Mechanism of Coronary Vasodilation Produced by Bradykinin

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Background. Bradykinin has been demonstrated to be an endothelium-dependent vasodilator in the cerebral circulation of the mouse, but the actions of bradykinin on regional tissue perfusion in the canine coronary circulation have not been studied.

Methods and Results. The mechanism of coronary vasodilation by bradykinin was studied in open-chest, anesthetized dogs. The role of cyclooxygenase stimulation, bradykinin B2 receptor activation, and endothelium-derived relaxing factor in bradykinin-mediated vasodilation was studied in separate groups of dogs. Bradykinin was infused intracoronarily so as to avoid changes in systemic hemodynamics capable of altering the regional distribution of coronary blood flow (radioactive microspheres). Bradykinin produced a preferential increase in subendocardial blood flow. Pretreatment with indomethacin had no effect on bradykinin-mediated increases in total left ventricular flow or the transmural distribution of coronary blood flow. Blockade of bradykinin B2 receptors with the competitive antagonist [ThiD-Phe]-bradykinin attenuated both the increase in total flow and redistribution of perfusion to the subendocardium produced by bradykinin. Inhibition of endothelium-derived relaxing factor with quinacrine, occlusion/reperfusion, or N6-monomethyl l-arginine attenuated the total increase in left ventricular flow and blocked the redistribution of flow to the subendocardium produced by bradykinin.

Conclusions. The present results demonstrate that intracoronary infusion of bradykinin produces a preferential increase in blood flow to the subendocardium via stimulation of B2 receptors and the release of an endothelium-dependent relaxing factor that may be nitric oxide. (Circulation 1991;83:2048–2056)

Since the original description by Furchgott and Zawadzki1 of the obligatory role of endothelial cells in the vascular relaxation produced by acetylcholine, many other vasoactive compounds have been shown to be dependent on an intact endothelium.2,3 Vascular endothelium has been demonstrated to produce potent vasodilator and vasoconstrictor substances. One of the most potent of the vasodilator substances is endothelium-derived relaxing factor (EDRF), which may be related to nitric oxide.4,5 Previous results from this laboratory6 have demonstrated that intracoronary infusion of the endothelium-dependent vasodilators acetylcholine, ATP, and arachidonic acid produces a preferential increase in subendocardial perfusion, resulting in an increase in the subendocardial/subepicardial blood flow ratio. These compounds stimulated the production and/or release of EDRF via different mechanisms, including specific receptor stimulation for acetylcholine and ATP or changes in membrane fluidity by arachidonic acid.6,7 In contrast, the endothelium-independent vasodilators, nitroprusside or nifedipine, produced uniform increases in blood flow across the left ventricular wall without an increase in the subendocardial/subepicardial blood flow ratio.6,8

Bradykinin has been demonstrated to be an endothelium-dependent vasodilator in vitro9 and in vivo in the cerebral circulation of the mouse,10 but the actions of bradykinin on regional tissue perfusion in the canine coronary circulation have not been studied. The objectives of this investigation were to characterize the direct effects of bradykinin on total coronary blood flow and the left ventricular transmural distribution of perfusion and to ascertain the mechanism of vasodilation by bradykinin. The bradykinin B2 receptor antagonist, [ThiD-Phe]-bradykinin, was used to determine whether vasodilation by bradykinin was receptor-mediated.11 Blockade of cyclooxygenase by indomethacin was used to determine

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the contribution of prostacyclin to increases in coronary blood flow produced by bradykinin, because bradykinin has been demonstrated to stimulate release of prostacyclin. Quinacrine, a phospholipase A2 inhibitor, which has been demonstrated to block endothelium-dependent relaxation, and Nω-monomethyl l-arginine (l-NMMA), a specific inhibitor of EDRF synthesis, were used as pharmacological tools to examine the role of EDRF in the redistribution of myocardial blood flow produced by bradykinin. Physical damage of coronary vascular endothelium produced by total coronary artery occlusion followed by reperfusion was used as an additional technique for investigation of the role of endothelium in bradykinin-mediated vasodilation in vivo.

**Methods**

**Experimental Preparation**

Adult mongrel dogs of either sex, weighing 15–25 kg, were anesthetized with sodium pentobarbital (25 mg/kg) and sodium barbitral (200 mg/kg) and ventilated by a respirator. Left ventricular and aortic pressures were recorded via a 7F dual pressure transducer–tipped catheter (model SPR-277, Millar Instruments, Houston) passed via the left carotid artery into the left ventricle and ascending thoracic aorta, respectively. Peak positive dP/dt was obtained by electronic differentiation of the left ventricular pressure pulse. The right femoral vein and left femoral artery were catheterized for drug administration and for withdrawing of reference arterial blood samples used in determining myocardial tissue blood flow, respectively.

Thoracotomy was performed at the left fifth intercostal space. A distal segment of a small-diameter diagonal branch of the left anterior descending coronary artery was cannulated with a heparin-filled catheter (PE-60) for intracoronary drug infusions. The site of the catheter placement was chosen to perfuse approximately equal regions of the left ventricle in all groups (the drug-perfused mass varied between 26% and 32% of the total left ventricular mass in the seven groups). The tip of the catheter was advanced to the origin of the branch to minimize trauma to the endothelium of the left anterior descending coronary artery. An electromagnetic flow probe (Statham 7515, Gould, Cleveland, Ohio) was also placed around the left anterior descending artery for measurement of coronary blood flow. A silk ligature positioned immediately distal to the flow probe was used to briefly occlude the vessel for production of the reactive hyperemic response. Preparations were not considered acceptable for use unless a 100% increase in flow occurred during the reactive hyperemic response after a 20-second occlusion.

Myocardial segment function (percent segment shortening [%SS]) was measured in subendocardial regions perfused by the left anterior descending and left circumflex coronary arteries using pairs of piezoelectric crystals. Using left ventricular dP/dt, end-systolic length (SL) was determined at −dP/dt max and end-diastolic length (DL) was determined just before the onset of systole. %SS was calculated using the equation: %SS = (SL − SL)/DL × 100. A catheter was placed in the left atrium for injection of radioactive microspheres. Hemodynamics were continuously recorded on a polygraph (model 7, Grass Instrument Co., Quincy, Mass.).

The drug-perfused region was identified by injection of 5–7 ml India ink via the coronary branch catheter at the end of each experiment. In experiments in which a coronary artery occlusion and reperfusion were used, infarcted myocardium was identified by a previously described method. Briefly, at the completion of these experiments, Patent Blue dye was injected into the left atrium to stain normal myocardium dark blue, while saline was simultaneously injected into the left anterior descending coronary artery. In this manner, the ischemic/reperfused area remained unstained. The heart was then removed and sliced transversely from apex to base in sections approximately 1 cm in width. The unstained drug-perfused region was separated from the blue-stained area, and the two regions were separately incubated at 37°C for 15 minutes in 1% 2,3,5-triphenyltetrazolium chloride in 0.1 M phosphate buffer. The triphenyl tetrazolium chloride stained noninfarcted myocardium a brick-red color, indicating the presence of dehydrogenase enzymes, while leaving infarcted tissue unstained. Infarcted tissue was not included for determination of regional myocardial perfusion.

Multiple transmural tissue samples were obtained from both the centers of the left anterior descending (drug-perfused) and left circumflex (normal) regions of all groups. Myocardial blood flow (Qm, in milliliters per minute per gram) in each sample was calculated from the equation:

\[ Q_m = Q_r \cdot C_m/C_r \]

where Qr is the rate of withdrawal (in milliliters per minute) of the reference blood sample, Cm is the true activity (in counts per minute) of the reference blood sample, and Cm is the true activity (in counts per minute per gram) of the tissue specimen. Myocardial blood flow values of tissue samples from the drug-perfused and control areas were pooled for calculation of flow in the subepicardium, midmyocardium, and subendocardium of either region. Transmural myocardial blood flow was the weighted average of flows in the subepicardium, midmyocardium, and subendocardium in drug-perfused or normal zones.

**Experimental Design**

After completion of surgery, sotalol (2.0 mg/kg i.v.) was administered to block myocardial and coronary vascular β-adrenoceptors as previously described. In one group of dogs (n = 12), the effects of cyclooxygenase stimulation by bradykinin were studied. In this group, changes in hemodynamics and
myocardial perfusion produced by bradykinin were measured before and after administration of indomethacin. In the remaining groups, indomethacin (1.5 mg/kg i.v.) was administered to all dogs to block cyclooxygenase stimulation. This dose of indomethacin was sufficient to prevent prostacyclin formation with minimal effects on systemic hemodynamics. After a 30-minute equilibration period, the peak reactive hyperemic response after a 20-second total left anterior descending coronary artery occlusion was obtained. Measurement of reactive hyperemia allowed selection of intracoronary doses of bradykinin that produced submaximal vasodilation of the left anterior descending perfusion territory to avoid non-specific changes in the transmural distribution of coronary flow that occur during maximal vasodilation. The effects of several antagonists were studied in separate groups of dogs (n=7–12). In all experiments, the first radioactive microsphere was administered during intracoronary infusion of drug vehicle (control state). Five-minute intracoronary infusions of bradykinin (1–30 μg/min) were then administered in random order to establish dose–response relations for increases in coronary blood flow. Subsequently, the second microsphere was administered at steady-state flow conditions during a 5-minute intracoronary infusion (6 μg/min) of a dose of bradykinin that produced an increase in coronary flow that was approximately 50% of the peak reactive hyperemic response. The following drugs or interventions were then administered in separate groups of dogs to assess the effects of each antagonist on the response to bradykinin: quinacrine (300 μg/min i.c. for 30 minutes), n=8; [Thi58, D-Phe7]-bradykinin acetate (25 μg/min i.c. for 15 minutes), n=12; l-NMMA (3 mg i.c. during 2-minute occlusion), n=7; normal saline (12 ml i.c. during 2-minute occlusion), n=6; 45-minute left anterior descending coronary occlusion followed by 60 minutes of reperfusion, n=7.

The third injection of radioactive microspheres was made after each of the above interventions to serve as a second control measurement of myocardial perfusion. The fourth radioactive microsphere was injected at steady-state flow conditions during intracoronary infusion of bradykinin at the same dose during which the second microsphere was injected. This allowed assessment of the effects of each intervention on the increase in myocardial perfusion produced by bradykinin.

In the experimental group subjected to 45-minute total left anterior descending coronary artery occlusion, regional segment function was assessed at several intervals during coronary occlusion to document passive systolic thickening as an indicator of the severity of ischemia. Dogs that displayed active shortening during occlusion were excluded from the study. After 45 minutes of occlusion, lidocaine (50 mg i.v.) was administered, and reperfusion was accomplished by gradual release of the occlusion. The third microsphere was administered 15 minutes after the onset of reperfusion.

### Statistical Analysis

All values are reported as mean±SEM. Analysis of variance with a randomized complete block design with four treatments was used to test for main effects. Simple effects were examined with the Waller-Duncan adaptive multiple comparisons procedure. Multiple analysis of variance followed by Bonferroni's modification of the t test was used to analyze differences between groups in control resting coronary flow, the flow response to bradykinin, and peak reactive hyperemia.

### Results

#### Hemodynamics

Six groups of dogs were included in the study and were treated with the following: 1) [Thi58, D-Phe7]-bradykinin; 2) indomethacin, 3) quinacrine, 4) 45-minute occlusion and 60-minute reperfusion; 5) l-NMMA, and 6) normal saline. Left ventricular weights ranged from 98±7 g to 131±13 g. The drug-perfused region was selected to achieve approximately equal-sized areas in all groups. The weight of the drug-perfused region ranged from 25±2 g to 41±5 g, corresponding to 26±1% to 33±4% of the left ventricular mass. The reactive hyperemic re-

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**TABLE 1. Peak Reactive Hyperemic Response After 20-Second Coronary Artery Occlusion in Six Treatment Groups of Dogs**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control response (ml/min)</th>
<th>Postintervention response (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Peak</td>
</tr>
<tr>
<td>Thi,Phe-BK</td>
<td>22±1</td>
<td>84±7†</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>28±3</td>
<td>115±10</td>
</tr>
<tr>
<td>Quinacrine</td>
<td>24±1</td>
<td>123±12</td>
</tr>
<tr>
<td>Occ-Rep</td>
<td>31±5</td>
<td>134±19</td>
</tr>
<tr>
<td>l-NMMA</td>
<td>26±4</td>
<td>93±12</td>
</tr>
<tr>
<td>Sham l-NMMA</td>
<td>24±2</td>
<td>74±8†</td>
</tr>
</tbody>
</table>

*Values are mean±SEM (n=6–12 dogs). Thi,Phe-BK, [Thi58, D-Phe7]-bradykinin; Occ-Rep, 45-minute occlusion and 60-minute reperfusion; l-NMMA, N6-monomethyl l-arginine; Sham l-NMMA, normal saline.

†p<0.05 compared with control peak flow in the same group.

*p<0.05 compared with control peak flow in Occ-Rep group.
response after a 20-second occlusion of the left anterior descending coronary artery was measured before and at the end of each experiment. Baseline and peak mean coronary blood flow data are summarized in Table 1. Inhibition of B₂ receptors and cyclooxygenase with [Thi₅⁸, d-Phe⁷]-bradykinin and indomethacin, respectively, had no effect on resting coronary blood flow or the peak reactive hyperemic response. Inhibition of EDRF with quinacrine or L-NMMA also had no effect on reactive hyperemia. However, coronary artery occlusion followed by reperfusion significantly attenuated the peak reactive hyperemic flow.

In all experimental groups, bradykinin produced dose-related increases in coronary blood flow but no systemic hemodynamic changes. None of the compounds or interventions used to antagonize the effects of bradykinin produced alterations in blood pressure, heart rate, or resting coronary blood flow. [Thi₅⁸, d-Phe⁷]-bradykinin, indomethacin, and quinacrine produced small decreases in peak positive dP/dt and/or regional segment shortening. Occlusion and reperfusion produced large decreases in segment shortening (from 13.2±3.4% to 0.6±2.8%). Changes in segment shortening and/or dP/dt reflected direct negative inotropic properties of the antagonists infused and not a nonspecific deterioration of the preparation, because no effects of L-NMMA or saline treatments (L-NMMA control series) were observed. Indomethacin, quinacrine, occlusion/reperfusion, and saline treatments (L-NMMA control series) had no effect on the bradykinin-mediated increases in total coronary blood flow (Table 2). Bradykinin B₂ receptor blockade with [Thi₅⁸, d-Phe⁷]-bradykinin blocked the total increase in coronary blood flow produced by bradykinin (Figure 1). L-NMMA also attenuated the increase in blood flow produced by bradykinin (Table 2).

**Regional Myocardial Perfusion**

Regional myocardial blood flow data from the normal (left circumflex) and bradykinin-perfused (left anterior descending) regions of all groups are summarized in Tables 3–7. Blood flow to the subepicardium, midmyocardium, and subendocardium of the left circumflex region was unchanged by bradykinin or antagonists of bradykinin. Transmural myocardial blood flow was increased approximately twofold by bradykinin in all experimental groups (Figures 2 and 3). None of the compounds or interventions that were used to antagonize the effects of bradykinin had any effect on resting transmural blood flow.

The increase in blood flow mediated by bradykinin was greatest in the subendocardium in all groups. Indomethacin had no effect on the total increase in flow or redistribution of flow to the subendocardium produced by bradykinin (Figure 2). [Thi₅⁸, d-Phe⁷]-bradykinin blocked both the transmural increase and the redistribution of myocardial perfusion produced by bradykinin (Figure 3). Inhibition of EDRF-mediated vasodilation with quinacrine, L-NMMA, or occlusion/reperfusion attenuated transmural increases in myocardial blood flow and completely blocked the redistribution of blood flow to the subendocardium produced by bradykinin (Figure 3). In a separate series, a saline infusion was used as a vehicle control.

### Table 2. Effects of Bradykinin on Mean Coronary Blood Flow Before and After Treatment With Various Antagonists in Five Groups of Dogs

<table>
<thead>
<tr>
<th>Antagonist group</th>
<th>n</th>
<th>Control</th>
<th>BK</th>
<th>Antagonist</th>
<th>Antagonist+BK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thi,Phe-BK (25 µg/min)</td>
<td>12</td>
<td>22±2</td>
<td>47±3*</td>
<td>22±2</td>
<td>33±3*†</td>
</tr>
<tr>
<td>Indomethacin (1.5 mg/kg)</td>
<td>12</td>
<td>29±3</td>
<td>59±4*</td>
<td>31±3</td>
<td>54±6*</td>
</tr>
<tr>
<td>Quinacrine (300 µg/min)</td>
<td>8</td>
<td>26±2</td>
<td>64±8*</td>
<td>29±2</td>
<td>53±11*</td>
</tr>
<tr>
<td>Occ-Rep</td>
<td>7</td>
<td>32±6</td>
<td>75±10*‡</td>
<td>32±6</td>
<td>64±9*</td>
</tr>
<tr>
<td>L-NMMA (3 mg)</td>
<td>7</td>
<td>20±4</td>
<td>58±7*</td>
<td>29±4</td>
<td>41±6*‡</td>
</tr>
</tbody>
</table>

Values are mean±SEM. BK, infusion of 6 µg/min bradykinin; Thi,Phe-BK, [Thi₅⁸, d-Phe⁷]-bradykinin; Occ-Rep, 45-minute occlusion and 60-minute reperfusion; L-NMMA, N⁶-monomethyl L-arginine.

*p<0.05 compared with control value; †p<0.05 compared with BK response; ‡p<0.05 compared with BK response of Thi,Phe-BK group.

### Table 3. Effects of Bradykinin on Regional Myocardial Blood Flow in the Drug-Perfused Region Before and After [Thi₅⁸, d-Phe⁷]-Bradykinin Administration in Dogs

<table>
<thead>
<tr>
<th>Region</th>
<th>MBF before Thi,Phe-BK (ml/min)</th>
<th>MBF after Thi,Phe-BK (ml/min/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (6 µg/min)</td>
<td>Thi,Phe-BK (25 µg/min)</td>
</tr>
<tr>
<td>Subepicardium</td>
<td>0.97±0.12</td>
<td>1.53±0.18*</td>
</tr>
<tr>
<td>Midmyocardium</td>
<td>0.94±0.12</td>
<td>1.57±0.18*</td>
</tr>
<tr>
<td>Subendocardium</td>
<td>0.95±0.13</td>
<td>3.08±0.26*</td>
</tr>
</tbody>
</table>

Values are mean±SEM (n=12 dogs). MBF, myocardial blood flow; Thi,Phe-BK, [Thi₅⁸, d-Phe⁷]-bradykinin.

*p<0.05 compared with control, †p<0.05 compared with bradykinin.
Bradykinin increased transmural flow to 1.41±0.09 ml/min/g before and 1.51±0.10 ml/min/g after the saline infusion and increased the subendocardial/subepicardial blood flow ratio to 2.38±0.22 before and 2.08±0.23 after the saline infusion, demonstrating that the preparation was responsive to successive infusions of bradykinin over time.

Discussion

The objectives of the present investigation were to characterize the left ventricular transmural distribution of perfusion during an increase in coronary blood flow produced by bradykinin and to determine mechanisms involved in the coronary vasodilation produced by bradykinin. Intracoronary bradykinin was administered to avoid changes in systemic hemodynamics capable of altering myocardial perfusion. Transmural myocardial blood flow was increased by bradykinin with a preferential increase in subendocardial blood flow, resulting in an increase in the subendocardial/subepicardial blood flow ratio. Antagonism of B2 receptors by the competitive antagonist, [Thi3,8, D-Phe2]-bradykinin, blocked the increase in coronary flow and the preferential subendocardial vasodilation produced by bradykinin. Inhibition of cyclooxygenase with indomethacin had no effect. Interventions that inhibited endothelium-dependent vasodilation attenuated the transmural increase in blood flow and blocked the preferential increase in subendocardial flow produced by bradykinin. Thus, bradykinin produced a preferential increase in subendocardial blood flow, presumably via B2 receptor-mediated stimulation of EDRF.

The biological effects of bradykinin, which include endothelium-dependent vasodilation and hypotension, stimulation of nociceptive neurons, increased vascular permeability, and increased intestinal motility and chloride secretion, are mediated by specific membrane receptors.19 Two classes of bradykinin

![Graph showing mean left anterior descending coronary artery blood flow during control (vehicle infusion) and intracoronary bradykinin infusion (3, 6, and 15 ug/min) before (open square) and after (solid square) administration of [Thi3,8, D-Phe2]-bradykinin. *p<0.05 compared with corresponding value after [Thi3,8, D-Phe2]-bradykinin blockade.](image1)

**Table 4. Effects of Bradykinin on Regional Myocardial Blood Flow in the Drug-Perfused Region Before and After Indomethacin Administration in Dogs**

<table>
<thead>
<tr>
<th>Region</th>
<th>MBF before indomethacin (ml/min/g)</th>
<th>MBF after indomethacin (1.5 mg/kg)</th>
<th>Bradykinin+ indomethacin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Bradykinin (6 ug/min)</td>
<td>Indomethacin</td>
</tr>
<tr>
<td>Subepicardium</td>
<td>0.78±0.08</td>
<td>1.16±0.13*</td>
<td>0.78±0.09</td>
</tr>
<tr>
<td>Midmyocardium</td>
<td>0.78±0.07</td>
<td>1.19±0.13*</td>
<td>0.80±0.06</td>
</tr>
<tr>
<td>Subendocardium</td>
<td>0.95±0.07</td>
<td>2.17±0.28*</td>
<td>0.93±0.08</td>
</tr>
</tbody>
</table>

Values are mean±SEM (n=12 dogs). MBF, myocardial blood flow.

*p<0.05 compared with control.
TABLE 5. Effects of Bradykinin on Regional Myocardial Blood Flow in the Drug-Perfused Region Before and After Quinacrine Administration in Dogs

<table>
<thead>
<tr>
<th>Region</th>
<th>MBF before quinacrine (ml/min/g)</th>
<th>MBF after quinacrine (ml/min/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Bradykinin (6 μg/min)</td>
</tr>
<tr>
<td>Subepicardium</td>
<td>0.61±0.06</td>
<td>1.12±0.20*</td>
</tr>
<tr>
<td>Midmyocardium</td>
<td>0.65±0.04</td>
<td>1.49±0.29*</td>
</tr>
<tr>
<td>Subendocardium</td>
<td>0.69±0.04</td>
<td>2.86±0.61*</td>
</tr>
</tbody>
</table>

Values are mean±SEM (n=8 dogs). *p<0.05 compared with control; †p<0.05 compared with bradykinin.

The role of coronary vascular endothelium in the redistribution of myocardial blood flow was investigated using several methods. Quinacrine, a phospholipase A2 inhibitor, has been shown to inhibit endothelium-dependent relaxation in vitro. In addition, a previous investigation from this laboratory has demonstrated that quinacrine attenuated increases in transmural flow and blocked the preferential increase in subendocardial perfusion produced by the endothelium-dependent compounds acetylcholine, ATP, and arachidonic acid in canine myocardium in vivo. In the present study, intracoronary infusion of quinacrine attenuated the increases in transmural flow and blocked the preferential increase in subendocardial blood flow produced by bradykinin. Although quinacrine has a consistent effect on the left ventricular transmural distribution of myocardial blood flow produced by endothelium-dependent vasodilators, it has other pharmacological properties that could interfere with vasodilation, including inhibition of calcium fluxes, antagonism of membrane depolarization, and blockade of Na+-Ca2+ exchange. Thus, a physical method of endothelial cell damage was also used in this investigation to attenuate vasodilation produced by bradykinin and confirm observations made with quinacrine. Previous studies have demonstrated impaired vasodilator responses of canine coronary arteries to acetylcholine and bradykinin in vitro and in vivo after 60 minutes of occlusion and 60 minutes of reperfusion. These authors concluded that endothelial cell damage contributed to the loss of coronary artery responsiveness after occlusion and reperfusion. A recent report by Mehta et al demonstrated preservation of epicardial coronary artery relaxation after occlusion.

TABLE 6. Effects of Bradykinin on Regional Myocardial Blood Flow in the Drug-Perfused Region Before and After Occlusion/Reperfusion in Dogs

<table>
<thead>
<tr>
<th>Region</th>
<th>MBF before Occ-Rep (ml/min/g)</th>
<th>MBF after Occ-Rep (ml/min/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Bradykinin (6 μg/min)</td>
</tr>
<tr>
<td>Subepicardium</td>
<td>0.71±0.07</td>
<td>1.12±0.10*</td>
</tr>
<tr>
<td>Midmyocardium</td>
<td>0.74±0.04</td>
<td>1.58±0.22*</td>
</tr>
<tr>
<td>Subendocardium</td>
<td>0.84±0.05</td>
<td>3.07±0.53*</td>
</tr>
</tbody>
</table>

Values are mean±SEM (n=7 dogs). MBF, myocardial blood flow; Occ-Rep, 45-minute occlusion and 60-minute reperfusion. *p<0.05 compared with control; †p<0.05 compared with bradykinin.

receptors designated as B1 and B2 have been described. Activation of B2 receptors on cultured bovine pulmonary endothelial cells has been demonstrated to be responsible for elevation of intracellular calcium and release of EDRF. In addition, bradykinin has been shown to be a potent vasodilator in isolated human basilar artery via stimulation of B2 receptors. There are no reports characterizing the bradykinin receptors responsible for vasodilation in the canine coronary circulation after stimulation by bradykinin. In the present study, antagonism of B2 receptors with [Thr58, D-Phe7]-bradykinin blocked the total increase in coronary blood flow as well as the redistribution of perfusion to the subendocardium mediated by bradykinin.

Prostacyclin formation in endothelial cells has been shown to be stimulated by many substances that are also capable of releasing EDRF, including bradykinin, acetylcholine, and the calcium ionophore, A23187. Indomethacin prevented bradykinin-stimulated generation of prostacyclin without affecting the magnitude of the vasodilator response in isolated perfused guinea pig hearts, thus indicating that vasodilation mediated by bradykinin was not dependent on prostacyclin release. The role of prostacyclin in bradykinin-mediated vasodilation in the present investigation was tested by measuring the increase in coronary blood flow before and after a dose of indomethacin shown to effectively block cyclooxygenase. There was no difference in the coronary vasodilator action of bradykinin before or after treatment with indomethacin, which is consistent with the results of Stewart and Piper. Thus, the increase in coronary blood flow produced by bradykinin was demonstrated to be independent of prostacyclin.
and reperfusion in vascular rings harvested from animals treated with superoxide dismutase during reperfusion. These authors concluded that endothelial cell damage produced by occlusion and reperfusion was attenuated by removal of superoxide anions during reperfusion.

In the present investigation, a modification of the occlusion/reperfusion techniques of others25-28 was used to study regional myocardial perfusion. The results of this study demonstrate that the distribution of resting coronary blood flow was unchanged after 45 minutes of occlusion and 60 minutes of reperfusion. Since no irreversibly damaged tissue was included in the samples analyzed for regional flow, measurements of tissue flow were limited to tissue previously ischemic but noninfarcted. The preferential subendocardial distribution of blood flow produced by bradykinin was attenuated after occlusion and reperfusion. Thus, the present results support the hypothesis that coronary occlusion followed by reperfusion produced functional damage to vascular intima, which prevented a preferential increase in subendocardial blood flow by bradykinin. Previous observations29 have suggested that reduction in endothelial cell function after occlusion and reperfusion may contribute to a decrease in vascular reserve as estimated by the reactive hyperemic response. In the present investigation, reactive hyperemia after occlusion and reperfusion was significantly reduced, supporting this observation. In contrast, treatment with quinacrine of l-NMMA had no effect on reactive hyperemia. It is possible, however, that higher doses of the pharmacological inhibitors of EDRF may produce a decrease in reactive hyperemia.

An additional pharmacological antagonist of EDRF production was used in the present study to ascertain the nature of EDRF stimulated by intracoronary bradykinin infusion. Nitric oxide has been shown to account for the biological activity of EDRF released from cultured porcine aortic endothelial cells stimulated by bradykinin in a bioassay system.5 Similarly, EDRF released from bovine intrapulmonary artery and vein in response to acetylcholine has identical biological and chemical properties as nitric oxide.6 Moncada et al29 have proposed a pathway for the biosynthesis of nitric oxide from L-arginine within vascular endothelial cells. The release of nitric oxide from endothelial cells in culture and the endothelium-dependent relaxation of rabbit aortic rings were demonstrated to be inhibited by l-NMMA, and this inhibition was reversed by L-arginine.30 L-NMMA has been reported to be a specific inhibitor of nitric oxide generation in vascular endothelium and has been demonstrated to inhibit endothelium-dependent relaxation by 55-76% in bioassay experiments.13 When administered to anesthetized rabbits, l-NMMA caused a significant increase in arterial pressure and inhibited the hypotensive action of acetylcholine without affecting that of the endothelium-indepen-

![Graphs showing transmural blood flow and transmural perfusion gradient](image)

**FIGURE 3.** Bar graphs showing transmural blood flow (panel A) and transmural perfusion gradient (ENDO/EPI, panel B) in the drug-perfused region. L-NMMA, N^6^-monomethyl l-arginine; OCC-REP, 45-minute occlusion and 60-minute reperfusion; THI, PHE-BK, [Thi^5^, D-Phe^7^]-bradykinin; BK, bradykinin infusion; ANTAG, treatment with endothelium-derived relaxing factor antagonist L-NMMA (3 mg), quinacrine (300 μg/min for 30 minutes), or OCC-REP or with bradykinin antagonist THI, PHE-BK (25 μg/min for 15 minutes). Values were measured during control (vehicle infusion), after 6 μg/min BK, after ANTAG, and during BK after ANTAG (ANTAG+BK). *p<0.05 compared with corresponding control value; †p<0.05 compared with corresponding BK value.

**TABLE 7.** Effects of Bradykinin on Regional Myocardial Blood Flow in the Drug-Perfused Region Before and After N^6^-Monomethyl l-Arginine in Dogs

<table>
<thead>
<tr>
<th>Region</th>
<th>MBF before l-NMMA (ml/min/g)</th>
<th>MBF after l-NMMA (ml/min/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Bradykinin (6 μg/min)</td>
</tr>
<tr>
<td>Subepicardium</td>
<td>0.76±0.06</td>
<td>1.21±0.10 *</td>
</tr>
<tr>
<td>Midmyocardium</td>
<td>0.93±0.07</td>
<td>1.72±0.11 *</td>
</tr>
<tr>
<td>Subendocardium</td>
<td>0.92±0.08</td>
<td>2.84±0.18 *</td>
</tr>
</tbody>
</table>

Values are mean±SEM (n=7 dogs). MBF, myocardial blood flow; l-NMMA, N^6^-monomethyl l-arginine.

*p<0.05 compared with control; †p<0.05 compared with bradykinin.
dent vasodilator nitroglycerin. In the present investigation, the effects of L-NMMA on resting coronary blood flow were not directly studied with dose–response relations. However, the increase in transmural blood flow and the preferential subendocardial redistribution of flow produced by bradykinin were antagonized by L-NMMA. Factors other than EDRF may be involved in the increase in total coronary blood flow produced by bradykinin and cannot be totally ruled out by the present experiments. In studies by Amezcue et al and Kelm and Schrader, L-NMMA produced dose-related sustained increases in coronary perfusion pressure in isolated perfused rabbit and guinea pig hearts, respectively. In addition, Chu et al have demonstrated sustained decreases in epicardial coronary artery diameter after infusion of L-NMMA. Measurements of regional myocardial blood flow reflect tissue perfusion rather than large vessel caliber and do not directly contradict the findings of Chu et al. The results of the present study did not address the role of nitric oxide in determining the levels of resting large vessel coronary tone, since L-NMMA was infused locally into the perfusion territory of the left anterior descending coronary artery as opposed to systemic administration in these other studies. Results of the present investigation do support the hypothesis that stimulation of bradykinin B receptors at least partially mediates the release of nitric oxide, which produces a preferential increase in subendocardial perfusion in the canine coronary circulation in vivo.

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References


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**KEY WORDS** • endothelium-derived relaxing factor • bradykinin • nitric oxide • regional myocardial blood flow • vasodilation • coronary circulation • l-arginine
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