogs, with all regions in each layer demonstrating flow reserve. At 50 mm Hg, there was greater regional flow reserve in the subepicardium than in the midwall, and the midwall had greater regional flow reserve than the subendocardium; Figure 2b shows data for the subendocardium and the subepicardium. Note also that several regions within the subendocardium demonstrated no flow response to adenosine at 50 mm Hg. At a coronary pressure of 40 mm Hg, the subepicardium again demonstrated both more regions with flow reserve and a greater maximum flow reserve than did the subendocardium. Although most regions within the subepicardium had persistent flow reserve at 40 mm Hg, several regions were maximally vasodilated and did not increase their flow during adenosine infusion. In the subendocardium, a majority of regions had no flow response to adenosine, whereas other regions maintained flow reserve up to a maximum of 0.5 ml/min/g. At 30 mm Hg, dog 3 demonstrated no flow reserve in
TABLE 4. Percent of Myocardial Regions With Significant Coronary Flow Reserve at Different Perfusion Pressures

<table>
<thead>
<tr>
<th>LMCA perfusion pressure</th>
<th>70 mm Hg</th>
<th>50 mm Hg</th>
<th>40 mm Hg</th>
<th>30 mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transmural</td>
<td>100</td>
<td>92±12</td>
<td>55±24</td>
<td>8±7</td>
</tr>
<tr>
<td>Subendocardium</td>
<td>100</td>
<td>78±15</td>
<td>35±32</td>
<td>1±2</td>
</tr>
<tr>
<td>Midwall</td>
<td>100</td>
<td>97±5</td>
<td>58±20</td>
<td>3±3</td>
</tr>
<tr>
<td>Subepicardium</td>
<td>100</td>
<td>100</td>
<td>72±27</td>
<td>19±22</td>
</tr>
</tbody>
</table>

Values are mean (±SEM where indicated); n=8 for all values except at 50 mm Hg, where n=4. Regional flow reserve was considered significant if it exceeded twice the total estimated error (95% confidence limit).

any myocardial layer (Figure 2a), whereas reserve persisted in the subepicardium in dog 5 (Figure 2b). Thus, we observed a nonuniform depletion of regional flow reserve both within each layer as well as between different layers as coronary pressure was decreased. The heterogeneous loss of regional flow reserve was similar in dogs with or without right coronary artery ligation. Data presented in Figures 1b and 2b were obtained from a dog with a ligated right coronary artery.

Using estimated errors, we determined whether flow reserve was significant in each small myocardial region. The percent of regions with statistically significant flow reserve at each LMCA pressure both in the left ventricle overall and within each myocardial layer was then calculated. These percentages were averaged for all animals and are presented in Table 4. At 70 mm Hg, 100% of regions showed significant coronary flow reserve. The amount of vasodilator reserve was highly variable, with flows during adenosine infusion ranging from 150% to 2,400% of resting flows. This heterogeneity of coronary reserve was distributed randomly throughout the left ventricle. Overall, regions in the subendocardium and subepicardium had comparable amounts of flow reserve (the ratio of subendocardial to subepicardial flow reserve was 1.2±1, Table 3).

At perfusion pressures of 50 and 40 mm Hg, 92% and 55% of pieces demonstrated significant flow reserve, respectively, with the remaining regions showing minimal or no flow reserve (Table 4). When perfusion pressure was further lowered to 30 mm Hg.

FIGURE 2. Panel a: Frequency histogram of pharmacological vasodilator reserve within different left ventricular layers at left main coronary artery (LMCA) pressures of 70, 40, and 30 mm Hg in dog 3. Arrows along the baseline show mean regional flow reserve. At 40 mm Hg, a greater maximum flow reserve and more regions with persistent flow reserve were observed in the subepicardium than in the subendocardium. Panel b: Frequency histogram of flow reserve within different ventricular layers at LMCA pressures of 70, 50, 40, and 30 mm Hg in dog 5. The distributions of flow reserve in this dog are similar to those for dog 3, except that reserve was also evaluated at 50 mm Hg, and subepicardial flow reserve persisted at 30 mm Hg.
only 8% of regions maintained flow reserve. Flow reserve was depleted in more regions of the subendocardium than the subepicardium at each of these perfusion pressures and was completely exhausted in the subendocardium at 30 mm Hg. Also, regions within individual layers did not all exhaust their flow reserve at the same perfusion pressure. For example, some regions of the subendocardium exhausted their flow reserve at or above an LMCA pressure of 50 mm Hg, and other regions within the same layer maintained significant flow reserve at or below a pressure of 40 mm Hg. Although the absolute number of regions with significant flow reserve at ischemic perfusion pressures varied from animal to animal (reflected in the high standard deviations shown in Table 4), vasodilator reserve was lost over a range of perfusion pressures in all dogs.

Changes in Baseline Myocardial Blood Flow with Reductions in LMCA Pressure

Mean regional blood flow at baseline (before adenosine administration) was determined at each LMCA pressure. At LMCA pressures of 70, 50, 40, and 30 mm Hg, mean regional blood flow (milliliters per minute per gram) decreased from 0.79±0.16 (mean±SD) to 0.62±0.21, 0.57±0.17, and 0.37±0.19, respectively. These flow reductions were significant with each decrease in coronary pressure (p<0.05, one-way ANOVA). Figure 3 demonstrates, in a representative dog, that baseline flow in regions found to have significant flow reserve at 50, 40, and 30 mm Hg was not different from baseline flow in regions whose flow reserve was exhausted (no flow increase during subsequent adenosine infusion).

Myocardial Oxygen Consumption

Table 5 shows mean left ventricular oxygen consumption preceding and during adenosine infusion at different coronary perfusion pressures. At 50 mm Hg, the flow increase seen during maximal vasodilatation tended to be associated with an increase in oxygen consumption, although this did not reach statistical significance (n=3). At 40 mm Hg, oxygen consumption remained unchanged despite a significant rise in coronary flow with pharmacological vasodilatation. Similarly, oxygen consumption did not increase with maximal vasodilatation at a coronary pressure of 30 mm Hg.

Discussion

The major finding of this study was the observation that only a subset of myocardial regions vasodilate in response to adenosine at coronary pressures low enough to cause ischemia. We noted also that the number of regions whose flow reserve was exhausted increased as perfusion pressure was reduced.

Critique of Methods

Our results are based on data obtained with radioactive microspheres. As discrete particles, microspheres accurately reflect blood flow to a tissue sample only when they lodge in that tissue sample in sufficient numbers. If too few spheres reach the sample, not only would the error of flow measurements increase but the heterogeneity of observed flows would also be expected to increase. In small sample sizes, this problem becomes more important. Bassingthwaighte et al.28 have shown that flow measurements with two different microspheres (109Ce and 113Ru) correlate well over a range of regional blood flows from 0.17 to 1.12 ml/g/min (mean r=0.976) using sample sizes of 54 mg, which is well below the sample size used in our experiment. They also demonstrated that coronary flows measured with radioactive microspheres and the diffusible indicator 2-iododesmethylimipramine are comparable (r=0.91). Tripp et al.29 also found a good correlation (r=0.94–0.96) between radioactive microspheres and tritiated

Table 5. Mean Left Ventricular Oxygen Consumption Before and During Pharmacological Vasodilatation at Different Left Main Coronary Artery Perfusion Pressures

<table>
<thead>
<tr>
<th>Coronary perfusion pressure (mm Hg)</th>
<th>Oxygen consumption (ml O2/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>−Ad</td>
</tr>
<tr>
<td>50</td>
<td>3.70±2.21</td>
</tr>
<tr>
<td>40</td>
<td>3.04±1.60</td>
</tr>
<tr>
<td>30</td>
<td>2.35±0.99</td>
</tr>
</tbody>
</table>

Values are mean±SEM. −Ad, before adenosine infusion; +Ad, during adenosine infusion. n=3; NS, not significantly different from before adenosine.
water at low coronary flows in 80-mg tissue samples. Although sphere entrapment error cannot be entirely excluded in our experiment, only dogs with adequate numbers of spheres in all myocardial regions (>400 spheres/region) with each radionuclide were considered acceptable for analysis. Three dogs were rejected for analysis because more than 1% of regions in their left ventricles had fewer than 400 microspheres of a given radionuclide.

Collateral blood flow, which is often incomplete, might be expected to cause an irregular distribution of flow reserve similar to that observed in our experiment. For this reason special steps were taken to exclude collateral flow. Only dogs with a suitable LMCA that allowed insertion of the Gregg cannula without kinking of the circumflex or left anterior descending arteries at their origin were used. Also, despite the very small amount of collateral flow from the right coronary artery reported to occur in canine hearts,30 special care was taken to ensure that it was completely ligated in three dogs, and the results from these dogs were compared with those from dogs without right coronary artery ligation. No differences between these two groups could be found.

Baseline blood flow measurements were made before the measurements during adenosine infusion. This sequence was followed after preliminary experiments revealed that LMCA flow would not return to baseline after adenosine had been administered at 70 mm Hg. In contrast, flow did return to baseline after brief subtotal ischemia. How this brief ischemic insult might affect blood flow to myocardial regions during subsequent measurements is unknown; therefore, hearts with visible subendocardial or subepicardial hemorrhage at the completion of the experiment (two dogs) were not analyzed. In these two dogs, heparin was given before the placement of segment-shortening crystals, and hemorrhage occurred in the myocardium surrounding these crystals. In subsequent dogs, heparin was administered after placement of the crystals, and no further myocardial hemorrhage was seen. Although microscopic damage remains a possibility, our observation that all regions demonstrated an increase in flow during adenosine infusion at 70 mm Hg (the last measurement) makes significant microscopic damage unlikely.

The mean weight of the small myocardial regions we analyzed was 201 mg. Using estimates of interarteriolar distances, Bassingthwaighte et al31 suggested that the functional microvascular unit of the myocardium weighs between 0.2 and 1 mg. Each of our regions, then, might have contained 200 functional units or more. The observed coronary vasodilator reserve for a given region, therefore, actually represents a mean flow reserve of all functional units in that region. Thus, a region labeled as having exhausted its flow reserve in our experiment would require maximal vasodilatation of virtually all its functional units, whereas a region labeled as having persistent vasodilator reserve would require increased blood flow in only enough units to increase mean regional flow signi-

cantly. This averaging process would tend to increase the number of regions observed to have persistent vasodilator reserve at low perfusion pressures and decrease the number of regions with no response to adenosine (maximal vasodilatation by endogenous stimuli).

Several investigators have suggested that blood flow to small regions of the ventricle during metabolic regulation normally is cyclical.32,33 Sestier et al33 estimated a cycling time of 30–90 seconds at normal coronary perfusion pressures. Therefore, in the current study, microspheres were injected continuously over 1 minute to average any cyclical changes in vasomotor tone. Despite this precaution, we do not know whether some of the heterogeneity of flow reserve observed was due to temporal changes in vasomotor tone.

Gratton et al8 suggested that pharmacological flow reserve persists until coronary flow drops to near zero. This might have been true in our experiment if those regions with no response to adenosine had negligible blood flow, that is, if regions with no pharmacological vasodilator reserve had reached their pressure-flow zero point. As shown in Figure 3, regions whose flow reserve was exhausted by ischemia had baseline blood flows significantly different from zero (p<0.05), and these regions showed a range of flows similar to that observed in regions with persistent vasodilator reserve. All dogs showed similar results. Thus, the heterogeneity of flow reserve at low perfusion pressures is not a manifestation of regional differences in the pressure at which flow ceases (pressure-flow zero).

By using conventional methods of analysis, our results were similar to those reported by others. When microspheres were used to measure blood flow to entire myocardial layers, we observed persistent flow reserve in the subendocardium, midwall, and subepicardium during constant-pressure perfusion at 40 mm Hg. At 30 mm Hg, vasodilator reserve persisted only in the subepicardium. Canty and Klocke7 noted an increase in blood flow to all myocardial layers during maximal vasodilatation with adenosine at a constant left circumflex artery perfusion pressure of 35 mm Hg; they also noted a flow increase only in the subepicardium at 25 mm Hg. Aversano and Becker6 found persistent flow reserve in all layers at left circumflex artery pressures of 50 and 35 mm Hg by using intracoronary adenosine as a maximal vasodilator. Pantely et al,9 using swine, demonstrated a flow increase with adenosine in both the subendocardium and the subepicardium at left anterior descending arterial pressures of 45 and 35 mm Hg. Gratton et al8 perfused the LMCA of dogs to minimize collateral flow and reported a persistence of flow reserve recruited by adenosine or carbochromes in all layers at coronary pressures of 40 and 30 mm Hg.

All of these studies averaged flow reserve within each myocardial layer, treating each layer as a homogeneous region, and based their conclusions on how the average flow reserve in a given myocardial layer
changed as perfusion pressure was reduced. Recent observations by Austin et al.\textsuperscript{17} showed a marked spatial heterogeneity of flow reserve in all layers of the canine left ventricle at normal perfusion pressures. This suggests that even within a given myocardial layer, some regions may exhaust their flow reserve sooner than other regions (i.e., flow reserve is exhausted at different coronary perfusion pressures). Our findings supported this concept and showed that regional flow reserve remained spatially heterogeneous at low perfusion pressures both between different myocardial layers and within individual myocardial layers. Thus, the customary practice of averaging flow reserve within entire myocardial layers may mask important regional differences and misrepresent the underlying physiology of coronary blood flow during ischemia.

Our study was able to demonstrate that vasodilator reserve was no longer present in a number of myocardial regions when global markers indicated ischemia. Technological limitations prevented us from demonstrating specifically that ischemia was present in those regions whose reserve was exhausted and absent in those regions with persistent reserve. Thus, the important question of whether ischemia is a maximal stimulus for coronary vasodilatation remains unanswered. However, our findings do suggest that the persistent pharmacological flow reserve observed during ischemia by others was not uniformly present in all regions within each myocardial layer. We believe our results are best explained by a model in which microregional ischemia is a maximal stimulus for local coronary vasodilatation and in which ischemia develops at different critical perfusion pressures both transmurally (subendocardium sooner than subepicardium) and among regions within a given myocardial layer. In this model, pharmacological agents would not vasodilate regional coronary resistance vessels to a greater degree than ischemia of that region. Thus, if pharmacological vasodilator reserve persists during ischemia, then endogenous vasodilator reserve should also be present. That some degree of endogenous flow reserve persists during ischemia is supported by our observation in dogs that perfusion of the LMCA with blood from the coronary sinus augmented left coronary artery flow at perfusion pressures low enough to cause ischemia.\textsuperscript{34} Support for the concept that ischemia develops heterogeneously within myocardial layers is provided by the observations of Steenbergen et al.,\textsuperscript{35} who used NADH fluorescence photography in rat hearts to demonstrate that ischemia produced by decreasing coronary flow resulted in a heterogeneous pattern of small discrete areas of anoxic tissue sharply bounded by areas with normal mitochondrial oxidative function across the epicardial surface of the heart.

If myocardial regions with persistent flow reserve at low coronary perfusion pressures were not ischemic, one would not expect overall cardiac function to improve when these regions were vasodilated with pharmacological agents. Although our data must be considered preliminary because of the small number of dogs tested with each different functional assessment, we found no significant increase in segment length shortening, intramyocardial pH, lactate extraction, or oxygen consumption despite a statistically significant increase in coronary flow during pharmacological vasodilatation at three different perfusion pressures. This result is similar to the findings of Pantely et al.,\textsuperscript{9} who observed no change in oxygen consumption, lactate extraction, or wall thickening with pharmacological vasodilatation during constant low-pressure perfusion of the left anterior descending artery. Our findings are also consistent with the clinical observation that pharmacological agents, such as adenosine or dipyridamole, which dilate resistance vessels but have no effect on larger epicardial vessels,\textsuperscript{36–38} provide little or no improvement in syndromes of ischemic heart disease even though they increase total coronary blood flow.\textsuperscript{39} In contrast to Pantely et al. and the present study, Aversano and Becker\textsuperscript{2} observed a significant increase in percent segment shortening with adenosine infusion at 35 mm Hg. However, the improvement was small, increasing from a mean of 3.4\% to 5.6\%. Recognizing that the different measures of left ventricular function mentioned above are relatively insensitive methods, it is possible that a small degree of functional recovery, which we were unable to detect, did occur in our experiments.

Canty and Klocke\textsuperscript{7} demonstrated that pharmacological flow reserve persists despite significant reduction in coronary blood flow. We also found a significant reduction in regional blood flow with decreases in perfusion pressure both in regions maximally vasodilated by ischemia and in regions with persistent flow reserve during ischemia. This finding suggests that coronary flow is affected in an important way by extravascular forces (such as heart rate and systolic compression) as perfusion pressure is decreased even before flow reserve is exhausted. Thus, flow reduction may not be an accurate marker for the exhaustion of endogenous vasodilator reserve. Possible mechanisms to explain flow reductions in the presence of vasodilator reserve include reduced myocardial contractile activity,\textsuperscript{40–42} or altered neurohumoral interactions during ischemia.\textsuperscript{43}

**Clinical Implications**

If ischemia of a myocardial region is indeed a maximal stimulus for coronary vasodilatation, then the heart is highly efficient in its metabolic regulation of vascular tone. The persistence of regional vascular tone during ischemia represents an effort by the heart to shunt blood to areas of the myocardium that need it most. Maximal vasodilatation of resistance vessels with pharmacological agents, then, may actually be deleterious by creating regional coronary steal phenomena. The striking clinical benefit of coronary vasodilators such as nitrates and Ca\textsuperscript{2+} channel blockers in the treatment of ischemia probably relies on their ability to reduce myocardial oxygen consumption by...
decreasing preload and afterload and on their ability to reverse and prevent coronary spasm. Pharmacological coronary vasodilatation, other than to relieve coronary spasm, may be of little or no benefit in the treatment of syndromes of ischemic heart disease.

Summary

Our results indicate that pharmacological coronary flow reserve observed during ischemia persists in only a portion of left ventricular regions. All remaining regions did not vasodilate in response to adenosine. As perfusion pressure was decreased, the number of regions responding to adenosine decreased, and flow reserve was exhausted in the subendocardium at 30 mm Hg. In addition, preliminary data from this study suggest that augmentation of coronary blood flow with adenosine during ischemia does not improve myocardial oxygen consumption, lactate metabolism, segment length shortening, or intramyocardial pH. These findings may best be explained by a model in which regional ischemia is a maximal coronary vasodilator and persistent pharmacological vasodilator reserve seen when global markers indicate ischemia simply reflects persistent endogenous flow reserve in myocardial regions not yet ischemic.

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**KEY WORDS**
- coronary reserve
- vasodilator reserve
- adenosine
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