Coronary Microvascular Responses to Reductions in Perfusion Pressure
Evidence for Persistent Arteriolar Vasomotor Tone During Coronary Hypoperfusion

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The goals of this study were to test the following hypotheses: 1) Coronary autoregulatory adjustments to decreases in perfusion pressure occur primarily in coronary arterioles (<150 μm in diameter). 2) Small coronary arteries (>150 μm in diameter) can be recruited to participate in the autoregulatory adjustments as perfusion pressure is progressively lowered. 3) Small arterioles are the location of vasodilator reserve in the coronary microcirculation during hypoperfusion. Studies were performed in anesthetized open-chest dogs in which coronary perfusion pressures were reduced to 80, 60, 40, and 30 mm Hg. During reductions in coronary perfusion pressure, measurements were made of systemic hemodynamics, myocardial blood flow (radioactive microspheres), and coronary microvascular diameters. Arterial pressure and heart rate were largely unchanged during the experimental maneuvers. Measurements of microvascular diameters in the beating heart were performed during epi-illumination via a stroboscopic light source synchronized to the cardiac cycle using fluorescence intravitral microscopy. Coronary autoregulatory adjustments were evident during reductions in perfusion pressure from control (96 mm Hg) to 80 and 60 mm Hg. Blood flow was unchanged from control, and active vasodilation of coronary arterioles was observed. At 80 mm Hg, only coronary arterioles dilated (4.4±1.2%), whereas at 60 mm Hg both small arteries (4.9±2.2%) and arterioles (6.9±1.2%) demonstrated significant vasodilation (p<0.05). The magnitude of dilation (i.e., percent increase in diameter) was inversely related to the initial diameter; that is, the arterioles dilated to a greater extent, percentagewise, than the small arteries. At 40 mm Hg, myocardial blood flow decreased slightly from that under control conditions, but coronary arterioles dilated to a greater extent than at 60 mm Hg (8.1±1.6%); yet, microvessels were incompletely vasodilated, because adenosine produced a further increase in microvessel diameter (12.5±2.1%) (p<0.05). At a perfusion pressure of 30 mm Hg, arterioles demonstrated a decrease in vascular diameter (~0.2±2.1%), which was reversed by adenosine (11.1±3.1%). From these results we concluded the following: 1) Coronary autoregulatory adjustments involve primarily coronary arteriolar vessels, but small coronary arteries can be recruited to participate in the autoregulatory response. 2) The magnitude of vessel dilation appears to be inversely related to vascular diameter. 3) Coronary arterioles are not maximally vasodilated during coronary hypoperfusion, and these vessels may be the source of persistent vasomotor tone during coronary insufficiency. (Circulation Research 1990;66:1227-1238)

Autoregulatory adjustments of coronary vasomotor tone are involved in the maintenance of coronary blood flow during reductions in coronary perfusion pressure.1,2 These adjustments in tone involve an appropriate decrease in coronary vascular resistance to maintain blood flow relatively constant during decreases in coronary perfusion pressure. Coronary autoregulation enables adequate nutrient and oxygen supply to the myocardium during reductions in perfusion pressure but fails in anesthetized animals at 60 and 40 mm Hg in the subendocardium and subepicardium, respectively.3 In the conscious animal, auto-
regulatory adjustments in coronary vasomotor tone enable maintenance of subendocardial perfusion to about 40 mm Hg.4

Although the microvascular sites of autoregulatory adjustments in the skin,5 intestine,6 and skeletal muscle7 have been observed to occur primarily in arterioles between 7 and 100 μm in diameter, there has been no information concerning the microvascular sites responsible for autoregulation in the coronary circulation. Compared with some of these tissues (e.g., skin), autoregulation in the heart is more efficient.8 This suggests the presence of greater vascular reserve in comparably sized vessels, greater sensitivity to the feedback signal, or more of the vascular network participating in autoregulatory adjustments. In view of our previous observations that approximately 25–30% of total coronary resistance resides in arteries greater than 150 μm in diameter,9,10 it is important to understand factors that regulate vasomotor responses throughout the coronary network, because relatively large coronary vessels have the potential to substantially influence total coronary resistance. The goal of this study was to test the hypotheses that coronary autoregulation occurs primarily in small arterioles and that, as perfusion pressure is progressively decreased, upstream arteries would be recruited to participate in the autoregulatory response. A related hypothesis tested if arterioles (vessels <150 μm in diameter) possess residual vasomotor tone at pressures below the autoregulatory range. This latter hypothesis was based on the numerous observations that indicate incomplete coronary vasodilation during coronary hypoperfusion.11−14 To test these hypotheses, we used a method that enabled measurement of left coronary microvascular diameters in arteries and arterioles of the beating left ventricle during reductions in coronary perfusion pressure. This methodology allowed precise documentation of the locations within the coronary microcirculation that respond actively to reductions in perfusion pressure.

Materials and Methods

**General Preparation**

Mongrel dogs (4−10 kg; n=43) of either sex were sedated intravenously with 0.04 mg/kg fentanyl plus 2.0 mg/kg droperidol (Innovar-Vet, Haver-Lockhart, Cutter Laboratories, Shawnee, Kansas) and anesthetized with 25 mg/kg i.v. sodium pentobarbital. Animals were placed on a homeothermic blanket system to maintain body temperature at 37°−38°C. Both femoral arteries and a femoral vein were catheterized for hemodynamic measurements, fluid and drug administration, arterial blood gas analyses, and blood reference samples for radioactive microspheres (refer to “Measurement of Myocardial Blood Flow”). A double-sensor solid-state transducer (Millar, Houston, Texas) was introduced into the right carotid artery and situated so that simultaneous measurements of left ventricular pressure and aortic pressure (ascending aorta) were obtained. Measurements of left ventricular dP/dt were obtained from the left ventricular pressure pulse.

Jet ventilation was used to eliminate cardiac movement due to pulmonary inflation. An 18-gauge cannula was introduced into the trachea and advanced to the carina. An expiratory tracheal tube was positioned under 1−3 cm water. A solenoid valve that was triggered from left ventricular dP/dt was connected to a pressure source (60% N2−40% O2) and regulated to 6−12 psi. The solenoid was open for only 20−35 msec during a respiratory cycle. With this ventilation system, pulmonary inflation did not produce discernible cardiac motion due to the small tidal volume. Arterial blood gases and pH were analyzed approximately every 30 minutes and were maintained within physiological limits by varying the duration that the solenoid valve was open, the position of the cannula in the trachea, the regulated pressure, and, if required, administration of sodium bicarbonate. Supplemental anesthesia was administered as required, usually about 10 mg/kg/hr.

The heart was exposed by a left thoracotomy through the fifth intercostal space and partially stabilized with a pericardial cradle. The left atrium was catheterized, and a snare was placed around the descending thoracic aorta to control arterial pressure. The coronary circulation was instrumented to allow controlled reductions in perfusion pressure and

![Figure 1](http://circres.ahajournals.org/)

**Figure 1.** Illustration of the experimental and microvascular preparation. A snare occluder is situated around the left main coronary artery, and/or a pneumatic occluder is situated around either the left interior descending or circumflex artery (shown). Distal to the pneumatic occluder, a coronary catheter is inserted for measurements of coronary perfusion pressure, administration of fluorescein isothiocyanate (FITC) dextran, and infusion of adenosine. A portion of the microvasculature is observed by using intravital microscopic techniques with epistroboscopic illumination (see text for details).
in intracoronary administration of fluorescent dextrans for illumination of the microcirculation (Figure 1).

Two different experimental procedures, global and segmental, were used to reduce coronary artery perfusion pressure. For global reductions in coronary perfusion pressure, the left main coronary artery was isolated and a micrometer-controlled snare was situated around the vessel. Since our preparations were not stable during global reductions in coronary perfusion pressure at 40 and 30 mm Hg, we had to additionally situate a pneumatic occluder around either the left anterior descending or circumflex artery. A 24-gauge catheter was introduced into the vessel downstream from the pneumatic occluder. The cross-sectional area of this catheter was 0.35 mm² and did not produce significant obstruction of the coronary artery because coronary pressures were within 5 mm Hg of aortic pressures. Our protocol entailed using the left main snare to reduce global perfusion pressure to 80 and 60 mm Hg and then reducing pressure in the instrumented left anterior descending or circumflex artery to 40 and 30 mm Hg by inflation of the pneumatic occluder with left main pressure held at 60 mm Hg. Segmental reductions in coronary perfusion pressure were performed if dissection of the left main coronary artery was too precarious or if the vessel was too short for adequate isolation. This procedure involved isolation of either the circumflex or left anterior descending artery and the placement of the pneumatic occluder around the dissected vessel. The coronary catheter was situated as described previously, and perfusion pressure was lowered by progressively inflating the occluder.

**Microvascular Preparation**

Coronary microvascular diameter measurements were accomplished in the beating heart by use of an intravital microscope (Leitz Ploemopak, Wild Leitz USA, Rockleigh, New Jersey) and a Dage silicon intensified tube video camera (model 66, Dage-MTI, Michigan City, Indiana). Illumination was accomplished with a computer-controlled stroboscopic light source (100 W xenon arc, Chadwick-Helmuth, El Monte, California) point-source bulb. A PDP 11/73 computer (Digital Equipment, Cambridge, Massachusetts) received the left ventricular dP/dt signal and was adjusted to flash the strobe once per cardiac cycle at the same point in late diastole during successive cardiac cycles. Under these conditions, the epicardial microvasculature appeared to be motionless when viewed through the microscope, because the heart was illuminated for a short period of time (15–30 µsec) at the same point during the cardiac cycles. A polarizing filter was used to reduce the glare from the epicardial surface. The microscope objectives used in this preparation were the Leitz EF4 (×4, numerical aperture=0.12), Leitz L10 (×10, numerical aperture=0.22), and Leitz L20 (×20, numerical aperture=0.32).

An area of the left ventricle having a large number of vessels was identified. Cardiac motion of this area was partially restrained by inserting four 22-gauge needles attached to a rod. The myocardium within this region still contracts vigorously with restraint primarily limiting the “up and down” movement. Our previous experience with this procedure has shown that neither resting blood flow nor vasodilator reserve are compromised9,10; thus, the microvasculature does not appear traumatized.

**Microvascular Diameter Measurements**

Measurements of diameters of coronary arteries and arterioles were made during late diastole. Images were directly digitized from the camera with a frame digitizer (Imaging Technology, Woburn, Massachusetts). These images were displayed on a high-resolution video monitor (Conrac, Covina, California) and stored on a hard disk or transferred to magnetic tape for permanent data storage and subsequent analysis. Diameter measurements were accomplished off-line from digitized images that were displayed on the monitor. Cursors were aligned with the vessel edges by use of a digitizing tablet (Summagraphics, Fairfield, Connecticut), and a computer program was used to calculate the vessel diameter in microns. The diameter-measuring system was calibrated by two independent methods. First, a standard micrometer grid (10-µm divisions) was used to calibrate the distance between cursors at different magnifications. Second, fluorescein-labeled (Fluoresbrite, Polysciences, Warrington, Pennsylvania) microspheres of different sizes (2, 3, 6, 10, 20, and 25 µm) were applied to the surface of different hearts. The diameters of the beads were measured “blindly,” and these values were correlated to the known values. A correlation of “actual” versus “measured” diameter yielded an equation for a line of \( y = 0.97x - 1.02 \) and a correlation coefficient of 0.98. Neither the slope nor the intercept of the line was different from the line of identity. This latter method of calibration demonstrated that our measurements using our stroboscopic intravital microscopic techniques were accurate under the experimental conditions using epifluorescence microscopy.

To obtain optimal visualization of coronary arteries and arterioles, fluorescein isothiocyanate dextran (molecular weight, 500,000–2,000,000) was injected into the cannulated coronary artery as a bolus. The volume of the fluorescein isothiocyanate dextran-physiological saline solution was usually about 0.05 ml, and the final fluorescein isothiocyanate dextran concentration was 50 mg/ml. We have recently reported that coronary diameters are equivalent when measured with either polarized light or a fluorescent dextran15; thus, the dextrans do not appear to be vasoactive. After injection of the fluorescent dextran, arteries and veins would illuminate sequentially; that is, arteries would illuminate initially, followed by venous illumination 1–2 seconds later. This procedure allowed identification of small coronary arteries and arterioles and enabled measurement of internal diameter, because the vessel lumen was completely illum-
nated by the fluorescent dextran (Figure 2). The fluorescein molecule was activated and visualized by using the Leitz H₂ filter in conjunction with the Ploemopak system. A particular vessel was measured four to eight times at the same point along the vessel by using various images of a particular vessel obtained over approximately a 5–30-second period. The range of these diameter measurements typically varied less than 3% from the average value.

**Experimental Protocols**

Experimental variables were measured during two experimental protocols: 1) microvascular responses to segmental or global reductions in coronary perfusion pressure, and 2) microvascular responses during adenosine-induced coronary vasodilation.

**Microvascular Responses to Reductions in Coronary Perfusion Pressure**

Measurements of hemodynamics, myocardial blood flow, and arterial and arteriolar diameters were accomplished at five different coronary perfusion pressures: 1) 100 mm Hg (control), 2) 80 mm Hg, 3) 60 mm Hg, 4) 40 mm Hg, and 5) 30 mm Hg. Reductions in perfusion pressure were performed sequentially from the highest (control) to the lowest pressure (30 mm Hg). Global reductions in coronary perfusion pressure were accomplished inde-
pended of aortic pressure by constricting the left main coronary snares with the micrometer occluder. As mentioned previously, most of the preparations would not tolerate left main coronary perfusion pressures of 40 and 30 mm Hg; consequently, left main coronary artery pressure was reduced to 60 mm Hg, and then the pneumatic occluder was inflated to further reduce circumflex or left anterior descending artery pressure to 40 and 30 mm Hg. In the segmental preparations, the protocol was the same as in the global preparation, except that only the pneumatic occluder was inflated to reduce coronary pressure in either the left anterior descending or circumflex artery. After reduction in coronary artery perfusion pressure, microvascular diameter measurements were accomplished after a 30–45-second steady-state period. Pressure was controlled to within 3 mm Hg of the desired value; otherwise, the measurement was not used. After measurements were obtained at each pressure, coronary artery pressure was restored to control, and diameters were remeasured. Any diameter that did not return to within 7.5% of control was not used in final data analysis; this ensured that the preparations were not deteriorating during the course of the experiment. Measurements of myocardial blood flow were made after the diameter measurements, except at 30 mm Hg.

**Coronary Microvascular Diameters During Adenosine-Induced Vasodilation**

Measurements of diameter and myocardial blood flow were made under the following experimental conditions: 1) control measurements at 100 mm Hg, 2) pressure at 100 mm Hg during adenosine infusion, 3) coronary perfusion pressure reduced to 80 mm Hg, 4) coronary perfusion pressure reduced to 60 mm Hg, 5) coronary perfusion pressure reduced to 40 mm Hg, and 6) coronary perfusion pressure reduced to 30 mm Hg during adenosine infusion. Adenosine (intracoronary) was infused at 0.1–0.2 mg/kg/min. Coronary perfusion pressures were reduced as described for the previous protocol. Measurements were repeated after return of coronary perfusion pressure to control levels during adenosine infusion to ascertain if the intense pharmacological dilation was maintained during the experimental protocol. Myocardial blood flow was not measured at a perfusion pressure of 30 mm Hg.

In 10 animals, both the autoregulation and adenosine protocols were completed; however, in seven, only the adenosine protocol was accomplished.

**Measurement of Myocardial Blood Flow**

Myocardial perfusion was measured with nuclide-labeled microspheres (15-μm diameter labeled with $^{68}$Sc, $^{85}$Sr, $^{113}$Sn, $^{95}$Nb, and $^{153}$Gd). Microspheres (1×10$^4$–3×10$^6$) were sonicated for 15 minutes, agitated on a vortex mixer, and injected into the left atrium. For 10–15 seconds before and for 1.5 minutes after the microsphere injection, arterial blood was collected with a constant withdrawal pump from the femoral arterial catheters at a rate of 0.97 ml/min. Reference samples of blood from both femoral arteries were placed in counting vials to determine nuclide activity with a sodium iodide detector (Nuclear Data, Schaumburg, Illinois) and a multichannel analyzer (model 65, Nuclear Data) to separate the gamma spectra of each nuclide. If activities of these blood samples differed by more than 15%, the measurement was excluded from analysis. After the animal was killed, the heart was removed. Tissue samples from the left ventricle were obtained, divided into the subepicardial, midmyocardial, and subendocardial thirds, and weighed. Tissue samples ranged in weight.
from 0.65 to 0.90 g. Myocardial blood flow per gram (MBF) was calculated from the following expression: 

\[ \text{MBF} = \frac{C_m \times W_t}{C_i} \]

where \( C_m \) is activity per gram weight of tissue, \( W_t \) is the withdrawal rate of the pump, and \( C_i \) is total nuclide activity in the blood reference sample. Nuclide activity for the different isotopes was corrected for overlap with standard computer formulas.

**Data Analysis**

All hemodynamic variables (systolic, diastolic, and mean arterial pressure, heart rate, left ventricular pressure, and dP/dt) were recorded on an oscillographic recorder. These hemodynamic variables and myocardial blood flow were compared by analysis of variance in conjunction with Scheffe's multiple-comparison test to detect differences at the various coronary perfusion pressures.16

Microvascular diameter responses to the decreases in coronary perfusion pressure were analyzed by using two approaches. First, by linear regression analysis,16 a relation was constructed with the initial diameter as the independent variable and percent change in coronary diameter from control during pressure reductions as the dependent variable. Second, the percent change in microvascular diameter from control at the different perfusion pressures during autoregulatory adjustments and adenosine-induced vasodilation in two distinct diameter classes of vessels was compared: arterioles (<150 \( \mu m \) in diameter) and arteries (>150 \( \mu m \) in diameter). Differences among the vessel classes were analyzed by analysis of variance in conjunction with Scheffe's multiple-comparison test to detect intergroup differences.16 No differences in diameter responses between the global and segmental studies were found; thus, these data were combined at the same coronary pressure and analyzed as a single group.

All data are presented as mean±SEM, and a value of \( p<0.05 \) was used as the criterion for statistical significance.

**Results**

**Systemic Hemodynamics and Arterial Blood Gases**

Table 1 shows systemic hemodynamics during the experimental protocols. Heart rate and arterial pressures were changed only at the lowest coronary perfusion pressure (30 mm Hg). Adenosine (intra-coronary) decreased arterial pressure slightly (7–8
mm Hg) but significantly \((p<0.05)\) at coronary pressures of 60 and 30 mm Hg. Arterial blood gases were unchanged during the experimental protocols.

**Myocardial Blood Flow**

Table 2 shows the effects of reducing coronary perfusion pressure on myocardial blood flow. Blood flow was reduced from control only at 40 mm Hg \((p<0.05)\). Table 2 also summarizes the effects of adenosine on myocardial blood flow at the different coronary pressures. Adenosine caused a significant increase in blood flow \((p<0.05)\) at all perfusion pressures.

**Microvascular Diameters**

Figures 3–6 illustrate the effects of reductions of coronary perfusion pressure on coronary microvascular diameters. In each figure, the initial diameter under control conditions is plotted on the abscissa, and the percent change in diameter during the reduction of pressure is plotted on the ordinate. Figure 3 shows the responses of coronary microvessels to reductions in perfusion pressure at 80 mm Hg. Generally, arterioles were characterized by modest vasodilation; the diameters of arteries were virtually unchanged. Figure 4 illustrates the responses to reductions in perfusion pressure to 60 mm Hg. Arterioles dilated to a greater extent than that observed at 80 mm Hg, and some arteries demonstrated significant vasodilation with these vessels dilating by 10–20% of their baseline diameter. Vasomotor responses to reductions in perfusion pressure to 40 mm Hg are shown in Figure 5. Coronary vessels dilated as an inverse function of their initial diameter; that is, coronary arterioles dilated more significantly than small coronary arteries. Note that the small arteries often exhibited significant vasodilator responses at pressures of 80 and 60 mm Hg; that is, this response was not confined to 40 mm Hg perfusion pressure. Figure 6 illustrates the responses of coronary microvascular diameter to reductions in perfusion pressure at 30 mm Hg. Note that at this pressure coronary arterioles were characterized by a reduction in diameter and that the regression equation was characterized by a positive slope and correlation coefficient. This is in contrast to the equations describing the relations at higher pressures, which
had negative slopes and correlation coefficients. Generally, the data show that within the pressure range of 80–40 mm Hg the percent increase in diameter was inversely related to the original size of the vessel. Moreover, as perfusion pressure was progressively lowered, the magnitude of coronary vasodilation was greater and occurred throughout the vasculature. Another aspect of the data that should be mentioned is the marked variation in microvascular responses during reductions in perfusion pressure. We also observed that not every microvessel dilates during the reductions in perfusion pressure; some vessels are characterized by passive reductions in diameter during decreases in pressure.

Figure 7 summarizes the effects of adenosine on the coronary microcirculation with arterial pressure maintained at baseline (96±1 mm Hg). The extent of vasodilation was inversely related to microvascular diameter: small arteriolar vessels dilated as much as 30–40%, whereas larger vessels only dilated 5–10%. Similar to the heterogeneous vasodilatory responses during reductions in perfusion pressure, the microvascular vasodilatory responses to adenosine were also variable; some vessels within a small diameter range (e.g., 50–100 μm) dilated as little as 4% or as much as 40%.

Figure 8 illustrates the autoregulatory responses of arterioles and small arteries to reductions in perfusion pressure. Note that with perfusion pressure at 80 mm Hg diameters of arterioles were significantly increased above control (100 mm Hg; no change in diameter) and dilation was maintained when perfusion pressure was reduced to 40 mm Hg (p < 0.05). Moreover, the proportionate increase in arteriolar diameter at 40 mm Hg (8.1±1.6%) was greater than at 80 mm Hg (4.4±1.2%) (p < 0.05). At 30 mm Hg, arteriolar diameters decreased. In contrast to arteriolar responses, small arteries did not demonstrate dilation with perfusion pressure at 80 mm Hg but significantly dilated at 60 mm Hg (p < 0.05). At 40 mm Hg, the amount of arterial vasodilation was less than that observed for arterioles (p < 0.05) but not significantly different at 30 mm Hg.
Figure 8. Graph showing the effects of reduction in coronary perfusion pressure on percent change in diameter (Δ%) of coronary arterioles (<150 μm in diameter) and small arteries (>150 μm in diameter). Data are presented as mean ± SEM.

Figure 9 shows the effects of adenosine on coronary microvascular diameters in the two different classes during the reductions in coronary perfusion pressure. In vessels less than 150 μm in diameter, adenosine significantly increased vessel diameters from control at all coronary perfusion pressures (p < 0.05). Adenosine infusion at 40 mm Hg produced vasodilation in 13 of 16 small arterioles (7–29% increase in diameter). It is also worth emphasizing that in small arterioles diameters decreased from control at the lowest perfusion pressure (30 mm Hg), but this was reversed to vasodilation in five of seven vessels by adenosine, with the dilator response ranging from 6% to 25% increases in diameter. Adenosine produced vasodilation in small arteries at 100 and 80 mm Hg, but these vessels did not respond to the vasodilator at coronary perfusion pressures of 60 mm Hg or below.

Discussion

There are three new observations elucidated by this study: 1) Autoregulatory responses in coronary arterioles occur primarily in vessels less than approximately 150 μm in diameter. These vessels undergo active vasodilation during reductions in coronary perfusion pressure. Moreover, the magnitude of the dilator response becomes greater as initial vessel size decreases. 2) Upstream arterial vascular segments are also involved in the autoregulatory response, because these vessels show significant vasodilation as perfusion pressure is progressively lowered. However, the extent of dilation (4.8%) in these vessels was less than that in smaller ones (10.1%). 3) Coronary arterioles are not fully dilated during reductions in coronary perfusion pressure, even at pressures below the autoregulatory range. Adenosine produced significant dilation of coronary arterioles even during coronary hypoperfusion at 30 and 40 mm Hg.

Our conclusions and interpretations depend on several factors which include 1) critique of the experimental approach, 2) microvascular locations of autoregulation, 3) mechanisms of coronary autoregulatory responses, and 4) persistent vasomotor tone during coronary hypoperfusion.

Critique of the Experimental Approach

All experimental preparations were characterized by systemic hemodynamics and arterial blood gases in the physiological range. Moreover, the preparations were stable hemodynamically, insofar as these variables did not change during the course of the experiment. The experimental design ensured that the preparation was not deteriorating during the course of the experiment, because all measurements of microvascular diameter had to return to within 7.5% of the original control. Using the latter experimental criterion, we rejected approximately 30% of all the experimental measurements.

The accuracy of our experimental measurements is also critical to the interpretations and conclusions. With the present system we can resolve pixel sizes of approximately 1.3 μm with a ×20 objective, 2.6 μm with the ×10 objective, and approximately 6 μm with the ×4 objective. Therefore, we can detect very small changes in microvascular caliber. Routinely, the larger objectives are used for any vessels less than 200 μm in diameter. Fluorescence microscopic techniques also enhance visualization of microvessels (Figure 2), because the fluorescent dextran illuminates the lumen and enables edge detection of the inside diameter.

The variability of the microvascular responses to changes in coronary perfusion pressure was greater than we initially expected but can be explained by several different reasons: 1) Inherent heterogeneity of responses could contribute to the disparate vascular reactions. For instance, we would frequently observe that reductions in perfusion pressure often caused substantial vasodilation (10–30% increase in
diameter) in one arteriole, whereas another in the same field of view would dilate only slightly (3–5%). Occasionally some vessels would behave passively, with a reduction in diameter during decreases in pressure, but would later dilate during adenosine infusion. There is a precedent in the literature for variable arteriolar responses to alterations in perfusion pressure. Nicoll and Webb reported that during reductions in perfusion pressure approximately 33% of all arterioles actively dilated, 33% showed no response, and 33% were characterized by passive reductions in vascular diameter. Moreover, we have observed biological variation of myogenic responses to reductions in perfusion pressure. In isolated subepicardial arterioles, active vasodilation was observed in about 80% of the vessels during reductions in intraluminal pressure, even though all arterioles generated spontaneous tone. Thus, even in carefully controlled in vitro experiments, there is some variation in arteriolar responses to reductions in intraluminal pressure. It is worth emphasizing that in the present study approximately 80% of all vessels studied (in a similar diameter range to the isolated arterioles) also demonstrated active vasodilatory responses to reductions in perfusion pressure. A simple assumption that we have made in the present study is that comparable reductions in intraluminal pressure and blood flow occurred in microvessels of similar size. This assumption may not be valid, especially in view of recent data showing sixfold variations in regional myocardial blood flow reserve and the well-acknowledged spatial variations in myocardial blood flow. Thus, it is likely that all arterioles of a particular size do not have similar pressure and flow profiles and that substantial redistribution of blood flow occurs within arteriolar networks when perfusion pressure is reduced.

3) Another source of variation relates to the nomenclature used to characterize microvascular responses. Vessel diameter was used as the baseline measurement from which vascular reactions were characterized. It is clear, however, that vessels of similar diameters but from different sizes of hearts may be of different vascular branching orders. This would undoubtedly cause the pressure-flow relations in arterioles of comparable size from two hearts of unequal size to be different.

4) It is conceivable that the superficial location of the microvessels in the subepicardium could influence their autoregulation. Since autoregulation is improved by enhanced metabolism in skeletal muscle and the metabolism of the subepicardium is reported to be lower than that in the subendocardium, superficial epicardial microvessels may not demonstrate marked autoregulatory capacity. Also, it is possible that the outermost portion of the epicardium was exposed to abnormally high levels of oxygen. This could also influence our data because it has been reported that high PO2 impaired autoregulatory responses in microvessels of skeletal muscle, thus, a high epicardial tissue PO2 may also have contributed to the variable responses during autoregulation in coronary microvessels. Collectively, there are many reasons for the variation of responses observed during reductions in coronary perfusion pressure. We emphasize that the microsphere measurements of blood flow showed adequate autoregulatory adjustments but that such measurements represented the average of vascular responses; thus, it was not possible to evaluate heterogeneous microvascular reactions from the measurement of myocardial blood flow.

**Microvascular Locations of Autoregulation in Organ Systems Other Than the Heart**

In the bat wing, arterioles between 7–70 μm in diameter have been reported to constrict in response to increased intraluminal pressure produced by retrograde infusion of saline. In the rat mesoappendix, autoregulatory adjustments were found to occur in both arterioles and precapillary sphincters. However, variable responses in all vessel groups were observed. The microvascular responses ranged from passive changes and maintenance of diameter to diameter changes inversely related to pressure. These latter autoregulatory adjustments were observed in approximately one third of the arterioles and in no metarterioles. In the mesentery of the cat, autoregulatory adjustments appeared to occur primarily in arterioles greater than 65 μm in diameter, because vessels smaller than this size respond passively to reductions in arterial pressure. Also, in the rat intestine, passive diameter changes in arterioles smaller than 65 μm in diameter were also observed to respond to reductions in perfusion pressure. In skeletal muscle, the relative contributions of large and small arterioles were noted to vary. Large arterioles were involved in autoregulatory adjustments at arterial pressures above 80 mm Hg, but at pressures below this level, smaller vessels appeared to be the dominant control mechanism. The contribution of these relatively large arterioles and small arteries can account for as much as 25% of the total resistance change during autoregulatory adjustments. Recently, however, Slaaf and coworkers reported relatively homogeneous autoregulatory responses of skeletal muscle arterioles to reductions in perfusion pressure. There was a tendency for the smaller arteriolar vessels to dilate more than larger arterioles.

In the aggregate, it appears that microvascular sites of autoregulation appear to be dependent on the particular organ system studied. Our observations appear to be most closely related to those in skeletal muscle in which autoregulatory adjustments occur at different levels in the microcirculation. Specifically, we observed autoregulatory adjustments in both arterioles and small arteries. We also observed an inverse response between percent changes in diameter and the initial diameter of the microvessel, which indicates that in the coronary microcirculation autoregulatory adjustments in smaller arterioles are the most important. Yet, at the lowest pressure (30 mm Hg), diameters in the smallest arterioles decreased passively whereas larger ves-
Coronary Autoregulatory Responses

Three hypotheses have been proposed to explain coronary autoregulation: the tissue pressure hypothesis, the myogenic hypothesis, and the metabolic hypothesis. Although the experimental results do not allow us to determine which factors are responsible for coronary autoregulation, some observations in our study provide some suggestive evidence for mechanisms of coronary autoregulation. We often observed disparate microvascular responses to reductions in perfusion pressure in arterioles of similar size within the same microvascular field. Such variable responses do not reconcile with the tissue pressure hypothesis, which proposes that changes in perfusion pressure result in directionally similar changes in tissue pressure due to capillary ultrafiltration. According to this hypothesis, decreasing perfusion pressure will decrease tissue pressure and produce a passive increase of vascular caliber. If this mechanism were to occur, we would expect uniform changes in vascular caliber because the changes in tissue pressure should be exerted uniformly on small vessels.

Although the present results cannot distinguish between metabolic and myogenic autoregulatory mechanisms, it is tempting to speculate that coronary autoregulatory adjustments may involve both metabolic and myogenic mechanisms. In this regard, during reductions in perfusion pressure, an increase in the concentration of a putative vasoactive metabolite in the interstitium would cause direct metabolic vasodilation. A provision of this scheme is that the diffusion distance between the myocardial parenchymal cells and arterioles should be sufficiently short to enable such autoregulatory adjustments. It is conceivable that the smallest arterioles, being in most intimate contact with the interstitium, may be under preferential metabolic control. Upstream arterioles, being located anatomically distant from the parenchymal cell metabolites, could be predominantly governed by myogenic mechanisms. During reductions in perfusion pressure, it is reasonable to assume that these vessels could also encounter a decrease in intraluminal pressure and undergo active vasodilation. Intraluminal pressures in these upstream arterioles would be affected by both the upstream perfusion pressure, as well as the downstream resistance. For instance, if a decrease in perfusion pressure caused a reduction in blood flow, metabolic vasodilation of the smallest arterioles would cause further reductions in pressure in the upstream arterioles, possibly causing direct myogenic recruitment of this segment for autoregulatory adjustments. A scheme involving series-coupled myogenic and metabolic mechanisms to explain autoregulation was recently proposed by Granger. It is worth noting that we have recently reported the existence of myogenic regulatory mechanisms in coronary arterioles ranging from 40–100 μm in diameter.

Another mechanism that may contribute to coronary autoregulation relates to adjustments in the periodic alterations in coronary tone at the microscopic level. Such variations in coronary tone are termed “vasomotion” and are hypothesized to be caused by periodic alterations in precapillary sphincter tone or arteriolar vasoconstriction, causing temporal changes in arteriolar or capillary blood flow. It is conceivable that the periodic episodes of constriction decrease during reductions in perfusion pressures, which, in essence, decrease coronary resistance and aid in the maintenance of coronary blood flow during autoregulation.

Persistent Vasomotor Tone During Coronary Hypoperfusion

Many laboratories have demonstrated pharmacological coronary vasodilator reserve at coronary perfusion pressures below the lower range of autoregulation. Adenosine infusion has been reported to increase transmural myocardial blood flow at pressures below 35 and 40 mm Hg and to improve left ventricular function. Our results have identified that small arterioles were a microvascular location of this vasodilator reserve in the subepicardial vasculature. We observed that adenosine produced substantial vasodilation in arterioles. At a perfusion pressure of 30 and 40 mm Hg, adenosine produced significant vasodilation of arterioles. Thus, these vessels are not completely dilated during hypoperfusion. An implication of this result is that dilation of this vascular segment during myocardial ischemia could improve oxygen delivery to the jeopardized myocardium and potentially reduce the amount of tissue necrosis.

Although adenosine did not dilate vessels greater than 150 μm in diameter during hypoperfusion, the possibility exists that these relatively large vessels also possess tone during hypoperfusion. It is difficult to interpret diameter changes in these vessels during adenosine administration because significant decreases in intraluminal pressure probably occur as a result of downstream vasodilation. Thus, an active dilator response could be masked by a reduction in distending pressure. In support of this argument, Kanatsuka and colleagues have found that nitroglycerin can dilate relatively large coronary microvessels during coronary hypoperfusion. Also, based on our measurements of myocardial blood flow, it would appear that the dose of adenosine we used did not produce maximal blood flow, because maximal coronary blood flow at a perfusion pressure of 100 mm Hg is usually in the range of 4–6 ml/min/g, whereas our measurements were in the range of 2.5–3.0 ml/min/g. Therefore, the possibility exists that with a higher dose of adenosine we may have observed greater vasodilation of all coronary vessels.

Another aspect of our data that should be considered was that we studied only subepicardial microvessels. It has been shown that the subepicard-
dium possessed greater vasodilator reserve than the subendocardium during hypoperfusion.\textsuperscript{13,14,19} In view of this important difference between different transmural left ventricular regions, our results, which demonstrated that small arterioles were the site of persistent tone in the subepicardial microcirculation during hypoperfusion, should be extrapolated to the subendocardium only with caution.

**Conclusions**

This study provides unequivocal evidence that the microvascular locations of coronary autoregulation, in response to reductions in perfusion pressure, occur in arterioles and small arteries. The relative importance of these microvascular autoregulatory adjustments is inversely related to the size of the coronary blood vessel, with arterioles experiencing proportionately the largest magnitude of vasodilation. We also demonstrate that during coronary hypoperfusion arterioles are not maximally vasodilated because these vessels exhibit substantial vasodilator responses to adenosine at pressures below the autoregulatory range.

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**References**

Coronary microvascular responses to reductions in perfusion pressure. Evidence for persistent arteriolar vasomotor tone during coronary hypoperfusion.

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