Acute Impairment of Endothelium-Dependent Relaxations to Aggregating Platelets Following Reperfusion Injury in Canine Coronary Arteries

Paul J. Pearson, Hartzell V. Schaff, and Paul M. Vanhoutte

Experiments were designed to determine whether endothelial injury contributes to augmented coronary vascular tone seen during myocardial reperfusion. Canine left anterior descending coronary arteries were exposed to ischemia followed by reperfusion (60 minutes each). Rings (3–4 mm) of the reperfused artery and of normal left circumflex (control) coronary artery segments were prepared. Rings were suspended for isometric force measurement in organ chambers containing modified Krebs’ Ringer bicarbonate solution (37°C, 95% O2-5% CO2). Endothelium-independent contractions to KC and prostaglandin F2α were unaltered after reperfusion. Endothelium-dependent relaxations to nitric oxide, sodium nitroprusside, and isoproterenol were comparable in control and reperfused arteries. However, reperfused coronary arteries contracted with prostaglandin F2α lost the ability to express endothelium-dependent relaxations to aggregating platelets. Reperfused arterial rings also exhibited impaired endothelium-dependent relaxations to acetylcholine, the calcium ionophore A23187, and the platelet-derived compounds ADP and serotonin. Quiescent (noncontracted) reperfused arterial rings exhibited larger contractions than controls when exposed to aggregating platelets. In such quiescent rings, the endothelium-dependent increase in tension to hemoglobin was unaltered after reperfusion. Thus, coronary reperfusion impairs the normal endothelium-dependent relaxations to aggregating platelets and vasoactive drugs. This impairment of platelet-mediated coronary relaxation could help explain the increased vascular tone and tendency toward vasospasm commonly observed after reperfusion of the coronary arteries. (Circulation Research 1990;67:385–393)

Coronary vasospasm can occur after reperfusion of previously ischemic coronary arteries.1–3 The mechanism of this increased vascular reactivity is not defined, but an impairment of endothelium-dependent relaxation is one possible explanation. Indeed, if reperfusion-induced injury to the coronary endothelium were to prevent it from exerting its protective role against platelet-induced spasm and thrombosis,4,5 this could play an important role in the expression of reperfusion injury to the heart. Altered endothelium-dependent responses in the large coronary arteries, favoring the occurrence of arterial spasm in particular in response to aggregating platelets, could severely impair reperfusion at the level of the myocardium. While previous studies have demonstrated that endothelium-dependent relaxations to thrombin,6 acetylcholine,7,8 and bradykinin8 are impaired after reperfusion, no information seems available on the effect of reperfusion on endothelium-dependent responses to platelets. The present study was designed to examine the effects of reperfusion injury to the canine coronary artery on endothelium-dependent responses to aggregating platelets and platelet products.

Methods

Animal Preparation

Heartworm-free mongrel dogs (25–30 kg) of either sex were anesthetized with intravenous pentobarbital sodium (30 mg/kg bolus injection; Fort Dodge Laboratories, Fort Worth, Tex.), intubated with a cuffed endotracheal tube, and ventilated with 100% oxygen. The respirator was adjusted to keep blood pH and PCO2 in the physiological range. The Po2 was always

From the Department of Physiology and Biophysics, Department of Surgery, Section of Thoracic and Cardiovascular Surgery, Mayo Clinic and Mayo Foundation, Rochester, Minn. Supported in part by Heart, Lung, and Blood Institute grants HL-31183 and 31547-04.

Address for correspondence: Paul M. Vanhoutte, MD, PhD, Director, Center for Experimental Therapeutics, Baylor College of Medicine, One Baylor Plaza, Room 802E, Houston, TX 77030.

Received March 27, 1989; accepted March 27, 1990.
above 120 mm Hg. The blood gas values were monitored every 15 minutes during the experiments (pH/Blood Gas Analyzer 813, Instrumentation Laboratory, Lexington, Mass.). The electrocardiogram was continuously monitored, and the animals' body temperature was maintained by hot water thermal blankets. A left lateral thoracotomy was performed in the fourth intercostal space to expose the heart. A 4-mm segment of the left anterior descending coronary artery (LAD) was carefully dissected free immediately distal to its first diagonal branch. After a 30- to 45-minute stabilization period following the dissection, a control electrocardiogram was recorded along with arterial blood gas values. Next, the LAD was occluded with a small vascular clamp. Cessation of blood flow was confirmed by darkening of the myocardium subtended by the artery, paradoxical wall motion of the ischemic myocardium, and electrocardiographic evidence of myocardial ischemia (typically ST segment depression in leads II and III). Dysrhythmias that occurred were not treated. At 15, 30, and 60 minutes after occlusion, the electrocardiogram was recorded again to document myocardial ischemia. After 60 minutes of ischemia, the occlusion clip was gently removed, and the artery was reperfused for 60 minutes. Reperfusion was confirmed by vascular and myocardial hyperemia, electrocardiographic changes, and the resumption of normal myocardial wall motion. If an animal had extensive collateral blood flow and did not exhibit ischemic changes 15 minutes after occlusion, it was excluded from the study (n=7). The time span for occlusion in the present study was chosen because 1) reperfusion accelerates the disintegration of irreversibly injured cells, and in the canine myocardium irreversible injury begins within 20 minutes after the onset of persistent ischemia; and 2) previous investigators, using a similar model, have used comparable periods of occlusion.6–8

After 60 minutes of reperfusion, the animal was exsanguinated, and the beating heart was quickly removed and immersed in cold, oxygenated modified Krebs' Ringer bicarbonate solution of the following millimolar composition: NaCl 118.3, KCl 4.7, MgSO4 1.2, KH2PO4 1.22, CaCl2 2.5, NaHCO3 25.0, CaEDTA 0.016, and glucose 11.1 (control solution). The procedures and the handling of the dogs were reviewed and approved by the Institutional Animal Care and Use Committee of the Mayo Foundation.

In Vitro Experiments

The LAD (reperfused) and left circumflex coronary artery (control) were carefully dissected free and placed in control solution. Care was taken to remove as much of the connective tissue as possible. A maximum of four rings (4–5 mm in length) of the reperfused LAD was taken at least 10 mm distal to the occlusion site. Rings of mid to distal left circumflex arteries were used as controls. Previous studies6,7 and preliminary experiments (data not shown) demonstrated no difference in endothelium-dependent responses between these vessels. In some rings, the endothelium was removed by gentle rubbing of the intimal surface with the tip of a pair of watchmaker's forceps. Previous histological studies have shown that this procedure successfully removes the endothelial cells in large canine arteries while preserving the ability of the vascular smooth muscle to contract.10,11

The rings were suspended in (25-ml) organ chambers filled with control solution maintained at 37°C and aerated with 95% O2-5% CO2 (pH 7.4). Each ring was suspended by two stainless steel clips passed through the lumen. One clip was anchored to the bottom of the organ chamber; the other was connected to a strain gauge (Statham UC2, Gould, Cleveland) for the measurement of isometric force. Rings were placed at the optimal point of their length-tension relation by progressively stretching them until the contraction to KCl (20 mM), imposed at each level of distension, was maximal.4 In all experiments, the presence or absence of endothelium was confirmed by the response to acetylcholine (10−6 M) of rings contracted with potassium ions (20 mM).10,12 After optimal tension was achieved, the rings were allowed to equilibrate for 45 minutes before the administration of drugs.

Protocols

Rings of reperfused and control arteries (with and without endothelium) from the same animal were studied in parallel. Rings were used for one of six protocols (Table 1). Indomethacin (10−5 M) was added to the organ bath for 40 minutes before contraction of rings with prostaglandin F2α to prevent synthesis of endogenous prostanoids. When concentration-response experiments to 5-hydroxytryptamine (5HT, serotonin) or platelets were perfused, the rings were first incubated with the SHT2-serotonergic antagonist ketanserin (10−4 M) for 30 minutes to inhibit direct SHT2-serotonergic effects of the monoamine on the vascular smooth muscle. An equilibration period of at least 30 minutes occurred between each experiment in the specific protocols.

Drugs and Platelets

The following drugs were used: acetylcholine chloride, ADP, the calcium ionophore A23187, catalase (bovine), hemoglobin (bovine), 5-hydroxytryptamine creatinine sulfate, (+)-isoproterenol hydrochloride, indomethacin, KCl, prostaglandin F2α sodium nitroprusside, superoxide dismutase (bovine) (all from Sigma Chemical Co., St. Louis), ketanserin tartrate (Janssen Pharmaceutical Inc., Piscataway, N.J.), and nitric oxide (Union Carbide, Chicago). All drugs were prepared daily with distilled water except for indomethacin, which was dissolved in Na2CO3 (10−3 M), and the calcium ionophore A23187, which was dissolved in dimethyl sulfoxide, with further dilutions obtained in distilled water. The concentrations are expressed as final molar concentration in the organ chamber. A platelet-rich solution was prepared by centrifugation as described previous-
ly,13,14 Briefly, autologous blood (360 ml) was drawn from the left carotid artery of the dog into citrate anticoagulant to yield final concentrations of 9.3 mM sodium citrate, 0.7 mM citric acid, and 14 mM dextrose.13,14 The blood was centrifuged for 40 minutes at 500 rpm (55g) at room temperature, and the platelet-rich plasma was drawn off with a pipette. An equal volume of cold citrate anticoagulant solution (in mM: sodium citrate 93, citric acid 7, dextrose 105, and KCl 5, pH 6.5) was added to the platelet-rich plasma, and the mixture was centrifuged for 20 minutes at 1,600 rpm (570g). The supernatant was discarded, and the remaining platelet pellet was resuspended in a small volume of the second citrate anticoagulant mixture. A platelet count of this suspension was then obtained (Coulter Electronics Inc., Hialeah, Fla.). Platelet aggregation on exposure to the collagen of the blood vessel wall and the calcium-containing modified Krebs’ Ringer bicarbonate solution was evidenced by clearing of the initially turbid solution and formation of visible platelet clumps15,16; under the experimental conditions of the present study, it is likely that the subsequent vascular responses to the platelet products are transmitted mainly through the adventitial wall. Earlier studies have demonstrated the release of 5HT and thromboxane B2 under these conditions.

Bovine hemoglobin (type 1) contains a mixture of oxyhemoglobin and the oxidized derivative, methemoglobin. Oxyhemoglobin, which inactivates endothelium-derived relaxing factor,17 was prepared by adding 600 mg bovine hemoglobin to 10 ml distilled water containing 70 mg sodium dithionite (Na2S2O4). Sodium dithionite was then removed by dialysis in 15 l distilled water (containing 0.001% EDTA) for 2 hours at room temperature. During dialysis, the water was bubbled with nitrogen. The percentage of oxyhemoglobin was determined spectrophotometrically.17

Nitric oxide from a cylinder was used to fill a glass gas bulb fitted with a silicon injection septum. By using a glass syringe, gas was removed from the bulb and injected into another glass bulb, which had been filled with 100 ml distilled water (which had been bubbled with helium for 3 hours) to give stock solutions of nitric oxide (4×10⁻⁵ M, 4×10⁻⁴ M, and 4×10⁻³ M).18

Data Analysis

The results are expressed as mean±SEM. In all experiments, n refers to the number of animals from which rings were taken. In rings contracted with prostaglandin F2α, responses are expressed as percent changes from the contracted levels, and in quiescent (noncontracted) rings responses are expressed as percent changes of the response to KCl (20 mM) unless otherwise stated. For relaxations, the negative logarithm of the effective molar concentration of agonist causing 50% inhibition of the contractions to prostaglandin F2α was calculated for concentration-response curves, and the mean of these values is presented. Statistical evaluation of the data was performed by Student’s t test for either paired or unpaired observations. Values of p<0.05 were considered to be statistically significant.

Results

Platelets

Quiescent rings. In both control and reperfused quiescent (noncontracted) rings without endothelium, aggregating platelets (single dose of 75,000

---

**TABLE 1. Experimental Protocols Performed on Control and Reperfused Coronary Artery Rings With and Without Endothelium**

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Order of agonists</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Acetylcholine* (10⁻⁵–10⁻⁴ M) Isoproterenol* (10⁻⁵–10⁻³ M) Platelets* (1,000–10,000 platelets/μl final bath concentration)</td>
</tr>
<tr>
<td>II</td>
<td>ADP* (10⁻⁵–10⁻⁴ M) Sodium nitroprusside* (10⁻⁵–10⁻³ M) A23187* (10⁻⁶–10⁻⁴ M)</td>
</tr>
<tr>
<td>III</td>
<td>5-Hydroxytryptamine* (10⁻⁵–10⁻⁴ M) ADP*† (10⁻⁵–10⁻³ M) KCl‡ (5–40 mM)</td>
</tr>
<tr>
<td>IV</td>
<td>Prostaglandin F₂α† (10⁻⁵–10⁻³ M) Acetylcholine*† (10⁻⁵–10⁻³ M) Platelets ‡ (single dose of 75,000 platelets/μl; final bath concentration) A23187† (10⁻⁶–10⁻⁴ M)</td>
</tr>
<tr>
<td>V</td>
<td>Hemoglobin†‡ (10⁻⁵ M)</td>
</tr>
<tr>
<td>VI</td>
<td>Nitric oxide* (10⁻⁵–10⁻⁴ M) ADP* (10⁻⁵–10⁻⁴ M) in presence of superoxide dismutase (150 units/ml) and catalase (1,200 units/ml)</td>
</tr>
</tbody>
</table>

*Relaxations to agonist in rings contracted with prostaglandin F₂α (2×10⁻⁶ M). The response to the Ca²⁺ ionophore A23187 is taken as last because previous experience has demonstrated that it is possibly irreversible.
†Experiment performed in the presence of indomethacin (10⁻⁷ M).
‡Experiment performed in the presence of ketanserin (10⁻⁶ M).
platelets/μl final organ chamber concentration) caused comparable contractions (47±21% and 40±20% of the contraction to 20 mM KCl for control and reperfused rings, respectively). In control rings with endothelium, the platelets induced a moderate contraction (13±7%), while a significantly greater contraction (35±11%) was induced in the reperfused rings (Figure 1).

**FIGURE 1.** Response to aggregating platelets (75,000/μl) of quiescent control and reperfused coronary artery rings with and without endothelium (n=6). Data are mean±SEM and are expressed as percent of contraction to potassium ions (20 mM). Asterisk denotes significance between control and reperfused rings with endothelium (Student’s t test for paired observation; p<0.05).

**FIGURE 2.** Concentration-response curves to aggregating platelets in control and reperfused coronary arterial rings with and without endothelium (n=6). Rings were contracted with prostaglandin F$_{2\alpha}$ (PGF$_{2\alpha}$) (2×10$^{-6}$ M) and incubated with the 5-hydroxytryptamine serotoninergic antagonist ketanserin (10$^{-6}$ M, 30 minutes). Values are mean±SEM. Asterisks denote significance between control and reperfused rings with endothelium (Student’s t test for paired observation; p<0.05). LCX, left circumflex coronary artery; LAD, left anterior descending coronary artery.

**Platelet Products**

**ADP.** ADP caused comparable concentration-dependent relaxations in control and reperfused rings without endothelium that did not reach the baseline value. In control and reperfused rings with endothelium, ADP caused concentration-dependent relaxations to baseline that were significantly greater than in rings without endothelium. However, in the reperfused rings with endothelium, there was a significant shift of the concentration-response curve to the right compared with control rings with endothelium (Figure 3, Table 2). In control rings without endothelium, indomethacin significantly attenuated the relaxation to 10$^{-5}$ M ADP; in reperfused rings, a significant attenuation was observed at 3×10$^{-5}$ and 10$^{-4}$ M ADP (Table 3). Indomethacin also attenuated relaxations to ADP in control arteries with endothelium (10$^{-7}$ to 3×10$^{-6}$ M). Indomethacin had no effect on reperfused arteries with endothelium.

**FIGURE 3.** Concentration-response curves to ADP in control and reperfused coronary arterial rings with and without endothelium (n=7). Rings were contracted with prostaglandin F$_{2\alpha}$ (PGF$_{2\alpha}$) (2×10$^{-6}$ M). Values are mean±SEM. Asterisks denote significance between control and reperfused rings with endothelium (Student’s t test for paired observation; p<0.05). LCX, left circumflex coronary artery; LAD, left anterior descending coronary artery.
Superoxide dismutase and catalase (150 units/ml and 1,200 units/ml, respectively, added together), potentiated endothelium-dependent responses in control and reperfused arteries but did not modify the difference in response between control and reperfused coronary segments (n = 3, data not shown).

5-Hydroxytryptamine. There was no significant difference between control and reperfused rings without endothelium in response to 5HT (serotonin). In control and reperfused rings with endothelium, serotonin caused concentration-dependent relaxations that were significantly greater than in rings without endothelium. However, in reperfused rings with endothelium, there was a significant shift of the concentration-response curve to the right, and the reperfused rings relaxed to only 38 ± 11% of the initial contraction to prostaglandin F₂α as opposed to 11 ± 4% for control rings with endothelium (Figure 4).

Other endothelium-dependent dilators. Acetylcholine (10⁻²–10⁻⁴ M) (Figure 5) and the calcium ionophore A23187 (10⁻⁹–10⁻⁶ M) (Figure 6) caused endothelium-dependent, concentration-dependent relaxations in control and reperfused arteries with endothelium. However, in the reperfused rings, the concentration-response curves to these agonists were shifted to the right (Table 2). Indomethacin had no significant effect on relaxations to acetylcholine in either control or reperfused arteries (Table 3). Indomethacin attenuated relaxations to the calcium ionophore A23187 (10⁻⁸ to 3 x 10⁻⁷ M) in control but not in reperfused rings (Table 3).

Basal release of endothelium-derived relaxing factor. In quiescent coronary rings with endothelium, hemoglobin (10⁻⁷ M) caused comparable increases in tension in control and reperfused vessels (14.0 ± 4.8%)

---

**Table 2. Values for 50% Inhibition (−Log M) of Control and Reperfused Rings With and Without Endothelium**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Reperfused</th>
</tr>
</thead>
<tbody>
<tr>
<td>With endothelium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>7.59±0.14</td>
<td>7.12±0.19*</td>
</tr>
<tr>
<td>ADP</td>
<td>6.98±0.12</td>
<td>6.11±0.16*</td>
</tr>
<tr>
<td>A23187</td>
<td>7.59±0.13</td>
<td>7.34±0.13*</td>
</tr>
<tr>
<td>Without endothelium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitric oxide</td>
<td>6.78±0.18</td>
<td>6.91±0.20</td>
</tr>
<tr>
<td>Sodium nitroprusside</td>
<td>7.78±0.14</td>
<td>7.47±0.15</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>7.54±0.19</td>
<td>7.33±0.15</td>
</tr>
</tbody>
</table>

*Significant difference from control rings by Student's t test for paired observation (p<0.05).

**Table 3. Concentration-Dependent Relaxesations in Control and Reperfused Coronary Arterial Rings With and Without Endothelium in the Presence and Absence of Indomethacin**

<table>
<thead>
<tr>
<th>Drug concentration (−log M)</th>
<th>9</th>
<th>8.5</th>
<th>8</th>
<th>7.5</th>
<th>7</th>
<th>6.5</th>
<th>6</th>
<th>5.5</th>
<th>5</th>
<th>4.5</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Reperfused</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With endothelium</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ach</td>
<td>100</td>
<td>98±2</td>
<td>90±9</td>
<td>74±10</td>
<td>42±11</td>
<td>17±8</td>
<td>8±4</td>
<td>4±2</td>
<td>4±2</td>
<td>3±2</td>
<td>3±2</td>
</tr>
<tr>
<td>Ach+Indo</td>
<td>100</td>
<td>84±14</td>
<td>35±10</td>
<td>6±6</td>
<td>5±5</td>
<td>5±5</td>
<td>5±5</td>
<td>5±5</td>
<td>5±5</td>
<td>5±5</td>
<td>5±5</td>
</tr>
<tr>
<td>A23187</td>
<td>100</td>
<td>82±16</td>
<td>42±16</td>
<td>6±4</td>
<td>3±4</td>
<td>3±4</td>
<td>3±4</td>
<td>3±4</td>
<td>3±4</td>
<td>3±4</td>
<td>3±4</td>
</tr>
<tr>
<td>A23187+Indo</td>
<td>100</td>
<td>67±8</td>
<td>23±13</td>
<td>8±8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ADP</td>
<td>100</td>
<td>95±2</td>
<td>72±7</td>
<td>36±10</td>
<td>15±9</td>
<td>6±4</td>
<td>0±4</td>
<td>0±4</td>
<td>0±4</td>
<td>0±4</td>
<td>0±4</td>
</tr>
<tr>
<td>ADP+Indo</td>
<td>100</td>
<td>98±1</td>
<td>85±6</td>
<td>62±11</td>
<td>33±11</td>
<td>17±11</td>
<td>7±11</td>
<td>6±11</td>
<td>6±11</td>
<td>6±11</td>
<td>6±11</td>
</tr>
<tr>
<td>Without endothelium</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADP</td>
<td>100</td>
<td>99±1</td>
<td>99±1</td>
<td>99±1</td>
<td>99±1</td>
<td>93±5</td>
<td>84±9</td>
<td>69±12</td>
<td>59±11</td>
<td>43±11</td>
<td>43±11</td>
</tr>
<tr>
<td>ADP+Indo</td>
<td>100</td>
<td>99±1</td>
<td>99±1</td>
<td>99±1</td>
<td>98±1</td>
<td>97±1</td>
<td>96±1</td>
<td>92±1</td>
<td>87±2*</td>
<td>77±4*</td>
<td>77±4*</td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With endothelium</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ach</td>
<td>100</td>
<td>97±2</td>
<td>82±8</td>
<td>46±14</td>
<td>13±8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ach+Indo</td>
<td>100</td>
<td>99±1</td>
<td>64±8</td>
<td>1±1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A23187</td>
<td>100</td>
<td>44±14</td>
<td>5±4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A23187+Indo</td>
<td>100</td>
<td>95±2*</td>
<td>33±9*</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ADP</td>
<td>100</td>
<td>96±3</td>
<td>91±5</td>
<td>49±12</td>
<td>15±8</td>
<td>2±1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ADP+Indo</td>
<td>100</td>
<td>97±1*</td>
<td>65±9*</td>
<td>13±6</td>
<td>1±1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Without endothelium</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADP</td>
<td>100</td>
<td>101±1</td>
<td>103±3</td>
<td>102±2</td>
<td>101±3</td>
<td>97±3</td>
<td>94±3</td>
<td>84±4</td>
<td>74±6</td>
<td>67±9</td>
<td>60±11</td>
</tr>
<tr>
<td>ADP+Indo</td>
<td>100</td>
<td>99±1</td>
<td>99±1</td>
<td>97±1</td>
<td>94±3</td>
<td>91±4*</td>
<td>87±5</td>
<td>84±5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Significant difference between rings in the presence and absence of indomethacin to a particular agonist (Student’s t test for unpaired observations; p<0.05).
and 12.4±3.0% of the contraction to 20 mM KCl, respectively (n=6, data not shown).

Endothelium-independent dilators. Nitric oxide (10⁻⁶–10⁻⁵ M) caused comparable concentration-dependent relaxations in control and reperfused rings without endothelium (Figure 7, Table 2). In addition, sodium nitroprusside (10⁻⁶–10⁻⁵ M) caused comparable, concentration-dependent relaxations in control and reperfused rings without endothelium (Table 2). Also, in control and reperfused rings without endothelium, there was no difference in the relaxations induced by ADP, serotonin, and isoproterenol (10⁻⁶–10⁻⁴ M) (Figures 3, 4, and 8).

Contractions. KCl (5–40 mM) and prostaglandin F₂α (10⁻⁹–10⁻⁵ M) caused comparable, concentration-dependent relaxations in control and reperfused rings with and without endothelium (Table 4). There was no difference in the maximal response to either agonist between the two groups. Within each group, there was no difference between rings with and without endothelium.

Discussion

The present study was undertaken to examine endothelium-dependent responses to aggregating platelets and vasoactive drugs in the canine coronary artery after acute occlusion and reperfusion. The major findings were 1) contracted reperfused coronary arteries with endothelium lose their ability to generate endothelium-dependent relaxations to aggregating
platelets and, as a consequence, exhibit additional contraction after exposure to lower numbers of aggregating platelets; 2) quiescent reperfused arteries with endothelium exhibit an exaggerated constrictor response to aggregating platelets; 3) reperfusion impairs endothelium-dependent relaxations to ADP, serotonin, acetylcholine, and the calcium ionophore A23187; 4) reperfusion apparently does not alter the basal release of endothelium-derived relaxing factor; and 5) the ability of the vascular smooth muscle to contract or relax is unaltered after 60 minutes of ischemia followed by 60 minutes of reperfusion.

As reperfused coronary arteries exhibit a severe attenuation of endothelium-dependent relaxations to aggregating platelets, reperfused arteries with endothelium act in a comparable manner to those without endothelium. Potiated contraction, as a consequence of attenuated relaxations to aggregating platelets and their products, is also apparent after endothelial regeneration and is a common finding in other types of vascular disease such as hypertension and atherosclerosis. The mechanism of this aberrant response could be multifactorial. First, release of endothelium-derived relaxing factor in response to the platelet-mediated vasodilator could be impaired. In the canine coronary artery, ADP (and ATP) and serotonin are the platelet products that evoke endothelium-dependent relaxations. In this species, the adenine nucleotides apparently exert the key role in mediating endothelium-dependent relaxation to aggregating platelets.

However, reperfusion impairs endothelium-dependent relaxations to both serotonin and ADP while leaving the direct effect of these agonists on smooth muscle unaltered. Thus, the impaired response to both platelet products could cause the aberrant platelet response. This is in agreement with data from atherosclerotic porcine blood vessels in which decreased responses to both of these platelet-mediated vasodilators are impaired. However, this differs from the findings in porcine coronary arteries with regenerated endothelium or in the aorta of the spontaneous hypertensive rat in which the altered endothelium-dependent responses to serotonin are the primary abnormality that underlies the abnormal response to the platelets.

Another possible mechanism of the impaired platelet response would be the production of a constricting prostaglandin or decreased production of a relaxing prostaglandin by the endothelium. Experiments performed in the presence of indomethacin

Table 4. Concentration-Dependent Contractions to Potassium Ions and Prostaglandin F₂α in Control and Reperfused Canine Coronary Arterial Rings

<table>
<thead>
<tr>
<th>Potassium ions (mM)*</th>
<th>Control</th>
<th>Reperfused</th>
<th>Without endothelium (n=5)</th>
<th>Control</th>
<th>Reperfused</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>8±3</td>
<td>7±3</td>
<td>9±4</td>
<td>7±4</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>33±7</td>
<td>28±6</td>
<td>33±8</td>
<td>26±7</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>78±3</td>
<td>78±3</td>
<td>76±5</td>
<td>71±3</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>94±1</td>
<td>94±1</td>
<td>96±2</td>
<td>93±1</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Maximal response (g)</td>
<td>12±2</td>
<td>12±1</td>
<td>11±1</td>
<td>12±1</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Prostaglandin F₂α (–Log M)</th>
<th>Control</th>
<th>Reperfused</th>
<th>Control</th>
<th>Reperfused</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.5</td>
<td>1±0.7</td>
<td>1±0.9</td>
<td>0.6±0.6</td>
<td>2±2</td>
</tr>
<tr>
<td>7</td>
<td>5±33</td>
<td>5±2</td>
<td>4±3</td>
<td>4±2</td>
</tr>
<tr>
<td>6.5</td>
<td>22±4</td>
<td>23±3</td>
<td>22±7</td>
<td>26±4</td>
</tr>
<tr>
<td>6</td>
<td>49±5</td>
<td>53±4</td>
<td>52±8</td>
<td>60±5</td>
</tr>
<tr>
<td>5.5</td>
<td>81±4</td>
<td>83±2</td>
<td>78±4</td>
<td>86±2</td>
</tr>
<tr>
<td>5</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Maximal response (g)</td>
<td>9±2</td>
<td>10±2</td>
<td>9±1</td>
<td>8±1</td>
</tr>
</tbody>
</table>

Data are presented as percent of maximal response to potassium ions (40 mM) or prostaglandin F₂α (10⁻⁵ M); mean±SEM.

*Concentration of potassium ions above control solution concentration.
support the hypothesis that reperfused arteries have a decreased production of a relaxing prostanoid because its presence attenuated the endothelium-dependent responses to ADP and the calcium ionophore in control but not reperfused rings. The endothelium-dependent responses to acetylcholine and the calcium ionophore A23187 were also attenuated by reperfusion. Such a general impairment of endothelium-dependent responses is also observed in atherosclerosis.23 This differs from changes in porcine coronary arteries with regenerated endothelium in which there is a selective decrease in endothelium-dependent response to some agonists, while the response to others remains intact.14

There are several possible explanations for the general impairment of endothelium-dependent relaxations in reperfused arteries. First, the smooth muscle of the arterial wall could lose its ability to respond to endothelium-derived relaxing factor. However, this is not the case as there was no difference between control and reperfused arteries in response to nitric oxide, which has been identified as one major chemical mediator of endothelium-dependent relaxations.18,24 Also, there was no difference between control and reperfused arteries in response to sodium nitroprusside which, like endothelium-derived relaxing factor, activates guanylate cyclase in the vascular smooth muscle to cause relaxation.25,26 In addition, there was no difference in the ability of control and reperfused arteries without endothelium to relax in response to either isoprotrenol or ADP, suggesting that the potential of the smooth muscle to relax is unaltered after reperfusion. Second, there could be a decreased production of relaxing factor(s) by the endothelial cell. This is possible because other types of cells exhibit impaired synthetic processes after sustaining reperfusion injury.9 Third, there could be impairment of the release of the factor from the endothelial cell because other cells also exhibit membrane transport and structural dysfunction after reperfusion.27 Finally, there could be an accelerated breakdown of endothelium-derived relaxing factor by the artery. As reperfusion promotes the generation of oxygen-derived free radicals in other tissues,28,29 and as superoxide anions inactivate the relaxing factor,30,31 it is possible that radicals continuously produced by reperfused coronary arteries scavenge endothelium-derived relaxing factor. However, this explanation is unlikely, because experiments done in the presence of scavengers of free radicals failed to alter the difference in response between control and reperfused arteries.

Clinical Implications

The present study suggests that a decrease in the ability of the endothelium to produce relaxing factor could become a reason for an exaggerated vasoconstrictor response to platelet products. Endothelium-derived relaxing factor not only relaxes vascular smooth muscle, but also inhibits platelet adhesion, and is itself a potent antiaggregatory substance.33,34 If the release of endothelium-derived relaxing factor is reduced in reperfused arteries, platelet adhesion will not be inhibited; since the antiaggregatory effect of endothelium-derived relaxing factor would also be curtailed, platelet aggregation would be favored in coronary arteries damaged by the reperfusion. This interpretation is strengthened by the demonstration that platelet deposition is promoted by damage to the endothelium and that reperfusion of the coronary arteries leads to injury of the endothelial lining.7 Thus, reperfusion could lead to unchecked platelet deposition and aggregation in coronary arteries. The present study demonstrates that the endothelium of the reperfused segments of the coronary artery is no longer capable of breaking the constrictor response to the platelet products, which should favor the occurrence of vasoconstriction. In fact, in vivo a correlation exists between quantitative platelet deposition and localized vasoconstriction at the site of endothelial injury secondary to trauma.34 The present results suggest that an augmented adhesion and aggregation of platelets, and constriction of the reperfused coronary arteries to the platelet products resulting from endothelial dysfunction, could play a key role in the local vasoconstrictions. Aggregating platelets have been implicated in the potentiation of myocardial ischemia,35 progressive reperfusion injury,36,37 and surgically induced myocardial ischemia.38

In conclusion, reperfusion impairs the normal relaxation to aggregating platelets and vasoactive drugs mediated by the coronary endothelium. This impairment of relaxation could help explain the increased vascular tone and tendency toward vasoconstriction commonly observed after reperfusion of the coronary vasculature.

Acknowledgments

The authors wish to thank Mr. R.R. Lorenz for preparing the figures and Mrs. J. Krage, Ms. Kathleen Kros, and Mrs. Cynthia Lindsay for secretarial assistance.

References

7. VanBenthuyzen KM, McMurtry IF, Horwitz LD: Reperfusion after acute coronary occlusion in dogs impairs endothelium-dependent relaxation to acetylcholine and augments contrac-
8. Mehta JL, Nichols WW, Donnelly WH, Lawson DL, Saldeen TGP: Impaired canine coronary vasodilator response to a-
9. Jennings RB, Reimer KA: Factors involved in salvaging isch-
11. DeMey JG, Vanhoutte PM: Heterogeneous behavior of the canine arterial and venous wall: Importance of the endo-
12. Furchgott RF, Zawadzki JV: The obligatory role of endo-
13. Houston DS, Shepherd JT, Vanhoutte PM: Adenine nucleo-
14. Shimokawa H, Aarhus LL, Vanhoutte PM: Porcine coronary arteries with regenerated endothelium have a reduced endothelium-dependent responsiveness to aggregating plate-
15. Luscher TF, Vanhoutte PM: Endothelium-dependent responses to platelets and serotonin in spontaneously hyper-
17. Martin W, Villani GM, Johianandan D, Furchgott RF: Selective blockade of endothelium-dependent and glyceryl
 triinitrate-induced relaxation by hemoglobin and methylene blue in the rabbit aorta. J Pharmacol Exp Ther 1985;232:
708–716
18. Palmer RMJ, Ferrige AG, Moncada S: The release of nitric
 oxide by vascular endothelial cells accounts for the activity of
 EDRF. Nature 1987;327:524–526
19. Heistad DD, Armstrong ML, Marcus ML, Piegers DJ, Mark
 AL: Augmented responses to vasoconstrictor stimuli in hyper-
cholesterolemic and atherosclerotic monkeys. Circ Res 1984;
54:711–718
20. Vanhoutte PM, Luscher TF: Vascular endothelium and hyper-
21. Miller VM, Vanhoutte PM: Endothelium-dependent contrac-
tions to arachidonic acid are mediated by products of cyclo-
22. Gryglewski FJ, Dembinska-Kiec A, Zumuda A, Gryglewski T:
Prostaglandin and thrombnoxone A2; biosynthesis capacities of
heart, arteries, and platelets at various stages of experimental
23. Frieman RC, Mitchell GG, Heistad DD, Armstrong ML, Harri-
24. Ignarro LJ, Byrns RE, Buga GM, Wood KS: Endothelium-
derived relaxing factor from pulmonary artery and vein pos-
sesses pharmacologic and chemical properties identical to
nitroprusside, nitroglycerin and sodium oxide on levels of
26. Furchgott RF: Role of endothelium in responses of vascular
27. Frame LH, Lopez JA, Khaw BA, Fallon JT, Haber E, Powell
WJ Jr: Early membrane damage during coronary reperfusion
in dogs: Detection by radiolabeled anticardiac myosin (Fab')2.
28. Hammond B, Hess ML: The oxygen free radical system: Poten-
tial mediator of myocardial injury. Basic Concepts Cardiol 1986;5:215–220
IW: Species may cause myocardial reperfusion injury. Biochem
Biophys Res Commun 1985;127:87–93
30. Rubanyi GM, Vanhoutte PM: Oxygen-derived radicals, endo-
thelium and responsiveness of vascular smooth muscle. Am J
Physiol 1986;250:H815–H821
31. Gryglewski FJ, Palmer RMJ, Moncada S: Superoxide anion
is involved in the breakdown of endothelium-derived vascular
32. Sneddon JH, Vane JR: Endothelium-derived relaxing factor
reduces platelet adhesion to bovine endothelial cells. Proc Natl
Acad Sci USA 1988;85:2800–2804
inhibition of platelet aggregation. Br J Pharmacol 1986;88:
441–445
34. Radomski MW, Palmer RMJ, Moncada S: Comparative phar-
macology of endothelium-derived relaxing factor, nitric oxide
aggravation of acute ischemia in an isolated rabbit heart
36. Yee ES, Price DC, Ahern T, Ebert PA: Intracoronary
platelet aggregation: Pattern of deposition after ischemia,
37. Rosenbaum D, Levitsky S, Silverman N, Kohler J, Lipowski J,
Le Breton G, Feinberg M: Cardioplegia does not prevent
reperfusion injury induced by intracoronary platelet deposi-
38. Feinberg H, Rosenbaum DS, Levitsky S, Silverman NA,
Kohler J, Le Breton G: Platelet deposition after surgically
induced myocardial ischemia: An etiologic factor for reperfu-

KEY WORDS • ADP • ischemia • serotonin • thrombosis •
vasospasm
Acute impairment of endothelium-dependent relaxations to aggregating platelets following reperfusion injury in canine coronary arteries.
P J Pearson, H V Schaff and P M Vanhoutte

Circ Res. 1990;67:385-393
doi: 10.1161/01.RES.67.2.385

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1990 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/67/2/385