Ischemia-Reperfusion Impairs Endothelium-Dependent Relaxation of Coronary Microvessels but Does Not Affect Large Arteries

James E. Quillen, MD, Frank W. Sellke, MD, Leonard A. Brooks, and David G. Harrison, MD

We examined the effects of ischemia with and without reperfusion on endothelium-dependent and -independent vascular relaxation in both conduit and resistance coronary arteries. Studies were performed on dogs under control conditions (n = 13) or after 1 hour of circumflex coronary artery occlusion with (n = 10) or without (n = 8) 1 hour of reperfusion. Rings of obtuse marginal branches of the left circumflex coronary artery (conduit arteries) were studied in organ chambers. Coronary microvessels (110–220-μm diameter) were studied in a pressurized state with an in vitro microvessel imaging apparatus. Relaxation was evaluated after preconstriction with prostaglandin F₂α and U46619 (a thromboxane A₂ analogue) in conduit and resistance vessels, respectively. Conduit vessel function was not altered by ischemia with or without reperfusion. Endothelium-dependent microvascular relaxation was depressed in response to acetylcholine, ADP, and calcium ionophore A23187 after ischemia with reperfusion compared with control relaxation (ED₅₀ as -log[M]: 6.0±0.2 [p<0.05], 5.1±0.4 [p<0.05], and 5.8±0.1 versus 6.8±0.2, 6.8±0.2, and 6.6±0.2, respectively). Ischemia without reperfusion modestly altered microvascular endothelium-dependent relaxation. Microvascular relaxation to nitroglycerin was not altered by ischemia with reperfusion. We conclude that 1) endothelium-dependent relaxation in large epicardial coronary arteries is relatively refractory to ischemia with or without reperfusion, 2) ischemia alone produces mild alterations of coronary microvascular reactivity, 3) ischemia followed by reperfusion produces a marked and selective impairment of endothelium-dependent responses in the coronary microcirculation. (Circulation 1990;82:586–594)

Control of coronary arterial tone may be influenced by circulating and locally released vasoactive compounds. The response to many of these vasoactive compounds is modulated by the endothelium.

Alterations in large vessel vascular tone have been reported after brief episodes of ischemia. Ku demonstrated altered vascular responses to thrombin in the left anterior descending coronary artery after ischemia followed by reperfusion, and VanBenthuyisen et al subsequently extended these observations in a similar model by demonstrating blunted endothelium-dependent relaxation to acetylcholine.

The effect of ischemia and reperfusion on the coronary microcirculation has been less well defined. Mehta et al reported impaired coronary flow reserve to acetylcholine and bradykinin after ischemia and reperfusion. Evaluation of the microcirculation in vivo is complicated by myocardial compressive forces, autoregulation, and metabolic influences. In addition, effects of ischemia on myocardial perfusion in vivo may at least in part be due to vascular occlusion secondary to white cell adhesion and platelet aggregation.

Thus, the direct effect of ischemia with or without reperfusion on coronary microvessels is unclear and to date has not been evaluated by an in vitro method. Because microvessels play a central role in regulation of myocardial perfusion, their function after ischemia...
and reperfusion may be particularly important. The goal of the present study was to evaluate the effects of ischemia with or without reperfusion on both the coronary conduit and resistance vessels in vitro.

Methods

Experimental Preparation

Mongrel dogs weighing between 18 and 24 kg were used. Anesthesia was obtained with intravenously administered thiopental (30 mg/kg) and pentobarbital (30 mg/kg). In the control group (n=13), a left thoracotomy was performed. The heart was immediately removed and placed in a cold Krebs' buffer of the following composition (mM): 118.3 NaCl, 4.7 KCl, 2.5 CaCl₂, 1.2 MgSO₄, 1.2 KH₂PO₄, 25 NaHCO₃, 11.1 glucose, and adjustment to a pH of 7.4. In the ischemia groups, the dogs were intubated and ventilated, and arterial blood gases were measured and adjusted by ventilatory rates, volumes, and inspired PO₂ to maintain PaO₂ greater than 100 mm Hg, pH greater than 7.35 and less than 7.45, and PaCO₂ greater than 30 and less than 40 mm Hg. The right femoral artery and vein were isolated and cannulated for vascular access. The arterial line was advanced to the thoracic aorta for central aortic blood pressure measurements. A left thoracotomy was performed in the fourth intercostal space followed by placement of a pericardial cradle and heparinization (500 units/kg). The left carotid artery was isolated and cannulated to provide the source for a carotid to left circumflex coronary artery shunt. The left circumflex coronary artery was isolated by blunt dissection approximately 1 cm proximal to the first obtuse marginal branch. After thorough flushing of the shunt, the distal end was placed in the left circumflex coronary artery, and the proximal left circumflex coronary artery immediately proximal to this site was ligated. A pressure port was available at the distal end of the shunt to allow measurement of distal left circumflex coronary pressure.

Experimental Protocols

Ischemia without reperfusion. Eight dogs were subjected to 1 hour of ischemia without reperfusion. After the experimental preparation, the shunt was clamped for 1 hour. In the case of hypertension secondary to the anesthesia, the animals were bled to maintain mean aortic blood pressure of approximately 100 mm Hg. The distal left circumflex coronary pressure was monitored (which reflected collateral flow), and the myocardium became noticeably cyanotic and dyskinetic. One dog in the ischemia without reperfusion group developed ventricular fibrillation, which was treated with lidocaine and direct current cardioversion. At the end of the ischemia period, the heart was removed and immediately placed in cold Krebs' buffer.

Ischemia with reperfusion. Eleven dogs were subjected to 1 hour of ischemia followed by 1 hour of reperfusion. The ischemia period was the same as the above group. At the end of the ischemia period, the shunt occlusion was slowly released during 10-15 seconds with return of distal left circumflex coronary pressure to preocclusion levels. Four dogs in the ischemia with reperfusion group developed ventricular fibrillation. One dog had refractory ventricular fibrillation, and the experimental protocol was not completed; the other three were successfully treated with lidocaine and direct current cardioversion. At the end of 1 hour of reperfusion, the heart was removed and immediately placed in cold Krebs' buffer.

Three dogs were subjectd to 3 hours of ischemia followed by 1 hour of reperfusion. One dog in this group developed ventricular fibrillation that was treated as above. With the exception of a 3-hour ischemia period, there was no change in the protocol in this group.

Conduit coronary artery studies. The epicardial obtuse marginal branch of the left circumflex coronary artery was dissected free and cut into 3-4-mm rings. The rings were 1-3 mm in diameter. Care was taken to avoid damage to the vascular endothelium. Six to eight ring segments were studied from each animal. These segments were studied in a 25-ml organ chamber containing oxygenated (95% O₂-5% CO₂) Krebs' buffer maintained at 37°C. Each ring was suspended on two steel clips passed through the lumen. One clip was anchored inside the organ chamber, and the other end was connected to a force transducer with a silk suture. Changes in isometric tension were processed through a bridge amplifier and recorded continuously on a multichannel oscillographic recorder. Each ring was exposed to 100 mM KCl at different resting tensions to determine optimum tension. Studies were subsequently performed at this optimal resting tension. After at least 1 hour of equilibration, some vascular rings were preconstricted with prostaglandin F₂α to attain a tension level between 30% and 70% of that attained with 100 mM KCl, whereas the others were studied without preconstriction. One or two dose-response curves were obtained for each vascular ring. Increasing concentrations of adenosine, calcium ionophore A23187, nitroglycerin, bradykinin, acetylcholine, or ADP were studied after preconstriction, and potassium chloride was studied without preconstriction. Studies were not performed on vessels after exposure to A23187.

Coronary microvessel studies. Microvessels from the obtuse marginal branches of the left circumflex coronary artery were carefully dissected with a x40 power dissecting microscope. Microvessels had internal diameters of 110-220 μm and were 300-500 μm in length. These were placed in an isolated Plexiglas organ chamber, cannulated with dual glass micropipettes measuring 50-100 μm in diameter, and secured with 10-0 nylon monofilament suture. Oxygenated Krebs' buffer warmed to 37°C was continuously circulated through the organ chamber. The vessels were pressurized to 20 mm Hg in a no-flow
state by a manometer filled with Krebs' buffer. In previous experiments, optimal distending pressure for contraction to potassium chloride was found to occur at 20 mm Hg. With a split screen microscope connected to a video camera, the vessel image was projected on a television monitor. A video electronic dimension analyzer was used to measure luminal diameter, and a pressure transducer measured distending pressures. Measurements were recorded with a strip chart recorder. After at least 1 hour of equilibration, the microvessels were preconstricted with U46619 (a stable thromboxane A2 analogue) by 15–60% of the resting baseline diameter. One to four dose-response curves were obtained for each vessel. Vessels were washed three times with Krebs' buffer and allowed to equilibrate 15 minutes between interventions. Increasing concentrations of acetylcholine, A23187, ADP, and nitroglycerin were studied after preconstriction. Studies were not performed on vessels after an exposure to A23187.

**Drugs**

Acetylcholine, ADP, adenosine, A23187, and bradykinin were obtained from Sigma Chemical Co. (St. Louis, Mo.). Nitroglycerin was obtained from DuPont Pharmaceuticals (Wilmington, Del.). U46619 and prostaglandin F2α were obtained from Upjohn (Kalamazoo, Mich.). All drugs were dissolved in water with the exception of A23187, which was initially dissolved in dimethyl sulfoxide. All drug solutions were made fresh for each experiment with the exception of U46619, which was stored at −20°C as a stock solution.

**Data Analysis**

Relaxation responses of microvessels and conduit arteries were expressed as percent relaxation from their preconstricted diameters and tensions, respectively. Constricting responses of conduit vessels were expressed as percent constriction as a percent of the maximal potassium chloride constriction. ED50 values were expressed as the log of the molar concentration. ED50 values were not calculated when a plateau at maximal relaxation was not attained with the highest dose of the specific agent being studied. Mean responses at each drug dose and their respective ED50 values were compared by analysis of variance. Whenever significance was indicated, Scheffé's F test for multiple comparison was used to compare between groups. Values were expressed as mean ± SEM. Significance was assumed when p was less than 0.05.

**Results**

Baseline mean aortic pressure was 119±4 and 117±5 mm Hg, and the heart rate was 172±5 and 175±6 beats/min in the ischemia and ischemia with reperfusion groups, respectively (p=NS). During ischemia, the aortic pressure decreased to 99±5 and 101±4 mm Hg, and heart rate decreased to 156±7 and 149±6 beats/min in the ischemia and ischemia with reperfusion groups, respectively (p=NS). Distal left circumflex coronary artery pressure during ischemia averaged 17±1.0 and 16±0.5 mm Hg in the ischemia and ischemia with reperfusion groups, respectively (p=NS). During reperfusion, distal left circumflex pressure returned to 95±3 mm Hg, and aortic pressure was 103±5 mm Hg.

**Vessel Characteristics**

Vascular ring resting tension for the control, ischemia, and ischemia with reperfusion groups was 3.7±0.2 (n=13), 3.0±0.1 (n=8), and 2.9±0.1 g (n=10), respectively. Preconstricted tension for the control, ischemia, and ischemia with reperfusion groups was 3.6±0.3, 2.8±0.4, and 3.1±0.3 g, respectively.

Microvessels from the obtuse marginals were between 110 and 220 μm in diameter, averaging 183±9 (n=7), 169±10 (n=6), and 167±6 μm (n=7) in the control, ischemia, and ischemia with reperfusion groups, respectively. Preconstriction diameters for the control, ischemia, and ischemia with reperfusion groups were 116±9, 115±11, and 109±7 μm, respectively (p=NS).

**Endothelium-Dependent Responses**

Endothelium-dependent conduit artery relaxations to acetylcholine, ADP, bradykinin, and A23187 were not altered by 1 hour of ischemia with or without reperfusion (Figures 1–3 and Table 1). Despite prolongation of ischemia to 3 hours followed by reperfusion, no alterations of endothelium-dependent relaxations were observed in conduit coronary arteries.

In control microvessels, acetylcholine produced 93% relaxation with an ED50 of −6.8. This was mildly, but not significantly, blunted by ischemia alone; however, ischemia followed by reperfusion substantially reduced relaxation to acetylcholine (Figure 1 and Table 2).

In control microvessels, ADP produced relaxations of 96% with an ED50 of −6.8. This response to ADP was not markedly altered with ischemia. In contrast, ischemia with reperfusion markedly decreased relaxations to ADP and shifted the dose-response curve rightward (Figure 2 and Table 2).

A23187 produced relaxations of 90% in control microvessels with an ED50 of −6.6. This response was only slightly altered by ischemia. Ischemia followed by reperfusion severely impaired relaxations to A23187 (Figure 3 and Table 2).

To determine whether alterations of endothelium-dependent vascular relaxation in the coronary microvessels of dogs with ischemia followed by reperfusion were due to lidocaine or direct current cardioversion, a separate analysis excluding these dogs was performed. Mean data for peak relaxations and ED50 values are presented in Table 3. There were no differences between endothelium-dependent relaxation regardless of this subgrouping with the exception of the peak relaxation to A23187. A23187 caused vasoconstriction in one vessel from a dog that had undergone defibrillation. Thus, exclusion of this animal by subgroup analysis increased the average peak
TABLE 1. ED50 Values and Peak Relaxations of Coronary Conduit Arteries in All Dogs

<table>
<thead>
<tr>
<th>Group</th>
<th>Acetylcholine</th>
<th>ADP</th>
<th>Bradykinin</th>
<th>A23187</th>
<th>Nitroglycerin</th>
<th>Adenosine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
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<tr>
<td>n</td>
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<td>13</td>
<td>7</td>
<td>8</td>
<td>10</td>
<td>13</td>
</tr>
<tr>
<td>ED50</td>
<td>-7.14±0.12</td>
<td>-630±0.14</td>
<td>-8.64±0.06</td>
<td>-6.44±0.15</td>
<td>-7.04±0.08</td>
<td>-5.40±0.19</td>
</tr>
<tr>
<td>Peak response (%)</td>
<td>100</td>
<td>98±1.8</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>81±5.1</td>
</tr>
<tr>
<td>Ischemia (1 hr)</td>
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<td>n</td>
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<td>8</td>
<td>7</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>ED50</td>
<td>-7.00±0.20</td>
<td>-6.46±0.18</td>
<td>-8.77±0.16</td>
<td>-6.29±0.16</td>
<td>-7.02±0.05</td>
<td>-5.45±0.10</td>
</tr>
<tr>
<td>Peak response (%)</td>
<td>97±2.1</td>
<td>99±1.1</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>87±4.5</td>
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<tr>
<td>Ischemia (1 hr) + reperfusion</td>
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<tr>
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<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>ED50</td>
<td>-6.93±0.15</td>
<td>-5.99±0.18</td>
<td>-8.57±0.12</td>
<td>-6.17±0.06</td>
<td>-6.98±0.08</td>
<td>-5.19±0.20</td>
</tr>
<tr>
<td>Peak response (%)</td>
<td>95±2.7</td>
<td>89±6.3</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>75±4.0</td>
</tr>
<tr>
<td>Ischemia (3 hr) + reperfusion</td>
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<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>ED50</td>
<td>-6.71±0.11</td>
<td>-5.99±0.14</td>
<td>-8.42±0.19</td>
<td>-6.08±0.08</td>
<td>-6.99±0.03</td>
<td>-5.41±0.13</td>
</tr>
<tr>
<td>Peak response (%)</td>
<td>90±8.6</td>
<td>99±5.4</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>70±14.2</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Peak responses are to 100 μm of the specified agent. ED50 expressed as log molar concentration.

relaxation by A23187 from 65±16% to 82±6%. Excluding this dog had no effect on the ED50 of A23187 in this group.

Endothelium-Independent Responses

Endothelium-independent conduit artery relaxation to nitroglycerin and adenosine was not altered by ischemia with or without reperfusion (Figure 4 and Table 2). Muscle contraction of conduit coronary arteries to potassium chloride was unaltered by ischemia with or without reperfusion in conduit coronary arteries, and peak constriction was 7.4±1.1, 8.4±0.6, and 7.7±0.8 g in the control, ischemia, and ischemia with reperfusion groups, respectively (p=NS). Subgroup analysis of endothelium-independent relaxation in dogs not requiring defibrillation (Table 3) was not different from above.

Figure 1. Plots of average relaxations to acetylcholine from baseline of vessels for control, 1 hour ischemia, and 1 hour ischemia with reperfusion. Panel A: Relaxations of conduit coronary arteries. Panel B: Relaxations of coronary microvessels. Values are mean±SEM.
TABLE 2. ED$_{50}$ Values and Peak Relaxations of Coronary Microvessels in All Dogs

<table>
<thead>
<tr>
<th>Group</th>
<th>Acetylcholine</th>
<th>ADP</th>
<th>A23187</th>
<th>Nitroglycerin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
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<tr>
<td>n</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>ED$_{50}$</td>
<td>-6.80±0.24</td>
<td>-6.79±0.07</td>
<td>-6.60±0.16</td>
<td>-5.42±0.08</td>
</tr>
<tr>
<td>Peak response</td>
<td>93±4.8%</td>
<td>96±1.8%</td>
<td>90±2.7%</td>
<td>71±3.2%</td>
</tr>
<tr>
<td>Ischemia (1 hr)</td>
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<tr>
<td>n</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>ED$_{50}$</td>
<td>-6.35±0.17</td>
<td>-6.53±0.20</td>
<td>-6.39±0.16</td>
<td>-5.74±0.18</td>
</tr>
<tr>
<td>Peak response</td>
<td>88±6.8%</td>
<td>89±6.1%</td>
<td>78±9.1%</td>
<td>77±4.8%</td>
</tr>
<tr>
<td>Ischemia (1 hr) +</td>
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<tr>
<td>reperfusion</td>
<td>7</td>
<td>6</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>ED$_{50}$</td>
<td>-5.96±0.17*</td>
<td>-5.10±0.37†</td>
<td>-5.77±0.09</td>
<td>-5.67±0.12</td>
</tr>
<tr>
<td>Peak response</td>
<td>68±11.2%</td>
<td>54±9.8%†</td>
<td>65±16.3%</td>
<td>79±6.7%</td>
</tr>
</tbody>
</table>

Values are mean±SEM.
Peak responses are to 10 $\mu$m of the specified agent. ED$_{50}$ expressed as log molar concentration.
*p<0.05 compared with control; †p<0.05 compared with ischemia.

Discussion

In this study, we found that coronary conduit vessels are relatively tolerant to myocardial ischemia with or without reperfusion. Neither endothelium-dependent nor -independent responses were altered with ischemia periods as long as 3 hours followed by reperfusion. In contrast, endothelium-dependent relaxations of coronary microvessels were markedly impaired after ischemia with reperfusion. The blunted responses were demonstrated with receptor-mediated and nonreceptor-mediated agents. Endothelium-independent relaxations to nitroglycerin were unaltered after ischemia with or without reperfusion in the microcirculation. Ischemia without reperfusion did not alter vascular responses in the large conduit arteries and only mildly altered relaxation in coronary microvessels.

Mechanism of Altered Microvascular Endothelial Relaxation

In this study, ischemia without reperfusion only mildly altered endothelium-mediated relaxations, even though these vessels were exposed to an oxygenated Krebs' buffer after the ischemic insult. In contrast, the ischemia with reperfusion group had markedly altered endothelium-dependent relax-

![Figure 2](http://circ.ahajournals.org/)

**Figure 2.** Plots of average relaxations to ADP from baseline of vessels for control, 1 hour ischemia, and 1 hour ischemia with reperfusion. Panel A: Relaxations of conduit coronary arteries. Panel B: Relaxations of coronary microvessels. Values are mean±SEM.
The observation suggests that the impaired endothelial responses are caused by blood products or myocar
dial metabolites that are released during the reperfu
sion period rather than by exposure to oxygen per
se. This differentiation between ischemia and isch-
emia with reperfusion was made possible by the use
of an in vitro approach to the study of coronary
microvessels.

Speculation about the mechanism of the microvas-
cular endothelial dysfunction after ischemia with
reperfusion must include consideration of the role of
oxygen free radicals in endothelial injury. Lamb et

al\textsuperscript{10} reported that electrical field stimulation to rat
tail vessels in vitro inhibited endothelium-dependent
relaxation to acetylcholine and A23187, which could
be prevented with the addition of antioxidants in the
organ chamber buffer. Stewart et al\textsuperscript{11} reported
blunted endothelium-dependent relaxation to acetyl-
choline and serotonin after free radical generation by
electrolysis in vivo rabbit hearts. Zweier et al\textsuperscript{12}
reported that endothelial cells subjected to anoxia and
reoxygenation generated large amounts of oxygen
free radicals. Together, these studies demonstrate
that oxygen free radicals can alter endotheli-
dum-dependent vascular relaxation. Oxygen free

B.

**Figure 3.** Plots of average relaxations to A23187 from baseline of vessels for control, 1 hour ischemia, and 1 hour ischemia with
reperfusion. Panel A: Relaxations of conduit coronary arteries. Panel B: Relaxations of coronary microvessels. Values are
mean±SEM.

**Table 3.** ED\textsubscript{50} Values and Peak Relaxations of Coronary Microvessels Excluding Dogs With Ventricular Fibrillation

<table>
<thead>
<tr>
<th>Group</th>
<th>Acetylcholine</th>
<th>ADP</th>
<th>A23187</th>
<th>Nitroglycerin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
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<td>n</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>ED\textsubscript{50}</td>
<td>-6.80±0.24</td>
<td>-6.79±0.07</td>
<td>-6.60±0.16</td>
<td>-5.42±0.08</td>
</tr>
<tr>
<td>Peak response</td>
<td>93±4.8%</td>
<td>96±1.8%</td>
<td>90±2.7%</td>
<td>71±3.2%</td>
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<tr>
<td><strong>Ischemia (1 hr)</strong></td>
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<td>n</td>
<td>5</td>
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</tr>
<tr>
<td>ED\textsubscript{50}</td>
<td>-6.35±0.17</td>
<td>-6.60±0.26</td>
<td>-6.56±0.08</td>
<td>-5.74±0.18</td>
</tr>
<tr>
<td>Peak response</td>
<td>95±3.8%</td>
<td>88±8.2%</td>
<td>83±10.1%</td>
<td>77±4.8%</td>
</tr>
<tr>
<td><strong>Ischemia (1 hr) +</strong></td>
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<td><strong>reperfusion</strong></td>
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<td>n</td>
<td>5</td>
<td>5</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>ED\textsubscript{50}</td>
<td>-5.86±0.13*</td>
<td>-5.26±0.41*†</td>
<td>-5.77±0.09</td>
<td>-5.65±0.10</td>
</tr>
<tr>
<td>Peak response</td>
<td>75±5.2%</td>
<td>57±11.2%*†</td>
<td>82±5.8%</td>
<td>77±8.1%</td>
</tr>
</tbody>
</table>

Values are mean±SEM.
Peak responses are to 10 μm of the specified agent. ED\textsubscript{50} expressed as log molar concentration.
*p<0.05 compared with control; †p<0.05 compared with ischemia.
radicals are generated by the myocardium after ischemia and could be responsible for the blunted relaxations that we observed after ischemia and reperfusion. An additional source of oxygen free radicals is endothelial production in which xanthine dehydrogenase may be converted to xanthine oxidase under hypoxic conditions. The release of adenosine from ischemic myocytes may be the precursor to hypoxanthine used by endothelial cells. The endothelial cells in the microcirculation may have greater access to the released pool of adenosine than larger vessels secondary to their proximity to the myocytes and, thus, may generate greater amounts of oxygen free radicals, leading to selective microvascular endothelial dysfunction.

Oxygen free radicals potentially may disrupt endothelium-dependent relaxation without endothelial cell injury. The endothelium-dependent relaxing factor may be degraded by superoxide anion and protected by oxygen free radical scavengers. One possible explanation for the altered relaxations seen in coronary microvessels after ischemia with reperfusion may be oxygen free radical degradation of the cellular endothelium-dependent relaxing factor. It is now known whether the relaxing factor is produced at the time of its release or whether there is a pool or reservoir where it is stored until its release. Ischemia with reperfusion may produce a prolonged depletion of endothelium-dependent relaxing factor, of both potentially stored and nonstored sources, secondary to its degradation by the generated oxygen free radicals.

Ischemia followed by reperfusion may alter plasma membrane fluidity. This change in fluidity may alter interactions of membrane receptors and second messenger systems. This may in part explain altered endothelium-dependent responses but cannot account for the entire defect observed. Relaxations to A23187, which act through a nonreceptor-mediated mechanism, were also abnormal. Changes in plasma membrane fluidity could, however, depress the release of endothelium-dependent relaxing factor from endothelial cells.

Anatomic endothelial injury from ischemia with reperfusion has been reported. Transmission and scanning electron microscopy of fixed perfused myocardium was performed on one representative dog after ischemia and on a second dog after ischemia with reperfusion (Figure 5). Practically no evidence of endothelial cell swelling or disruption was present. Furthermore, no white cell or platelet deposition was observed.

The blunted endothelium-dependent responses observed in the microvessels might have been secondary to a constricting factor produced in response to ischemia with reperfusion. This seems unlikely, however, because the basal release of a constrictor would impair relaxations to nitroglycerin. The receptor-mediated release of an endothelium-derived constricting factor cannot be excluded.

Endothelium-dependent relaxations require the activation of guanylate cyclase. This enzyme functions within a narrow range of redox potentials that may be altered by ischemia and reperfusion. This, however, is unlikely to explain the altered endothelium-dependent vascular responses. Relaxations to nitroglycerin, which also produces vascular relaxation by the activation of guanylate cyclase, were not altered.

Altered endothelial function after reperfusion may also be due to sudden changes in perfusion pressure. Ischemia causes profound dilation of the resistance vasculature. On reperfusion, the microvasculature is exposed to higher-than-normal perfusion pressures.
that may cause endothelial injury to the microvasculature. Acute hypertension has been shown to produce endothelial damage in the cerebral and coronary circulations.\textsuperscript{22,23}

Comparison With Previous Studies

Vascular injury by ischemia with or without reperfusion has been examined in multiple experimental models; however, only two in vitro studies have evaluated the effects of ischemia and reperfusion on large coronary vessels, and no study to date has examined the effects of ischemia and reperfusion on coronary microvessels in vitro.

Ku\textsuperscript{2} and VanBenthuyzen et al\textsuperscript{3} reported that ischemia followed by reperfusion resulted in altered vascular responses to thrombin and acetylcholine in the left anterior descending coronary artery, respectively. In addition, VanBenthuyzen et al\textsuperscript{3} observed normal endothelium-independent relaxation to nitroprusside after ischemia and reperfusion.

In contrast to these earlier reports, we were unable to detect abnormal endothelium-dependent vascular relaxations to multiple agonists in large conduit coronary arteries even after 3 hours of ischemia. In contrast to prior studies, we studied obtuse marginal branches of the left circumflex coronary artery to avoid the confounding effects of endothelial damage within the left circumflex coronary artery produced by surgical manipulation. We also used prostaglandin F\textsubscript{2\alpha} as a preconstricting agent rather than potassium chloride so that the hyperpolarization effects of endothelium-dependent relaxing factor would be demonstrated.

Collateral blood flow may vary substantially in mongrel dogs.\textsuperscript{24} It is conceivable that endothelial function was preserved in animals with ischemia alone because of high collateral blood flows in this group. This seems unlikely, however, because distal coronary pressure, an estimate of collateral blood flow,\textsuperscript{25} was similar between groups and all pressures were comparable to prior studies of acute coronary occlusion.\textsuperscript{25}

In vivo studies by Mayhan et al\textsuperscript{26} demonstrated that 10 minutes of ischemia followed by reperfusion blunted cerebral microvascular relaxation to acetylcholine but not to adenosine. Mehta et al\textsuperscript{4} reported impaired coronary flow reserve after intracoronary administration of acetylcholine and bradykinin after 1 hour of ischemia followed by 1 hour of reperfusion; however, they did not evaluate an endothelium-independent agent. No previous in vitro study has evaluated coronary microvascular relaxation after ischemia with or without reperfusion. Our in vitro study is in agreement with these prior in vivo studies.

In the present experiments, we examined only epicardial coronary microvessels. Thus, the results cannot be extrapolated to the subendocardial microvessels. More pronounced abnormalities may

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure5}
\caption{Transmission electron micrographs ($\times$600) of coronary microvessels from one dog after 1 hour of coronary occlusion (approximately 130 m in diameter) and from one dog after 1 hour of coronary occlusion followed by 1 hour of reperfusion (approximately 180 m in diameter). Coronary vasculature was perfusion-fixed with gluteraldehyde at 80 mm Hg. Endothelial cell layer is intact, and leukocyte deposition is not apparent.}
\end{figure}
actually occur in endocardial microvessels, because ischemia is known to be more severe in the endocardium. Preliminary studies in our laboratory have demonstrated that porcine endocardial and epicardial microvessels responded similarly to endothelium-dependent relaxing agents. This suggests that the intrinsic properties of the selected vessels themselves would not have affected the vascular responses.

Clinical Implications

Ischemia followed by reperfusion occurs in several clinical situations. These include variant angina, unstable angina, myocardial infarction with either spontaneous or therapeutic recannulization, and after cardiopulmonary bypass surgery. Microvascular endothelial dysfunction after ischemia followed by reperfusion may alter neurohumoral regulation of myocardial perfusion and may be a predisposing factor to recurrent ischemia.

Endothelium-dependent relaxing factor has potent platelet antiaggregating properties. Endothelial cell injury secondary to ischemia with reperfusion may decrease the amount of endothelium-dependent relaxing factor released in the above clinical situations. Diminished release of endothelium-dependent relaxing factor may lead to microvascular platelet aggregation and contribute to a nonreflow type of injury.

The limited vascular dysfunction after ischemia without reperfusion suggests that reperfusion causes the majority of the endothelium-dependent dysfunction. This observation suggests that maintaining endothelial function may be possible by altering the composition of the reperfusate.

References


Key Words: ADP, myocardial ischemia, coronary vessels, microvessels, acetylcholine, nitroglycerin, bradykinin, endothelium
Ischemia-reperfusion impairs endothelium-dependent relaxation of coronary microvessels but does not affect large arteries.

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