Evidence for Decreased Coronary Flow Reserve in Viable Postischemic Myocardium

Johan Vanhaecke, Willem Flameng, Marcel Borgers, Ik-Kyung Jang, Frans Van de Werf, and Hilaire De Geest

To try to unravel the complexity and heterogeneity of the “no-reflow” phenomenon and its underlying mechanisms, we studied tissue perfusion in reperfused heart muscle by using tracer microspheres in an anesthetized dog model of 90-minute coronary occlusion followed by reperfusion for 2½ hours, 24 hours, or 1 week. Regional myocardial blood flow was determined both in basal flow conditions and during reactive hyperemia. The effect of intracoronary adenosine administration was examined, and the ultrastructure of postischemic myocardium was analyzed. In viable reperfused tissue (as delineated by triphenyltetrazolium chloride staining), reflow in basal conditions is unimpaired. Coronary flow reserve (as approximated by peak reactive hyperemic flow) is intact at the start of reperfusion, decreases by more than half after 2½ hours, and recovers completely within 1 week. This impairment of coronary reserve can be relieved by intracoronary adenosine administration. On ultrastructural examination, the capillaries are patent. On the other hand, in irreversibly damaged myocardium, both the basal reflow impairment and the decrease in coronary flow reserve are severe and permanent. Coronary flow reserve is already decreased at the start of reperfusion, and the pharmacological intervention has no beneficial effect. Ultrastructurally, extracellular and intracellular edema invariably are present, whereas the vascular endothelium is damaged and the capillaries are packed with red blood cells. We conclude that the no-reflow phenomenon (i.e., mechanical obstruction to blood flow) is limited to infarcted tissue. In viable myocardium, however, coronary flow reserve is transiently diminished, probably because of washout and subsequent insufficient availability of the chemical mediator adenosine after breakdown and slow recovery of the precursor ATP pool. (Circulation Research 1990;67:1201–1210)

When arterial flow is restored after a prolonged period of interrupted blood supply, tissue perfusion does not regain the preischemic level. This impairment of tissue reperfusion has been called the “no-reflow” phenomenon, although in most instances reflow is not absent but merely is reduced to a variable degree. This phenomenon of reduced reflow has been demonstrated in kidney, brain, and skin. It was described in canine myocardium by Kloner et al, and there is some evidence for its occurrence in humans.

Several mechanisms for the no-reflow phenomenon have been proposed, including capillary damage, cell swelling, red blood cell packing, neutrophil plugging, and compression by cardiac contraction. All of these assume a mechanical obstruction to blood flow. Leaf hypothesized a vicious circle of ischemia causing cell swelling, resulting in further impairment of tissue perfusion and obstruction to reflow, ultimately leading to cell death. Support for the idea that tissue edema is responsible for reduced reflow came from the work of Willerson et al, who improved myocardial reflow by administration of hypertonic mannitol in dogs on right ventricular heart bypass. Tranum-Jensen et al obtained similar results using selective hypertonic reperfusion in isolated porcine hearts. These earlier studies did not make a distinction between reflow in irreversibly damaged myocardium and reflow in tissue that remained viable after a period of ischemia. Also, the term no-reflow was restricted to blood flow in basal conditions.

Data on coronary flow reserve in reperfused heart muscle are scarce and conflicting. One hour after release of a 2-hour coronary ligation, Bloor and White in five unanesthetized dogs found an unchanged peak reactive hyperemia but a decreased flow debt repayment, as measured with an electromagnetic flow probe. Blumenthal et al reported no change in reactive hyperemia during reperfusion in

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two dogs without a rise in serum enzyme levels after 1–1½ hours of coronary occlusion; reactive hyperemia was reduced in nine dogs with enzymatic evidence of infarction. Cobb et al.12 related flows in reperfused canine myocardium to the extent of histological necrosis. Basal flow was decreased in samples containing more than 50% infarction. Transient ischemic stimulation and intravenous adenosine infusion effected increases in myocardial blood flow that were inversely proportional to the extent of infarction. These reactive hyperemic and adenosine flows, however, were not compared with measurements in control conditions. Finally, a recent abstract13 reports decreases in coronary flow reserve after a transient (15-minute) period of ischemia in open-chest dogs.

We studied myocardial blood flow in a canine model of coronary occlusion and reperfusion. Viable postschismic myocardium was analyzed separately from irreversibly damaged tissue, and the temporal evolution of reflow was determined not only in basal conditions but also during reactive hyperemia, reflecting the evolution of coronary flow reserve in the occlusion–reperfusion setting. The mechanisms responsible for reflow impairment were explored by pharmacological manipulation of reflow and by ultrastructural tissue analysis.

Materials and Methods

Experimental Model and Protocol

Basically, in our canine experimental preparation, a coronary artery was occluded for 90 minutes and thereafter reperfused for 2½ hours (group I), 24 hours (group II), or 1 week (group III) (see Figure 1). Forty-three mongrel dogs of either sex, weighing 18–25 kg, were premedicated with Hypnorm (10 mg fluanisone/0.2 mg fentanyl per ml) (Duphar, Amsterdam, The Netherlands) (0.25 ml/kg i.m.) and were anesthetized with sodium pentobarbital (15 mg/kg i.v.). After endotracheal intubation, the lungs were ventilated with a 50/50 mixture of oxygen and room air using a Bird Mark 7 respirator (Bird Electronic Corp., Cleveland). Arterial blood gases and pH were determined repeatedly throughout the experiment (pH/blood gas 166 microanalyzer, Corning Glass Works, Corning, N.Y.), and ventilation was adjusted if necessary to keep these values within the normal range. Additional doses of pentobarbital were administered as required to maintain anesthesia. Coronary occlusion was produced either by ligation or thrombosis.

Ligation protocol. In the ligation protocol (group I, n=20), the chest was opened through the left fifth intercostal space, and the heart was suspended in a pericardial cradle. The left anterior descending coronary artery (LAD) was isolated from the surrounding tissue and encircled by a snare. Coronary occlusion and reperfusion were produced by tightening and release of the snare.

In this group of dogs studied up to 2½ hours of reperfusion, LAD occlusion was produced by ligation to ensure the accuracy of some of the regional myocardial blood flow (RMBF) measurements that were planned (testing of coronary flow reserve through reactive hyperemia and intracoronary adenosine infusion, see below). In six of the 20 experiments of group I, a 24-gauge needle connected to a small tubing was inserted in the LAD at the site of future ligation. At the desired times during the experiment, adenosine was infused through this needle into the coronary artery in a dose of 0.5 mg/min; the infused volume was 0.5–1.0 ml/min. This dose previously has shown to produce maximal vasodilation,14 and the intracoronary route of administration was chosen to minimize alterations in peripheral hemodynamics.

Thrombosis protocol. In the thrombosis protocol (groups II and III, n=23), a copper coil attached to a guide wire was advanced under fluoroscopic control via the carotid artery into the LAD. The formation of an occlusive thrombus took 5–10 minutes and was confirmed by coronary angiography using a 5F Leh-
man catheter (USCI, Billerica, Mass.). Reperfusion was achieved by coronary thrombolysis, using an intravenous infusion of a thrombolytic agent. As thrombolytic substances, urokinase (10 µg·kg⁻¹·min⁻¹), recombinant tissue-type plasminogen activator (10 µg·kg⁻¹·min⁻¹), or recombinant single-chain uroki-nase-type plasminogen activator (20 µg·kg⁻¹·min⁻¹) were used. The thrombolytic infusion was started 1 hour after thrombus formation. Reperfusion occurred within 15–30 minutes and was documented by coronary angiography. Then the carotid artery was ligated, skin wounds were sutured, and the dog was allowed to recover. Twenty-four hours (group II, n = 8) or 1 week (group III, n = 15) later, a final open-chest experiment was performed, at the end of which the animal was killed and the heart removed for further processing.

In groups II and III (24 hours and 1 week of reperfusion, respectively), LAD occlusion was produced by coronary thrombosis to avoid two consecutive open-chest experiments on the same animal. Coronary flow reserve in these animals was studied only during the final open-chest experiment.

**Regional Myocardial Blood Flow Measurements**

**Methodology.** RMBF was measured with the tracer microsphere technique. We used 15-µm microspheres labeled with ¹⁴¹Ce, ¹²⁵Sn, ¹⁰³Ru, or ⁹⁵Nb (NEN Chemicals GmbH, Dreieich, FRG). Microsphere handling, dilution, and mixing were performed as described previously.

At different times during an experiment, microspheres were injected in basal flow conditions as well as during intracoronary adenosine infusion and during peak reactive hyperemia after release of a 60-second LAD occlusion. The hyperemic response was monitored by means of an electromagnetic flowmeter to ensure good timing of the microsphere injection.

At the end of an experiment, left ventricular slices were cut into multiple tissue samples after epicardial fat and vessels were removed. The left ventricular wall was divided into three layers of equal thickness (epimyocardium, midmyocardium, and endomyocardium). The area at risk (LAD area) and the control area (circumflex area) were separated, and LAD samples were labeled by their macroscopic appearance after triphenyltetrazolium chloride (TTC) staining as either viable, patchy infarcted, or homogeneously infarcted (see below). This procedure typically resulted in 60–80 tissue samples of ±1 g (range, 0.5–2.0 g) per experiment. Tissue samples were put into test tubes and immersed in 3% glutaraldehyde solution. Radioactivity in reference blood samples and tissue samples was counted (5 min/sample) using an automatic gamma counter and sample changer system (analyzer model 45, Molsgaard Medical, Hørsholm, Denmark) connected to an ND 680 programmable analyzer/computer system (Nuclear Data GmbH, Frankfurt/Main, FRG).

Values for blood flow in every myocardial sample were computed using a slight adaptation of the MIP II program of Schosser et al.

RMBF in a specific region of the left ventricle (e.g., epimyocardium in the circumflex area and viable epimyocardium in the LAD area) was calculated by averaging the values of all tissue samples belonging to that region.

**Timing.** Under preischemic control conditions, basal myocardial blood flow measurements were obtained in 32 animals from groups I, II, and III; all data were pooled. Coronary flow reserve was determined by RMBF measurement at peak reactive hyperemia in all 20 animals of group I. In six of these dogs, coronary reserve also was tested by RMBF measurement during infusions of adenosine.

Under coronary occlusion conditions, RMBF measurement was performed in 40 animals from groups I, II, and III at the end of 90 minutes of LAD occlusion.

Under reperfusion conditions, RMBF was measured in six dogs of group I at 1 minute of reperfu-
sion, during the reactive hyperemia immediately following the release of the snare after 90 minutes of coronary occlusion. At 2½ hours of reperfusion, RMBF was measured in 15 dogs of group I. Coronary flow reserve was determined by RMBF measurement at peak reactive hyperemia in 18 dogs of group I. In six of these dogs, coronary reserve also was tested by RMBF measurement during intracoronary adenosine infusion. At 24 hours of reperfusion (group II), RMBF was measured in all eight dogs. Coronary flow reserve was determined by RMBF measurement at peak hyperemic response in five dogs. At 1 week of reperfusion (group III), RMBF was measured in all 15 dogs in basal flow conditions as well as during peak reactive hyperemia.

**Hemodynamic Measurements**

Systolic and diastolic aortic pressures and left ventricular end-diastolic pressure or left atrial pressure were monitored through fluid-filled catheters connected to a Siemens pressure transducer 746 (Siemens Elema, Solna, Sweden). These catheters were inserted via carotid and/or femoral arteries, or for the left atrial pressure (open-chest experiments), directly into the left atrium via an incision in the left atrial appendage. The pressure signals together with electrocardiographic lead II were displayed on an oscilloscope and recorded on a multichannel ink jet recorder (Siemens Corp., Iselin, N.J.) throughout the experiment.

**Delineation of Viable and Infarcted Tissue in the Area at Risk**

For this purpose, we used a dye perfusion and fixation technique. At the end of each experiment, the heart was removed and both right and left coronary ostia were cannulated as well as the LAD distal to the site of previous occlusion. The LAD was perfused with Ringer’s solution, and the ostia were perfused at the same pressure with a mixture of Ringer’s solution and Evans blue. Two minutes later, the LAD perfusion was switched to a TTC solution at 37° C for 10 minutes. Finally, the heart was fixed by
perfusing the LAD area for another 5 minutes with 2% glutaraldehyde and both coronary ostia with a mixture of 2% glutaraldehyde and Evans blue.

After the right ventricle, the atria, and the valvar structures were removed, the isolated left ventricle was cut in 1-cm-thick slices perpendicularly to the long axis.

As a result of the above-described dye perfusion technique, myocardium supplied by nonoccluded coronary arteries (referred to as the circumflex area) was colored blue, whereas in the occluded bed (the LAD area), viable tissue stained brick red, and infarcted tissue was white.

Ultrastructural Analyses

After perfusion staining and fixation, small samples of myocardial tissue were immersed in a fixative containing 3% glutaraldehyde and 90 mmol/potassium oxalate, adjusted to pH 7.4 with sodium potassium hydroxide. These samples then were washed thoroughly in the same buffer supplemented with 0.22% sucrose. Postfixation was done in 1% osmic acid, buffered to pH 7.4 with 0.05 M veronal acetate containing 0.093 M sucrose, for 1 hour at 4°C. After a rinse in the buffer, samples were dehydrated in a graded series of ethanol and routinely embedded in epoxy resin. Ultrathin sections were examined with a Philips EM300 electron microscope (Philips Nederland, Eindhoven, The Netherlands) after staining with uranyl acetate and lead citrate.

Statistical Methods

Statistical analyses were done using the SAS statistical package. Student’s t test (paired or unpaired) was used for comparisons between two groups. When more than two groups were compared, one-way analysis of variance was used to assess overall significance of group differences. If a statistically significant F value was observed, an appropriate multiple comparison method (Tukey’s modification of the honestly significant difference test) was applied to evaluate the difference between any two groups. Values are given as mean±SD unless stated otherwise.

Results

Time Dependency of the No-Reflow Phenomenon

The data on RMBF measurements at different times during reperfusion are presented separately for viable subepicardium in Table 1 and for homogeneously infarcted subendocardium in Table 2.

### Table 1. Temporal Evolution of Regional Myocardial Blood Flow in Viable Subepicardium

<table>
<thead>
<tr>
<th>Group</th>
<th>Basal conditions (ml/min/100 g)</th>
<th>PRH in LAD area (ml/min/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cx n</td>
<td>LAD</td>
</tr>
<tr>
<td>Control</td>
<td>32</td>
<td>49±17</td>
</tr>
<tr>
<td>Occlusion</td>
<td>40</td>
<td>55±19</td>
</tr>
<tr>
<td>Reperfusion</td>
<td></td>
<td>...</td>
</tr>
<tr>
<td>1 min</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>2½ hr</td>
<td>15</td>
<td>60±19</td>
</tr>
<tr>
<td>24 hr</td>
<td>8</td>
<td>53±20</td>
</tr>
<tr>
<td>1 wk</td>
<td>15</td>
<td>48±25</td>
</tr>
</tbody>
</table>

Dogs underwent a 90-min occlusion of the left anterior descending artery followed by reperfusion. Values are mean±SD. PRH, peak reactive hyperemia; LAD, left anterior descending; Cx, circumflex.

### Table 2. Temporal Evolution of Regional Myocardial Blood Flow in Infarcted Subendocardium

<table>
<thead>
<tr>
<th>Group</th>
<th>Basal conditions (ml/min/100 g)</th>
<th>PRH in LAD area (ml/min/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cx n</td>
<td>LAD</td>
</tr>
<tr>
<td>Control</td>
<td>24</td>
<td>68±21</td>
</tr>
<tr>
<td>Occlusion</td>
<td>25</td>
<td>74±27</td>
</tr>
<tr>
<td>Reperfusion</td>
<td></td>
<td>...</td>
</tr>
<tr>
<td>1 min</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>2½ hr</td>
<td>12</td>
<td>66±19</td>
</tr>
<tr>
<td>24 hr</td>
<td>6</td>
<td>66±14</td>
</tr>
<tr>
<td>1 wk</td>
<td>9</td>
<td>62±23</td>
</tr>
</tbody>
</table>

Dogs underwent a 90-min occlusion of the left anterior descending artery followed by reperfusion. Values are mean±SD. PRH, peak reactive hyperemia; LAD, left anterior descending; Cx, circumflex.

*p<0.001 vs. Cx flow at the same time.
†p<0.02 vs. Cx flow at the same time.
‡p<0.001 vs. control PRH in LAD area.
During occlusion, collateral flow in the LAD area is about 45% of the preischemic flow. Reflow in the subepicardial LAD area after reperfusion for 2½ hours, 24 hours, and 1 week is slightly but not significantly higher than the preischemic value. As compared with flow in the circumflex area, there is initially a small but significant flow deficit ($p<0.02$) because of a small increase in circumflex flow. This flow deficit disappears by 24 hours and 1 week of reperfusion.

The coronary flow reserve as assessed by the peak reactive hyperemic (PRH) flow is intact at the start of reperfusion. PRH flow during the first minute of reflow after 90 minutes of occlusion is similar to the preischemic control PRH flow elicited by transient ischemic stimulation: 292±73 and 338±137 ml/min/100 g, respectively ($p=NS$). Thus, a 90-minute coronary occlusion per se does not adversely affect coronary flow reserve in viable tissue. The decrease in PRH flow (to about 45% of control values) observed after 2½ hours of reflow occurs during the initial phase of reperfusion. After 24 hours of reperfusion, the PRH flow still is depressed (47% of control values), but at the end of 1 week of reperfusion, there is a complete recovery of the coronary flow reserve in viable subepicardium: PRH flow returned to a value of 345±145 ml/min/100 g.

Infarcted subendocardium. In homogeneously infarcted subendocardium, the preischemic control flow is 15% lower than in the subepicardial circumflex area because of real and/or apparent microsphere loss.21

Collateral flow during occlusion is very low (4% of the preischemic LAD flow). Reflow after 2½ hours of reperfusion is severely impaired and amounts to only 33% of preischemic LAD flow. In the subsequent days of reperfusion, there is no recovery of this flow deficit: at 1 week, reflow still is 34% of preischemic LAD flow. Thus, in infarcted tissue, the basal flow deficit is permanent, whereas in viable myocardium, basal flow normalizes after reperfusion.

The coronary flow reserve in homogeneously infarcted subendocardium also behaves differently in several aspects. First, the vasodilatory capacity is already severely compromised during the 90-minute period of coronary occlusion: PRH flow at the start of reperfusion has decreased to 25% of preischemic control values. So, in contrast to the viable subepicardium, here the adverse effects have occurred primarily during the period of ischemia. During the subsequent hours of reperfusion, there is a trend toward a further decrease of PRH flow ($p=NS$). At 2½ and 24 hours of reperfusion, a transient ischemic stimulation elicits very little hyperemic response (flows increase slightly over the basal values), and the PRH flow does not even reach the level of basal flow in the corresponding circumflex area. Finally, there is no significant recovery of PRH flow in infarcted subendocardium after 1 week of reperfusion (22% of control values).

<table>
<thead>
<tr>
<th>Duration of reperfusion</th>
<th>$n$</th>
<th>Heart rate (beats/min)</th>
<th>Mean aortic pressure (mm Hg)</th>
<th>LVEDP (mm Hg)</th>
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<tbody>
<tr>
<td>2½ hr</td>
<td>18</td>
<td>117±29</td>
<td>93±15</td>
<td>6.3±2.7</td>
</tr>
<tr>
<td>24 hr</td>
<td>5</td>
<td>161±33</td>
<td>81±19</td>
<td>4.8±1.0</td>
</tr>
<tr>
<td>1 wk</td>
<td>15</td>
<td>148±32</td>
<td>102±16</td>
<td>2.2±1.3</td>
</tr>
</tbody>
</table>

Values are mean±SD. LVEDP, left ventricular end-diastolic pressure.

Hemodynamics during reperfusion. It is appropriate to consider briefly the most important hemodynamic parameters at these different times during reperfusion (Table 3). After 24 hours of reperfusion, the animals are not in a very stable condition: they often have severe arrhythmias, accounting for the high heart rate, and their blood pressure is usually rather low. Together with the relatively small number of observations at this time, these considerations call for some caution in the interpretation of the data on PRH flow at this time of reperfusion. The important point, however, is that hemodynamics after 2½ hours and after 1 week of reperfusion are comparable. Mean aortic pressure is on average 9 mm Hg higher after 1 week, and heart rate is 31 beats/min faster. The combination of these factors permits a safe exclusion of a major role of hemodynamic variables in the observed changes in PRH flow.

Intracoronary Adenosine Infusion. In six open-chest dogs of group I, instrumented as described in “Materials and Methods,” RMBF was measured during peak reactive hyperemia and during intracoronary adenosine infusion, both in preischemic control conditions and after 2½ hours of reperfusion. The results are shown in Figure 2. During intracoronary infusion of adenosine, mean aortic blood pressure fell slightly but not significantly. To correct for these differences in perfusion pressure during reactive hyperemia and intracoronary adenosine infusion, we also calculated coronary resistance (diastolic blood pressure divided by coronary flow).

In viable subepicardium, blood flows during intracoronary adenosine infusion in preischemic control conditions tended to be higher than PRH flows (456±153 and 414±184 ml/min/100 g, respectively; $p=NS$), and coronary resistance was lower during intracoronary adenosine infusion than during peak reactive hyperemia (0.16±0.08 versus 0.21±0.07; $p<0.05$). This confirms previous evidence that coronary vasodilatation during reactive hyperemia is not maximal.14

After 2½ hours of reperfusion, PRH flow dropped expectedly to 200±71 ml/min/100 g, or 48% of control values ($p<0.02$ versus control). The adenosine flow at this time, however, remained unchanged (463±150 ml/min/100 g). Comparison of coronary resistance after 2½ hours of reperfusion with preischemic control values gave similar results: coronary resistance during reactive hyperemia doubled (0.21±0.07 to 0.42±0.17; $p<0.02$), whereas it re-
mained unchanged during intracoronary adenosine infusion (0.16±0.08 versus 0.17±0.08).

Only three of the six dogs had homogeneously infarcted subendocardial tissue blocks, which makes statistical analysis of these data not very meaningful. It is evident from Figure 2, however, that in these three animals, the very distinct decrease in PRH flow after 2½ hours of reperfusion is matched by a similar decrease in flows during intracoronary adenosine infusion (430±174 to 76±10 and 502±260 to 102±35 ml/min/100 g, respectively). The rises in coronary resistance in infarcted subendocardium during both reactive hyperemia and intracoronary adenosine infusion are roughly comparable (0.19±0.03 to 1.01±0.17 and 0.15±0.05 to 0.80±0.24, respectively) and even statistically significant (p<0.02 and p>0.05) despite the small number of observations. Thus, although the hyperemic response to a transient ischemic stimulus is markedly blunted in viable reperfused myocardium, the coronary vasodilatory capacity as such is preserved, because exogenous adenosine administration restores maximal flows. On the other hand, in irreversibly damaged tissue, adenosine infusion cannot relieve the severe reflow impairment.

**Ultrastructural Analysis of Reperfused Myocardium**

Tissue samples from viable and infarcted portions of the LAD area (as indicated by TTC staining) and samples from the control circumflex area were processed as described above. At least three ultrathin sections from different sites were analyzed per experiment. These sections were scanned systematically for mitochondrial, nuclear, and myofibrillar changes; for the integrity of the cell membrane; and for the presence of intracellular and/or extracellular edema. Vascular structures (i.e., almost exclusively capillaries) were analyzed separately in a number of experiments. A normal appearance of the control myocardium from the circumflex bed should testify to the appropriate processing of the tissue samples. In four dogs (2½ hours, n=1; 24 hours, n=2; and 1 week, n=1, of reperfusion) in which control myocardium was analyzed, the ultrastructure looked normal. In two of these animals, confluent mitochondrial cristae were present, suggesting minimal signs of tissue injury. The capillary lumen was always patent, and endothelial cells were intact.

The characteristic signs of irreversible myocardial damage include the presence of amorphous dense bodies in the mitochondrial matrix, pyknosis of the cell nucleus, the development of contraction bands in the myofilaments, and rupture of the cell membrane. Hallmarks of irreversible injury were absent in TTC-stained tissue of 16 dogs (2½ hours, n=6; 24 hours, n=2; and 1 week, n=8, of reperfusion), but they were invariably present in TTC-unstained tissue of five dogs (2½ hours, n=4; and 1 week, n=1, or reperfusion).

As for the relation of ultrastructural changes to reflow impairment, the absence of edema in viable tissue samples is remarkable. Except for a small pericapillary rim, which is a known artifact after sequential perfusion staining and fixation, extracellular and intracellular edema were completely absent in 15 of 16 and 13 of 16 animals, respectively. On the other hand, edema was an almost universal feature of infarcted tissue samples. Representative electron micrographs are shown in Figures 3 and 4. In this context it should be pointed out that the perfusion media used in the postmortem staining and fixation
FIGURE 3. Ultrastructure of viable subepicardium after 2½ hours of reperfusion. Panel A: Aspect of the contractile system and the mitochondria is essentially normal. The nucleus shows some degree of chromatin clumping but is not pyknotic. Note the complete absence of edema (×24,820). Panel B: Transverse section of a capillary vessel shows a patent lumen; endothelium and intercellular junctions are intact (×15,470).

Vascular integrity always was preserved in viable tissue after 2½ hours (n=5), 24 hours (n=2), and 1 week (n=4) of reperfusion. The capillary lumen invariably was patent and endothelial cells were intact, although in about half of the experiments, some degree of nuclear chromatin clumping and cytoplasmic changes was observed. In infarcted tissue (2½ hours, n=10; 1 week, n=1, of reperfusion), the microvascular damage was variable. Occasionally, the capillaries had a normal appearance. In most experiments, a variable portion of the capillaries was
FIGURE 4. Ultrastructure of infarcted subendocardium after 2½ hours of reperfusion. Panel A: Myofilaments are relaxed and partially distorted; the mitochondrial matrix is cleared and there is fragmentation of the cristae; numerous intramitochondrial amorphous dense bodies are present; the cell membrane is ruptured; edema is evident (x24,820). Panel B: A capillary vessel typically is packed with red blood cells. The capillary lumen also contains some cellular debris (x15,470).

Discussion

The perfusion bed of an acutely occluded coronary artery is a very inhomogeneous entity. For a variable period of time, it contains all degrees of tissue injury, varying from completely normal myocardial cells to necrosis; the capillary network may be intact or obstructed to a variable degree. This inhomogeneity results from inhomogeneities in collateral blood supply and oxygen demand. A priori, one could therefore expect the reflow pattern in this tissue to be inhomogeneous too. Previous studies documenting...
the no-reflow phenomenon have failed to highlight its complexity and inhomogeneity. Several mechanisms have been invoked to explain the no-reflow phenomenon; their common feature is that they all assume some form of mechanical obstruction to blood flow, a “shut down” of capillaries, caused by damage to the vascular endothelium, edema, plugging by packed red blood cells or neutrophils, and compression by cardiac contraction. Finally, the term no-reflow largely has been restricted to blood flow in basal conditions, without regard to the coronary flow reserve of the myocardium under study. The data presented here provide evidence that after coronary reperfusion, different mechanisms are responsible for the observed flow patterns in viable postischemic heart muscle as opposed to irreversibly damaged myocardium.

In viable tissue, reflow in basal conditions is similar to the preischemic flow at all times after reperfusion (2½ hours, 24 hours, and 1 week). Because changes in hemodynamics between the preischemic control condition and the reperfusion phase may influence myocardial blood flow, it may be more appropriate to compare flow in the postischemic LAD area with flow in the circumflex bed at the same time point. Thus, after 2½ hours of reperfusion, we found a slightly lower basal reflow in viable postischemic tissue. The presence of a zone of “low reflow” in the viable subepicardium overlying an infarct also has been shown by Kloner and Alker. In that study, the authors found that no-reflow was associated with irreversibly injured myocardium (TTC-negative), whereas low reflow (mild reperfusion abnormality) occurred in viable TTC-positive tissue. Because reperfused myocardium is stunned and thereby has lower metabolic requirements, the reduced reflow in basal conditions suggests that autoregulation is intact, that is, that the resistance vessels are not “stunned.” An alternative explanation would be that the difference between circumflex and LAD basal flows at 2½ hours of reperfusion indicates increased circumflex flow, caused by hyperkinesis of the circumflex territory, to overcome the hypokinesis of the postischemic zone. However, after 24 hours, when stunning of the postischemic zone probably is still present, the basal circumflex and LAD flows are similar.

When PRH flow is used as a measure of coronary flow reserve, two points need to be addressed. First, from previous evidence as well as from our own data, it appears that PRH flow to some extent underestimates coronary flow reserve as measured after intracoronary adenosine administration. Thus, PRH flow is only a useful approximation of true flow reserve. Second, we assume that a brief coronary occlusion causes the same ischemic insult in viable postischemic myocardium versus control myocardium. Although we are not aware of data to the contrary, this point is hard to verify. In three dogs not included in this study, extension of the coronary occlusion up to 180 seconds did not further enhance the hyperemic response in postischemic tissue.

With these caveats in mind, our results indicate that coronary flow reserve in viable postischemic tissue is preserved at the onset of reperfusion. It decreases during the initial phase of reperfusion, remains depressed for at least 24 hours, and has completely recovered after 1 week of reperfusion. The ultrastructure of this viable postischemic myocardium is intact at the different times of reperfusion. Thus, the evolution of coronary flow reserve during reperfusion must be explained either by a reduced availability of vasodilating mediator substances or by the inability of the vasculature to vasodilate maximally. The results with intracoronary adenosine infusion rule out the latter and indicate that the vasculature in this tissue remains not only structurally but also functionally intact after reperfusion. The transient decrease in coronary flow reserve occurs probably because vasoactive metabolites responsible for the autoregulation of coronary flow (primarily adenosine), which are formed by the rapid breakdown of ATP during ischemia, are washed out from the postischemic bed on reperfusion and thereafter are insufficiently available for a period of time because of depletion of their precursor pool of nucleotides, which has a slow rate of recovery. In a recent study, Reimer et al demonstrated that even short periods of ischemia followed by reperfusion are associated with a considerable loss of adenine nucleotides.

Undoubtedly, this hypothetical scheme oversimplifies a complex interplay of factors that regulates blood flow in viable postischemic myocardium. For example, the amount of adenosine needed for coronary vasodilation is probably small, and although tissue levels of ATP after 2½ hours of reperfusion are low, they are by no means negligible (data not shown). Therefore, one has to call on the concept of compartmentalization of ATP stores to explain the observed flow patterns on the basis of insufficient availability of adenosine. The question of whether this compartmentalization is intracellular, within the cardiac myocyte, or intercellular, between different cell types (myocytes, pericytes, vascular endothelium), further complicates the matter. An alternative explanation for the observed flow patterns would be a decrease in sensitivity and/or number of vascular adenosine receptors in postischemic viable tissue. More experimental work is needed to corroborate or refute these conjectures.

In irreversibly damaged myocardium, the severity and permanence of the basal reflow impairment persist do not permit any conclusion about mechanisms of no-reflow. Indeed, dead myocytes do not require oxygen, so there is no reason to suspect that basal reflow should ever return to control levels in infarcted tissue. However, the ultrastructural findings (edema, capillary plugging, and vascular damage) and the fact that intracoronary adenosine effects only a very minor relief of the reflow impairment are
consistent with the current idea that no-reflow largely is a mechanical phenomenon.

In conclusion, it would be preferable if the term no-reflow were restricted to its original definition, that is, basal reflow impairment due to physical restriction of capillary flow. This phenomenon is limited to irreversibly damaged myocardium. In viable postischemic heart muscle, the vasculature remains functionally and structurally intact; the transient decrease of flow reserve in this tissue possibly is due to a reduced production of the chemical mediator adenosine.

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