Correlation between Acute Reductions in Myocardial Blood Flow and Function in Conscious Dogs

Stephen F. Vatner

SUMMARY Decreases in regional endocardial function (ultrasonic dimension technique) and blood flow (radioactive microsphere technique) were correlated in 14 conscious dogs with acute graded levels of coronary stenosis. Coronary stenosis affected overall ventricular function only slightly, but induced gradual reductions in regional blood flow (BF) and segment length (SL) shortening in the ischemic zone. The relationship was best fit by an exponential function relating % change in SL to % change in BF; i.e., \( SL(\%) = -161.6 e^{-0.76BF(\%)} (r = 0.92) \). In 14 segments, where no change in function was observed, blood flow fell by 6 ± 1%. However, 10–20% reductions of blood flow impaired function significantly. Severe reduction of blood flow was required to reduce function completely. In 12 segments exhibiting paradoxical motion, blood flow fell by 95 ± 2%, a value greater \( (P < 0.01) \) than in four akinetic segments, i.e., those with no shortening or lengthening (−82 ± 4%). These data, which show on one hand that only 10–20% reductions in blood flow impair function significantly and that, on the other hand, severe reductions in blood flow are required to abolish active shortening completely, indicate sensitive coupling between blood flow and function in the conscious dog with acute myocardial ischemia. Circ Res 47: 201-207, 1980

MYOCARDIAL mechanical function is dependent primarily on its blood supply, since arteriovenous oxygen extraction is near-maximal in the normal heart and anaerobic reserves are minimal (Berne and Rubio, 1979). Accordingly, it is recognized generally that reduction in myocardial blood flow results in impaired mechanical function. However, the precise relationship between reductions of blood flow and function that are induced by coronary stenosis is not known, particularly in the intact conscious animal in which myocardial function is not already depressed by general anesthesia and the surgery required for an open-chest preparation (Vatner and Braunwald, 1975; Manders and Vatner, 1976; Vatner and Smith, 1974). The advent of techniques to measure regional changes in blood flow to discrete areas of ischemic myocardium (radioactive microspheres) and myocardial function at the same sites (ultrasonic dimension gauge) permitted this study to be conducted in intact, conscious animals.

The goal of this investigation was to discern the relationship between reductions in regional myocardial blood flow and mechanical function in conscious dogs with acute, graded levels of coronary stenosis. In addition to defining this relationship, it was of particular interest to determine (1) how much blood flow can be reduced without an apparent reduction in function, (2) at what level of flow reduction myocardial function is impaired significantly, (3) at what level of flow reduction myocardial function is completely lost, and (4) whether segments demonstrating paradoxical bulging are characterized by greater flow deprivation than akinetic segments. These goals were attained by gradually constricting a coronary arterial branch while function was continuously monitored in the distribution of that branch and radioactive microspheres were administered during control conditions, and (1) at the point of the first detectable reduction in function, (2) at the point at which function was completely lost, and (3) at an intermediate point. It was judged particularly important to correlate regional myocardial blood flow and function at endocardial sites (since ischemia is manifested in endocardial layers initially and ultimately in its most intense form).

Methods

Fourteen dogs, weighing 25–35 kg, were anesthetized with pentobarbital sodium, 30 mg/kg, iv. Through a thoracotomy in the 5th left intercostal space, miniature pressure gauges (P22, Konigsberg Instruments) were implanted within the left ventricle through a stab wound in the apex, pacing electrodes were implanted on the right ventricle and left atrium, and hydraulic occluders were placed around the left anterior descending coronary artery or one of its major branches, 3–5 cm from its origin. Heparin-filled Tygon catheters (Norton Co.) were implanted in the left atrium and aorta. Up to four pairs of miniature ultrasonic transducers were im-
planted endocardially, parallel to the muscle fibers, 1-2 cm apart in potentially ischemic zones.

The miniature pressure gauges were calibrated in vitro and in vivo against Statham P23Db strain gauge manometers (Statham Instruments) connected to the left atrial and aortic catheters. An improved ultrasonic transit-time dimension gauge (Heyndrickx et al., 1975; Pagani et al., 1978a) was used to measure regional myocardial segment length (SL). This instrument generates a voltage linearly proportional to the transit time of acoustic impulses traveling at the sonic velocity of approximately $1.5 \times 10^6$ mm/sec between the 3 MHz piezoelectric crystals, thereby giving a record of instantaneous myocardial fiber length. At a constant room temperature, the thermal drift of the instrument is minimal, i.e., less than 0.01 mm in 6 hours. The frequency response is flat to 60 Hz. Any drift in the measuring system, i.e., the instrument electronics, the data tape recorder, and the oscillograph that displayed data, was eliminated during the experiment by periodic calibrations. This involved substitution of pulses of precisely known duration from a crystal-controlled pulse generator with a basic stability of 0.001%. The position of the miniature ultrasonic transducers was confirmed at autopsy and minimal fibrosis extending for <1 mm from the transducer was observed at the site of implantation. If transducers were not in the endocardial third of the myocardial wall, the data were discarded.

Regional myocardial blood flow was measured by the radioactive microsphere technique. The microspheres were shipped from the manufacturer (3M Co.) in dry form, in multiple vials for each isotope. The solutions were prepared individually as needed, and the microspheres were in contact with the solution for less than 7 days. The concentration of microspheres per milliliter of solution was adjusted appropriately to account for natural radioactive decay. The microspheres were suspended in 0.01% Tween 80 solution (10% dextran) and placed in an ultrasonic bath for 60 minutes. Subsequently, they were agitated by direct application of an ultrasonic probe to insure dispersion of the spheres just before injection. Absence of microsphere aggregation was verified by microscopic examination. Several hours before injection of microspheres, 0.7 ml of Tween 80-dextran solution (without microspheres) was injected to determine whether the diluent for the microsphere suspension had an adverse effect on cardiac dynamics (Millard et al., 1977). One to three million microspheres (15 ± 1 µm) labeled with either $^{46}$Sc, $^{68}$Nb, $^{85}$Sr, or $^{141}$Ce were injected through the catheter implanted in the left atrium for four separate determinations of blood flow; during control conditions, and at three levels of coronary stenosis. A reference sample of blood was withdrawn beginning 10 seconds before microsphere injection and continuing for at least 60 seconds after the injection had been completed. After the dog had been killed with an overdose of pentobarbital, Na, myocardial samples were obtained from the sites at which function was measured, by sectioning the myocardium into approximately equal epi, mid, and endocardial components. When it was determined that the ultrasonic transducers were in the endocardial third of the ventricular wall, these endocardial samples were weighed, placed in a multichannel γ well counter (Searle Analytic, Inc.), and counted with appropriately selected-energy windows for 10 minutes.

Experiments were conducted 1-4 weeks after operation. While the conscious, unsedated dogs rested quietly, control records of left ventricular pressure, the rate of change of pressure (dP/dt), mean arterial pressure, and segment length shortening and the rate of segment length shortening (dSL/dt) were recorded in 10 dogs with heart rate held constant 20-40 beats/min above the spontaneous rhythm, and in four dogs with spontaneous rhythm. After control measurements were recorded and the first injection of microspheres was made, the coronary vessel was constricted. Stenosis was induced gradually by means of a threaded, gas-tight, 1-ml syringe (Hamilton). The second dose of microspheres was injected after one of the segments showed a sustained (5-15 minute) detectable reduction in shortening. At this time, other segments in the ischemic zone frequently failed to show significant reductions in function (Fig. 1). Then, the vessel was constricted further until an intermediate reduction in function was observed. At this point, the third dose of radioactive microspheres was injected. This level of stenosis was also maintained.

**Figure 1** Phasic waveforms for left ventricular (LV) pressure and two segments in the ischemic zone along with mean arterial pressure are shown during control conditions, then after mild stenosis when function fell slightly in the top segment, but not the other, and finally with severe stenosis when paradoxical bulging was observed in the top segment and minimal shortening was preserved in the bottom segment.
for 5-15 minutes. Then the vessel was constricted further until function was completely lost. At this point the fourth radioactive microsphere was administered. Finally, the animal was killed to verify placement of the transducers and measurements of regional myocardial blood flow. As mentioned above, data were discarded if the transducers were not endocardial, or if the myocardial samples for these two transducers did not show similar flow reductions (±20%).

Data were recorded on a multichannel tape recorder and played back on two multichannel, direct-writing oscillographs at a paper speed of 100 mm/sec. A cardiotachometer, triggered by the pressure pulse signal, provided instantaneous and continuous records of heart rate. Continuous records of dP/dt and dSL/dt were derived from the pressure and segment length signals with Philbrick (Teledyne Philbrick) operational amplifiers connected as differentiators, having frequency responses of 700 and 140 Hz, respectively. A triangular wave signal with known slope (rate of change) was substituted for the pressure and segment length signals to calibrate the differentiators directly.

The data-correlating reductions in regional blood flow and myocardial function were fitted to an exponential function with a PDP 11/34 computer (Digital Equipment). Blood flow in the ischemic zone was normalized for changes in nonischemic zone flow, i.e., ischemic zone flow was multiplied by the quotient of the control and response blood flow in the average of three normal zone endocardial samples. This minimized variations due to the microsphere technique. Decreases in regional blood flow were correlated with decreases in regional segment length shortening, velocity of segment length shortening, and increases in regional end-diastolic and end-systolic lengths. Decreases in segment length shortening were, in some instances, greater than 100%, due to paradoxical lengthening. When a segment failed to shorten during systolic ejection, its velocity of shortening was considered to be zero.

Results

Effects of Graded Coronary Stenosis on Overall Hemodynamics (Table 1)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Level 1</th>
<th>Level 2</th>
<th>Level 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate</td>
<td>116 ± 9 beats/min</td>
<td>1.3 ± 1.0</td>
<td>1.3 ± 1.3</td>
<td>4.9 ± 2.7</td>
</tr>
<tr>
<td>Mean arterial pressure</td>
<td>160 ± 3 mm Hg</td>
<td>0.4 ± 1.0</td>
<td>2.6 ± 1.5</td>
<td>7.7 ± 3.8</td>
</tr>
<tr>
<td>LV systolic pressure</td>
<td>121 ± 3 mm Hg</td>
<td>-0.4 ± 0.7</td>
<td>2.1 ± 1.1</td>
<td>5.1 ± 2.5</td>
</tr>
<tr>
<td>LV end-diastolic pressure</td>
<td>7.8 ± 0.6 mm Hg</td>
<td>-1.0 ± 1.0</td>
<td>4.2 ± 2.8</td>
<td>15.1 ± 5.9</td>
</tr>
<tr>
<td>LV dP/dt</td>
<td>3520 ± 120 mm Hg/sec</td>
<td>-1.0 ± 1.2</td>
<td>-1.9 ± 1.9</td>
<td>0.8 ± 3.2</td>
</tr>
<tr>
<td>Normal zone endocardial coronary blood flow</td>
<td>1.44 ± 0.14 ml/min per g</td>
<td>-3.0 ± 9.0</td>
<td>4.4 ± 8.3</td>
<td>27.5 ± 11.3</td>
</tr>
</tbody>
</table>

* Significant change, P < 0.05.

Relationship between Reductions in Blood Flow and Segment Length Shortening (n = 68)

The relationship between the decreases in blood flow (BF) and segment length shortening was best fit by an exponential expression. The exponential form was selected for the following reasons: first, visual inspection of the data indicates a distinct curvature (Fig. 2); second, using the exponential, there was a substantial improvement from 0.86 to 0.92 in the correlation coefficient over the values for a linear fit; and third, the behavior of the exponential in the extremes of the range of blood flow changes matched the data better than either polynomial or power models. In addition, the plot of % decrease of regional blood flow vs. % change of regional segment length was distinctly linear, and the residuals of this plot were well scattered and unskewed, in contrast to those for the power and polynomial models.

The relationship between % changes in segment length and blood flow was best described as SL(%)Δ = -161.6 e-0.047BF(%)Δ (r = 0.92), (standard error of the estimate [SEE] = 23.7). This relationship was not affected substantially by correcting ischemic zone blood flow for changes in blood flow in the normal zone. Without correcting for changes in blood flow in the normal zone, the relationship was: SL(%)Δ = -168.9 e-0.047BF(%)Δ (r = 0.85), (SEE = 27.9). The lack of substantial difference between the relationships with and without correction for normal zone flow can be attributed to the fact that blood flow did not change significantly in the normal zone, except with the most intense level of stenosis.

These data indicate a very good correlation between reductions in blood flow and function with acute coronary stenosis. In 14 segments, in which no change in function was detected, blood flow fell by 6.4 ± 1.3% (Fig. 3). However, 10-20% reductions...
of blood flow impaired function significantly (Fig. 4). Severe reduction in blood flow (>90%) was required to eliminate function completely (Figs. 2–4). This is based on the exponential function relating blood flow and function. Finally, in the 12 segments that showed paradoxical motion, blood flow fell by 95 ± 2%, a value greater ($P < 0.01$) ($t$-test for unpaired comparisons, Armitage, 1973) than was observed in four akinetic segments with neither shortening nor lengthening (82 ± 4%) (Fig. 3).

Relationship between Reductions in Blood Flow and End-Diastolic and End-Systolic Lengths (Fig. 5)

Responses of end-diastolic and end-systolic length differed in that an increase in end-systolic length was a more sensitive index of ischemia than an increase in end-diastolic length (Fig. 5). The relationship between changes in end-systolic length (ESL) and blood flow was: $\text{ESL}(\% \Delta) = 25.7 e^{-0.033 \text{BF}(\% \Delta)} (r = -0.69)$, (SEE = 7.4), whereas the same relationship for changes in end-diastolic length (EDL) was: $\text{EDL}(\% \Delta) = 5.5 e^{-0.021 \text{BF}(\% \Delta)} (r = -0.75)$, (SEE = 2.6). These relationships were significantly different ($P < 0.01$).
Blood Flow (% of control)

**Figure 5** The exponential relationships between % increases in end-diastolic length (EDL) and end-systolic length (ESL) vs. regional blood flow are shown: $EDL(%\Delta) = 5.5 e^{-0.030BF(%A)}$ ($r = -0.75$); $ESL(%\Delta) = 25.7 e^{-0.033BF(%A)}$ ($r = -0.69$).

**Relationship between Reductions in Blood Flow and Velocity of Segment Length Shortening (Fig. 6)**

The relationship between a reduction in velocity ($V$) of shortening and blood flow was similar to that for segment length shortening and blood flow: $V(%\Delta) = -110.5 e^{-0.043BF(%A)}$ ($r = 0.87$), (SEE = 19.5).

Discussion

In this investigation, in which an exponential function was used, an excellent correlation ($r = 0.92$) between reductions in regional myocardial blood flow and function was observed. Regional myocardial mechanical function was closely coupled to reductions in regional myocardial blood flow. Segments that did not exhibit loss of function were characterized by reductions in blood flow of only $6.4 \pm 1.3\%$. These data, indicating such a sensitive coupling between blood flow and mechanical function, differ from the majority of data collected in open-chest, anesthetized preparations (Banka et al., 1977; Kerber et al.; 1975, Lekven et al.; 1973, Stowe et al.; 1978, Waters et al.; 1977, Wyatt et al.; 1975). In these latter experiments, relatively large reductions in blood flow, i.e., approximately 50%, were observed before abnormalities in function were detected consistently. In the present investigation, significant impairment of function was found with only 10–20% reductions in blood flow (Fig. 4). A recent study by Downey (1976) conducted in open-chest, anesthetized dogs found data similar to those presented here, in that endocardial function, measured by a deep strain gauge arch, began to fall with only slight reductions in blood flow. Moreover, the data of Downey (1976) are similar in that the relationship between decreases in deep contractile force and decreases in coronary blood flow does not appear to be linear. However, the data from Downey differ in that they do not show the preservation of some contractile function with severe reductions in coronary blood flow.

There are several important differences between this investigation and prior studies in open-chest, anesthetized preparations. First of all, general anesthesia and surgical manipulation of the heart depresses baseline myocardial contractility profoundly (Vatner and Smith, 1974; Manders and Vatner, 1976). Second, these interventions interfere substantially with autonomic control of the circulation (Vatner and Braunwald, 1975). Thus, baseline levels of end-diastolic cardiac size and myocardial contractility are lower, whereas levels of heart rate and sympathetic tone are higher in an open-chest, anesthetized animal. These important differences between the present and prior investigations could reconcile any discrepancies. However, other important differences should be considered.

In the present study, measurements of regional myocardial function and blood flow were localized to endocardial zones where ischemia is manifested first and eventually in its most intense form. Thus, the sensitivity of the ultrasonic dimension technique, which allows measurements of endocardial segment function in discrete ischemic zones, permitted the finding of changes in myocardial function with relatively small decrements in blood flow. Prior studies in open-chest preparations used either
less-sensitive indicators of regional myocardial function (Banka et al., 1977; Kerber et al., 1975; Lekven et al., 1973; Waters et al., 1977; Wyatt et al., 1975), or a less sensitive indicator of regional myocardial blood flow (Banka et al., 1977; Lekven et al., 1973; Stowe et al., 1978; Waters et al., 1977; Wyatt et al., 1975). This is a critical consideration for the correlation of regional myocardial blood flow and function in the presence of acute myocardial ischemia, where there is often juxtaposition of ischemic and normal areas of myocardium. For example, two transducers comprising one segment occasionally were found to be in severely ischemic and almost totally normal areas of myocardium, respectively, even though they were only 1–2 cm apart. By measuring blood flow to these separate regions, it was possible to discard these data, since the myocardial segment subtended by the two transducers was not homogeneous in its response to coronary stenosis. Similarly, blood flow is frequently sharply reduced in endocardial but not epicardial layers with ischemia. Therefore, if the measuring device is not sensing only endocardial function, the measurement would tend to be less sensitive. Finally, measurement of blood flow in a coronary artery (Banka et al., 1977; Lekven et al., 1973; Stowe et al., 1978; Waters et al., 1977; Wyatt et al., 1975) does not indicate only the change in blood flow to the endocardium of the central ischemic zone, since the artery also supplies epicardium, border zone, and perhaps areas of the opposite, nonischemic wall of the left ventricle, as well as part of the right ventricle. Some of these criticisms can be directed toward a previous preliminary report from our laboratory in which measurements of regional function were correlated with measurements of transmural blood flow (Millard et al., 1975). Under these conditions, the changes in blood flow are underestimated, since changes in epicardial flow occur later in the response to ischemia. For this reason, these data were discarded and the present study was undertaken to examine regional blood flow and function in the endocardium only. In this connection, it is of interest to note a preliminary report by Gallagher et al. (1978) indicating a poorer correlation between changes in epicardial and regional function, whereas a preliminary report by Genain et al. (1979) indicates almost no correlation between changes in epicardial flow and regional function with acute myocardial ischemia.

Whereas only slight reductions in blood flow were required to reduce function initially, some regional shortening was maintained until blood flow fell severely. Using the exponential expression, it was found that 90% reduction in blood flow was required for complete loss of segmental shortening. It was also of interest to note that segments exhibiting paradoxical bulging were characterized by greater flow reductions (95%) than segments with neither shortening nor bulging, i.e., akinetic, where blood flow fell by 82%. This suggests that paradoxical bulging is the most intense form of regional myocardial ischemic function and that this occurs because of a local phenomenon rather than a secondary effect from increased contraction in the nonischemic zone. However, whether the bulging is due to greater deprivation of regional blood flow or, on the other hand, induces a greater reduction in blood flow, could not be determined.

Acute regional ischemia was associated with earlier and, ultimately, greater increases in end-systolic length as opposed to end-diastolic length, as has been shown previously (Pagani et al., 1978b). This finding, i.e., the relative insensitivity of end-diastolic length as an index of ischemia, might be interpreted as further support for the contention that myocardial relaxation is impaired in the presence of myocardial ischemia (Matthey et al., 1974; Grossman and Mann, 1978; Weisfeldt et al., 1978; Serizawa et al., 1980). However, it is important to keep in mind that left ventricular end-diastolic dimensions increase only slightly in the conscious dog despite large increases in left ventricular end-diastolic pressure in response to volume loading or methoxamine infusion, as well as during acute myocardial ischemia (Boettcher et al., 1978). This occurs because end-diastolic myocardial length is near maximal in the conscious, reclining dog.

It must be mentioned that the present experiments involved responses to acute myocardial ischemia. Although stable levels of reduced function were maintained using these protocols, it is possible that relationships may have differed between chronic reductions in blood flow and mechanical function—in particular, where myocardial infarction intervened.

In conclusion, in the conscious dog, sensitive coupling between blood flow and function exists with acute myocardial ischemia. When blood flow fell by only 10–20%, regional function was significantly impaired. Severe reduction of blood flow (>90%) was required to reduce function completely. Finally, segments exhibiting paradoxical bulging were characterized by greater flow reductions than were akinetic segments.

Acknowledgments

The author gratefully acknowledges the advice on curve fitting from the Harvard School of Public Health, Statistics Consulting Program, the help of Stanley Letovsky, and preparation of the manuscript by D. Reichman and D. Dragon.

References

Boettcher DH, Vatner SF, Heyndrickx GR, Braunwald E (1978)
Lekven J, Mjos OD, Kjekshus JK (1973) Compensatory mechanisms during graded myocardial ischemia. Am J Cardiol 31: 467-473
Waters DD, Da Luz P, Wyatt HL, Swan HJC, Forrester JS (1977) Early changes in regional and global left ventricular function induced by graded reductions in regional coronary perfusion. Am J Cardiol 38: 537-543
Correlation between acute reductions in myocardial blood flow and function in conscious dogs.
S F Vatner

*Circ Res.* 1980;47:201-207
doi: 10.1161/01.RES.47.2.201

*Circulation Research* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1980 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/47/2/201