Transmural Variation in Autoregulation of Coronary Blood Flow in Hyperperfused Canine Myocardium

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SUMMARY We assessed transmurally the coronary autoregulatory response to elevated perfusion pressure in 85 anesthetized, open-chest dogs. Steady state and transient flow distributions were measured with $9 \pm 1 \mu m$ radioactive microspheres. Steady state distributions of myocardial blood flow were uniform transmurally ($P > 0.05$) at perfusion pressures of $103 \pm 1$ (n = 81) and $146 \pm 2$ (n = 34) mm Hg, whereas Endo:Epi ratios of $1.26 \pm 0.05$ and $1.51 \pm 0.07$ at perfusion pressures of $194 \pm 1$ (n = 60) and $221 \pm 3$ (n = 14) mm Hg, respectively, indicated progressive redistribution ($P < 0.05$) of flow toward subendocardium. As perfusion pressure was increased, autoregulation failed first in subendocardium. At high perfusion pressures, subendocardial flow exceeded subepicardial flow ($P < 0.05$) whether or not subepicardial flow was autoregulated. The initial, transient response to elevation of coronary perfusion pressure from $99 \pm 1$ to $145 \pm 2$ (n = 5) mm Hg was an increase ($P < 0.05$) in Endo: Epi ratio from $1.05 \pm 0.06$ to $1.30 \pm 0.06$. By 2 minutes this ratio had returned to near control. In contrast, the initial Endo:Epi ratio of $1.32 \pm 0.05$ caused by elevation of perfusion pressure to $190 \pm 5$ (n = 6) mm Hg did not wane ($P > 0.05$). A selective increase in subendocardial oxygen demand could not explain greater subendocardial flow since (1) elevated perfusion pressure had no effect on aortic and left ventricular pressures, cardiac output, and heart rate, (2) left ventricular oxygen consumption was constant in the presence of preferential subendocardial perfusion, (3) regional myocardial oxygen tension increased in parallel with blood flow to subepicardium and subendocardium. Also, elevated perfusion pressure caused a greater steady state increase in subendocardial flow in four empty, nonworking hearts. The results demonstrate transmural variation in coronary autoregulatory potential; the subepicardial vasculature appears better adapted to autoregulate blood flow at elevated perfusion pressures. Circ Res 47: 599-609, 1980

UNDER conditions of stable cardiac function and, therefore, unchanging metabolic requirements of the myocardium, total coronary blood flow remains relatively constant despite moderate variations in coronary perfusion pressure, a phenomenon termed autoregulation (Mosher et al., 1964; Berne et al., 1964). When perfusion pressure exceeds the autoregulatory capability of the coronary circulation, total coronary flow increases markedly with pressure, but it is unknown if this autoregulatory failure occurs uniformly throughout the myocardium. It does appear, however, that total coronary blood flow is autoregulated on a regional basis in order to achieve uniform transmural perfusion of the left ventricular free wall at normal perfusion pressure. To compensate for limitation of flow to subendocardium during systole, excess flow during diastole is required. If this is accomplished by a transmural gradient of vascular tone (Moir, 1972), subepicardial vessels must be more constricted in the face of higher transmural pressures throughout the cardiac cycle. Consequently, it would not be surprising if the coronary vasculature in the subepicardial region evolved with greater ability to withstand elevated perfusion pressures. To test this hypothesis, the present investigation was undertaken. Radioactive microspheres were used to measure subendocardial and subepicardial blood flows during elevations in coronary perfusion pressure to levels that were within and above the autoregulatory range. Both transient and steady state responses to elevations in coronary perfusion pressure were examined.

Methods

Experiments were conducted on mongrel dogs, 11–30 kg, which were anesthetized with sodium pentobarbital, 30 mg/kg, administered intravenously. Supplementary anesthetic was administered through a catheter positioned in a femoral vein. After tracheotomy, the dogs were ventilated with oxygen-enriched room air by a positive-pressure, constant-volume respirator, which was adjusted to maintain arterial blood gases within physiological limits.
The heart was exposed through a thoracotomy in the 4th left intercostal space. Catheters were positioned in the aorta via the left carotid artery and in the left ventricle via the left atrial appendage in order to measure aortic and left ventricular pressures, respectively. The left common coronary artery was dissected free, and a loose ligature was placed under it. When surgical procedures had been completed, heparin, 500 U/kg, was administered intravenously. A metal cannula was introduced into the left subclavian artery and advanced, via the aorta, into the ostium of the left common coronary artery, where it was secured with the previously placed ligature. The left common coronary artery was perfused by a controlled pressure system supplied with arterial blood diverted from a femoral artery. The basic perfusion system has been described previously (Downey et al., 1975). In this investigation, the blood reservoir was connected to one of two large pressurized air chambers. One chamber was pressurized at control pressure (approximately equal to mean aortic pressure) and the other at an elevated pressure. By switching rapidly from one chamber to the other, coronary perfusion pressure was increased abruptly. The perfusion line to the coronary artery was equipped with a mixing chamber, a pressure transducer, and an electromagnetic flow transducer (Micron flowmeter, model RC 1000). Perfusion pressure was corrected for pressure dissipated across the coronary cannula at all flow rates.

Pressures were measured with Statham transducers (model P23Db), and heart rate was monitored with a Beckman cardiotachometer coupler (model 9857). Electrocardiogram, pressures, coronary blood flow, and heart rate were recorded with a Beckman recorder (model R411).

The significance of differences between observations made at different perfusion pressures within groups was tested with an analysis of variance and the Student-Newman-Keuls procedure (Zar, 1974). Comparisons at corresponding perfusion pressures between autoregulating and non-autoregulating groups (Fig. 1) were tested with the Student's t-test for unequal data (Zar, 1974). In this report, the term "significant" reflects \( P < 0.05 \). Other probabilities are stated explicitly.

Myocardial blood flow was measured from tissue trapping of 9 ± 1 \( \mu \)m microspheres (3M Co.), labeled with \( \gamma \)-emitting radionuclides, \(^{44}\)Sc, \(^{51}\)Cr, \(^{85}\)Sr, or \(^{111}\)Ce. Prior to injection, microspheres were dispersed in a solution of 10% dextran and agitated in an ultrasonicator and in a vortex mixer. Verification of size and dispersion of microspheres was accomplished by visual examination of a drop of the dose suspension with a light microscope. Approximately 10\(^5\) microspheres were injected as a bolus into the perfusion line proximal to the mixing chamber. Beginning simultaneously with injection, two pairs of consecutive 1-minute reference blood samples were collected at a constant rate (8–12 ml/min) from two points along the perfusion line that were distal to the mixing chamber and 30–35 cm from the point of microsphere injection. Adequacy of microsphere mixing in the blood perfusate was verified by finding similar radioactivities in the duplicate reference blood samples.

Injection of India ink into the perfusion line at the end of each experiment demonstrated that the left ventricular free wall and the interventricular septum were perfused in all preparations. Four samples, weighing approximately 5 g each, were obtained from the perfused left ventricular free wall and divided transmurally into thirds.

Tissue samples and blood reference samples were analyzed for radioactivity with a \( \gamma \) spectrometer equipped with a multichannel analyzer (Nuclear Chicago, model 4233, or Packard Instrument Company, model 9015). A mini-computer (DEC PDP-8E) was used to carry out isotope separation using standard techniques of \( \gamma \) spectroscopy (Rudolph and Heymann, 1967). Blood flow required to account for the radioactivity in myocardial tissue samples was calculated from the equation of Mackowski et al. (1968): \( \text{MBF} = (F_b \times (R_t/R_b)) \), where MBF is myocardial blood flow (ml/min), \( F_b \) is the reference blood-sampling rate (ml/min), \( R_t \) is the radioactivity in the tissue sample (counts/min), and \( R_b \) is the average radioactivity in the duplicate sets of reference blood-samples (counts/min). Myocardial blood flow per unit weight was obtained by dividing the calculated myocardial blood flow by the weight of the tissue sample. Myocardial blood flow values determined for the outer and inner thirds of each of the four left ventricular samples were averaged to compute mean subepicardial and subendocardial blood flows, respectively. The ratio of subendocardial blood flow to subepicardial blood flow (Endo:Epi) was calculated for each transmural sample, and the four values obtained in each heart were averaged.

To attribute changes in myocardial blood flow to elevated coronary perfusion pressure per se, it was necessary to establish that cardiac function and, therefore, myocardial metabolic requirements for flow were stable. To this end, measurements of the following variables were made: (1) cardiac output, (2) left ventricular oxygen consumption, and (3) transmural myocardial \( \text{Po}_2 \).

Cardiac output was measured with an indicator-dilution technique involving systemic injection of 9 ± 1 \( \mu \)m radioactive microspheres. A dose of \( 2 \times 10^6 \) microspheres of known radioactivity was introduced via a catheter into the left atrium. Simultaneous with injection of microspheres, duplicate samples of arterial blood \( \text{Po}_2 \) were collected at a constant rate from catheters positioned in the aorta. Cardiac output was computed according to the equation developed by Archie et al. (1973): \( \text{CO} = (F_b \times (R_t/R_b)) \), where CO is cardiac output (ml/
Studies in Working Hearts

The effects of elevated perfusion pressure on regional myocardial blood flow were studied in 81 working hearts. In all studies, total left coronary blood flow (measured electromagnetically), left ventricular and mean aortic blood pressures, and heart rate were recorded. In some studies, cardiac output, left ventricular oxygen consumption, and regional myocardial \( P_02 \) were also measured. After control measurements were made, the left common coronary circulation was subjected to one or more elevations of coronary perfusion pressure. When more than one pressure elevation was applied to the same coronary circulation, the perfusion pressure was returned to the control pressure until total coronary flow stabilized before pressure was again elevated. When multiple variations of coronary perfusion pressure were imposed on the same coronary circulation, these variations were imposed in random order.

**Steady State Distributions of Myocardial Blood Flow**

Myocardial perfusion pressure was set equal to mean aortic pressure for control measurements of myocardial blood flow and other parameters. Coronary perfusion pressure was then abruptly elevated, and, when coronary blood flow measured electromagnetically had stabilized, the measurements were repeated. One to three elevations of coronary perfusion pressure were studied in each of 81 hearts.

**Transient Distributions of Myocardial Blood Flow**

In eleven hearts, transmural distribution of myocardial blood flow was measured (1) under control conditions, (2) immediately after abrupt elevation of coronary perfusion pressure, (3) 6 seconds later, and (4) in the steady state, 2 minutes after the increase in perfusion pressure. For the second and third determinations of myocardial blood flow, the radioactive microspheres were injected upstream into the coronary perfusion line approximately 3 cm from the coronary cannula, so they would quickly reach the coronary vasculature. Duplicate reference samples were withdrawn from the coronary perfusion line 1 cm from the coronary cannula. Although the mixing chamber was not used in these experiments, adequate mixing of the microspheres was demonstrated by the similarity of radioactivity in duplicate reference samples (duplicate samples differed by less than 5%). The volume of the perfusion tubing and coronary cannula from the point of injection to the coronary artery was approximately 2 ml. With total coronary flows of approximately 1 ml/sec, the delay from injection to the coronary circulation was approximately 2 seconds.

To make sequential measurements of the transmural distribution of myocardial flow immediately after elevation of coronary perfusion pressure and 6 seconds later, two syringes were loaded with differently labeled microspheres and their needles inserted into the perfusion line. When these preparations were complete, an investigator administered one dose of microspheres, and, exactly 2 seconds later, another investigator abruptly increased cor-
ony perfusion pressure. This procedure corrected for the cannula delay, although a few more seconds elapsed before the microspheres were trapped in the coronary microcirculation.

The third administration of radioactive microspheres was made 4 seconds after elevation of the perfusion pressure. These microspheres reached the coronary circulation approximately 6 seconds after the change in perfusion pressure. The fourth administration of microspheres performed 2 minutes after elevation of perfusion pressure was made proximal to the mixing chamber, as was done for other steady state determinations. Sequential measurements of the transmural distribution of myocardial blood flow were made in five dogs after increasing coronary perfusion pressure to approximately 150 mm Hg and in six dogs after the pressure had been increased to approximately 190 mm Hg.

Studies in Non-Working Hearts

In a further effort to ensure unchanging myocardial metabolism during coronary pressure elevations, we conducted studies in four empty beating dogs hearts (Scott et al., 1960). The brachiocephalic trunk and the aortic arch distal to the left subclavian artery were clamped. The coronary circulation was supplied with blood introduced retrogradely into the aorta through the left subclavian artery. In these experiments, arterial blood for the perfusion system was provided by a support dog. The left ventricle was vented and coronary venous return was siphoned from the right ventricle and returned to the support dog. Aortic pressure, which in this case was equal to coronary perfusion pressure, left ventricular pressure, and heart rate were monitored. Myocardial blood flow measurements were made by administration of microspheres into the perfusion line at normal and elevated coronary perfusion pressures.

Results

Working Hearts

Steady State Changes in Coronary and Myocardial Blood Flows

Total left coronary blood flow measured electromagnetically in 58 dogs is reported in Table 1. Under control conditions, with coronary perfusion pressure at 102 ± 1 mm Hg, coronary blood flow averaged 0.84 ± 0.05 ml/min per g. Average increases in coronary perfusion pressure of 43%, 94%, and 102% above the control pressure caused significant average increases in flow of 45%, 181%, and 339%, respectively. The magnitude of these flow increases indicate that, in many animals, total coronary flow was not autoregulated when perfusion pressure was elevated to 146 mm Hg or greater; i.e., steady state increases in flow were proportional to or in excess of increases in coronary perfusion pressure. At the higher perfusion pressures, the exponential increase in total flow is consistent with distention of the coronary vasculature.

Regional myocardial blood flow was measured with radioactive microspheres in 81 dogs at normal and elevated coronary perfusion pressures (Table 2). Under control conditions, coronary perfusion pressure averaged 103 ± 1 mm Hg, subepicardial blood flow averaged 0.84 ± 0.03 ml/min per g, and subendocardial blood flow averaged 0.81 ± 0.03 ml/min per g. A 41% increase in coronary perfusion pressure in 34 hearts resulted in significant parallel increases in both subepicardial and subendocardial flows of 36%, but no change in the Endo:Epi ratio. An 88% increase in coronary perfusion in 60 hearts significantly increased subepicardial and subendocardial flows by 123% and 188%, respectively. Redistribution of myocardial blood flow toward the endocardium was reflected by a 29% increase (P < 0.01) in the Endo:Epi flow ratio. A 114% increase in perfusion pressure in 14 hearts significantly increased subepicardial flow by 217%, subendocardial flow by 333%, and caused a further increase (P < 0.05) in the Endo:Epi ratio. In these animals, mean aortic blood pressure averaged 97 ± 1 mm Hg, left ventricular systolic and end-diastolic pressures averaged 111 ± 1 and 5 ± 1 mm Hg, respectively, and heart rate averaged 164 beats/min during control conditions. Elevations in the coronary perfusion pressure had no significant effects (P > 0.05) on these hemodynamic parameters (Table 2).

Examination of the results from individual experiments revealed marked variation in the ability of coronary circulations to autoregulate either total coronary flow or regional myocardial flow at the elevated pressures used in this investigation. Frequently, steady state measurements of total left coronary blood flow showed no autoregulation, whereas steady state measurements of regional flow reflected autoregulation of subepicardial but not of subendocardial blood flow. Thus, in these hearts, overperfusion of the subendocardium obscured autoregulation in measurements of total coronary blood flow. Because of our interest in defining regional differences in the coronary response to elevated perfusion pressure, the data were partitioned into two groups on the basis of the presence or absence of subepicardial autoregulation (with or without concurrent subendocardial autoregulation). In this analysis, an increase in steady state flow in the subepicardium which was less than proportional to the elevation in perfusion pressure was the criterion for subepicardial autoregulation. The results of this analysis are presented in Figure 1.

Although the two groups clearly differed in their responses to elevated perfusion pressure, they had similar regional flows at normal control perfusion pressures. Of 34 coronary circulations subjected to moderate perfusion pressure (approximately 145 mm Hg), 59% autoregulated subepicardial blood flow; flow in this region tended to increase but was
not significantly above control (Fig. 1). In this group, subendocardial flow was also autoregulated, although flow to this region increased significantly above control. Among the 41% lacking subepicardial autoregulation, subendocardial autoregulation also was absent. In these non-autoregulating hearts, flows in both regions significantly exceeded control values as well as flows measured in corresponding regions of the autoregulating hearts. At this moderate elevation of perfusion pressure, the transmural distribution of myocardial blood flow, as reflected by the Endo:Epi ratio, was similar to control in both autoregulating and non-autoregulating hearts.

The percentage of coronary circulations capable of autoregulating subepicardial flow decreased at higher perfusion pressures. Of the 60 coronary circulations subjected to high perfusion pressure (approximately 195 mm Hg), 42% autoregulated subepicardial blood flow. Among these dogs, subendocardial flow significantly exceeded subepicardial flow, and the increase in flow to the subendocardium exceeded the increase in perfusion pressure; at this pressure, subepicardial but not subendocardial autoregulation was evident. Only 36% of coronary circulations were able to autoregulate subepicardial blood flow at very high (approximately 221 mm Hg) coronary perfusion pressure, and these circulations failed to autoregulate subendocardial flow. Non-autoregulating hearts perfused at high and very high pressures had flows to both regions that significantly exceeded flows measured in corresponding regions of autoregulating hearts. At these pressures, the Endo:Epi ratios in the non-autoregulating hearts were significantly greater than control and increased with pressure; they were not different, however, from the ratios measured in corresponding regions of autoregulating hearts.

From these results, two points should be emphasized. First, elevations in coronary perfusion pressure caused consistently greater increases in subendocardial blood flow than in subepicardial blood flow. Second, when autoregulation failed, it failed first in subendocardium.

In the subepicardial autoregulating group, mean aortic pressure averaged 99 ± 2 mm Hg, left ventricular systolic and diastolic pressure averaged 113 ± 2 and 5 ± 1 mm Hg, respectively, and heart rate averaged 167 ± 3 beats/min under control conditions with coronary perfusion pressure set at 102 ± 1 mm Hg. These hemodynamic parameters were similar in non-autoregulating hearts, and elevations

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**TABLE 1** Total Left Coronary Blood Flow Measured Electromagnetically at Normal and Elevated Coronary Perfusion Pressures

<table>
<thead>
<tr>
<th>Coronary perfusion pressure (mm Hg)</th>
<th>Control</th>
<th>Moderate</th>
<th>High</th>
<th>Very high</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>102 ± 1</td>
<td>146 ± 2</td>
<td>193 ± 2</td>
<td>224 ± 4</td>
</tr>
<tr>
<td>Coronary blood flow (ml/min per g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.84 ± 0.05</td>
<td>1.22 ± 0.10†</td>
<td>2.36 ± 0.21†</td>
<td>3.69 ± 0.45†</td>
</tr>
<tr>
<td>Observations</td>
<td>58</td>
<td>26</td>
<td>41</td>
<td>10</td>
</tr>
</tbody>
</table>

Values are means ± SE. Data from working hearts of 58 dogs. Although flow was measured electromagnetically in all dogs, weight of the total perfused myocardium was measured in only 58 dogs.

* Different from control, *P < 0.05.
† Different from measurement at next lower perfusion pressure, *P < 0.05.

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**TABLE 2** Regional Myocardial Blood Flows Measured with Radioactive Microspheres and Hemodynamic Parameters at Normal and Elevated Coronary Perfusion Pressure

<table>
<thead>
<tr>
<th>Coronary perfusion pressure (mm Hg)</th>
<th>Control</th>
<th>Moderate</th>
<th>High</th>
<th>Very high</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>103 ± 1</td>
<td>146 ± 2</td>
<td>194 ± 1</td>
<td>221 ± 3</td>
</tr>
<tr>
<td>Myocardial blood flows (ml/min per g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epi</td>
<td>0.84 ± 0.03</td>
<td>1.14 ± 0.07†</td>
<td>1.87 ± 0.13†</td>
<td>2.67 ± 0.44†</td>
</tr>
<tr>
<td>Endo</td>
<td>0.81 ± 0.03</td>
<td>1.10 ± 0.06†</td>
<td>2.34 ± 0.18†</td>
<td>3.51 ± 0.40†</td>
</tr>
<tr>
<td>Endo:Epi ratio</td>
<td>0.98 ± 0.02</td>
<td>0.98 ± 0.03</td>
<td>1.28 ± 0.05†</td>
<td>1.51 ± 0.17†</td>
</tr>
<tr>
<td>Mean aortic pressure (mm Hg)</td>
<td>97 ± 1</td>
<td>95 ± 3</td>
<td>97 ± 2</td>
<td>96 ± 3</td>
</tr>
<tr>
<td>Left ventricular pressure (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>111 ± 1</td>
<td>108 ± 3</td>
<td>111 ± 2</td>
<td>111 ± 3</td>
</tr>
<tr>
<td>End-diastolic</td>
<td>5 ± 1</td>
<td>4 ± 1</td>
<td>5 ± 1</td>
<td>4 ± 1</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>164 ± 3</td>
<td>163 ± 4</td>
<td>164 ± 3</td>
<td>164 ± 6</td>
</tr>
<tr>
<td>Observations</td>
<td>81</td>
<td>34</td>
<td>60</td>
<td>14</td>
</tr>
</tbody>
</table>

Values are means ± SE. Data from working hearts of 81 dogs. Epi and Endo refer to epicardial and endocardial thirds of the left ventricular free wall.

* Different from control, *P < 0.05.
† Different from measurement at next lower perfusion pressure, *P < 0.05.
TABLE 3

Effect of Coronary Perfusion Pressure on Myocardial Oxygen Consumption (MVO₂)

<table>
<thead>
<tr>
<th>Coronary perfusion pressure (mm Hg)</th>
<th>Control</th>
<th>Moderate</th>
<th>High</th>
<th>Very high</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>103 ± 1</td>
<td>150 ± 3</td>
<td>197 ± 3</td>
<td>212 ± 3</td>
</tr>
<tr>
<td>MVO₂ (ml/min per 100 g)</td>
<td>8.18 ± 0.55</td>
<td>8.06 ± 0.60</td>
<td>8.93 ± 1.14</td>
<td>9.40 ± 0.53</td>
</tr>
<tr>
<td>Observations</td>
<td>33</td>
<td>16</td>
<td>17</td>
<td>4</td>
</tr>
</tbody>
</table>

Non-autoregulating Coronary Circulation

<table>
<thead>
<tr>
<th>Coronary perfusion pressure (mm Hg)</th>
<th>Control</th>
<th>Moderate</th>
<th>High</th>
<th>Very high</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>105 ± 3</td>
<td>192 ± 2</td>
<td>192 ± 2</td>
<td>229 ± 4</td>
</tr>
<tr>
<td>MVO₂ (ml/min per 100 g)</td>
<td>7.55 ± 0.48</td>
<td>7.57 ± 0.66</td>
<td>10.26 ± 0.95†</td>
<td>10.17 ± 1.24*</td>
</tr>
<tr>
<td>Observations</td>
<td>35</td>
<td>11</td>
<td>26</td>
<td>8</td>
</tr>
</tbody>
</table>

Values are means ± se. Data from working hearts of 64 dogs. In four dogs, multiple observations at different perfusion pressures revealed subepicardial autoregulation at moderate, but not at high, pressures. Control findings for these four dogs are included among the baseline observations of both autoregulating and non-autoregulating groups. Regional flows for these groups were similar to that shown in Figure 1.

* Different from control, P < 0.05.
† Different from value at next lower perfusion pressure, P < 0.05.
Table 4  Transient Changes in Myocardial Blood Flow in Response to a Sudden Elevation in Coronary Perfusion Pressure

<table>
<thead>
<tr>
<th>Coronary perfusion pressure (CPP) elevated from 99 ± 1 mm Hg to 154 ± 2 mm Hg (n = 5)</th>
<th>Epi</th>
<th>Endo</th>
<th>Endo/Epi ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.67 ± 0.09</td>
<td>0.69 ± 0.08</td>
<td>1.05 ± 0.06</td>
</tr>
<tr>
<td>Upon abrupt elevation of CPP</td>
<td>1.09 ± 0.12†</td>
<td>1.44 ± 0.21†</td>
<td>1.30 ± 0.06†</td>
</tr>
<tr>
<td>6 seconds after elevation of CPP</td>
<td>0.98 ± 0.14*</td>
<td>1.11 ± 0.21*</td>
<td>1.15 ± 0.06†</td>
</tr>
<tr>
<td>2 minutes after elevation of CPP (steady state)</td>
<td>0.77 ± 0.10</td>
<td>0.86 ± 0.07*</td>
<td>1.15 ± 0.08</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Coronary perfusion pressure elevated from 96 ± 2 mm Hg to 190 ± 5 mm Hg (n = 6)</th>
<th>Epi</th>
<th>Endo</th>
<th>Endo/Epi ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.60 ± 0.06</td>
<td>0.55 ± 0.06</td>
<td>0.93 ± 0.08</td>
</tr>
<tr>
<td>Upon abrupt elevation of CPP</td>
<td>1.61 ± 0.15†</td>
<td>2.12 ± 0.20†</td>
<td>1.32 ± 0.05†</td>
</tr>
<tr>
<td>6 seconds after elevation of CPP</td>
<td>1.60 ± 0.24*</td>
<td>1.97 ± 0.43*</td>
<td>1.18 ± 0.11*</td>
</tr>
<tr>
<td>2 minutes after elevation of CPP</td>
<td>1.46 ± 0.25*</td>
<td>2.40 ± 0.79*</td>
<td>1.47 ± 0.28*</td>
</tr>
</tbody>
</table>

Epi and Endo refer to epicardial and endocardial thirds of the left ventricular free wall.
* Different from control, P < 0.05.
† Different from preceding measurement, P < 0.05.

by 168% in subepicardial tissue and by 285% in subendocardial tissue (P < 0.01). The Endo:Epi ratio increased from 0.93 to 1.32 (P < 0.01). Approximately 6 seconds after elevation of the perfusion pressure, these flows were essentially unchanged. Two minutes after elevation of coronary perfusion pressure, subepicardial flow was 143% above control and subendocardial flow was 336% above control. These values were not significantly different from those measured immediately or 6 seconds after elevation of perfusion pressure, but they suggest a tendency for subepicardial flow to decrease and a tendency for subendocardial flow to increase with time following such a large elevation of perfusion pressure. The Endo:Epi ratio of 1.47 at 2 minutes after elevation of perfusion pressure was significantly greater than control (P < 0.05), but not different from ratios measured immediately and 6 seconds after elevation of perfusion pressure (P < 0.05).

In comparing the results of these two groups, we see that these animals were able to autoregulate blood flow in both subepicardial and subendocardial regions when their coronary circulations were subjected to a moderate (55%) increase in perfusion pressure. However, at this pressure, subendocardial flow significantly exceeded control flow. A larger, 94%, increase in coronary perfusion pressure exceeded the autoregulatory capability of both subepicardial and subendocardial regions, but also caused preferential perfusion of the subendocardium.

Myocardial Oxygen Tensions

In nine dogs, regional myocardial PO2, as well as regional blood flow, was measured at normal and elevated coronary perfusion pressures. In these experiments, cardiac output and myocardial oxygen consumption also were measured. The findings are presented in Table 5. Under control conditions in nine dogs, coronary perfusion pressure averaged 105 ± 2 mm Hg, mean aortic pressure averaged 90 ± 3 mm Hg, left ventricular systolic and diastolic pressures averaged 105 ± 3 and 5 ± 1 mm Hg, respectively, and heart rate averaged 157 ± 6 beats/min. These values were not significantly altered by elevations of coronary perfusion pressure. Under control conditions, subepicardial flow averaged 0.74 ± 0.06 ml/min per g, subendocardial flow averaged 0.62 ± 0.04 ml/min per g, and myocardial PO2 averaged 28 ± 2 mm Hg in subepicardium and 27 ± 4 mm Hg in subendocardium. Myocardial oxygen consumption averaged 6.24 ± 0.48 ml O2/min per 100 g.

Elevation of coronary perfusion pressure by 29% to 136 mm Hg in five dogs resulted in a 45% increase (P < 0.01) in subepicardial blood flow and a 56% increase (P < 0.01) in subendocardial flow. The Endo:Epi ratio tended to increase, but was not significantly different from control. This moderate increase in perfusion pressure was associated with increased PO2 (P < 0.05) in both subepicardium and subendocardium, but with no change in myocardial oxygen consumption or in cardiac output.

Increasing coronary perfusion pressure by 84% to 193 mm Hg in nine dogs caused a 103% increase (P < 0.01) in subepicardial flow and a 168% increase (P < 0.01) in subendocardial flow. The Endo:Epi ratio was 29% greater than control (P < 0.05). Myocardial oxygen tension increased (P < 0.01) by 54% in subepicardium and by 100% in subendocardium, whereas neither myocardial oxygen consumption nor cardiac output were altered significantly.
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TABLE 5 Effects of Elevated Coronary Perfusion Pressure on Regional Myocardial Blood Flow, Left Ventricular Oxygen Consumption, and Cardiac Output in Experiments in which Regional Myocardial Oxygen Tension was Measured

<table>
<thead>
<tr>
<th>Coronary perfusion pressure (mm Hg)</th>
<th>Control</th>
<th>Moderate</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myocardial blood flow (ml/min per g)</td>
<td>Epi 0.74 ± 0.06</td>
<td>1.07 ± 0.09*</td>
<td>1.50 ± 0.22*</td>
</tr>
<tr>
<td></td>
<td>Endo 0.62 ± 0.04</td>
<td>0.97 ± 0.09*</td>
<td>1.66 ± 0.26*</td>
</tr>
<tr>
<td>Endo: Epi ratio</td>
<td>0.86 ± 0.05</td>
<td>0.91 ± 0.05</td>
<td>1.11 ± 0.06*</td>
</tr>
<tr>
<td>Myocardial oxygen tension (mm Hg)</td>
<td>Epi 28 ± 2</td>
<td>40 ± 2*</td>
<td>43 ± 5*</td>
</tr>
<tr>
<td></td>
<td>Endo 27 ± 4</td>
<td>38 ± 2*</td>
<td>54 ± 7*</td>
</tr>
<tr>
<td>Myocardial oxygen consumption (ml/min per 100 g)</td>
<td>6.24 ± 0.48</td>
<td>6.18 ± 0.83</td>
<td>7.59 ± 0.55</td>
</tr>
<tr>
<td>Cardiac output (ml/min)</td>
<td>990 ± 160</td>
<td>1170 ± 150</td>
<td>1030 ± 190</td>
</tr>
<tr>
<td>Observations</td>
<td>9</td>
<td>5</td>
<td>9</td>
</tr>
</tbody>
</table>

Values are means ± SE. Data from working hearts of nine dogs. Epi and Endo refer to epicardial and endocardial thirds of the left ventricular free wall.
* Different from control, P < 0.05.
† Different from measurement at next lower perfusion pressure, P < 0.05.

Non-Working Hearts

With coronary perfusion pressure set at 103 ± 2 mm Hg, peak left ventricular pressure averaged 5 ± 1 mm Hg and heart rate averaged 133 ± 7 beats/min. Elevations of perfusion pressure to 147 ± 1 and 196 ± 2 mm Hg caused no significant changes in these parameters. During control conditions, subepicardial flow averaged 0.46 ± 0.05 ml/min per g, subendocardial flow averaged 0.70 ± 0.16 ml/min per g, and the Endo:Epi flow ratio averaged 1.65 ± 0.48. A 43% increase in perfusion pressure to 147 ± 1 mm Hg caused subepicardial flow to increase significantly by 57% to 0.72 ± 0.13 ml/min per g and subendocardial flow to increase significantly by 53% to 1.07 ± 0.16 ml/min per g. These parallel increases in regional flows resulted in no significant change in the Endo:Epi ratio. A 90% increase in perfusion pressure to 196 ± 2 mm Hg caused subepicardial flow to increase significantly by 196% to 1.24 ± 0.31 ml/min per g and subendocardial flow to increase significantly by 288% to 2.72 ± 0.56 ml/min per g. This preferential perfusion of the subendocardium was reflected in a significant increase in the Endo:Epi ratio to 2.49 ± 0.72.

Discussion

The most important findings of this investigation are the redistribution of myocardial blood flow toward the endocardium with elevated coronary perfusion pressure and the subsequent greater vulnerability of subendocardial autoregulation. Redistribution of blood flow was observed in both steady state and transient phases of the coronary response to elevated perfusion pressure. Furthermore, since myocardial flow redistribution occurred without evidence of changes in total or regional cardiac performance or metabolism, these findings suggest that arteriolar resistance vessels in the subendocardium are less well adapted to regulate flow in the face of increases in perfusion pressure than are those in the subepicardium.

Nine-micron microspheres were selected for measuring the transmural distribution of myocardial blood flow in this study because their distribution agrees more closely with that of diffusible indicators (Yipintsoi et al., 1973; Utley et al., 1974); larger microspheres tend to be preferentially distributed in the subendocardial region (Domenech et al., 1969; Utley et al., 1974). However, the possibility of arteriovenous shunting of the 9-µm microspheres must be considered. Accurate measurements of total regional blood flow with microspheres depends on their complete entrapment in small vessels during their initial passage. We reported recently that shunting of 9 ± 1 µm microspheres increased with elevation of perfusion pressure (Crystal et al., 1979); at a coronary perfusion pressure of 200 mm Hg, approximately 10% of microspheres entering the coronary circulation passed directly into the coronary venous effluent. Therefore, due to shunting of microspheres, the total myocardial flows were slightly underestimated when coronary perfusion pressure was elevated. The magnitude of these errors can be estimated by comparing the measurements of total coronary flow determined electromagnetically (Table 1) with measurements of regional flow determined with microspheres (Table 2). If we assume that subepicardial and subendocardial flows each represent one-half of total left coronary flow, the 8, 11, and 16% differences at moderate, high, and very high pressures agree satisfactorily with predictions of our earlier study. We have no information on the transmural location of...
microsphere shunting, nor have we any reason to believe that it occurs preferentially in either subepicardial or subendocardial tissue. However, even if all shunting were through subepicardial vessels, it could not account for the magnitude of the transmural differences in regional blood flow measured during elevated coronary perfusion pressure.

The possible influence of selective subepicardial edema on regional flow during increased coronary perfusion pressure merits consideration. Increased perfusion pressure may have caused a greater increase in capillary filtration in the subepicardial region because of a gradient of systolic tissue pressure across the left ventricular wall (Downey and Kirk, 1975). However, Salisbury et al. (1961) found that edema did not accumulate in myocardium perfused with arterial blood unless the coronary perfusion pressure was in excess of 200 mm Hg for several minutes. In our investigation, coronary hyperperfusion was brief, and failure of subendocardial autoregulation was observed at perfusion pressures less than 200 mm Hg. Furthermore, edema did not appear to limit subepicardial flow in hearts incapable of regional autoregulation, since in those hearts both subepicardial and subendocardial flows were markedly elevated (Fig. 1). Thus, it seems unlikely that regional myocardial edema could have accounted for the apparent regional differences in autoregulation observed in this investigation.

To evaluate pressure-flow autoregulation in an organ bed, its metabolic requirements must remain constant (Shaw et al., 1962; Mosher et al., 1964). Therefore, we monitored various indices of cardiac performance and metabolic activity during elevations in coronary perfusion pressure. These results suggest that redistribution of myocardial blood flow toward the endocardium at high coronary perfusion pressure was not in response to a selectively greater increase in metabolic requirement for flow to this region. First, aortic pressure, left ventricular pressure, cardiac output, and heart rate were not changed by elevations in coronary perfusion pressures. Second, preferential perfusion of subendocardium was observed without concurrent increase in myocardial oxygen consumption. Third, the change in transmural flow distribution during elevated coronary perfusion pressure also was observed in non-working (empty), beating hearts. Finally, the parallel changes in myocardial PO2 and blood flow observed in subepicardium and subendocardium during elevation of coronary perfusion pressure are consistent with no change in oxygen consumption in either region.

Myocardial PO2 estimates were made with bare-tipped platinum electrodes. This method has been used previously to evaluate transmural variations in PO2 across the wall of the left ventricle (Moss, 1968; Winbury et al., 1971). Although protein coating (Cater et al., 1959) and tissue damage (Moss, 1968) may limit quantitative accuracy of myocardial PO2 measurements obtained with bare-tipped platinum electrodes, relative changes in regional PO2 should be reliable (Winbury et al., 1971).

The Krogh cylinder model provides a framework for evaluating parameters of oxygen supply and demand in an organ bed (Krogh, 1936). In the present investigation, the relationship between myocardial blood flow and PO2 responses observed during elevation in coronary perfusion pressure was analyzed with the Kety equations (Kety, 1957; Winbury et al., 1971), which are based on the Krogh model. We observed an increase of 103% in flow to subepicardium and of 168% in flow to subendocardium when coronary perfusion pressure was raised 84% to 193 mm Hg. The Kety equations predict that flow increases of this magnitude should result in increases in tissue PO2 of 50% and 75%, respectively. Since the observed changes in regional myocardial PO2, 54% and 100%, were at least as great as the predicted changes, it appears that increases in myocardial PO2 followed directly from increases in flow, and that myocardial oxygen consumption was unchanged in both subepicardium and subendocardium.

The hypothesis that the autoregulatory capability of arteriolar resistance vessels is less in the subendocardium than in the subepicardium is supported also by observations obtained during the transient phase of coronary blood flow autoregulation. The quantitative accuracy of myocardial blood flow values during the transient phase of the autoregulatory response may be questioned, since microspheres were administered and reference blood samples were obtained when coronary flow was changing. However, the transmural distribution of myocardial blood flow, as reflected by the Endo:Epi ratio, should be valid since this index is affected only by the relative number of microspheres trapped in each region. The initial response to moderately increased coronary perfusion pressure was redistribution of flow to the subendocardium before mechanisms that autoregulate flow were fully operative. Then, once autoregulation ensued, flow became more uniformly distributed. However, with sufficiently large increases in coronary perfusion pressure, flow remained preferentially distributed toward the endocardium. It appears that the ability of subendocardial resistance vessels to autoregulate flow may be compromised by their greater initial hyperperfusion.

The elevated coronary perfusion pressures studied in this investigation significantly exceeded left ventricular pressures throughout the cardiac cycle. Thus, they probably exceeded systolic tissue pressure in the subendocardium (Downey and Kirk, 1975). Under these conditions, subendocardial, as well as subepicardial, flow would have been possible during systole. However, according to the waterfall hypothesis (Downey and Kirk, 1975), the driving pressure (perfusion pressure-tissue pressure) would
have remained less in subendocardium compared to subepicardium. Therefore, preferential perfusion of subepicardium would have been favored unless the cross-sectional area of resistance vessels in subendocardium was greater than that in subepicardium. Since this is hypothesized to be the case, elevation of perfusion pressure without change in left ventricular pressure could have resulted in excessive subendocardial flow due to a systolic component of flow to that region. Lack of subendocardial autoregulation may reflect inability of vascular smooth muscle of subendocardial resistance vessels to mediate sufficient increases in vascular tone to limit flow appropriately but not necessarily distention of the vasculature. This may have been the case in hearts capable of autoregulating subepicardium but not subendocardial flow. In these hearts, subendocardial flow significantly exceeded subepicardial flow at high and very high perfusion pressures, but the amount of subendocardial flow above that proportional to the increase in pressure was small, approximately 0.2 ml/min per g, and well within the range that could have entered this region during systole. However, this appears not to have been the case in hearts incapable of autoregulating subepicardial flow, where, for similarly high perfusion pressures, excess subendocardial flows of 1.3 and 2.7 ml/min per g occurred. These flows were well beyond the possible systolic contribution. Distention of the subendocardial, as well as subepicardial, vessels must have occurred; although the transmural differences in flow, 0.44 and 0.99 ml/min per g at high and very high pressures, respectively, may have been due to systolic perfusion of the subendocardium at these pressures.

However, the question remains as to why vessels in the subendocardium are more vulnerable to autoregulatory failure following elevation in perfusion pressure. A speculative explanation can be proposed on the basis of the Law of Laplace: \( T = \pi R P \), where \( T \) is the wall tension opposing the distending force \( \pi R P \); \( P \) is the vascular transmural pressure; and \( R \) is the vessel radius. Moir (1972) postulated that a diastolic gradient in vascular tone compensated for reduced flow in subendocardium during systole. This gradient requires that subepicardial vessels, which are perfused throughout the cardiac cycle, be more constricted than subendocardial vessels. This increased subepicardial tone forces more flow to the subendocardium during diastole, and thereby results in uniform transmural perfusion at normal coronary perfusion pressures (Moir and Debra, 1967; Buckberg et al., 1972). With relatively less tone, the radii of subendocardial vessels would normally be greater than those of subepicardial vessels. Thus, it would follow from the Law of Laplace that an increase in vascular transmural pressure induced by elevation of coronary perfusion pressure would cause a greater increase in tension in the wall of subendocardial vessels, and more vigorous contraction of vascular smooth muscle would be required in subendocardial vessels in order to restore flow to its initial value. Autoregulation of flow in subepicardium would require less force because of smaller vessel diameters. Furthermore, since subepicardial vessels normally have more tone (Moir, 1972), these vessels may be better adapted to constrict in response to increased perfusion pressure. This adaptation might include more smooth muscle fibers, a possibility that should be examined histologically. It is also possible that subepicardial vessels have increased sensitivity to chemical signals of excess perfusion such as increased myocardial \( \mathrm{PO}_2 \) (Gellai et al., 1973; Bourdeau-Martini et al., 1974) and decreased interstitial concentrations of adenosine (Berne et al., 1971).

Alternatively, increased coronary perfusion pressure may recruit additional vascular capacity (Morgenstern et al., 1973). Under conditions of maximal coronary vasodilation, we demonstrated a transmural gradient of vascularity favoring the subendocardium, and we suggested that this gradient provides a reserve to ensure uniform transmural flow at normal perfusion pressure even in the absence of subepicardial tone (Downey et al., 1975). It is possible that, as elevation of coronary perfusion pressure overcomes the autoregulatory process, it also unmasks this vascularity gradient.

In conclusion, the results of this study suggest that, under conditions of elevated perfusion pressure, the subepicardial vasculature is better adapted to limit blood flow in accordance with the oxygen requirements of myocardium than is the subendocardial vasculature. This adaptation may have evolved from continuous exposure of the subepicardium to perfusion throughout the cardiac cycle and the resulting transmural gradient of vascular tone responsible for the uniform distribution of blood flow under normal conditions.

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