Impaired Canine Coronary Vasodilator Response to Acetylcholine and Bradykinin After Occlusion-Reperfusion


Previous studies indicate impairment of coronary arterial ring relaxation in response to acetylcholine (ACh) following coronary reperfusion, mediated via loss of endothelium-derived relaxing factor (EDRF). To examine if coronary vasodilator reserve is reduced following coronary occlusion-reperfusion in intact animals, 16 open-chest mongrel dogs were subjected to 1 hour of total left circumflex (Cx) coronary artery occlusion followed by reperfusion for 1 hour. Prior to Cx occlusion, coronary blood flow increased and vascular resistance decreased (both p<0.01) in response to ACh and bradykinin (BK). Following reperfusion, increase in Cx coronary flow in response to both vasodilators was significantly (p<0.01) impaired. Myocardial histology showed extensive neutrophil infiltration and capillary plugging by neutrophils in the Cx compared with the left anterior descending coronary artery-supplied myocardium. Myocardial myeloperoxidase activity was also increased in the Cx compared with the left anterior descending region (p<0.02). Pretreatment of four dogs with indomethacin partially reduced the vasodilator response to BK but not to ACh. However, indomethacin did not affect reperfusion-induced attenuation of BK or ACh's coronary vasodilator effects. To determine if calcium blocker verapamil would modify reperfusion-induced impairment in coronary vasodilator reserve, six dogs were treated with verapamil. Although verapamil enhanced coronary vasodilator effects of ACh and BK, it did not modify reperfusion-induced attenuation of coronary vasodilator reserve. Myocardial neutrophil accumulation and myeloperoxidase activity was also similar in control, indomethacin, and verapamil-treated dogs. These observations suggest that coronary reperfusion impairs coronary vasodilator reserve in intact dogs. This impairment is not modified by prostaglandin inhibition or by calcium blockade. Besides loss of EDRF, capillary plugging by neutrophils may contribute to the altered coronary flow reserve observed in the immediate post-reperfusion period. Furthermore, indomethacin or verapamil are not effective in modifying the reperfusion-related impairment of coronary vasodilator reserve.

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Endothelium participates in the maintenance of coronary arterial tone by releasing vasodilator and vasoconstrictor substances. One of the most potent vasodilator substances, endothelium-derived relaxing factor (EDRF), is released from the endothelial cells and may be related to nitric oxide. Furchgott first described the obligatory role of endothelial cells in vascular relaxation evoked by acetylcholine (ACh). Subsequent studies showed that the coronary artery ring relaxation by thrombin is converted to constriction when dog coronary arteries are subjected to total occlusion followed by reperfusion. Van-Benthuyzen et al have also shown attenuation of ACh-induced relaxation of coronary arterial rings from dogs subjected to temporary coronary occlusion. In those studies, electron microscopy demonstrated focal areas of endothelial cell injury with partial detachment of the cells from the underlying subendothelium. Recent studies have shown extensive capillary plugging by neutrophils in the myocardium of dogs following coronary occlusion and reperfusion, which may relate to the microvascular origin of the "no-reflow" phenomenon. Neutrophil capillary plugging reduces the cross-sectional area of the microvascular bed and could reduce coronary vasodilator reserve.
Bradykinin (BK) dilates coronary arteries in a dose-dependent manner by stimulating release of EDRF as well as another potent vasodilator from the vessel wall, prostacyclin (PGI2). Pretreatment of the animals with cyclooxygenase inhibitors partially decreases the vasodilation, indicating that PGI2 release plays a role in BK-induced vasorelaxation.

The purpose of this study was to examine if temporary coronary artery occlusion followed by reperfusion would diminish coronary vasodilator response to ACh and BK in the intact animal. Accordingly, studies were conducted to determine the coronary hemodynamic effects of ACh and BK in intact dogs subjected to coronary artery occlusion followed by reperfusion. To determine the role of prostaglandins in coronary vasodilator responses to ACh and BK, studies were also conducted in animals treated with indomethacin. Since calcium blocker verapamil modifies coronary artery ring relaxation after temporary coronary occlusion, we also examined the coronary effects of ACh and BK in the presence and absence of verapamil.

Materials and Methods

Experimental Preparation

Sixteen healthy, adult mongrel dogs of either sex ranging in weight from 20 to 32 kg (average weight, 25.3 kg) were used for this study. The animals were anesthetized with intravenous sodium pentobarbital at a dose of 30 mg/kg body weight. Small maintenance doses of the anesthetic agent were administered periodically when needed during the experiment. Once the animal was anesthetized, a cuffed endotracheal tube was inserted and respiration maintained with an intermittent positive pressure respirator (Harvard Apparatus, South Natick, Massachusetts). The rate and volume per stroke of the respirator were adjusted to maintain arterial blood gases within the physiological range (pH 7.35-7.45, PCO2 30-40 mm Hg, and P02 85-100 mm Hg). The heart and great vessels were exposed through the fifth intercostal space. The pericardium was opened and reflected in order to form a cradle for suspending the heart. Approximately 2 cm of the left anterior descending (LAD) and circumflex (Cx) coronary arteries were dissected free from the adjacent tissues. Flow in the two arteries were measured with a dual-channel 20-MHz ultrasonic Doppler flowmeter (Valpey-Fisher) and rigid flow probes with an inside diameter ranging from 2.0 to 3.5 mm. Probes were carefully matched to the diameter of the artery in each case and were chosen so that the probe slightly constricted the vessel. The Doppler flowmeter and probes were calibrated by matching the output signal to a calibrated electromagnetic flowmeter (BL 613, Biotronex) signal. A pressure catheter (Millar, Houston, Texas) was passed through a carotid artery and positioned so that the tip was in the ascending aorta. A small Teflon catheter (3F) was inserted into the first diagonal branch of the LAD and advanced to the left main coronary artery. A large bore catheter was placed in the coronary sinus via the right atrium. Blood flow in LAD and Cx coronary arteries increased equally after a 50 μg bolus injection of nitroglycerin, indicating catheter position in the left main coronary artery. The catheter’s position was also confirmed at the termination of the experiment by opening the vessel. A small plastic occluder was positioned around the Cx artery and used for total occlusion of the vessel.

Two additional dogs were anesthetized and instrumented precisely as the other 16 dogs and served as sham controls.

Experimental Protocol

After a stabilization period of at least 15 minutes, heart rate, ascending aortic pressure, and coronary blood flows (LAD and Cx) were measured. ACh and BK were then infused in a random fashion into the left main coronary artery over 10 seconds. The amount of ACh dissolved in saline varied from 0.125 to 1 μg, always in a volume of 0.2 ml. BK (0.12 to 1.25 μg), dissolved in 0.2 ml of saline, was administered in a manner similar to ACh. In all experiments, coronary blood flows and arterial pressure were allowed to return to baseline before administration of ACh or BK. The vehicle (normal saline) was frequently infused (0.2 ml) over 10 seconds to demonstrate only minimal (<10%) change in coronary blood flow.
A group of six animals (group A) was then subjected to total Cx coronary occlusion for 1 hour followed by reperfusion for 1 hour (see below).

In four other animals (group B), after making the measurements described above, indomethacin (Merck Sharp & Dohme Research Laboratories, West Point, Pennsylvania) was administered intravenously (5 mg/kg). After 30–45 minutes, ACh and BK were again administered. The animals were then subjected to Cx coronary occlusion and reperfusion.

In another group of six animals (group C), after control measurements were made, verapamil 0.2 mg/kg was administered intravenously. When the coronary blood flows and arterial pressure had stabilized for approximately 15 minutes, ACh and BK administration was repeated.

After all variables had returned to control, Cx coronary artery was totally occluded in all 16 animals for 1 hour followed by reperfusion for 1 hour. To prevent serious arrhythmias and possible ventricular fibrillation, reperfusion was accomplished by gradual release of the occluder over 15 minutes. After 1 hour of reperfusion, ACh and BK administration was repeated as before. In group C animals, verapamil (0.2 mg/kg/hr) was continued throughout the period of occlusion, reperfusion, and subsequent time during which ACh and BK were administered. If the arterial pressure after reperfusion was lower than in the preocclusion state, phenylephrine was administered intravenously to increase the mean arterial pressure to the preocclusion value.

The two control dogs were subjected to sham coronary occlusion and reperfusion and were given ACh and BK in the same fashion as the other dogs.

**Histology**

After the completion of the experiments, dogs were killed by rapid injection of excess of KCl, and the hearts were quickly removed. Full thickness myocardial sections from the center of regions supplied by the LAD and Cx, as well as segments of epicardial LAD and Cx coronary arteries, were collected and placed in 10% formalin. Histological processing was done by conventional methods, and sections were stained with hemotoxylin and eosin and Mallory’s PTAH stain. The person (W.H.D.) evaluating myocardial and coronary histology was blinded as to the treatment of the animal.

**Myocardial Myeloperoxidase Assay**

As a specific enzymatic marker of neutrophil infiltration into the reperfused myocardium, approximately 500 mg of left ventricular tissue (including endocardium) was removed from the center of the regions supplied by the Cx and LAD coronary arteries. The myeloperoxidase activity was measured by modification of the method of Bradley et al. The person performing the myeloperoxidase assay...
TABLE 1. Effect of Acetylcholine and Bradykinin on Circumflex Coronary Vascular Resistance (Group A Animals)

<table>
<thead>
<tr>
<th></th>
<th>Circumflex coronary vascular resistance (mm Hg/ml/min)</th>
<th>% change</th>
<th>Postreperfusion (mm Hg/ml/min)</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Preocclusion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetylcholine (μg)</td>
<td>0</td>
<td>4.12±0.91</td>
<td>3.61±0.34</td>
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<tr>
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<td>0.125</td>
<td>2.56±0.61</td>
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<td>3.08±0.29</td>
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<td>0.25</td>
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<td>2.41±0.44</td>
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<td></td>
<td>1.0</td>
<td>1.75±0.32</td>
<td>-58</td>
<td>2.32±0.51</td>
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<tr>
<td>Bradykinin (μg)</td>
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<td>4.40±0.72</td>
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<td>3.39±0.30</td>
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<td>1.99±0.13</td>
<td>-54</td>
<td>3.04±0.44</td>
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<tr>
<td></td>
<td>0.50</td>
<td>1.86±0.15</td>
<td>-57</td>
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<tr>
<td></td>
<td>1.25</td>
<td>1.68±0.21</td>
<td>-61</td>
<td>2.82±0.54</td>
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</tbody>
</table>

Summary of five experiments, mean±SEM.

*P<0.01 compared with preocclusion value.

Circumflex coronary preocclusion (mm Hg/ml/min): 4.12±0.91, 2.56±0.61, 2.29±0.54, 2.06±0.51, 1.75±0.32.
Circumflex coronary postreperfusion (mm Hg/ml/min): 4.32±0.71, 2.34±0.28, 1.99±0.13, 1.86±0.15, 1.68±0.21.

(D.L.L.) was blinded as to the treatment of the animal and was not aware of the histological assessment.

Coronary Venous PGI2 Measurement

To determine the effect of coronary occlusion and reperfusion on PGI2 release and to examine cyclooxygenase inhibition with indomethacin, 5 ml of coronary venous blood was collected before Cx occlusion and immediately after reperfusion in indomethacin-EDTA containing anticoagulant as described previously.17 6-keto-PGF1α, a stable hydrolysis product of PGI2, was then measured in the coronary venous plasma by radioimmunoassay with supplies from New England Nuclear, Boston, Massachusetts.18

Calculations and Statistical Analysis

Coronary vascular resistance was calculated as the ratio of mean arterial pressure and coronary blood flow. All data presented are peak changes and are expressed as mean±SEM. Student's t test (for paired data) and analysis of variance with repeated measurements followed by protected t test were used for statistical analysis. A value of p<0.05 was considered significant.

Results

Effects of ACh and BK on Coronary Hemodynamics

Prior to Cx occlusion, coronary blood flow (Cx and LAD) increased in response to ACh and BK in a dose-dependent fashion (p<0.01) (Figures 2 and 3). Coronary vascular resistance decreased (p<0.01) in both LAD and Cx, and the reductions were dependent on the amount of ACh or BK infused (Tables 1 and 2). Heart rate and arterial pressure were not significantly affected by either of the two agents at the peak coronary blood flow effect.

TABLE 2. Effect of Acetylcholine and Bradykinin on Left Anterior Descending Coronary Vascular Resistance (Group A Animals)

<table>
<thead>
<tr>
<th></th>
<th>Before Cx occlusion (mm Hg/ml/min)</th>
<th>% change</th>
<th>After Cx reperfusion (mm Hg/ml/min)</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylcholine (μg)</td>
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<td>5.37±0.63</td>
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<td>5.80±0.43</td>
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<tr>
<td></td>
<td>0.12</td>
<td>3.81±0.47</td>
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<td>0.25</td>
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<td>3.45±0.45</td>
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<td>3.15±0.36</td>
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<td>1.0</td>
<td>2.20±0.29</td>
<td>-57</td>
<td>2.50±0.40</td>
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<tr>
<td>Bradykinin (μg)</td>
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<td>5.48±0.62</td>
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<td>5.44±0.80</td>
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<td>0.12</td>
<td>2.97±0.28</td>
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<td>0.50</td>
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<td>-56</td>
<td>2.36±0.26</td>
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<tr>
<td></td>
<td>1.25</td>
<td>2.33±0.18</td>
<td>-57</td>
<td>2.09±0.20</td>
</tr>
</tbody>
</table>

Summary of five experiments, mean±SEM.

Reduction in left anterior descending (LAD) coronary vascular resistance in response to acetylcholine or bradykinin was not affected by circumflex (Cx) occlusion-reperfusion.
Indomethacin Pretreatment and Coronary Hemodynamic Effects of ACh and BK

Administration of indomethacin caused significant, but small, increases in mean arterial pressure (145±13 vs. 127±11 mm Hg, p=0.05) and coronary blood flows (LAD, 27±2 vs. 22±2 ml/min; Cx, 35±7 vs. 30±6 ml/min; both p=0.05) without affecting either the heart rate or coronary vascular resistances. Cx coronary blood flow increased (p<0.01) in response to ACh in a dose-dependent manner, and the magnitude of increase was similar to that observed before indomethacin (Figure 4). Cx coronary blood flow also increased in response to BK, but the magnitude of increase was smaller (p=0.05) than that before indomethacin (Figure 4). Decrease in Cx coronary vascular resistance with ACh was not affected by indomethacin, whereas that with BK was attenuated (p=0.05) (Table 3). Similar effects of ACh and BK were observed in the LAD coronary artery (data not shown).

Verapamil Pretreatment and Coronary Hemodynamic Effects of ACh and BK

After administration of verapamil, heart rate was lower (128±3 vs. 137±5 beats/min, p=0.02), and PR interval was greater (0.103±0.002 vs. 0.088±0.002 seconds, p=0.02). Mean Cx coronary blood flow (25±2 vs. 26±2 ml/min) and vascular resistance (5.00±0.45 vs. 4.96±0.56 mm Hg/ml per min) were unchanged.

Administration of ACh and BK increased Cx coronary blood flow (Figure 5) and decreased coronary vascular resistance (Table 4) in a dose-dependent fashion (p<0.01). These changes in coronary flow and resistance were always greater (p<0.02) than before treatment of the animals with verapamil. Similar effects of ACh and BK were observed in the LAD as in the Cx coronary artery (data not shown).

Coronary Occlusion-Reperfusion and Coronary Hemodynamic Effects of ACh and BK

Following 1 hour of Cx occlusion and 1 hour of reperfusion, mean arterial pressure was lower (mean decrease 12±3 mm Hg) than in the control state in five animals (two saline-treated, two verapamil-treated, and one indomethacin-treated). As per protocol, mean arterial pressure in these animals was increased to that before coronary occlusion by infusion of phenylephrine. Resting hemodynamic table:

<table>
<thead>
<tr>
<th>Table 3. Effect of Indomethacin on Cx Coronary Vascular Resistance: Response to Acetylcholine and Bradykinin (Group B Animals)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Before indomethacin</strong></td>
</tr>
<tr>
<td>-------------------------</td>
</tr>
<tr>
<td><strong>Acetylcholine (µg)</strong></td>
</tr>
<tr>
<td>0.125</td>
</tr>
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<td>0.25</td>
</tr>
<tr>
<td>0.50</td>
</tr>
<tr>
<td>1.00</td>
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<tr>
<td><strong>Bradykinin (µg)</strong></td>
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<td>0.12</td>
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<td>0.25</td>
</tr>
<tr>
<td>0.50</td>
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<td>1.25</td>
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</table>

Data from four experiments, mean±SEM.

Cx, Circumflex coronary artery; after indomethacin (pre-Cx occlusion), reduction in Cx in response to bradykinin, but not acetylcholine, is less than that observed before Cx occlusion (p=0.05). However, post-Cx reperfusion, there is significant (*p<0.01) attenuation of decrease in Cx coronary vascular resistance in response to both acetylcholine and bradykinin.

Figure 4. Cx coronary blood flow responses to acetylcholine (left) and bradykinin (right) in group B animals. Before indomethacin (O), Cx coronary blood flow increased. After indomethacin (●), Cx coronary blood flow increase was blunted (p<0.05) in response to bradykinin, but not in response to acetylcholine. After Cx reperfusion (A), increase in Cx coronary blood flow in response to both acetylcholine and bradykinin is significantly (p<0.01) attenuated.
variables, including coronary blood flows, coronary vascular resistance, heart rate and mean arterial pressure were similar to pre-Cx occlusion values in all three groups of animals.

**Group A Animals**

Increase in Cx coronary blood flow in response to both ACh and BK was significantly \((p<0.01)\) attenuated (Figures 2 and 3). Reduction in Cx coronary vascular resistance with ACh as well as BK was also significantly \((p<0.01)\) blunted (Table 1). In contrast, increase in LAD coronary blood flow in response to both ACh and BK was preserved (Figures 2 and 3). LAD coronary vascular resistance decreased upon administration of ACh and BK, similar to that before Cx occlusion (Table 2).

**Group B Animals**

Increase in Cx coronary blood flow in response to ACh was significantly \((p<0.01)\) attenuated (Figure 4), and this attenuation was comparable to that in group A animals. Reduction in Cx coronary vascular resistance was also decreased \((p<0.01)\) as in group A animals (Table 3). Increase in Cx coronary blood flow and reduction in vascular resistance in response to BK were further attenuated, beyond the inhibitory effect of indomethacin (Figure 4 and Table 3). Increase in LAD coronary blood flow and decrease in LAD coronary vascular resistance were similar to those before Cx coronary occlusion (data not shown).

**Group C Animals**

Cx coronary blood flow response to ACh and BK was significantly \((p<0.01)\) attenuated (Figure 5). Coronary vascular resistance response was also blunted (Table 4). These changes in the reperfused region were comparable to those in groups A and B after reperfusion and were not affected by verapamil. In contrast, alterations in coronary blood flow and vascular resistance in the nonoccluded LAD were similar to those before Cx occlusion.

**Effect of ACh and BK in Control Animals**

In two control animals subjected to sham coronary occlusion and reperfusion, administration of ACh and BK caused a dose-dependent increase in coronary blood flow and a decrease in coronary vascular resistance. When ACh and BK were readministered after 2 to 3 hours (time of administration of ACh and BK in the first 16 dogs), there were no significant differences in coronary blood flow response to ACh or BK. This indicates that the alteration in coronary vasodilator reserve following

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**Table 4. Effect of Continuous Verapamil Treatment on Cx in Response to Acetylcholine and Bradykinin (Group C Animals)**

<table>
<thead>
<tr>
<th>Acetylcholine (μg)</th>
<th>Before Cx occlusion</th>
<th>% change</th>
<th>After Cx reperfusion</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.51±0.21</td>
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<td>3.52±0.51</td>
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</tr>
<tr>
<td>0.125</td>
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<td>2.30±0.40</td>
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<td>2.63±0.42</td>
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<tr>
<td>0.5</td>
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<td>2.40±0.81</td>
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</tr>
<tr>
<td>Bradykinin (μg)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>4.70±0.35</td>
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<td>4.06±0.41</td>
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<td>0.25</td>
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<td>1.25</td>
<td>1.55±0.21</td>
<td>−67</td>
<td>2.31±0.38</td>
<td>−43*</td>
</tr>
</tbody>
</table>

Data expressed as mean±SEM.

Despite verapamil treatment, reduction in circumflex (Cx) coronary vascular resistance after reperfusion in response to both acetylcholine and bradykinin was significantly attenuated \((p<0.01)\) compared with before Cx occlusion.
coronary reperfusion is not related to spontaneous change in the animal preparation. This has been observed by us when peak reactive hyperemia was used as an index of coronary vasodilator reserve. 19

Histopathology

The myocardial tissues taken from the center of the Cx-supplied myocardial region supplied by reperfused Cx coronary artery had evidence of early myocardial damage, that is, wavy fibers and cell separation (Figure 6), cytoplasmic eosinophilia, and intense neutrophil infiltration of the intercellular spaces (Figure 7). Cx-supplied myocardium consistently contained greater than 30 neutrophils per high power field (×40) compared with fewer than 10 neutrophils per high power held in the LAD region. In the sham-operated control animals, the number of neutrophils in the myocardium was similar to that in the patent LAD region of the other 16 dogs. Capillary plugging by neutrophils with interspersed erythrocytes, mimicking rouleaux formation, was the most characteristic feature of the reperfused myocardium (Figure 8), particularly in the subendocardial region. Neutrophils often adhered to the endothelial lining of the arterioles, completely occluding several vascular lumina. Similar changes were not observed in the LAD-supplied myocardium or the myocardium of the sham-operated dogs (Figure 9). The same pathological features were found in dogs treated with indomethacin or verapamil. There were no important changes in the large epicardial Cx (beyond the site of occlusion) coronary arteries subjected to reperfusion compared with the LAD. Under light microscopy, endothelium appeared intact with no cellular disruption or attachment of cellular material (Figure 10).

Myeloperoxidase Assay (Table 5)

Neutrophil-specific myeloperoxidase activity in the Cx reperfused region was three to five times greater than in the LAD perfused region (p<0.02). In the indomethacin- or verapamil-treated animals, myeloperoxidase activity was also higher (p<0.02) in the Cx perfused regions than in the LAD perfused regions. These observations were similar to those in the saline-treated animals.

Plasma 6-keto-PGF<sub>1α</sub> Concentrations (Table 6)

Cx coronary artery occlusion-reperfusion had no significant effect on plasma 6-keto-PGF<sub>1α</sub> concentrations. Indomethacin administration significantly (p<0.02) decreased 6-keto-PGF<sub>1α</sub> concentrations by over 90% before Cx occlusion, with no change in the reperfusion period. Verapamil administration had no effect on 6-keto-PGF<sub>1α</sub> levels either before or after Cx occlusion-reperfusion.
FIGURE 7. Myocardial histology from the reperfused region demonstrating intense neutrophil infiltration (hematoxylin and eosin stain, original magnification, ×200).

FIGURE 8. Capillary plugging by neutrophils in the reperfused region (hematoxylin and eosin stain, original magnification, ×100).
FIGURE 9. Absence of capillary plugging or myocardial neutrophil infiltration in region supplied by the left anterior descending coronary artery, which was not subjected to occlusion-reperfusion.

FIGURE 10. Histology of the reperfused circumflex coronary artery (2 cm beyond the site of occlusion) from a representative group A animal. The endothelium in both arteries is intact without cellular detachment or attachment of circulating blood cells (hematoxylin and eosin stain).
Cardium in large numbers. Myocardial histology showed extensive neutrophil infiltration and plug-
acin confirms that the vasodilator effects of BK are neutrophils appear in the reperfused canine myo-
response to BK in dogs pretreated with indometh-
sion. Nonetheless, diminished coronary blood flow is not impaired following temporary coronary occlu-
Cx region. This suggests that PGI2 biosynthesis is of coronary vasodilator reserve in the reperfused 

As our studies indicate, 6-keto-PGFα, a levels were measured its release in the coronary venous blood. 

Data (units/100 mg net weight) expressed in mean±SEM. Cx, circumflex; LAD, left anterior descending coronary artery. *p≤0.02 compared with LAD-supplied myocardium. There were no differences among the three groups of animals.

### Discussion

Although reperfusion of the myocardium after coronary occlusion may reduce infarct size and improve myocardial function, several studies point to a potentially detrimental effect of reperfusion on endothelial function, myocardial structure, and coronary vascular reactivity. Specifically, EDRF-dependent coronary arterial ring relaxation following reperfusion is impaired. Our study shows that ACh- as well as BK-induced increases in coronary blood flow is significantly attenuated in the intact animals subjected to temporary coronary occlusion and reperfusion. The reduction in coronary vascular resistance in response to ACh and BK is also impaired, indicating loss of coronary vasodilator reserve.

This loss of coronary vasodilator reserve after reperfusion in the intact animal may be related to the loss of EDRF in coronary arteries subjected to reperfusion. Indeed, we have observed reduction in relaxation of Cx coronary artery rings in response to ACh in our preliminary experiments. Generation of superoxide radicals during reperfusion may cause degradation of EDRF and account for the altered coronary arterial relaxation. Impairment of PG12 release, mechanical plugging of the microcirculation by neutrophils, release of a vasoconstrictor substance from the endothelium, or a combination of these factors may also participate in the decrease in coronary vasodilator reserve and "no-reflow phenomenon" following coronary occlusion and reperfusion.

To examine if the attenuation of vasodilator effects of ACh and BK was due to loss of PG12, we measured its release in the coronary venous blood. As our studies indicate, 6-keto-PGFα levels were not reduced by Cx occlusion-reperfusion. In addition, indomethacin did not modify the attenuation of coronary vasodilator reserve in the reperfused Cx region. This suggests that PG12 biosynthesis is not impaired following temporary coronary occlusion. Nonetheless, diminished coronary blood flow response to BK in dogs pretreated with indomethacin confirms that the vasodilator effects of BK are in part prostaglandin-mediated.

Our study confirms previous observations that neutrophils appear in the reperfused canine myocardium in large numbers. Myocardial histology showed extensive neutrophil infiltration and plug-ging of a large number of capillaries in the reperfused Cx region in contrast to the myocardium supplied by LAD, which was not subjected to occlusion. Threefold to fivefold increase in Cx myocardial myeloperoxidase activity also suggests the presence of large numbers of neutrophils in the reperfused myocardium. Intense capillary plugging by neutrophils can reduce the microvascular cross-sectional area and result in attenuated coronary blood flow response to ACh and BK. It is noteworthy that there were no important changes in the reperfused epicardial coronary artery under light microscopy in our studies. Engler et al showed that reduction in neutrophil numbers in the dog before reperfusion significantly improves the "no-reflow" phenomenon, implying an important role of neutrophils in reperfusion-induced injury. In our preliminary studies, we have found attenuated coronary blood flow response to the EDRF-independent vasodilator nitroglycerin following temporary coronary occlusion and reperfusion. In addition, coronary vasodilator response to several EDRF-independent vasoactive peptides is also diminished (authors' unpublished observations). In particular, impaired coronary blood flow response to exogenous nitroglycerin, which may be similar to endogenous EDRF, implies that mechanical obstruction in the coronary microvasculature may play a very important role in diminished coronary vasodilator reserve. These observations also refute the notion that reduction in EDRF is the sole basis of altered vasodilator reserve following coronary occlusion and reperfusion.

In some previous studies, calcium channel blockers were shown to reduce myocardial infarct size following coronary occlusion and reperfusion, to restore coronary contractile response to "normal" in the reperfused coronary rings, and to partially restore EDRF-dependent relaxation. Beneficial effects of calcium blockers are presumably related to suppression of myocyte calcium accumulation and neutrophil migration. We hypothesized that verapamil would alter coronary flow responses to ACh and BK following reperfusion. Our results indicate that verapamil, given before coronary occlusion, enhances the coronary vasodilator effects of ACh and BK. However, it does not modify the attenuation of coronary flow responses to either agent after reperfusion. Although we did not quantify arrhythmias, verapamil did not seem to inhibit

### Table 5. Myocardial Myeloperoxidase Activity

<table>
<thead>
<tr>
<th>Group</th>
<th>Cx-supplied myocardium</th>
<th>LAD-supplied myocardium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline-treated dogs</td>
<td>0.21±0.03*</td>
<td>0.05±0.01</td>
</tr>
<tr>
<td>Indomethacin-treated dogs</td>
<td>0.18±0.02*</td>
<td>0.07±0.02</td>
</tr>
<tr>
<td>Verapamil-treated dogs</td>
<td>0.19±0.04*</td>
<td>0.03±0.01</td>
</tr>
</tbody>
</table>

Data (pg/ml) expressed in mean±SEM. Cx, circumflex; LAD, left anterior descending coronary artery.

### Table 6. Coronary Venous 6-keto-PGFα Concentrations

<table>
<thead>
<tr>
<th>Group</th>
<th>Before Cx occlusion</th>
<th>After Cx-reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline-treated dogs</td>
<td>193±14 (5)</td>
<td>180±18 (4)</td>
</tr>
<tr>
<td>Indomethacin-treated dogs</td>
<td>≥50* (3)</td>
<td>≥50 (3)*</td>
</tr>
<tr>
<td>Verapamil-treated dogs</td>
<td>223±6 (3)</td>
<td>195±16 (3)</td>
</tr>
</tbody>
</table>

Data (pg/ml) expressed in mean±SEM. Number in parenthesis represents number of observation, each measurement made in duplicate. *p=0.02 compared with saline-treated animals.
reperfusion arrhythmias, in accordance with some previous studies.33 Furthermore, myocardial neutrophil accumulation and myocardial myeloperoxidase activity were similar in verapamil-treated and untreated dogs. It is noteworthy that verapamil was continued throughout the period of coronary occlusion, reperfusion, and subsequent administration of ACh and BK. The adequacy of calcium blockade was apparent since heart rate decreased and PR interval increased with treatment of animals.

These observations suggest that calcium blockade does not protect against reperfusion-associated acute reduction in coronary vasodilator reserve. While verapamil may modify EDRF-dependent impairment of reactivity of coronary arterial rings,6 this is not evident in the intact animal. Lack of inhibitory effect on myocardial neutrophil infiltration also suggests that verapamil in clinically used dosages does not decrease myocardial neutrophil accumulation. Indeed, recent studies have questioned the presumed benefit of calcium blockers in reducing reperfusion-associated myocardial infarct size.34–36

The vagaries of coronary circulation and the presence of large numbers of collateral vessels in the canine heart are known to result in a variable extent of myocardial injury and function after coronary occlusion and reperfusion.7 The variations in coronary circulation also could have influenced the presence of small number neutrophils in the myocardium supplied by patent LAD in our experiments. However, the number of neutrophils in the LAD region was consistently severalfold smaller (less than 10 neutrophils/high power field) than in the reperfused Cx region. Although it is difficult to rule out the possibility that these few neutrophils may have migrated from the Cx region via collaterals, the myocardial histology in the LAD perfused regions was similar to that in control animals subjected to sham coronary occlusion and reperfusion.

In summary, we have documented attenuation of coronary vasodilator reserve immediately following reperfusion in the intact animals. This alteration in coronary vasodilator reserve is observed in response to both ACh and BK. Intense capillary plugging by neutrophils in the reperfused myocardium suggests that reduced microvascular cross-sectional area may play an important role in blunted response to ACh and BK. However, the precise role of reduction in EDRF or release of endothelium-derived vasoconstrictor substance cannot be excluded as partial explanations of the impaired coronary vasodilator reserve upon reperfusion. Furthermore, pretreatment of the animals with indomethacin or verapamil provides no benefit in this setting.

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34. Smith HJ, Goldstein RA, Griffith JM, Kent KM, Epstein S: Regional contractility and selective depression of ischemic myocardium by verapamil. *Circulation* 1976;54:629-638

**Key Words** • acetylcholine • bradykinin • coronary blood flow • coronary vascular resistance • coronary vascular reserve • indomethacin • verapamil
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