Prolonged Impairment of Coronary Vasodilation After Reversible Ischemia
Evidence for Microvascular “Stunning”

Roberto Bolli, J. Fernando Triana, and Mohamed O. Jeroudi

Reperfusion after brief, reversible myocardial ischemia is associated with prolonged depression of contractile function (myocardial “stunning”); however, the effect on coronary vascular function has not been defined. Thus, open-chest dogs \((n=14)\) underwent a 15-minute left anterior descending coronary artery (LAD) occlusion followed by reflow. Four hours after reperfusion, regional myocardial blood flow (microspheres) was significantly \((p<0.01)\) lower and coronary vascular resistance significantly \((p<0.01)\) higher in the postischemic as compared with the nonischemic endocardium. Furthermore, during maximal vasodilation elicited by intravenous adenosine \((n=6)\), myocardial blood flow was lower \((p<0.05)\) and coronary vascular resistance higher \((p<0.05)\) in the postischemic as compared with the nonischemic myocardium, both in the endocardial and in the epicardial layers. Similarly, during maximal dilation elicited by intravenous papaverine \((n=8)\), myocardial blood flow was lower \((p<0.05)\) and vascular resistance higher \((p<0.05)\) in the postischemic as compared with the nonischemic endocardium; a directionally similar trend was observed in the epicardium. Four hours after reperfusion, all indexes of reactive hyperemia after a 40-second coronary occlusion were significantly lower in the LAD than in the control circumflex coronary artery \((n=8)\). There was no appreciable correlation between systolic wall thickening in the stunned myocardium and 1) the resting myocardial perfusion, 2) the hyperemia attained during adenosine or papaverine, and 3) the hyperemic response to a 40-second coronary occlusion. In control dogs that did not undergo a 15-minute LAD occlusion \((n=15)\), there were no differences in myocardial blood flow or vascular resistance between the LAD-dependent and the circumflex-dependent bed, either before or during adenosine \((n=7)\) or papaverine \((n=8)\). Furthermore, reactive hyperemia after a 40-second occlusion did not differ between the LAD and the circumflex artery \((n=8)\). In conclusion, a brief (15 minute), reversible ischemic insult causes a prolonged increase in resting vascular resistance and a prolonged impairment in vasodilator responsiveness, both of which persist for at least 4 hours. The severity of these vascular derangements is not related to the severity of contractile depression, suggesting that they may represent a relatively independent phenomenon. It is proposed that, in addition to myocardial “stunning,” reversible ischemia also causes a microvascular “stunning.” (Circulation Research 1990;67:332–343)

It is well established that reperfusion after brief, reversible myocardial ischemia is associated with prolonged depression of contractile function (myocardial “stunning”) and with a variety of ultrastructural, metabolic, electrophysiological, and other functional abnormalities.\(^1\)–\(^{10}\) Whether reversible ischemia also affects the function of the coronary microvasculature, however, remains uncertain. Previous studies\(^2\)–\(^{10}\) have shown that subendocardial resting perfusion in the stunned myocardium after a 15-minute coronary occlusion is reduced; this could reflect injury of the coronary vessels or may simply represent an adjustment of coronary flow to the decreased oxygen demands of the depressed, stunned myocardium. Measurements of resting perfusion, in themselves, do not allow one to discern between these two possibilities. Therefore, to assess the functional integrity of the coronary vasculature, in the present investigation we evaluated the response of
coronary blood flow in the stunned myocardium to three different vasodilator stimuli, namely, adenosine, papaverine, and ischemia.

Methods

The experimental preparation used in this study has been described in detail previously. Briefly, pentobarbital-anesthetized dogs were instrumented with a snare around the mid–left anterior descending coronary artery (LAD), a Doppler flow velocity probe around the LAD adjacent to the snare, a Millar pressure transducer (Millar Instruments, Houston) in the left ventricle, Doppler ultrasonic wall thickening probes in the ischemic and non-ischemic regions, and left atrial and aortic catheters. Particular care was taken to ensure that fundamental physiological parameters were within normal limits for the duration of the protocol. Only dogs with hematocrit greater than 30% were admitted to the study. Supplements of potassium chloride (10–20 meq) were given intravenously to maintain plasma potassium concentration in the range of 4.0–5.0 meq/l. The rate of fluid replacement was standardized at approximately 80 ml normal saline per hour. Throughout the study, body temperature was kept within physiological limits by adjusting a heating blanket, while arterial pH and Po2 were maintained in the normal range by adjusting the ventilatory parameters. Dogs in which arterial Po2 was less than 60 mm Hg or arterial pH less than 7.36 in spite of these adjustments were not used.

Studies With Adenosine

Group I. In these dogs (n=6), the LAD was occluded for 15 minutes and then reperfused. This duration of ischemia was selected because it is well established that it does not cause any irreversible injury in the dog. In this group, as well as in group III, animals that developed ventricular fibrillation during coronary occlusion or after reperfusion were excluded. Coronary collateral blood flow was measured 10 minutes after occlusion by injecting radioactive microspheres, as described previously. Briefly, 1.5–2.0×10⁶ spheres labeled with ⁵¹Nb, ⁹⁵ᵐTc, or ⁵¹Cr were injected into the left atrium in 3 ml normal saline over 10–15 seconds, after which the catheter was flushed with an additional 20 ml saline. Beginning 15 seconds before and continuing for 2 minutes after the end of injection, a reference blood sample was withdrawn from the aorta at a constant rate of 4.05 ml/min. After 4 hours of reperfusion, adenosine was administered intravenously as a continuous infusion at a rate of 1.34 mg/kg/min for 15 minutes. Previous studies have shown this dose to effect maximal coronary vasodilation. Myocardial blood flow was measured before and 10 minutes after start of adenosine infusion by injecting radioactive microspheres.

Group II (controls for adenosine). To determine whether there was any difference in the coronary vasodilator response to adenosine between the LAD and the circumflex territories, a separate group of control dogs (n=7) was instrumented as group I but did not undergo ischemia and reperfusion. Five hours after instrumentation (the interval corresponding to the time elapsed between instrumentation and adenosine infusion in group I), regional myocardial blood flow was measured with microspheres before and 10 minutes after the start of intravenous adenosine infusion (same rate as in group I).

Studies With Papaverine and Reactive Hyperemia

For this part of the experiment, dogs were instrumented as described above; in addition, a Doppler flow velocity probe and a snare were placed around the proximal left circumflex coronary artery.

Group III. In these dogs (n=8), the LAD was occluded for 15 minutes and then reperfused. The hyperemic response to a 40-second coronary occlusion was determined before the 15-minute LAD occlusion (“baseline”), both in the LAD and in the circumflex vascular beds (the sequence of the 40-second LAD and circumflex occlusions was randomized; each artery underwent two 40-second occlusions, and the two measurements were averaged). After 4 hours of reperfusion, the hyperemic response to a 40-second coronary occlusion was determined again in the LAD and circumflex beds as described above. After these studies, a minimum of 15 minutes was allowed to ensure that hemodynamic variables and coronary blood flow returned to values indistinguishable from those present before the 40-second occlusions. Papaverine was then infused intravenously at a rate of 0.6 mg/kg/min for 15 minutes. Myocardial blood flow was measured with radioactive microspheres before and 10 minutes after the start of papaverine infusion.

Group IV (controls for papaverine and reactive hyperemia). These dogs (n=8) were instrumented as group III but did not undergo ischemia and reperfusion. The hyperemic response to a 40-second occlusion was determined, as described for group III, soon after instrumentation (“baseline”) and 5 hours later (this interval corresponded to the time elapsed between baseline and 4-hour postreperfusion measurements in group III). After the 5-hour hyperemic response measurements, papaverine was infused intravenously at the same rate as in group III; regional myocardial flow was determined with radioactive microspheres before and 10 minutes after the start of papaverine infusion.

The doses of adenosine and papaverine used in this study were selected to produce maximal coronary vasodilation. This was confirmed in each dog by the complete absence of hyperemic response to a 40-second circumflex occlusion during infusion of either vasodilator; furthermore, in pilot studies, higher doses of adenosine or papaverine failed to elicit additional vasodilation. The rationale for giving the vasodilators intravenously rather than by the intracoronary route was to be able to compare, in the same animal, the vasodilator response in the post-
The protocol, the occluded vascular bed was identified with a previously described post-
mortem dual perfusion technique. To assess the presence of irreversible injury, left ventricular slices were incubated in triphenyltetrazolium chloride. Four transmural samples (1.0–1.5 g) were obtained from both the occluded and nonoccluded vascular beds. To avoid admixture of ischemic and non-
ischemic tissue, ischemic samples were obtained at least 1 cm inside the margins of the unstained region. Each specimen was divided into epicardial and endocardial halves and weighed. The radioactivity of the tissue and reference blood samples was determined with a sodium iodide crystal well counter. Regional myocardial blood flow was calculated by standard methods. Regional coronary vascular resistance was determined by dividing mean arterial pressure by myocardial blood flow.

Reactive hyperemia after a 40-second coronary occlusion was quantified by measuring several variables: 1) the peak coronary flow; 2) the ratio between peak flow and baseline flow (i.e., the quotient of the maximal flow attained after release of occlusion and the flow measured immediately before occlusion); 3) the ratio between the area of hyperemia and the area of flow deprivation (the area of hyperemia was measured with a planimeter and was defined as the area under the mean coronary flow tracing during the interval in which coronary flow exceeded baseline flow by at least 15%; the area of flow deprivation was calculated by multiplying baseline flow by occlusion duration); 4) the minimal coronary vascular resistance, calculated as the ratio between mean aortic pressure and peak coronary flow; and 5) the duration of reactive hyperemia, defined as the time elapsed between the release of coronary occlusion and the moment when coronary flow returned to values within 15% of the preocclusion (baseline) value.

All values are reported as mean ± SEM. Analysis of variance was used to compare means among different groups of dogs and to analyze intragroup variation. If analysis of variance showed an overall difference, pairwise comparisons were performed with the two-tailed Student’s t test, and the resulting p values were adjusted by the Bonferroni method. The paired Student’s t test was used for intragroup comparisons of sequential measurements and of simultaneous measurements in the LAD and in the circumflex vascular beds. The correlation between wall thicken-

Results
Tetrazolium staining confirmed the absence of irreversible injury in every animal. This finding is consistent with numerous previous reports documenting that the damage associated with a 15-minute coronary occlusion is completely reversible in the dog.

Hemodynamic and Wall Thickening Data
Table 1 summarizes the hemodynamic and wall thickening measurements in all groups. In groups I and II (studies with adenosine), heart rate, systolic, diastolic, and mean arterial pressures, and peak positive left ventricular dP/dt did not change significantly during the protocol until the infusion of adenosine was started. Adenosine effected a significant reduction of heart rate and of systolic, diastolic, and mean arterial pressures, the magnitude of which was similar in the two groups. In group I (15-minute ischemia), systolic wall thickening in the LAD-dependent territory was replaced by paradoxical wall thinning during coronary occlusion with minimal recovery after reperfusion, indicating severe myocardial stunning. Infusion of adenosine was associated with a marked increase of wall thickening in the stunned myocardium (group I), whereas no appreciable change in wall thickening was observed during adenosine in control dogs that did not undergo a 15-minute LAD occlusion (group II).

In groups III and IV (studies with papaverine and reactive hyperemia), hemodynamic variables also did not change significantly until the infusion of papaverine was started, with the exception of diastolic and mean arterial pressures, which declined transiently during coronary occlusion in group III; however, by 4 hours of reperfusion, both variables had returned to values similar to baseline and to group IV. Papaverine produced a significant increase in heart rate, systolic arterial pressure, and left ventricular dP/dt and a significant decrease in diastolic and mean arterial pressures; the magnitude of these changes was comparable in the two groups. Similar to group I, in group III (15-minute ischemia) there was very little recovery of wall thickening 4 hours after reperfusion in the LAD-dependent territory. Analogous to the results obtained with adenosine, papaverine effected a striking improvement of wall thickening in the stunned myocardium (group I) but had no appreciable effect on the nonischemic myocardium (group IV). This selective enhancement of function by vasodilators in stunned myocardium agrees with previous reports.

Myocardial Blood Flow During Coronary Occlusion
In group I, epicardial, endocardial, and transmural blood flows to the ischemic region during coronary occlusion averaged 0.13 ± 0.05, 0.09 ± 0.03, and 0.11 ± 0.04 ml/min/g, respectively (Figure 1). In group II (control dogs that did not undergo LAD occlu-
### TABLE 1. Hemodynamic and Wall Thickening Data

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<tr>
<th></th>
<th>HR</th>
<th>SAP</th>
<th>DAP</th>
<th>MAP</th>
<th>CBF (LAD)</th>
<th>LV dP/dt</th>
<th>% ThF (IZ)</th>
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<td>7.3±3.4*</td>
<td>Group III 27.9±2.2</td>
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<td>Group IV 24.0±3.3</td>
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<td>21.9±3.2</td>
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</table>

Values are mean±SEM.

HR, heart rate (beats/min); SAP, systolic arterial pressure (mm Hg); DAP, diastolic arterial pressure (mm Hg); MAP, mean arterial pressure (mm Hg); CBF (LAD), coronary blood flow (ml/min) in the occluded/reperfused left anterior descending coronary artery; LV dP/dt, peak positive left ventricular dP/dt (mm Hg/sec); % ThF (IZ), percent thickening fraction in the ischemic/reperfused zone.

* p<0.05 vs. 4 hours.
† p<0.001 vs. 4 hours.
‡ p<0.01 vs. 4 hours.
§ p<0.01 vs. baseline.
myocardial blood flow was measured at "baseline," a time corresponding to LAD occlusion in group I. As illustrated in Figures 1 and 2, in group II the measurements of blood flow and vascular resistance obtained at baseline were similar to those obtained 5 hours after instrumentation, indicating that coronary resistance remains stable in this preparation over the duration of the experimental protocol. Collateral flow was not measured again in group III because the purpose of this study was to document persistence of postischemic contractile and vascular dysfunction rather than to precisely quantify the magnitude of collateral perfusion. Previous experience with this canine model indicates that, on the average, low ischemic zone flows are observed during coronary occlusion in animals that exhibit dyskinesis after reperfusion.11–19 Accordingly, the persistence of dyskinesis in group III 4 hours after reperfusion (Table 1) suggests that regional myocardial blood flow during LAD occlusion was severely reduced. In fact, the reduction of wall thickening at 4 hours was more severe in group III (thickening fraction, −12% of baseline values) than in group I (thickening fraction, +5% of baseline) (Table 1), suggesting that collateral flow in group III did not exceed that measured in group I (0.11 ± 0.04 ml/min/g for transmural flow). In any case, the precise values of collateral flow during LAD occlusion are not essential to the goal of this study; the important point is that in group III, wall thickening after reperfusion was strikingly abnormal, demonstrating the presence of marked myocardial stunning.

Coronary Blood Flow and Vascular Resistance After Reperfusion

Studies with adenosine (groups I and II). The measurements of regional myocardial blood flow and coronary vascular resistance 4 hours after reperfusion (before and during adenosine infusion) in group I are summarized in Figures 1 and 2. Four hours after reperfusion, endocardial blood flow was significantly lower in the previously ischemic territory as compared with the nonischemic territory; epicardial flow also tended to be lower, but the difference did not attain statistical significance (Figure 1). During infusion of adenosine, both endocardial and epicardial flows were significantly lower in the stunned as compared with the nonischemic myocardium (Figure 1). Similarly, 4 hours after reperfusion, endocardial vascular resistance was significantly higher in the LAD-dependent territory than in the nonischemic territory, whereas the difference in epicardial resistance was not statistically significant (Figure 2). During adenosine administration, coronary vascular resistance fell in both territories, but the values in the stunned myocardium were significantly higher than in the nonischemic myocardium, both in the epicardial and in the endocardial halves of the left ventricular wall (Figure 2).
In the control dogs that did not undergo ischemia and reperfusion (group II), regional myocardial blood flow and coronary vascular resistance did not differ in the LAD-dependent and in the circumflex-dependent territories before or during infusion of adenosine (Figures 1 and 2). Thus, the differences in coronary flow and resistance noted between the ischemic and reperfused region and the nonischemic region in the dogs that underwent ischemia and reperfusion cannot be ascribed to inherent differences between the LAD and the circumflex vascular beds.

Studies with papaverine (groups III and IV). Similar to the results obtained in group I, in group III at 4 hours of reperfusion, endocardial blood flow was significantly lower in the previously ischemic territory as compared with the nonischemic territory, whereas epicardial flow did not differ (Figure 3). During papaverine, endocardial flow remained demonstrably depressed in the LAD-dependent region; epicardial

**FIGURE 2.** Coronary vascular resistance in group I (dogs that underwent a 15-minute occlusion of the left anterior descending coronary artery [LAD]) (n=6) and in group II (dogs that did not undergo LAD occlusion) (n=7) at the following times: during LAD occlusion (group I) or at a corresponding time (Baseline, group II), 4 hours after reperfusion (group I) or at a corresponding time (5 hours after instrumentation, group II), and during adenosine. Epicardial (Epi), endocardial (Endo), and mean transmural (Mean) coronary resistance are depicted. Solid bars indicate resistance in the LAD-dependent bed; open bars indicate resistance in the circumflex-dependent bed. Mean and one SEM are depicted. *p<0.05; **p<0.01; §p<0.001 vs. circumflex bed. In group I, coronary vascular resistance was higher in the previously ischemic LAD territory as compared with the nonischemic circumflex territory both 4 hours after reperfusion and during adenosine. In contrast, in control dogs (group II), coronary vascular resistance did not differ in the two territories before or during adenosine.

**FIGURE 3.** Regional myocardial blood flow in group III (dogs that underwent a 15-minute occlusion of the left anterior descending artery [LAD]) (n=8) and in group IV (control dogs that did not undergo LAD occlusion) (n=8) at the following times: 4 hours after reperfusion (group III) or at a corresponding time (5 hours after instrumentation, group IV), and during infusion of papaverine. Epicardial (Epi), endocardial (Endo), and mean transmural (Mean) flows are depicted. Solid bars indicate flows in the LAD-dependent bed; open bars indicate flows in the control circumflex-dependent bed. Mean and one SEM are depicted. *p<0.05; **p<0.01 vs. circumflex bed. In group III, regional myocardial blood flows were lower in the previously ischemic LAD territory as compared with the nonischemic circumflex territory both 4 hours after reperfusion and during papaverine. In contrast, in controls (group IV), regional myocardial blood flow did not differ in the LAD-dependent territory and in the circumflex-dependent territory before or during papaverine.
flow was also lower, but the difference did not achieve statistical significance (Figure 3). The differences in coronary vascular resistance paralleled those in coronary flow (Figure 4). In the control dogs that did not undergo coronary occlusion (group IV), both regional blood flow and vascular resistance were similar in the LAD-dependent and in the circumflex-dependent territories before and after papaverine infusion (Figures 3 and 4).

Studies with reactive hyperemia (groups III and IV). Table 2 shows that in group III, all indexes of reactive hyperemia (peak hyperemic flow, ratio between peak hyperemic flow and baseline flow, ratio between area of hyperemia and area of flow deprivation, duration of hyperemia, and minimal coronary vascular resistance) were markedly abnormal in the LAD 4 hours after reperfusion as compared with baseline measurements. The ratio of the area of hyperemia to area of flow deprivation averaged only 46% of the value observed before the 15-minute ischemic episode ($p<0.005$). This striking reduction was the result of a reduction both in the maximal level of flow attained (ratio of peak flow to baseline flow, 77% of that measured before LAD occlusion [$p<0.005$]) and in the duration of the hyperemic response (56% of that measured before LAD occlusion [$p<0.005$]) (Table 2). In the control circumflex coronary artery, none of these indexes of reactive hyperemia exhibited a significant change between baseline and 4 hours after reperfusion. In contrast to group III, in the control dogs that did not undergo coronary occlusion (group IV), there was no discernible change in the hyperemic response of the LAD vascular bed over the 5-hour interval (Table 3).

**Correlation Between Myocardial Flow and Function**

To gain insights into the significance of the vascular abnormalities observed in the stunned myocardium, the relation between regional myocardial flow and function in the postischemic region was system-

![Figure 4. Coronary vascular resistance in group III (dogs that underwent a 15-minute occlusion of the left anterior descending coronary artery (LAD)) (n=8) and in group IV (dogs that did not undergo LAD occlusion) (n=8) at the following times: 4 hours after reperfusion (group III) or at a corresponding time (5 hours after instrumentation, group IV), and during papaverine. Epi (endocardial), Endo (endocardial), and mean transmural (Mean) coronary resistance are depicted. Solid bars indicate resistance in the LAD-dependent bed; open bars indicate resistance in the circumflex-dependent bed. Mean and one SEM are depicted. *p<0.05 vs. circumflex bed. In group III, coronary vascular resistance was higher in the previously ischemic LAD territory as compared with the nonischemic circumflex territory both 4 hours after reperfusion and during papaverine. In contrast, in control dogs (group IV), coronary vascular resistance did not differ in the two territories before or during papaverine.](http://circres.ahajournals.org/)

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**TABLE 2. Measurements of Reactive Hyperemia After a 40-Second Coronary Occlusion in Group III (15-Minute Ischemia) (n=8)**

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<th>Baseline</th>
<th>4 hours postreperfusion</th>
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<tbody>
<tr>
<td><strong>Left anterior descending coronary artery</strong></td>
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</tr>
<tr>
<td>Peak hyperemic flow (ml/min)</td>
<td>87.0±6.4</td>
<td>58.9±9.0*</td>
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<td>Peak flow/baseline flow ratio</td>
<td>3.35±0.11</td>
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<td>Area of hyperemia/area of flow deprivation ratio</td>
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<td>Duration of hyperemia (sec)</td>
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<td>Minimal coronary resistance (mm Hg/ml/min)</td>
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<td><strong>Left circumflex coronary artery</strong></td>
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<td>Peak hyperemic flow (ml/min)</td>
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<td>Peak flow/baseline flow ratio</td>
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<td>Area of hyperemia/area of flow deprivation ratio</td>
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<tr>
<td>Duration of hyperemia (sec)</td>
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<tr>
<td>Minimal coronary resistance (mm Hg/ml/min)</td>
<td>1.00±0.12</td>
<td>1.06±0.17</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

* $p<0.01$ vs. baseline.

*† $p<0.005$ vs. baseline.

‡ $p<0.05$ vs. baseline.
Use during infusion of vasodilators, including papaverine, caused an enhancement of the absolute blood flow in the postischemic LAD-dependent territory; the use of normal vasodilators of myocardial thickening and flow should enhance the accuracy of the analysis. We also examined the relation between the hyperemic response of the reperfused LAD bed to a 40-second occlusion and the mechanical performance of the LAD-dependent myocardium in group III. In Figure 7, the normalized values of thickening fraction in the LAD territory 4 hours after reperfusion were plotted against the ratio of peak flow to baseline values. There was no correlation between the normalized values of thickening fraction and average transmural flow ($r = -0.35$ for groups I and III together; $r = -0.04$ and $-0.43$ for groups I and III, respectively). When endocardial and transmural flows were expressed in absolute units, a weak, nonsignificant negative correlation was observed ($r$ values between $-0.50$ and $-0.60$), both when thickening fraction was expressed in absolute units and when it was normalized to baseline values.

In Figure 6, the normalized values of thickening fraction in the LAD territory 4 hours after reperfusion were plotted against the normalized values of endocardial blood flow in the same territory during maximal vasodilation induced by adenosine (group I) or papaverine (group III). Again, there was no discernible correlation between these two variables, either when groups I and III were pooled ($r = -0.26$) or when they were analyzed separately ($r = -0.47$ and $-0.43$, respectively). There was also no significant correlation between the normalized values of thickening fraction and average transmural flow ($r = -0.35$ for groups I and III together; $r = -0.04$ and $-0.43$ for groups I and III, respectively). When endocardial and transmural flows were expressed in absolute units, a weak, nonsignificant negative correlation was observed ($r$ values between $-0.50$ and $-0.60$), both when thickening fraction was expressed in absolute units and when it was normalized to baseline values.

**TABLE 3. Measurements of Reactive Hyperemia after a 40-Second Coronary Occlusion in Group IV (No Ischemia) ($n=8$)**

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<tr>
<td>Peak hyperemic flow (ml/min)</td>
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<td>Peak flow/baseline flow ratio</td>
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<td>80±5</td>
</tr>
<tr>
<td>Minimal coronary resistance (mm Hg/ml/min)</td>
<td>1.34±0.27</td>
<td>1.37±0.15</td>
</tr>
<tr>
<td><strong>Left circumflex coronary artery</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak hyperemic flow (ml/min)</td>
<td>97.4±13.6</td>
<td>108.0±20.3</td>
</tr>
<tr>
<td>Peak flow/baseline flow ratio</td>
<td>3.86±0.37</td>
<td>3.73±0.42</td>
</tr>
<tr>
<td>Area of hyperemia/area of flow deprivation ratio</td>
<td>2.54±0.37</td>
<td>2.43±0.35</td>
</tr>
<tr>
<td>Duration of hyperemia (sec)</td>
<td>83±9</td>
<td>78±9</td>
</tr>
<tr>
<td>Minimal coronary resistance (mm Hg/ml/min)</td>
<td>1.08±0.15</td>
<td>1.19±0.24</td>
</tr>
</tbody>
</table>

Values are mean±SEM.
baseline flow in the LAD 4 hours after reperfusion (normalized to the corresponding ratio in the circumflex artery). It is apparent that there was no correlation ($r=0.20$). Similar results were obtained when either normalized or absolute thickening fraction was related to the absolute values of the ratio of peak flow to baseline flow ($r=0.18$ and $0.35$, respectively). There was also no relation between normalized or absolute wall thickening and the other indexes of reactive hyperemia examined. For example, linear regression analysis of the normalized thickening fraction versus the ratio of area of hyperemia to area of flow deprivation (normalized to the corresponding ratio in the circumflex artery) yielded an $r$ value of $-0.08$; when the absolute ratio of area of hyperemia to area of flow deprivation was used, the $r$ value was $-0.05$. Thus, surprisingly, the hyperemic response to a 40-second coronary occlusion was unrelated to the degree of active systolic wall thickening.

In summary, we found no significant relation between the impairment of regional contractile performance in the stunned myocardium and 1) the resting myocardial perfusion, 2) the hyperemia attained during pharmacological vasodilation induced by adenosine or papaverine, and 3) the hyperemic response to a 40-second coronary occlusion.

**Discussion**

This study demonstrates that a brief, reversible ischemic insult causes prolonged abnormalities in vascular function. Even as late as 4 hours after a single 15-minute coronary occlusion, vascular resis-

![Figure 6](http://circres.ahajournals.org/)

**FIGURE 6.** Relation between systolic thickening fraction in the left anterior descending (LAD)–dependent territory 4 hours after reperfusion and endocardial blood flow measured in the LAD-dependent territory during infusion of vasodilators (approximately 4.5 hours after reperfusion). Thickening fraction is expressed as percent of preocclusion (baseline) values; endocardial flow is expressed as percent of simultaneous endocardial flow to the nonischemic, circumflex-dependent region. $\circ$, Dogs in group I, which received adenosine; $\bullet$, dogs in group III, which received papaverine. There was no discernible correlation between normalized thickening fraction and normalized endocardial flow during vasodilator injection ($r=-0.26$).

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

**FIGURE 7.** Relation between thickening fraction in the left anterior descending (LAD)–dependent territory 4 hours after reperfusion and peak flow/baseline flow ratio in the LAD measured at the same time point. The peak flow/baseline flow ratio is the quotient of maximal LAD flow after release of a 40-second occlusion and LAD flow immediately before the 40-second occlusion. Thickening fraction is expressed as percent of preocclusion (“baseline”) values; peak flow/baseline flow ratio is expressed as percent of the corresponding ratio measured at the same time in the left circumflex coronary artery. All dogs included in this plot were in group III (studies with papaverine and reactive hyperemia). There was no significant correlation between normalized thickening fraction and normalized peak flow/baseline flow ratio (an index of reactive hyperemia after a 40-second coronary occlusion) ($r=0.20$). Similar results were obtained when the absolute value of peak flow/baseline flow ratio was used.

Tance was significantly higher in the stunned than in the control myocardium. More importantly, the vasodilator response to three different stimuli (adenosine, papaverine, and ischemia) was consistently found to be impaired, that is, the minimal coronary vascular resistance attained was higher in the postischemic than in the nonischemic region, despite the fact that all interventions were given in maximal vasodilator doses. The impairment in resting flow and dilator response of the postischemic vasculature did not correlate with the impairment in mechanical performance of the postischemic myocardium, suggesting that it may be a relatively independent phenomenon. Taken together, these results indicate that, in addition to myocardial “stunning,” reversible ischemia also causes a microvascular “stunning.”

Reduced vasodilation was more consistently noted in the endocardial layers, in which both adenosine and papaverine elicited a demonstrably abnormal response. In the epicardial layers, the response to adenosine was definitely abnormal, whereas the response to papaverine exhibited only a trend toward impairment. Because the severity of ischemic injury during coronary occlusion increases from epicardium to endocardium, it is conceivable that the severity of the vascular derangements observed after reperfusion may also vary across the left ventricular wall in a similar fashion.
It could be argued that the hyperemic response to a 40-second coronary occlusion was reduced in the postischemic vascular bed because the myocardium supplied by this bed was dysfunctional and, therefore, accumulated a lower oxygen “debt” during ischemia compared with normally contracting myocardium. However, the absence of a positive correlation between reactive hyperemia and regional wall thickening strongly suggests that this was not the case. As shown in Figure 7 and elaborated in the “Results,” 4 hours after reperfusion we found no correlation between contractility and any measure of hyperemic response to a 40-second occlusion; hence, reduced vasodilation after a 40-second occlusion cannot be ascribed solely to decreased mechanical performance. Our finding that a brief occlusion caused the same hyperemic responses in dyskinetic regions as compared with hypokinetic regions may seem at first paradoxical. However, data from several laboratories28–33 have demonstrated that the stunned myocardium has normal oxygen consumption despite complete loss of active contraction. Apparently, there is either inefficient conversion of energy into contractile work or increased oxygen consumption for processes other than contraction.33 In either case, the oxygen “debt” accumulated during a brief coronary occlusion would be unrelated to the level of mechanical performance.

Previous investigations34,35 have shown that prolonged (60–90 minutes) coronary occlusions followed by reperfusion, which result in myocardial infarction, are associated with abnormal vasomotor responsiveness of large coronary arterial rings, as assessed by in vitro studies. The present findings extend these observations by indicating 1) that abnormalities of coronary vascular resistance and vasodilator responsiveness occur after brief episodes of ischemia that are unassociated with irreversible injury, 2) that such abnormalities can be demonstrated in the intact coronary circulation in vivo, and 3) that they involve the small arteries and arterioles of the coronary microcirculation, which are crucial for the control of coronary vascular resistance.

Although it has long been known that the process of myocardial infarction, with the attendant vascular destruction, causes a decrease in coronary flow36 and coronary flow reserve,37 only recently has investigative interest focused on the setting of reversible ischemia. A recent study by Nicklas and Gips38 supports our conclusions. Using open-chest dogs, Stahl et al25 reported that blood flow in the stunned myocardium did not differ significantly from control myocardium under resting conditions or during infusion of vasodilators (dipyridamole, papaverine, and nitroglycerin). These results are not necessarily in conflict with ours for several reasons. First, the experimental models of stunned myocardium are different because we used a 15-minute coronary occlusion, whereas Stahl et al25 used 12.5-minute occlusions separated by 10-minute reflow periods. It is possible that the vascular injury associated with brief, repeated ischemic episodes alternating with reperfusion is milder than that associated with a single, longer ischemic episode. (In this regard, the postischemic myocardium was hypokinetic or akinetic in the Stahl study, whereas at corresponding times in our protocol [90 minutes after reperfusion] the postischemic myocardium was still dyskinetic.) Second, differences in collateral flow (and therefore in severity of ischemia) may contribute to the apparent differences between the two investigations. Third, vasodilator stimulation was less intense in the Stahl study, as documented by the lower dose of papaverine (1 mg/min versus an average of 12 mg/min in our study) and by the lower blood flows during vasodilator infusion (nonischemic zone flows, 2.1–2.8 ml/min/g versus 4.4–6.6 ml/min/g in the present study). Finally, in this prior experiment25 there was a trend for blood flow to be reduced in the stunned myocardium, particularly during vasodilator-induced hyperemia. In two of the three groups of dogs studied by Stahl et al25 resting subendocardial flow was somewhat less in the stunned as compared with the nonischemic territory (1.1 versus 1.3 ml/min/g and 0.8 versus 1.3 ml/min/g), although in the third group subendocardial flows were the same. During infusion of vasodilators, subendocardial flow was less in the stunned than in the nonischemic region in all three groups (2.1 versus 2.6 ml/min/g with dipyridamole, 1.7 versus 2.1 ml/min/g with papaverine, and 1.0 versus 1.4 ml/min/g with nitroglycerin), a pattern that is consistent with our observations.

A recent report by Laxson et al32 in conscious dogs found that after three 10-minute coronary occlusions separated by 30-minute reperfusion periods, there was no significant reduction in resting myocardial blood flow, in vasodilator response to adenosine, or in peak hyperemia after 10–20-second coronary occlusions. Using a single 10-minute coronary occlusion in open-chest dogs, Jeremy et al39 have recently reported that the hyperemic response of the stunned myocardium to a 100-second coronary occlusion and to adenosine is normal. Our results do not necessarily conflict with these data, for the duration of the ischemic period in the present study (15 minutes) was longer than that used in both of the aforementioned studies (10 minutes). Because the severity of the ischemic insult associated with a 10-minute occlusion may not be sufficient to produce the functional alterations responsible for microvascular stunning, a direct comparison between our present findings and those described above32,39 is not possible.

That ischemia was milder in the study by Laxson et al32 than in ours is also suggested by the facts that isoproterenol was needed in approximately half of the dogs to induce mechanical stunning,32 and the degree of such stunning was less than that seen in the present study (segment shortening at 3 hours of reperfusion recovered to approximately 70% of baseline,32 whereas in our dogs wall thickening at 3 hours of reflow was close to or below zero). It should also be noted that in this prior investigation,32 suben-
docardial flows during adenosine were approximately 20% lower 1 hour after reperfusion as compared with baseline, suggesting perhaps a trend toward reduced vasodilation. Furthermore, the duration of reactive hyperemia and the blood flow debt repayment after 10- or 20-second coronary occlusions was significantly diminished in the stunned myocardium, in accordance with our results.

Our finding that resting subendocardial perfusion in the stunned myocardium is impaired agrees with two previous studies in conscious dogs, both of which used a 15-minute coronary occlusion: the first by Heyndrickx et al., and the second from our laboratory. Reduced myocardial blood flow has also been reported in rats after six consecutive 10-minute coronary ligations separated by 20-minute reperfusion periods. These studies assessed only resting myocardial perfusion. The present investigation expands these prior observations by demonstrating that abnormalities of resting subendocardial perfusion persist as long as 4 hours after reflow, that they do not correlate with mechanical abnormalities, and most important, that the vasodilator responsiveness of the coronary vasculature is also markedly abnormal.

In a recent study from our laboratory, resting blood flow to the stunned myocardium was found to be decreased only in one of three groups of dogs. This apparent discrepancy with the present results could be due to the fact that, in this prior investigation, we measured average transmural flow. Because the decrease in resting perfusion in the stunned myocardium occurs mostly in the subendocardial layers, with little or no change in the subepicardial layers (Figures 1 and 3), it is possible that in our previous study the differences in subendocardial flow were “diluted” by the lack of differences in subepicardial flow. Furthermore, in that investigation, blood flow was measured 1 hour after reperfusion, compared with 4 hours in the present experiment. The time course of the development of vascular abnormalities in the stunned myocardium is uncertain; it is possible that regional perfusion declines progressively with time.

The significance of the persistent increase in resting vascular resistance in the stunned myocardium remains to be determined. It seems unlikely that this phenomenon reflects merely an autoregulatory adjustment of arteriolar vasomotor tone to the reduced oxygen demands of the dysfunctional myocardium for two major reasons. First, as discussed above, oxygen consumption in the stunned myocardium is normal or increased rather than decreased. Second, we found no correlation between blood flow and wall thickening in the postischemic region (Figure 5), indicating that reduced subendocardial perfusion cannot be explained solely as a consequence of reduced mechanical function. On the other hand, we cannot exclude that a defect in myocardial perfusion might contribute to the impaired contractile performance. However, the lack of association between severity of contractile dysfunction and severity of hypoperfusion (Figure 5) would suggest that a deficit in flow is not the major cause of the mechanical derangements.

The impaired vasodilator response of the reperfused coronary vasculature could be due to functional and/or structural abnormalities in the microcirculation. Potential mechanisms include morphological alterations of capillaries, depression of vascular smooth muscle reactivity, interstitial edema (which is known to occur in the stunned myocardium and which could impede maximal vasodilation), and endothelial dysfunction, which could decrease formation of prostacyclin and/or interfere with production of endothelium-derived relaxing factors. These abnormalities could be initiated or amplified by oxygen-derived free radicals, which are known to cause vascular dysfunction and have been shown to be produced in the stunned myocardium in this same canine preparation. Whatever the mechanism, the possibility should be considered that microvascular stunning is pathophysiologically distinct from myocardial stunning, because no appreciable correlation was observed between the severity of vasodilator impairment and the severity of contractile impairment.

The implications of microvascular stunning differ profoundly from those of the microvascular abnormalities previously described in necrotic myocardium. If applicable to humans, the concept that the flow reserve of reperfused, viable myocardium remains abnormal for prolonged periods of time would be of considerable interest because the heart is exposed to transient, reversible ischemia in numerous clinical settings. In these situations, the existence of a prolonged postischemic microvascular stunning could impair the ability of the coronary vasculature to respond appropriately to recurrent ischemic episodes and to compensate for the obstruction caused by a critical proximal stenosis. In addition, microvascular stunning may interfere with the assessment of coronary flow reserve. When coronary reserve is measured, it is generally assumed that, in a given patient, this is a relatively fixed quantity. The present results suggest that coronary reserve is a dynamic entity that may be continuously modulated by the occurrence of transient ischemic episodes.

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Microvascular “Stunning” After Reversible Ischemia

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**Key Words** • myocardial reperfusion • coronary blood flow • adenosine • papaverine • coronary flow reserve
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