Transmural Distribution of Myocardial Blood Flow during Systole in the Awake Dog

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ABSTRACT. This study was designed to quantitate transmural myocardial blood flow when coronary arterial inflow was limited to systole and during the subsequent reactive hyperemic response. Studies were performed in 10 awake dogs chronically prepared with electromagnetic flowmeters and pneumatic occluders on the left circumflex coronary artery. Intermittent coronary perfusion, confined to the interval of left ventricular systole or an equivalent period during diastole, was effected by an R wave-triggered solenoid valve connected to the occluder. To measure regional myocardial blood flow we injected radionuclided-labeled microspheres, 7-10 μm in diameter, into the left atrium. When arterial inflow was limited to systole, flow was normal in the subepicardial layers and was decreased as a linear function of tissue depth in the subendocardial layers. When coronary arterial inflow was limited to an equivalent interval in diastole, the transmural distribution of flow was uniform. When coronary flow was confined to systole for more than 20 seconds, the blood flow debt incurred elicited a reactive hyperemia similar to that following a total occlusion of equivalent blood flow debt. However, regional myocardial blood flow during the peak of reactive hyperemia following systolic perfusion was preferentially directed to the subendocardium, where underperfusion was most marked, whereas reactive hyperemia flow following a total occlusion of equivalent blood flow debt was distributed more evenly across the left ventricular wall. Thus, when coronary inflow was limited to systole, ventricular contraction produced a transmural gradient in myocardial blood flow resulting in subendocardial underperfusion.

ALTHOUGH numerous investigations over the past century have demonstrated that ventricular contraction impedes coronary blood flow,1-4 few studies of the effect of cardiac contraction on transmural myocardial perfusion are available. In 1964 Kirk and Honig5 reported a decrease in transmural myocardial perfusion during systole in the anaesthetized dog. The results of this study indicate that transmural myocardial perfusion during systole in the awake dog differs from that in the anaesthetized dog.

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References

systolic compression decreased subendocardial flow relative to subepicardial flow. Subsequently, Downey and Kirk,\(^8\) using the \(^{22}RbCl\) wash-in technique in open-chest dogs, demonstrated that when coronary flow was limited to systole by perfusing a cannulated coronary artery directly from the left ventricular cavity, the subendocardium accumulated only one-half as much indicator as the subepicardium. Since absolute regional flows were not measured, however, the transmural gradient of tracer reported in these studies did not quantitate the degree of myocardial underperfusion. Consequently, our study was designed to evaluate quantitatively the transmural myocardial blood flow when coronary inflow is limited to the interval of ventricular systole.

It has been shown previously that total coronary artery inflow during reactive hyperemia bears a quantitative relationship to the degree of preceding ischemia;\(^7\)\(^9\) however, the relationship between regional myocardial ischemia and myocardial blood flow during the subsequent reactive hyperemia has not yet been studied. In our study we anticipated that when coronary inflow was limited to systole, ventricular contraction would produce a transmural gradient of underperfusion with flow decreasing from epicardium to endocardium. To determine whether differing degrees of underperfusion would elicit differing degrees of hyperperfusion during the subsequent reactive hyperemia, we evaluated transmural myocardial blood flow during the reactive hyperemia that followed an interval of systolic perfusion. All studies were performed in chronically instrumented unanesthetized dogs to avoid possible interfering effects associated with general anesthesia and acute surgical trauma.

**Methods**

Ten adult mongrel dogs weighing 18–32 kg were anesthetized with sodium thiamylal (30–40 mg/kg, \(\times 3\)) and subjected to left thoracotomy. A heparin-filled polyvinyl chloride catheter with an outside diameter (o.d.) of 3 mm was introduced into the arch of the aorta via the left internal mammary artery. A similar catheter was introduced into the left atrial cavity through the left atrial appendage and secured in place with a purse-string suture. The proximal 1.5 cm of the circumflex branch of the left coronary artery was dissected free and a pneumatic occluder, constructed in our laboratory of polyvinyl tubing with a 2.7-mm o.d., was placed around the artery proximal to any branches. In seven dogs an electromagnetic flowmeter probe (Statham Instruments, Inc.) was positioned around the left circumflex coronary artery proximal to the pneumatic occluder as previously described.\(^8\) The catheters, pneumatic occluder tube, and electromagnetic flowmeter leads were tunneled dorsally into a subcutaneous pouch at the base of the neck but were not exteriorized to protect them from damage. On the morning before the study the catheters, occluder tube, and flowmeter leads were exteriorized through a 1-cm skin incision, under 2% lidocaine infiltration anesthesia.

Throughout this paper the term “coronary blood flow” will denote measurements of flow through the circumflex branch of the left coronary artery. To measure coronary flow we used a Statham M-4000 electromagnetic flowmeter. Flowmeter calibrations performed by passing measured flows of normal saline through the flowmeter probes remained within a standard deviation of not more than \(\pm 8\)% during the period of study. Statham P23Db pressure transducers were used to measure aortic and left atrial blood pressures. Lead II of a standard electrocardiogram (ECG) was obtained. Data were recorded on an eight-channel magnetic tape recorder (Hewlett-Packard model 3917A) and an eight-channel direct-writing oscillograph (Hewlett-Packard model 8800).

Studies were conducted 12–30 days after the initial surgery. All animals were active and fully recovered from surgery without fever or other evidence of ill health. At the time of study hematomics ranged from 38% to 46%. Animals were trained to lie quietly on their right sides during study. The laboratory was dimly illuminated and kept free of noise or other activity that might disturb the dog. After all recording instruments were connected, a 60-second left circumflex coronary artery occlusion was produced while lead II of the ECG was monitored. All dogs exhibited marked elevation of the S-T segment during the occlusion, as well as increased heart rate, indicating the absence of an effective coronary collateral circulation that might impair the ischemic stimulus produced by coronary artery occlusion during subsequent studies or falsely elevate total coronary inflow during periods of intermittent coronary artery perfusion.\(^11\) Subsequently, a 60-minute interval was allowed for the animal to adjust to the laboratory conditions. During this time hemodynamic variables were recorded continuously to ensure that a control steady state of heart rate, arterial pressure, and coronary blood flow had been achieved.

To selectively control the interval and timing of blood flow in the left circumflex coronary artery, an electrical circuit consisting of an R wave trigger coupled to a variable delay circuit was used. This circuit was used to actuate a solenoid valve that was connected to the pneumatic occluder with a short length of thick-walled rubber tubing. When triggered by the circuit the valve inflated the occluder at a pressure of 1000 mm Hg. By adjusting the variable delay circuit we could confine coronary perfusion to any part of the cardiac cycle for any time interval. The recordings in Figure 1, made at a paper speed of 100 mm/sec, demonstrate the effectiveness of this technique in converting normal phasic coronary flow into precisely timed intermittent intervals of perfusion. As shown in Figure 1B, the term “systolic perfusion” will denote that condition in which coronary artery inflow was limited to the period of ventricular ejection, defined as the interval beginning with the upstroke of aortic pressure and continuing to the dicrotic notch. The term “diastolic perfusion” (Fig. 1C) will refer to that situation in which coronary inflow begins 100 msec after the dicrotic notch for an interval equal in length to ventricular ejection. We accomplished sustained coronary artery occlusions by manually triggering the solenoid valve. Proper functioning of the trigger device and pneumatic occluder was monitored by observing the coronary blood flow signal from the electromagnetic flowmeter probe. In the three dogs without electromagnetic flow probes we verified proper functioning of the occluder by demonstrating at autopsy that inflow of normal saline from a pressure head of 200 mm Hg into the coronary system was prevented by the inflated pneumatic cuff.
In animals with electromagnetic flow probes the reactive hyperemic response was observed after coronary artery occlusions of 5, 10, 15, 20, and 30 seconds' duration. All responses were observed in duplicate and a minimum of 3 minutes was allowed from the end of the reactive hyperemic response until the subsequent coronary artery occlusion was performed. The reactive hyperemic responses that followed periods of systolic perfusion of 10, 15, 20, 30, 45, and 60 seconds were then observed. If heart rate or arterial pressure during the occlusion and to the end of the reactive hyperemic response differed by more than 5% from the control values, studies were discarded. The volume of flow occurring during the reactive hyperemia was determined by electrical integration. The duration of the hyperemic period was taken as the time required for flow to fall to within 5% of the control measurement. Calculations of blood flow debt incurred during arterial occlusion, reactive hyperemia flow, and blood flow debt repayment were made as described by Freeman. Blood flow debt (ml) = control flow rate (ml/sec) \times duration of occlusion (sec). Reactive hyperemic flow (ml) = [total flow during reactive hyperemia (ml)] - [control flow rate (ml/sec) \times duration of reactive hyperemia (sec)]. Blood flow debt repayment (%) = (reactive hyperemic flow/blood flow debt) \times 100.

Measurements of distribution of myocardial blood flow were made by injecting carbonized microspheres (Nuclear Products Division, 3M Co.) into the left atrium. The microspheres were 7–10 \mu m in diameter and were labeled with gamma-emitting radionuclides \(^{141}\)Ce, \(^{51}\)Cr, \(^{85}\)Sr, and \(^{35}\)Sc. The microspheres were obtained as 1.0 mCi of each nuclide in 10 ml of 10% dextran and 0.05% polysorbate 80. This stock solution was diluted in 10% dextran so that 1.0 ml, the volume injected, contained approximately 3 million microspheres. Injection of this quantity of microspheres resulted in no change in heart rate, arterial pressure, or left atrial pressure. Before injection microspheres were mixed by alternate agitation for at least 15 minutes in an ultrasonic bath (3M Co.) and a Vortex agitator (Scientific Industries). We verified complete dispersion of microspheres by examining a drop of microsphere suspension with a light microscope before injection.

Transmural distribution of myocardial blood flow was studied during four different conditions. (1) In order to quantify the effect of cardiac contraction on transmural blood flow, we injected the 1.0-ml microsphere suspension into the left atrium of each dog 30 seconds after the onset of systolic perfusion. The left atrial catheter was then flushed with 5 ml of normal saline, and systolic perfusion continued for an additional 30 seconds. This and all subsequent microsphere injections were made over a 3-second interval. (2) To serve as a control and ensure that any transmural gradient observed during systolic perfusion was related to ventricular contraction and was not an artifact related to intermittent coronary artery perfusion, a second injection of microspheres was made 30 seconds after diastolic perfusion was started. The left atrial catheter was flushed as before and diastolic perfusion was continued for an additional 30 seconds. (3) To evaluate transmural flow during the reactive hyperemia that followed an interval of systolic perfusion, microspheres were injected at the peak of the reactive hyperemia following 60 seconds of systolic perfusion. If the subendocardium were selectively underperfused when inflow was limited to systolic, the resulting ischemia would be expected to cause preferential flow to the subendocardium during the subsequent reactive hyperemia. (4) To serve as a control for flow observed during the reactive hyperemia following systolic perfusion, microspheres were injected at the peak of the reactive hyperemia following a total coronary occlusion of equivalent blood flow debt. A 24-second total occlusion was required to incur a debt equal to 60 seconds of systolic perfusion.

Beginning simultaneously with each microsphere injection and continuing for 90 seconds, we collected reference samples of arterial blood from the aortic catheter at a constant rate of 15 ml/min, using a withdrawal pump (Harvard Apparatus Co.). The blood was collected into counting vials, and the vials were changed at 15-second intervals during the collection so that a total of six vials was obtained. In every case, radioactivity above background appeared in the first two vials only, indicating that complete dispersion of microspheres within the circulation had occurred within the first 30 seconds. After completion of study, the animal was killed with a lethal dose of pentobarbital and the heart was removed and fixed in 10% buffered formalin. The atria, right ventricle, aorta, and large epicardial vessels were dissected from the left ventricle and discarded. The left ventricle was then sectioned into four transverse sections of approximately
equal thickness in a plane parallel to the mitral valve ring as previously described. These will be referred to as rings 1 through 4. The two central sections (rings 2 and 3), which constituted 60 ± 5% of the left ventricular weight, were used for the study. Four full-thickness blocks, each encompassing approximately 1.5 cm of the outer circumference of each of the two central sections, were removed from the anterior left ventricular free wall, anterior papillary muscle region, posterior left ventricular free wall, and posterior papillary muscle region. Since injections of methylene blue into the left circumflex coronary artery demonstrated myocardial staining within a distribution encompassing the posterior left ventricular free wall and posterior left ventricular papillary muscle blocks, these specimens were taken to represent the myocardial regions under study, the two corresponding anterior specimens serving as controls. Each region was then divided into four equal transmural layers from the epicardial surface to the endocardial surface and these layers were weighed and placed in vials for counting. For the remainder of this paper, these layers will be referred to as layers 1 through 4, layer 1 being closest to the epicardium and layer 4, closest to the endocardium. Individual sample weights ranged from 0.523 to 2.67 g, with most samples weighing 1.00–1.40 g. The myocardial and blood samples were counted in a Beckman gamma spectrometer (model 167776) at window settings that corresponded to the peak energies emitted by each radioactive nuclide. The activities recorded in each energy window and the corresponding sample weights were then entered into a digital computer (IBM model 1 130) programmed to correct activity recorded in each window for contaminant activity contributed by the associated nuclides and for background activity, and to compute the corrected counts/min/g of myocardium. Knowing the rate of withdrawal of the reference sample (Q_r) and the radioactivity (C_r) in the reference sample, we used myocardial activity (C_m) to compute myocardial blood flow (Q_m) as: Q_m = Q_r • C_m/C_r.

To compare regional blood flows to rings 2 and 3 we used multiple paired t-tests, comparing each region and layer in ring 2 with the corresponding specimen in ring 3. The P values were adjusted by the Bonferroni inequality method, which corrects for multiple tests performed on correlated data; i.e., each P value was multiplied by the number of paired t-tests performed on each set of data and a P value of <0.05 was required for statistical significance. Since no significant difference in myocardial blood flow was found between any region in ring 2 and the corresponding region in ring 3, data from corresponding regions in rings 2 and 3 were pooled for subsequent analysis. Similarly, paired t-tests between corresponding layers of the posterior papillary muscle region and the posterior left ventricular free wall demonstrated no significant difference in flow between these regions. Consequently, the data from these areas were pooled. Likewise, no significant difference was found between corresponding layers of the anterior left ventricular free wall and anterior papillary muscle region, and the data from these two regions were combined to represent the control region. For each microsphere injection paired t-tests were performed between each layer in the region under study and the corresponding layer in the control region. The transmural distribution of flow from epicardium to endocardium was compared by performing paired t-tests between layers 1 and 4, and expressed as the endocardial/epicardial flow ratio (flow to layer 4/flow to layer 1). Mean myocardial blood flow during each intervention was determined by averaging the flow to all layers of a given region.

Results

Figure 2 illustrates hemodynamic data obtained from a 22-kg awake dog during control conditions, systolic perfusion, and diastolic perfusion. During control conditions coronary blood flow was 26.5 ml/min, and a 10-second total coronary artery occlusion resulted in a reactive hyperemic response (blood flow debt repayment = 440%). When the electrical circuit was activated for systolic perfusion (Fig. 2B), heart rate, aortic pressure, and left atrial pressure remained at control values, but mean coronary flow fell immediately to 8.5 ml/min. During the next 10 beats coronary flow increased in a stepwise manner to achieve a new steady state of 16.9 ml/min. A reactive hyperemic response occurred when systolic perfusion was discontinued (blood flow debt repayment = 420%). When the circuit was adjusted for diastolic perfusion (Fig. 2C), heart rate, aortic pressure, and left atrial pressure again remained unchanged. Coronary flow initially dropped to 14.7 ml/min and then increased to 21.3 ml/min. In this animal there was a small reactive hyperemia when diastolic perfusion was terminated (blood flow debt repayment = 65%). The initial decrease in flow followed by a stepwise increase to a steady state was a consistent finding at the onset of both systolic perfusion and diastolic perfusion.

Table 1 summarizes the hemodynamic data from 10 dogs during these same conditions. During quiet control conditions mean heart rate was 69 ± 3 beats/min, aortic pressure was 101 ± 4 mm Hg, left atrial pressure was 2.8 ± 0.5 mm Hg, and coronary flow was 21.5 ± 1.7 ml/min. During systolic perfusion the heart rate, aortic pressure, and left atrial pressure did not change significantly, but coronary flow at the steady state was decreased 33 ± 3% below control (P < 0.05). During diastolic perfusion coronary blood flow was decreased 19 ± 3% below control (P < 0.05), with no significant change in the other hemodynamic parameters.

Figure 3 illustrates myocardial blood flow to each transmural layer during resting control conditions, systolic perfusion, and diastolic perfusion. Mean myocardial blood flow during control conditions was 0.79 ± 0.06 ml/g/min. Endocardial flow was significantly greater than epicardial flow with an endocardial/epicardial flow ratio of 1.29, significantly greater than 1.00 (P < 0.01). Mean myocardial blood flow during systolic perfusion was 0.57 ± 0.04 ml/g/min, significantly less than control (P < 0.01). During systolic perfusion myocardial blood flow to layers 1 and 2 was not different from control values, but flow to layers 3 and 4 was significantly decreased below control levels (P < 0.01), resulting in a significant decrease of the endocardial/epicardial flow ratio to 0.38. During diastolic perfusion mean myocardial blood flow was 0.66 ± 0.04 ml/g/min, 16% below the control measurement (P < 0.01). The reduction of myocardial blood flow during diastolic perfusion was uni-
Figure 2. ECG, phasic aortic pressure, phasic and mean coronary blood flow, and mean left atrial pressure in a 22-kg awake dog. A (control): After a 10-second total occlusion a reactive hyperemic response 28 seconds in duration resulted in 440% blood flow debt repayment. B (systolic perfusion, 30 seconds): When coronary inflow was limited to systole for 30 seconds, termination of systolic perfusion resulted in a reactive hyperemic response 32 seconds in duration with a blood flow debt repayment of 420%. C (diastolic perfusion, 30 seconds): After 30 seconds of diastolic perfusion a small reactive hyperemia 12 seconds in duration resulted in a 65% blood flow debt repayment. Paper speed = 2.5 mm/sec.
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TABLE I. Hemodynamic Data

<table>
<thead>
<tr>
<th></th>
<th>Heart rate (beats/min)</th>
<th>Aortic pressure (mm Hg)</th>
<th>Left atrial pressure (mm Hg)</th>
<th>Coronary blood flow (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>69 ± 3</td>
<td>101 ± 4</td>
<td>2.8 ± 0.5</td>
<td>21.5 ± 1.7</td>
</tr>
<tr>
<td>Systolic perfusion</td>
<td>71 ± 4</td>
<td>102 ± 5</td>
<td>2.8 ± 0.4</td>
<td>14.3 ± 1.0*†</td>
</tr>
<tr>
<td>Diastolic perfusion</td>
<td>75 ± 6</td>
<td>97 ± 3</td>
<td>2.4 ± 0.5</td>
<td>17.7 ± 1.9*</td>
</tr>
</tbody>
</table>

Heat rate, aortic pressure, left atrial pressure, and coronary blood flow of all dogs studied during control conditions, systolic perfusion, and diastolic perfusion. Values are mean ± SEM.
* P < 0.05 in comparison with control values.
† P < 0.05 in comparison with values during diastolic perfusion.

Behrm across the wall of the left ventricle, thus maintaining a transmural distribution of flow similar to that during control conditions (endocardial/epicardial ratio = 1.29). Comparison of the transmural flow during diastolic perfusion with that during systolic perfusion revealed that the flow to layers 3 and 4 was significantly decreased during systolic perfusion (P < 0.02). Thus, the decrease in absolute flow in layers 3 and 4 during systolic perfusion was related to ventricular contraction and was not merely an artifact due to intermit- tent coronary artery perfusion.

During systolic perfusion it appeared that at some tissue position between layers 2 and 3 flow began to decrease below control values, and beyond that point flow was inversely related to the depth of the tissue layer. Consequently, we normalized the blood flow to transmural layers 2, 3, and 4 by dividing the value for each transmural layer by the corresponding control value, and the resultant normalized flows were plotted against relative myocardial depth. As shown in Figure 4, a significant inverse correlation existed between flow and depth of the layer (r = -0.91).

Table 2 summarizes the reactive hyperemia data obtained following varying periods of total coronary artery occlusion or systolic perfusion in seven dogs with electromagnetic flowmeter probes. The time required to accumulate a given blood flow debt was longer with systolic perfusion than with total occlusion, because significant arterial inflow occurred during systolic perfusion. Reactive hyperemias of equivalent accumulated blood flow debt were compared. There was no significant difference in the duration of reactive hyperemia following total occlusion as compared to that following an interval of systolic perfusion of equivalent blood flow debt. However, for debts of less than 4 ml, the volume of reactive hyperemia flow was significantly greater following systolic perfusion than following total occlusion, resulting in a greater blood debt repayment. Debts larger than 4 ml, whether incurred during systolic perfusion or total occlusion, resulted in similar blood flow debt repayments. A small reactive hyperemic response was generally seen follow- ing periods of diastolic perfusion. Although this response was not systematically evaluated, the mean blood flow debt repayment following 30 seconds of diastolic perfusion was 125 ± 40%.

Figure 5 illustrates phasic coronary artery flow during control conditions and during the maximum flow rates...
achieved in the reactive hyperemia following 24 seconds of total coronary artery occlusion and 60 seconds of systolic perfusion. It is apparent that systolic flow was markedly greater during the reactive hyperemia following total occlusion than following systolic perfusion. Mean coronary blood flow per beat and the systolic and diastolic components of flow during control conditions and during maximum hyperemic flow for all dogs with flowmeters are shown in Table 3. During control conditions 9.8% of coronary flow occurred in systole. At the peak of the reactive hyperemia following a 24-second total occlusion, absolute flow during systole had increased 6-fold over the control levels, while the systolic portion of flow had increased to 11.9%. In contrast to this, at the height of the reactive hyperemia following 60 seconds of systolic perfusion, systolic flow had increased to only 2½ times above the control level, so that the systolic contribution to flow had decreased significantly to $6.1 \pm 0.9\%$ ($P < 0.01$). During the reactive hyperemia following systolic perfusion, absolute flow during diastole and total flow per beat were also significantly less than following total occlusion.

Despite the lower peaks flow rates, this prolongation of the interval of maximum reactive hyperemic flow following systolic perfusion resulted in debt repayments similar to those following total occlusion.

Figure 6A illustrates the mean transmural distribution of blood flow during the maximum reactive hyperemia flow following 60 seconds of systolic perfusion. At this time flow to layer 1 was not significantly different from the control value for that layer, whereas flows to the deeper layers of myocardium were significantly and progressively increased above the control values. The marked preferential flow to the subendocardium was indicated by an endocardial/epicardial flow ratio of 6.4. In contrast to this, during the reactive hyperemia following total coronary artery occlusion of equivalent blood flow debt, flows to all transmural layers were significantly greater than control values ($P < 0.01$), and the endocardial/epicardial flow ratio was 1.73 (Fig. 6B). Flow to layer 1 during the reactive hyperemia following total occlusion was greater than the flow to layer 1 during the reactive hyperemia of systolic perfusion ($P < 0.01$).

To examine the relationship between the degree of underperfusion during systolic perfusion and the subsequent reactive hyperemia flow, we plotted the normalized flow for each myocardial layer against the corresponding reactive hyperemia flow. As shown in Figure 7, a significant negative correlation existed between the flow during systolic perfusion and flow during the ensuing reactive hyperemia ($r = -0.71$).

**Discussion**

In a previous study of Downey and Kirk, coronary flow was limited to systole by perfusing the cannulated left main coronary artery of open-chest dogs directly from the left ventricle. When $^{45}$RbCl was injected into the coronary cannula during systolic perfusion, the subendocardium accu-

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**Table 2. Reactive Hyperemia Data**

| Duration of total occlusion (sec) | Blood flow debt (ml) | Reactive hyperemia duration (sec) | Debt repayment (%) | Duration of systolic perfusion (sec) | Blood flow debt (ml) | Reactive hyperemia duration (sec) | Debt repayment (%) | $P$  
|---------------------------------|----------------------|----------------------------------|------------------|----------------------------------|---------------------|----------------------------------|------------------|------  
| 5.0                            | 1.89 ± 0.13          | 38.9 ± 4.3                       | 506 ± 40         | 10.0                             | 2.31 ± 0.26         | 55.7 ± 5.9                       | 745 ± 83         | < 0.05 |
| 10.0                           | 3.58 ± 0.25          | 58.7 ± 7.1                       | 524 ± 64         | 15.0                             | 2.63 ± 0.34         | 52.1 ± 7.5                       | 747 ± 92         | —     |
| 15.0                           | 5.11 ± 0.44          | 71.2 ± 12.8                      | 597 ± 67         | 20.0                             | 3.60 ± 0.53         | 69.6 ± 7.2                       | 720 ± 57         | < 0.05 |
| 20.0                           | 6.98 ± 0.58          | 85.0 ± 12.1                      | 513 ± 76         | 25.0                             | 4.53 ± 0.68         | 73.0 ± 8.2                       | 717 ± 65         | > 0.10 |
| 30.0                           | 10.5 ± 0.70          | 103 ± 13.7                       | 527 ± 49         | 30.0                             | 9.77 ± 1.30         | 112 ± 15.1                       | 563 ± 68         | > 0.10 |

Reactive hyperemia data tabulated for seven dogs with electromagnetic flowmeter probes. $P$ values represent comparison of blood flow debt repayments for reactive hyperemias of similar blood flow debts.
TABLE 3. Systolic and Diastolic Flow Data

<table>
<thead>
<tr>
<th></th>
<th>Systolic flow (ml/beat)</th>
<th>Diastolic flow (ml/beat)</th>
<th>Total flow (ml/beat)</th>
<th>Systolic flow/total flow × 100 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.034 ± 0.004</td>
<td>0.312 ± 0.03</td>
<td>0.345 ± 0.03</td>
<td>9.8 ± 8</td>
</tr>
<tr>
<td>Reactive hyperemia after total occlusion</td>
<td>0.206 ± 0.02*</td>
<td>1.54 ± 0.15*</td>
<td>1.75 ± 0.16*</td>
<td>11.9 ± 0.7</td>
</tr>
<tr>
<td>Reactive hyperemia after systolic perfusion</td>
<td>0.084 ± 0.02*†</td>
<td>1.23 ± 0.14*†</td>
<td>1.31 ± 0.15*†</td>
<td>6.1 ± 0.9*†</td>
</tr>
</tbody>
</table>

Systolic flow, diastolic flow, total flow per beat, and the percentage of total flow occurring during systole for each dog with a flowmeter probe during control conditions and during maximum reactive hyperemia flow following 24 seconds of total occlusion and 60 seconds of systolic perfusion.

* $P < 0.01$ in comparison to control values.
† $P < 0.01$ in comparison to values during the reactive hyperemia following total occlusion.

mulated one-half as much indicator as did the subepicardium. A second study by Downey et al. examined the transmural distribution of systolic flow in the isolated heart during isovolumetric contraction. Coronary flow was limited to systole and the flow distribution was examined by the radioisotope wash-in technique. $^{86}$RbCl was used to measure the flow distribution when coronary perfusion occurred throughout the entire cardiac cycle, and $^{4}$KCl was used to study the distribution when flow was limited to systole. During control conditions radioactive tracer uptake was slightly greater in the subendocardium than in the subepicardium, whereas during systolic perfusion a marked transmural gradient occurred with subendocardial uptake 37% of subepicardial uptake. Although these studies demonstrated a transmural perfusion gradient during systole, inability to calibrate the $^{86}$RbCl wash-in technique prevented determination of absolute transmural flow values. In addition, the studies were performed on open-chest animals and isolated heart preparations, in which the stresses of anesthesia and acute surgical trauma are known to affect the transmural distribution of myocardial blood flow in dogs.

In our present study the radioactive microsphere technique allowed computation of absolute flow values; in addition, by using different radionuclides we could make several consecutive interventions, allowing each animal to serve as its own control. Also, studies were made in awake dogs to avoid any possible influence of anesthesia and acute surgical trauma on myocardial blood flow. However, there were four possible sources of error in this study:

1. The flows measured with the electromagnetic flowmeter during systolic perfusion may have underestimated total arterial inflow because of the inability to measure inflow that occurred between systolic pulses from intercoronary collateral channels. This error was minimized by attempting to use only dogs with minimal intercoronary circulation; i.e., only dogs demonstrating marked S-T elevation during a 60-second total occlusion of the left circumflex coronary artery were studied. Since the microsphere technique measures both circumflex arterial inflow and collateral inflow, significant collateral inflow would have resulted in disproportionately greater flows being measured with the microsphere technique than with the coronary artery flowmeter. In fact, no such difference was found. During systolic perfusion, flow measured with the microsphere technique was 72% of control (0.57/0.79 ml/g/min), while the simultaneous flow measured with the electromagnetic flowmeter was 67% of control (14.3/21.5 ml/min). These similar values suggest little collateral inflow during systolic perfusion.

2. Systolic perfusion may alter the mechanics of ventricu-

![Graph A](http://circres.ahajournals.org/article-figs/6Control-ReactiveHyperemiaSystolicPerfusion.png)

**Figure 6.** Mean left ventricular myocardial blood flow (ml/g/min) ± SEM to all layers during control conditions (solid lines) and during the maximum reactive hyperemia flow following 60 seconds of systolic perfusion (A, broken line) and 24 seconds of total occlusion (B, broken line).
lar contraction, and consequently change the effect of ventricular systole on transmural blood flow. In our present study systolic perfusion for periods of as long as 2 minutes resulted in no change in heart rate, left atrial pressure, aortic pressure, or the ECG. Nevertheless, subtle alterations of regional myocardial function during the period of systolic perfusion cannot be ruled out.

3. The observed gradient in transmural myocardial blood flow during systolic perfusion may have been related to the intermittent nature of coronary perfusion. However, such an effect was unlikely since intermittent perfusion for an equivalent interval during diastole resulted in a transmural distribution similar to that found during control conditions.

4. Total inflow measured during systolic perfusion may have overestimated the actual flow occurring during systole, since blood may have been stored in the epicardial vessels during the systolic pulse to be discharged in early diastole. The distribution of flow resulting from the discharge of this stored blood would depend on the dynamic relationship between the instantaneous perfusion pressure and the transmural vascular resistance. The instantaneous perfusion pressure is represented by the pressure difference between the epicardial coronary artery and the left ventricular cavity.

To study this relationship, we measured coronary artery pressure distal to the occluder and left ventricular pressure in three awake dogs not included in the present study, using a technique previously described.14 Coronary artery pressure was similar to aortic pressure during normal perfusion, and fell to approximately 20 mm Hg during total coronary artery occlusion in all three dogs. During systolic perfusion the coronary artery pressure during systole was similar in contour to the left ventricular pressure, and fell to the pre-systolic level in parallel with left ventricular pressure during isovolumetric relaxation. The pressure throughout diastole was 20 mm Hg, identical with that during a total coronary artery occlusion. Because coronary artery pressure fell in phase with left ventricular pressure, the discharge of stored blood into the myocardium from the passive recoil of epicardial vessels occurred totally within the isovolumetric relaxation phase of left ventricular systole. Since, during both left ventricular ejection and isovolumetric relaxation, ventricular pressure equaled or exceeded coronary artery pressure, flow occurring during either of these two intervals was subject to similar intramuscular pressure relationships. The transmural distribution of stored blood discharged during isovolumetric relaxation would therefore be similar to the distribution of flow occurring during ejection. Thus, although the effect of epicardial artery capacitance may have slightly elevated total coronary inflow during systolic perfusion, the relative transmural distribution should not have been altered.

The results of our study indicate that the transmural gradient of flow observed during systolic perfusion was a reflection of the effect of ventricular contraction. The observed gradient in flow during systole (endocardial/epicardial ratio = 0.38) compares favorably with the value reported by Downey13 (endocardial/epicardial ratio = 0.37) in the isolated heart preparation. Since the absolute flow values obtained in our study demonstrated that subepicardial perfusion was normal when inflow was limited to systole, the observed gradient was a direct reflection of the degree of subendocardial underperfusion.

An explanation for the observed gradient during systole requires consideration of the three main factors that determine impedance to myocardial blood flow during systole: (1) vasomotor tone, (2) myocardial shear forces, and (3) intramyocardial tissue pressure. Vasomotor tone appears to be the major determinant of myocardial blood flow during diastole, but probably is effective in determining flow during systole only in the layers closest to the epicardium. Shear forces generated by ventricular contraction could affect coronary flow by altering the geometry of intramyocardial vessels. However, as demonstrated by Downey et al.,14 these forces appear to be a minor factor in determining vascular resistance during systole. In addition, shearing forces are uniformly distributed across the left ventricular wall and thus would be unlikely to contribute significantly to the observed gradient.16 The major factor controlling the resistance across the left ventricular wall during systole is the intramyocardial pressure developed during myocardial contraction. Whenever intramyocardial tissue pressure exceeds capillary outflow pressure, flow within the myocardium may be described by the vascular waterfall.17,18

The classical theory of flow dynamics computes flow as follows: \( Q = (p_i - p_e) \times 1/R \), where \( Q \) = flow, \( p_i \) = inflow pressure, \( p_e \) = outflow pressure, and \( R \) = resistance of the system. In the case of the vascular waterfall, the equation changes slightly to account for extravascular pressure: \( Q = (p_i - p_e) \times 1/R \), where \( p_e \) = extravascular pressure. Flow in the presence of a vascular waterfall demonstrates several interesting characteristics. First, flow is independent of outflow pressure provided that extravascular pressure exceeds outflow pressure. Second, resistance in such a system is determined by the dynamic interaction of \( p_i \) and \( p_e \), i.e., resistance is not a simple function of the radius of the conducting vessel but rather is determined by the degree of constriction of the vessel created by the two opposing pressures, \( p_i \) and \( p_e \). This interaction can be explained if one visualizes an indistensible, completely collapsible tube, with an inflow pressure of 120 mm Hg, an outflow pressure of 20

![Figure 7](http://circres.ahajournals.org/)

**Figure 7.** On the abscissa is plotted the normalized left ventricular myocardial blood flow during systolic perfusion, and on the ordinate the normalized reactive hyperemia flow following systolic perfusion.
mm Hg, and a pressure surrounding the tube of 80 mm Hg. Somewhere along the tube intraluminal pressure will drop below 80 mm Hg; at this point \( p_c \) exceeds \( p_t \) and the tube will begin to collapse. However, a dynamic equilibrium will be reached when the narrowing created by collapse of the tube generates sufficient proximal intraluminal pressure to prevent further closure. As long as \( p_t \) equals or exceeds \( p_c \), the conducting vessel can never fully collapse, for it would first develop an orifice small enough to generate a pressure equal to \( p_c \). Thus, flow in this system is determined by the size of the orifice created by the interaction of the two pressures, and not determined by the intraluminal diameter upstream from this point. Obviously, if \( p_c \) exceeds \( p_t \), complete collapse of the conducting vessel will occur and flow will cease.

Several investigators have provided evidence that intramyocardial pressure increases from the subepicardium to the subendocardium during ventricular contraction.\(^7\) If the vascular waterfall operates in those areas where intramyocardial pressure exceeds capillary outflow pressure, a series of waterfalls would be present throughout the left ventricular wall. The size of the orifice created in the conducting vessels would decrease from the subepicardium to the subendocardium as intramyocardial pressure \( (p_c) \) increased. Hence, resistance to flow would increase from the subepicardium to the subendocardium, and could account for the transmural gradient observed when flow was limited to systole. Consequently, the flow distribution in myocardial layers subjected to a waterfall phenomenon \( (p_c > p_t) \), would reflect the intramyocardial pressure distribution.

From the data of our experiment it was noted that blood flow was a linear function of myocardial depth beginning with myocardial layer 2. This relationship could be explained if the distribution of pressure within the wall was linear over this range. Since flow to layer 1 was identical to the flow to layer 2, either \( p_c \) was similar at the two layers and flow-limiting, or \( p_t \) was a relatively minor factor in this region and flow was controlled principally by active coronary vasomotion. The latter hypothesis appears to be the best explanation because, after the onset of systolic perfusion, there was an increase in the volume of flow per systolic pulse, indicating that vasodilatation was occurring. As noted earlier, such vasodilatation can increase flow only in regions where the waterfell does not function \( (p_c > p_t) \), and the epicardial regions would best fit this condition.

Several investigators have demonstrated that reactive hyperemia flow was a function of the preceding ischemic stimulus.\(^5\) Recently Bache et al.\(^6\) observed an endocardial/epicardial ratio of 1.8 during the reactive hyperemia following a 5-second coronary artery occlusion in awake dogs. This was similar to the endocardial/epicardial ratio \( (1.7) \) observed in our present study during maximum reactive hyperemia flow following a 24-second coronary artery occlusion. The greater subendocardial flow during reactive hyperemia was a consequence of the transmural gradient in ischemia that occurred during total occlusion.\(^2\) However, selective underperfusion of the subendocardial layers during systolic perfusion produced a more marked gradient in ischemia than that which occurred during total occlusion. Consequently, 85% of the flow during reactive hyperemia following systolic perfusion was directed preferentially to the subendocardial region. This resulted in a significant inverse relationship between the degree of underperfusion during systolic perfusion and the subsequent reactive hyperemia flow. Thus, the myocardial vasculature was able to selectively vasodilate and specifically direct reactive hyperemia flow to areas of ischemia.

The phasic coronary blood flow pattern during the reactive hyperemia following systolic perfusion also reflected preferential flow to the subendocardium. Since flow during systole was directed primarily to the subepicardium, little or no underperfusion of the subepicardial layers occurred when inflow was limited to systole. Consequently, vasodilation during the reactive hyperemia following systolic perfusion was confined principally to the subendocardium. Therefore, during this reactive hyperemia, flow during systole was only slightly increased, because the area principally perfused in systole had incurred only a small blood flow debt. Diastolic flow, however, was substantially increased as a result of the marked vasodilation in the subendocardium. In contrast, the more or less uniform underperfusion during a total occlusion would result in transmural vasodilatation; hence, following a total occlusion, flows in both systole and diastole were markedly increased.

Thus, during systolic perfusion, a transmural gradient in myocardial blood flow existed, the subepicardial layers of the left ventricular wall were normally perfused, and the subendocardial layers were underperfused. This gradient was a function of tissue depth and could be explained by the vascular waterfall theory. The transmural distribution of myocardial blood flow during the reactive hyperemia following systolic perfusion was a function of the degree of underperfusion during systolic perfusion and demonstrated the selective ability of the coronary vasculature to respond to localized ischemia.

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References

ATRIAL RECEPTORS AND HEART RATE—Zucker and Gilmore

IRVING H. ZUCKER, PH.D., AND JOSEPH P. GILMORE, PH.D.

ABSTRACT The discharge characteristics of type B left atrial receptors were analyzed during alterations in heart rate. Recordings were made from single-fiber preparations of the left cervical vagus of pentobarbital-anesthetized, open-chest dogs. The heart was paced following a sinoatrial crush at frequencies ranging from 60 to 240 beats/min. Left atrial transmural pressure was varied at each heart rate by the intravenous infusion of warm isotonic NaCl. As heart rate was increased there was a progressive decrease in the level of peak "v" wave left atrial pressure. Concomitantly with the decrease in left atrial pressure, the number of spikes per cardiac cycle decreased as did the maximal instantaneous frequency of discharge. A significant positive relationship could be demonstrated with either the discharge per minute \([\text{spikes per cardiac cycle}} \times \text{heart rate}]\) or discharge per cycle \(\times \text{vs. the peak "v" wave of the left atrial pressure, regardless of heart rate. The number of impulses that entered the central nervous system per unit of time remained relatively constant at heart rates between 90 and 240/min. It is concluded from these data that the reflex effects which have been attributed in the past to atrial stretch receptor stimulation during clinical episodes of atrial tachyarhythms may be better correlated with some aspect of receptor discharge other than frequency or the number of discharges per cycle.

ATRIAL STRETCH receptors have been located in both the right and left atria in a variety of species. Two types of atrial receptors whose fibers traverse the vagus have been identified by Paintal, who termed them type A and type B on the basis of the timing of their discharge in relation to the cardiac cycle. Their discharge characteristics have been investigated by several workers and these receptors have been implicated in reflexes that may be involved in the control of fluid and electrolyte balance, heart rate, and systemic resistance. Dur-

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The Response of Atrial Stretch Receptors to Increases in Heart Rate in Dogs

IRVING H. ZUCKER, PH.D., AND JOSEPH P. GILMORE, PH.D.

ABSTRACT The discharge characteristics of type B left atrial receptors were analyzed during alterations in heart rate. Recordings were made from single-fiber preparations of the left cervical vagus of pentobarbital-anesthetized, open-chest dogs. The heart was paced following a sinoatrial crush at frequencies ranging from 60 to 240 beats/min. Left atrial transmural pressure was varied at each heart rate by the intravenous infusion of warm isotonic NaCl. As heart rate was increased there was a progressive decrease in the level of peak "v" wave left atrial pressure. Concomitantly with the decrease in left atrial pressure, the number of spikes per cardiac cycle decreased as did the maximal instantaneous frequency of discharge. A significant positive relationship could be demonstrated with either the discharge per minute \([\text{spikes per cardiac cycle}} \times \text{heart rate}]\) or discharge per cycle \(\times \text{vs. the peak "v" wave of the left atrial pressure, regardless of heart rate. The number of impulses that entered the central nervous system per unit of time remained relatively constant at heart rates between 90 and 240/min. It is concluded from these data that the reflex effects which have been attributed in the past to atrial stretch receptor stimulation during clinical episodes of atrial tachyarhythms may be better correlated with some aspect of receptor discharge other than frequency or the number of discharges per cycle.

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