Effects of Acute Cellular Injury on Coronary Vascular Reactivity in Awake Dogs

FREDERICK R. COBB, M.D., PHILIP A. MCHALE, PH.D., and JUDITH C. REMBERT, PH.D.

SUMMARY The study was designed to examine effects of acute cellular injury on regional myocardial blood flow (RMBF) and coronary vascular reactivity. Before myocardial infarction in 14 dogs, RMBF was measured using 7–10 μm microspheres during the hyperemic response following a 60 sec transient ischemic stimulation (TIS). Myocardial infarction was induced by complete occlusion for two hours and then inflow to the injured area was re-established. RMBF was measured four hours later during basal conditions, following a 60 sec TIS and during infusion of adenosine, 1.0 mg/kg/min. Effects of acute cellular injury were examined by measuring RMBF in multiple myocardial samples, grouped according to extent of histologic necrosis.

Four hours after reperfusion, RMBF was decreased when infarction exceeded 50%. The decrements in flow were directly proportional to the extent of infarction. The vasculature was capable of delivering additional flow to the injured area since both TIS and adenosine infusion effected increases in RMBF in excess of 100% in each region of the ischemic zone. Blood flow responses to these stimuli, however, fell in proportion to the extent of infarction. RMBF responses to TIS and adenosine infusion were comparable, indicating ischemia which effects irreversible myocardial injury also directly alters vasmotor properties of the intramural vasculature.

ISCHEMIA OF THE MYOCARDIUM sufficient to initiate acute cellular injury effects local responses in the zone of ischemia which directly alter tissue perfusion.1,2 Studies from this laboratory3 have demonstrated that the alterations in regional myocardial perfusion which result from acute cellular injury are directly proportional to the extent of eventual histologic myocardial necrosis.

The objectives of the present study were to determine 1) whether factors which effect reduction in blood flow to an acutely injured area also prevent increases in blood flow in response to the metabolic stimuli resulting from transient ischemia, and 2) whether ischemia sufficient to effect irreversible myocardial injury also directly alters the vaso-motor properties of the intramural vasculature. Regional myocardial blood flow was measured after re-establishing inflow to an area subjected to two hours of ischemia. Regional myocardial blood flow was measured during basal conditions, immediately following the metabolic stimulation resulting from transient ischemia, and during direct vascular stimulation effected by intravenous adenosine. Effects of acute cellular injury on myocardial perfusion during these interventions were assessed by determining regional blood flow in multiple myocardial samples grouped according to subsequent histologic myocardial infarction. The study was performed in awake, chronically prepared animals to avoid variables introduced by general anesthesia and acute surgery.

Methods

Complete studies were performed in 14 mongrel dogs weighing 25–34 kg. The dogs were anesthetized with thi-amyal sodium (30–40 mg/kg, i.v.) and underwent a left thoracotomy. The proximal 1 cm of the left circumflex coronary artery was dissected free and a pneumatic cuff occluder was placed around the vessel. Heparin-filled catheters were inserted into the left atrial cavity and the aortic root. The catheters and snare were tunneled to a subcutaneous pouch at the base of the neck.

Studies were performed 7–10 days after the surgical procedure with the dogs lying quietly on a laboratory table as previously described.4,5 The mean hematocrit at the time of study was 42%, range 38–52%. To assure proper function of...
the coronary occluder, electrocardiographic and hemo-
dynamic responses to a 45–60 sec occlusion were observed. 
Proper function of the pneumatic occluder was verified by 
absence of abnormal Q waves before occlusion, elevation of 
ST segment, and an increase in heart rate and left atrial 
pressure within 30 sec after occlusion, and returned to pre-
occlusion values of each parameter within approximately 15 
sec after release of the occlusion. If the above responses were 
not observed, the animals were not included in the study. 
Coronary occlusion was then performed over a 15 min inter-
val by gradually increasing the pressure in the occluder tub-
ing. Coronary occlusion was maintained for 2 hours. Blood 
flow was then re-established to the ischemic area by com-
pletely deflating the occluder. Lidocaine, 2 mg/kg, was 
administered intravenously before the occlusion and at 15 min 
intervals for 1 hour after occlusion to reduce early arrhyth-
mas. Morphine sulfate, 10 mg, was injected intravenously 
as the snare was inflated to minimize any discomfort result-
ing from the coronary occlusion. Antiarrhythmic or anal-
gesic agents were not administered after the first hour of the 
study. Using the procedure, three dogs developed ventricu-
lar fibrillation after the occlusion and were excluded from 
the study.

Measurements of myocardial blood flow were made by 
serial injections of carbonized microspheres, 7–10 μm in 
diameter, labeled with gamma-emitting nuclides 51Cr, 54Ce, 
85Sr, and 46Sc as previously described. The initial myo-
cardial blood flow measurement was made to assess the 
response of the normal coronary vasculature to the met-
abolic stimulus resulting from transient ischemic stimula-
tion. Microspheres were injected over a 10–15 sec interval 
beginning 10 sec after release of a 60 sec complete circum-
flex occlusion. Studies performed in this laboratory using 
awake dogs with chronic implanted electromagnetic flow 
probes demonstrated that a 60 sec transient occlusion effects 
a reactive hyperemic response characterized by sustained 
maximum increase in blood flow measurements. Sub-
sequent blood flow measurements were made 4 hours follow-
ing release of the 2 hour occlusion. At this point blood flow 
was measured during basal conditions, following release of a 
60 sec complete occlusion as described above and during a 
constant intravenous infusion of adenosine 1.0 mg/kg/min. 
In previous studies in this laboratory this dose of adenosine 
produced maximum increases in phasic coronary flow and 
4–5 fold increases in transmural myocardial blood flow.

Six days after the initial study the animals were anes-
thesitized with thiamylal sodium and the hearts fibrillated 
with concentrated potassium chloride. The mean left ven-
tricular weight was 104 ± 5 g (SEM). As illustrated in figure 1 
and described in previous studies, the left ventricle was 
sectioned into four transverse sections, circumferential 
regions and finally into four transmural layers of approx-
imately equal thickness, samples size 1–2 g. Ten percent 
buffered formalin was added to the vials to preserve the 
tissue for histological sections after measurement of the 
tissue radioactivity.

![Diagram of the left ventricle with sections labeled 1 to 4.](image)

**Figure 1.** This schematic diagram illustrates 
the technique for sectioning the left ventricle. The 
atral tissue and right ventricle were removed as 
indicated by the stippled and lined area. The left 
ventricle was sectioned into four transverse rings. 
Each ring was sectioned into circumferential 
regions, i.e., anterior (A), septal (S), posterior (P), 
posterior papillary (PP), lateral (L), and anterior 
papillary (AP). Each circumferential region in 
rings 1, 2, and 3 was divided into four equal trans-
mural layers. Regions in ring 4 were divided into 
equal epicardial and endocardial layers.
Table 1. Hemodynamic Measurements

<table>
<thead>
<tr>
<th></th>
<th>Before occlusion</th>
<th>Four hours after reperfusion</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Reactive hyperemia</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SEM</td>
<td>82</td>
<td>91</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AP (mm Hg)</td>
<td>100</td>
<td>103</td>
</tr>
<tr>
<td>SEM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LAP (mm Hg)</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>SEM</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Measurement of mean heart rate (HR), arterial pressure (AP), and left atrial pressure (LAP) before occlusion and 4 hr after release of 2 hr occlusion. The P values associated with the reactive hyperemia response before occlusion and control after reperfusion compare the hemodynamic responses to the control before occlusion. The P values associated with the reactive hyperemia and adenosine response after reperfusion compare the responses to the control after reperfusion. Values are mean ± SEM.

The radioactivity in each myocardial sample and reference blood sample was determined in a gamma spectrometer (Model 167776, Beckman Instruments Inc.), using window settings selected to correspond to the peak energies of each radioactive nuclide. Blood flow to each myocardial sample in milliliters per minute per gram was calculated as previously described. After measurement of tissue radioactivity and calculation of blood flow to each myocardial sample, the samples were prepared for histological sectioning and the percentage of infarcted myocardium in each small myocardial sample was determined as previously described. Thus, blood flow and the extent of myocardial infarction were determined in multiple small tissue samples of the entire region subjected to ischemia. Since the effects of acute cellular injury on myocardial perfusion are a function of the extent of eventual necrosis and the extent of necrosis varies between animals, myocardial perfusion was analyzed in multiple myocardial samples grouped according to comparable degrees of histologic myocardial necrosis, i.e., 0–5, 6–25, 26–50, 51–75, 76–89, 90–100, and 100%.

The Student’s t-test for paired data was used to compare blood flow measurements in the same myocardial samples.

Table 2. Relationship Between Infarcted Myocardium and Blood Flow (ml/g/min)

<table>
<thead>
<tr>
<th></th>
<th>Anterior region</th>
<th>Left circumflex coronary artery region</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0%</td>
<td>0–5%</td>
</tr>
<tr>
<td>A. Before infarction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Reactive hyperemia</td>
<td>1.16 ± 0.09</td>
<td>5.33 ± 0.73</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.01 &lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>B. Four hours after reperfusion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Control</td>
<td>1.23 ± 0.10</td>
<td>1.27 ± 0.13</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>2. Reactive hyperemia</td>
<td>1.35 ± 0.16</td>
<td>3.88 ± 0.58</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.01 &lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>3. Adenosine</td>
<td>4.13 ± 0.31</td>
<td>4.37 ± 0.43</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Myocardial blood flow, ml/min/g, before infarction during the reactive hyperemic response and 4 hr after release of a 2 hr occlusion during control, the reactive hyperemic response and infusion of adenosine 1.0 mg/kg/min. Blood flow in the left circumflex coronary artery region is grouped according to the percent of infarcted myocardium in each sample. Values are mean ± SEM. P values compare blood flow in the anterior to that in the circumflex region.

NS = P >0.05.

Results

The results of hemodynamic measurements during control resting conditions and at each measurement of regional myocardial blood flow are tabulated in table 1. Average resting hemodynamics were as follows: heart rate 82 beats per minute; mean arterial pressure 100 mm Hg; and mean left atrial pressure 5 mm Hg. The initial blood flow measurement was carried out immediately following transient ischemic stimulation produced by a 60 sec occlusion of the circumflex coronary artery. During the occlusion the heart rate and mean arterial and left atrial pressures increased. Ten to fifteen seconds following deflation of the cuff, during the maximum reactive hyperemic response, heart rate, arterial and left atrial pressures were 91 beats/min, 103 and 7 mm Hg. The predominant rhythm during the 2 hour circumflex artery occlusion was sinus tachycardia with occasional premature ventricular contractions. Approximately 20 seconds after release of the two hour occlusion, the
rhythm was interrupted by ventricular tachycardia which lasted 10–15 min. Four hours after reperfusion the mean heart rate was 125 beats per minute and the mean arterial and mean left atrial pressures were 95 and 9 mm Hg, respectively. At this time the rhythm was predominantly ventricular in 9 animals. Fours hours after reperfusion, 60 second occlusion of the circumflex coronary artery did not produce significant changes in heart rate or arterial or left atrial pressure. At this time adenosine infusion increased heart rate to 143 beats/min and decreased arterial and left atrial pressure to 70 and 6 mm Hg, respectively.

The relationship between acute cellular injury and perfusion during each intervention was measured by determining mean blood flow in myocardial samples grouped according to the extent of histologic myocardial infarction, 0–6, 6–25, 26–50, 51–75, 76–89, 90–100 and 100% (table 2, figs. 2–6). Four hours after reperfusion blood flow to the acutely injured area was decreased in regions in which the subsequent extent of histologic myocardial infarction was greater than 50% (fig. 2). The decrements in flow were proportional to the extent of myocardial infarction. Significant levels of blood flow, however, remained in regions in which the extent of infarcted myocardium was 100%.

Four hours after reperfusion transient ischemic stimulation produced by a 60 second occlusion effected an increase in blood flow in all regions in the ischemic zone (fig. 3). Thus, even in regions which eventually developed complete infarction, the vasculature was capable of augmenting blood flow in response to the metabolic stimulus effected by transient ischemia. The response to transient ischemic stimulation, however, was significantly different from that recorded prior to the two hour occlusion (fig. 4). Prior to the two hour occlusion transient ischemic stimulation resulted in 4.5–5.0 fold increases in blood flow in all regions supplied by the circumflex coronary artery. Although a reactive hyperemic response could be elicited in all regions four hours after reperfusion, the response was decreased in each infarct range. The magnitude of reactive hyperemic response was reduced in direct proportion to the extent of subsequent myocardial infarction.

Four hours after reperfusion intravenous adenosine infu-
sion effected an increase in blood flow to all regions in the ischemic zone (fig. 5). Although blood flow increased in each infarct range, the magnitude of the blood flow response was inversely related to the extent of subsequent myocardial infarction. The response during adenosine infusion was significantly different in the region supplied by the non-occluded anterior descending coronary artery and in the infarcted regions. In figure 6 blood flow to the infarcted regions is expressed as the ratio of infarct to anterior nonischemic region flow. As compared to blood flow in the anterior region which increased during the adenosine infusion, blood flow to the infarcted region during adenosine infusion was less in regions with greater than 25% infarction. The relative reductions in the response to adenosine were directly proportional to the extent of infarcted myocardium. The magnitude of blood flow response in the infarcted regions following transient ischemic stimulation and during adenosine infusion was not significantly different in regions with greater than 5% myocardial infarction. However, in regions with 5% or less myocardial infarction, the blood flow response during adenosine infusion was significantly greater than that following transient ischemic stimulation.

Discussion

Several previous studies have demonstrated that prolonged myocardial ischemia may initiate local responses in the zone of acute cellular injury which alter perfusion of the myocardium. In previous studies from this laboratory, regional perfusion was measured immediately, 15 minutes, 4 hours, and 3 days after re-establishing blood flow to an area subjected to prolonged ischemia and related to the extent of eventual histologic infarction. Immediately following reperfusion, blood flow was increased in each region of the zone subjected to prolonged ischemia, but the magnitude of the hyperemic response in a given region was reduced in direct proportion to the extent of eventual histologic myocardial infarction. Fifteen minutes after reperfusion, the hyperemic response had subsided and blood flow to the acutely injured zone was equal to or slightly in excess of flow to noninjured areas. Four hours and 3 days after reperfusion blood flow was decreased in regions with greater than 50% infarcted myocardium. The decrements in flow were proportional to the extent of histologic myocardial infarction. The reductions in blood flow 4 hours and 3 days after reperfusion were comparable indicating that local tissue responses which effected reductions in blood flow were completed within 4 hours of reperfusion.

In the present study, four hours after reperfusion blood flow was reduced in myocardial regions which subsequently demonstrated greater than 50% histologic myocardial infarction. The decrements in flow were proportional to the extent of myocardial infarction. Similar relationships were observed in a previous study. Although blood flow was reduced in areas of extensive myocardial injury, transient ischemic stimulation elicited a residual vasodilator response with blood flow, increasing in excess of 100% in each region of the acutely injured zone. Thus, even in regions which subsequently demonstrated complete histologic myocardial infarction, the vasculature was capable of delivering additional blood flow to the injured area in response to the metabolic stimulation elicited by transient ischemia. As compared to the response to transient ischemic stimulation prior to acute infarction, the magnitude of the response 4 hours after reperfusion was reduced in direct proportion to the extent of myocardial infarction.

Basal myocardial blood flow and the blood flow response which follows transient ischemic stimulation, the reactive hyperemic response, are coupled to the metabolic activity of the myocardium. The precise factors which link vascular tone and thus blood flow to metabolic activity remain controversial. Rubio et al. have presented evidence that adenosine, a potent vasodilator, is continuously released by the normal myocardium and is released in increased quantities in response to myocardial ischemia. These investigators concluded that adenosine provides the link between coronary vascular tone and metabolic activity of the myocardium and is the mediator of the vascular response to ischemia. ATP, a high energy product of aerobic metabolism and precursor of adenosine, is rapidly depleted during ischemia. Acute cellular injury resulting from prolonged ischemia would be expected to severely reduce metabolic capabilities, including the ability to synthesize vasoactive metabolites. It may thus be reasoned that the reductions in basal blood flow and the blood flow response to transient ischemic stimulation may represent simply the loss of metabolic stimulus to blood flow and/or inability to synthesize vasoactive compounds which link blood flow to myocardial metabolic needs. The hyperemic response which follows transient ischemia is also dependent on a vasculature capable of vasodilating when appropriately stimulated. Factors which directly alter vascular reactivity or the ability of the vasculature to vasodilate may reduce basal blood flow and/or the blood flow response to ischemic stimulation independent of myocardial injury.

The reactivity of the vasculature to direct stimulation was tested by measuring the blood flow response during an intravenous infusion of adenosine. In previous studies from the laboratory, intravenous infusion of adenosine in awake animals resulted in a four to five fold increase in transmural blood flow. The vasodilator response to adenosine appears to be mediated by a direct effect on the intramural coronary vasculature. It was thus reasoned that the vasodilator response to adenosine should remain intact if the alteration in perfusion to an acutely injured area resulted from loss of metabolically active myocardium surrounding an intact and reactive intramural vasculature.

![Graph](http://circ.ahajournals.org/content/57/5/966/F6)

**Figure 6.** Myocardial blood flow 4 hours after release of a 2 hour occlusion during intravenous adenosine 10 mg/kg/min. Blood flow is expressed as the ratio of flow in samples from the anterior region. Values are mean ± SEM.
Coronary artery occlusion there is significant loss of microspheres from the infarcting myocardium.\textsuperscript{17, 18} Approximately 25% of the microspheres injected prior to infarction were lost from the infarcted region at 24 hours. No further loss occurred at 4\textsuperscript{17} or 8 days.\textsuperscript{19} In the present study the three blood flow measurements made after infarction were performed during a period of 20–30 min. Since the microspheres in a given tissue region should be lost randomly, the relationships between blood flow measured at the same interval after infarction should not change but each of the actual blood flow values may have been 20–30% higher than the values measured. Loss of microspheres after infarction would also be expected to reduce the blood flow measurements made during the reactive hyperemic response before infarction. Blood flow during the hyperemic response before infarction was increased five fold in samples from each infarct range indicating that microsphere loss in this model of infarction was not a function of the amount of infarction in the tissue samples and/or microsphere loss was minimal.

In the present study acute cellular injury which resulted in subsequent histologic myocardial necrosis reduced basal blood flow and reduced but did not entirely eliminate the vasodilator response to transient ischemic and direct vascular stimulation. Even in areas which eventually developed 100% histologic infarction, significant levels of basal blood flow were present and the vasculature was capable of delivering additional blood flow in response to direct vascular stimulation or the metabolic stimulus elicited by transient ischemia. This study was not designed to examine the specific factors, i.e., cell swelling, release of vasoactive compounds, or interstitial hemorrhage, which may have effected the alterations in perfusion. However, since prolonged ischemia altered the vasodilation response to direct and indirect vascular stimulation in a comparable fashion, the data indicate that ischemia sufficient to effect irreversible myocardial injury also directly alters the vascular reactivity of the intramural vasculature in the regions of irreversible myocardial injury.

Acknowledgments

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References

Unexplained In-Hospital Fever Following Cardiac Surgery

Natural History, Relationship to Postpericardiotomy Syndrome, and a Prospective Study of Therapy with Indomethacin versus Placebo

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SUMMARY In Part I of this study, the in-hospital course of 219 patients who had undergone a cardiac operation is analyzed. Fever (≥ 37.8°C, rectal) was present after postoperative day 6 in 159 patients (73%) and was of unexplained cause in 118. Fever decay in the population of unexplained fever patients was exponential. All patients with unexplained postoperative fever were afebrile by postoperative day 19. In-hospital pericardial rub and pleuritic chest pain, widening of the mediastinum on chest film, and pleural effusion were not specifically associated with unexplained postoperative fever. In Part II, 67 patients with unexplained postoperative fever were given indomethacin (100 mg per day) or placebo for 7 days by a randomized, double-blind protocol. Indomethacin resulted in a shorter duration of fever (2.4 vs 3.5 days, P < 0.01) and in a shorter duration of chest pain, malaise, and myalgias compared to placebo. Sixty-seven percent of the patients in Part I and all of the patients in Part II were contacted 2–8 months following hospital discharge. Five percent had experienced an illness that we considered to be acute pericarditis, but its occurrence was unrelated to whether the patient had had in-hospital unexplained postoperative fever, in-hospital rub or chest pain, or in-hospital administration of indomethacin.

FEVER AND CLINICAL SIGNS of a pleuropericardial process sometimes develop days to months after cardiac surgery, a combination of events widely referred to as the "postpericardiotomy syndrome."1 2 Opinions about what constitutes postpericardiotomy syndrome — when it begins, its relationship to the more general problem of unexplained postoperative fever, and its clinical importance and natural history — are extremely diverse. The incidence of the syndrome has been reported to be as low as 1% and as high as 64%,3-10 and recommendations for therapy have differed widely.4-8, 11-15 Cardiologists and cardiac surgeons in our institution have frequently administered indomethacin to such patients when fever has been accompanied by pleu-
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