Does Preconditioning Protect the Coronary Vasculature From Subsequent Ischemia/Reperfusion Injury?

Barbara Bauer, MD; Boris Z. Simkhovich, MD, PhD; Robert A. Kloner, MD, PhD; Karin Przyklenk, PhD

Background. "Preconditioning" with brief episodes of coronary artery occlusion reduces infarct size caused by subsequent sustained ischemia. However, the effects of preconditioning on the coronary vasculature are poorly understood. We sought to determine whether preconditioning would attenuate "low reflow" (ie, the deterioration in resting myocardial perfusion) and blunt the loss in coronary vasodilator reserve after sustained occlusion/reperfusion in the anesthetized open-chest canine model.

Methods and Results. Thirty-two dogs underwent 1 hour of sustained left anterior descending (LAD) coronary artery occlusion and 4 hours of reperfusion. Each dog was randomly assigned to the preconditioned group (four episodes of 5 minutes of LAD occlusion plus 5 minutes of reperfusion before sustained ischemia) or control group (no intervention). Submaximal vasodilator reserve was determined by measuring the increase in CBF in response to 0.01 mg acetylcholine (an endothelium-dependent dilator) and 0.05 mg nitroglycerin (an endothelium-independent dilator); low reflow was assessed by measurement of regional myocardial blood flow at 30 minutes and 4 hours after reflow; and infarct size was delineated by triphenyltetrazolium staining. In protocol 1 (n=14), vasodilator reserve was measured at baseline and at 30 minutes and 4 hours after reflow. There was no change in the response to acetylcholine and nitroglycerin at 30 minutes after reperfusion compared with baseline. However, all dogs exhibited a loss in vasodilator reserve during the subsequent 3.5 hours of reflow, with no difference between control and preconditioned groups. That is, in control dogs, acetylcholine increased CBF from a baseline value of 10.1±1.3 mL/min to 18.0±2.6, 18.2±2.1, and 15.4±1.7 mL/min before occlusion, 30 minutes after reflow, and 4 hours after reperfusion, respectively (P<.05 for 30 minutes vs 4 hours after reperfusion). Similarly, in the preconditioned group, acetylcholine increased CBF from a baseline value of 12.0±2.9 mL/min to 19.6±3.8, 23.6±5.3, and 15.6±3.5 mL/min, respectively (P<.01 for 30 minutes vs 4 hours after reperfusion; P=NS between groups). In addition, all dogs exhibited low reflow, with no difference between control and preconditioned groups: subendocardial blood flow deteriorated between 30 minutes and 4 hours after reflow, from 0.91±0.20 to 0.40±0.03 mL·min⁻¹·g⁻¹ in control animals (P=.05 for 30 minutes vs 4 hours after reperfusion) and from 1.03±0.25 to 0.35±0.02 mL·min⁻¹·g⁻¹ in the preconditioned group (P<.05 for 30 minutes vs 4 hours after reperfusion). However, all dogs in protocol 1 had small infarcts (3±1% and 2±1% of the risk region in control and preconditioned groups; P=NS), suggesting that control dogs may have been "preconditioned" by the vasodilators. An additional 18 dogs were entered into protocol 2, which was identical to protocol 1 except that acetylcholine and nitroglycerin were given only after reperfusion. In this case, we observed the expected reduction in infarct size in preconditioned dogs vs control dogs (2±1% vs 11±3% of the risk region; P<.01). However, the loss in vasodilator reserve was similar to that observed in protocol 1, with no difference between groups. Subendocardial blood flow at 30 minutes after reperfusion was higher in control animals than in preconditioned dogs (1.84±0.50 vs 0.74±0.08 mL·min⁻¹·g⁻¹; P<.05), but subendocardial flow then deteriorated during the subsequent 3.5 hours to a similar value in both groups (0.55±0.11 and 0.50±0.06 mL·min⁻¹·g⁻¹ in control and preconditioned dogs; P<.05 vs 30 minutes after reperfusion for both groups).

Conclusions. The protective effects of preconditioning do not extend to the coronary vasculature in this canine model: Preconditioning neither prevented the deterioration in resting myocardial perfusion nor blunted the loss in submaximal vasodilator reserve observed between 30 minutes and 4 hours after reperfusion. (Circulation 1993;88:659-672)

Key Words • ischemia • reperfusion • blood flow • acetylcholine • nitroglycerin • vasodilation • endothelium

Numerous recent studies have demonstrated that "preconditioning" the myocardium with brief episodes of coronary artery occlusion significantly reduces infarct size produced by subsequent sustained ischemia.¹⁻¹³ That is, brief episodes of ischemia/reperfusion paradoxically protect the myocytes, by an as yet unidentified mechanism, from a subsequent and more prolonged ischemic insult.

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From the Heart Institute, Hospital of the Good Samaritan and University of Southern California, Los Angeles, Calif.

Correspondence to Karin Przyklenk, PhD, Heart Institute/Research, Hospital of the Good Samaritan, 616 South Witmer St, Los Angeles, CA 90017.
The deleterious consequences of sustained ischemia, however, are not restricted to the myocytes. For example, a mild progressive decrease in resting myocardial perfusion, called “low reflow,” has been observed in viable previously ischemic tissue during the initial hours after restoration of blood flow.14-18 In addition, the ischemic/reperfused region exhibits a progressive decrease in endothelium-dependent and endothelium-independent coronary vasodilator reserve; that is, a reduction in the capacity for increased myocardial blood flow in response to metabolic or pharmacological stimuli.17-30

Do the benefits of ischemic preconditioning extend beyond the myocyte and protect vascular endothelium from injury and dysfunction after subsequent sustained occlusion/reperfusion? With the exception of two previous studies,13,31 virtually all preconditioning protocols have focused on the concept of infarct size limitation; the effects of ischemic preconditioning on the coronary vasculature remain poorly understood. Thus, our objective in the present study was to determine whether ischemic preconditioning would attenuate low reflow and blunt the loss of vasodilator reserve after 60 minutes of sustained coronary occlusion in the anesthetized canine model.

Methods

This study was approved by the Institutional Animal Care and Use Committee of the Hospital of the Good Samaritan (an AAALAC-accredited institution) and conforms to the Position of the American Heart Association on Research Animal Use (Circulation. 1985;71:849).

Surgical Preparation

Fifty mongrel dogs weighing between 16 and 29 kg were lightly sedated with morphine sulfate (15 mg SC) and anesthetized with sodium pentobarbital (30 mg/kg IV). The dogs were then intubated and ventilated with room air. After the left jugular vein was cannulated for administration of drugs and fluid and the carotid artery cannulated for measurement of systemic hemodynamic parameters, the heart was exposed by a left lateral thoracotomy through the fifth intercostal space and suspended in a pericardial cradle. A fluid-filled catheter was inserted in the left atrium for later injection of radiolabeled microspheres for measurement of regional myocardial blood flow (RMBF). A segment of the left anterior descending coronary artery (LAD) was isolated, usually just distal to its first major diagonal branch, for later placement of occlusive vascular clamps, and a second segment of the LAD was isolated for placement of a Doppler ultrasonic flow probe for measurement of mean coronary blood flow (CBF). Arterial pressure and CBF were monitored continuously throughout each experiment with a Gould recorder.

Protocol 1: Vasodilators Administered Before and After Sustained Occlusion

After baseline measurements of hemodynamic parameters, CBF, and vasodilator reserve (the details of which are described below) were obtained, the first 18 dogs in the study were randomly assigned to either the preconditioned group or the control group. Animals in the preconditioned group underwent four repeated 5-minute LAD occlusions, each separated by 5 minutes of reflow. The LAD was then occluded for 1 hour and reperfused for 4 hours. This preconditioning regimen was chosen on the basis of previous studies in the canine model in which four episodes of 5-minute circumflex coronary occlusion effectively limited infarct size caused by a subsequent 40-minute period of sustained circumflex occlusion.1 Dogs in the control group also underwent 1 hour of LAD occlusion and 4 hours of reperfusion but received no intervention during the 40 minutes before sustained ischemia. To control the incidence of lethal arrhythmias, lidocaine (1.5 mg/kg IV) was administered to all dogs before the first brief occlusion (preconditioned group only), before the sustained occlusion, and at the time of sustained reperfusion. Dogs that developed ventricular fibrillation were not resuscitated. Hemodynamic parameters and CBF were monitored during each brief occlusion/reperfusion (preconditioned group only), throughout sustained LAD occlusion, and at frequent intervals after sustained reflow.

Regional myocardial blood flow was assessed by injection of microspheres labeled with 141Ce, 103Ru, or 59Nb. The severity of ischemia in each dog was determined by measurement of RMBF at 30 minutes into the sustained LAD occlusion, and low reflow was assessed by measurement of RMBF at 30 minutes and 4 hours after sustained reperfusion.

Coronary vasodilator reserve was determined at baseline, immediately before sustained LAD occlusion, and at 30 minutes and 4 hours after reperfusion by measurement of the maximum increase in mean CBF in response to injections of acetylcholine perchlorate (an endothelium-dependent dilator; obtained from Sigma Chemical Co) and nitroglycerin (an endothelium-independent dilator; Tridil, obtained from Du Pont Pharmaceuticals Inc). Specifically, 0.01 mg of acetylcholine and 0.05 mg of nitroglycerin were each dissolved in saline and injected in randomized order over approximately 10 seconds into the jugular vein. The doses were chosen on the basis of preliminary experiments, in which we observed a consistent increase in CBF in normal, nonischemic myocardium to approximately 150% to 200% of baseline values. Although these doses of acetylcholine and nitroglycerin did not elicit maximal vasodilation, the response was comparable to that observed in a previous study in our laboratory using the same doses of the dilators administered via an intracoronary catheter.29 The intravenous route of administration was used to ensure that we did not cause brief ischemia (and perhaps precondition the hearts) during insertion of an intracoronary needle or cannula. CBF and hemodynamic parameters were measured before and throughout each injection. Changes in CBF and arterial pressure produced by the first injection were allowed to normalize before the second drug injection was begun.

After 4 hours of reperfusion, the LAD was ligated at the site of previous occlusion, and Uniparse blue pigment (CIBA-GEIGY Inc; 0.25 to 0.5 mL/kg) was injected into the coronary vasculature via the atrial catheter to delineate the in vivo extent of the occluded LAD bed or area at risk (AR). Under deep pentobarbital anesthesia, cardiac arrest was produced by intra-cardiac injection of KCl. The hearts were rapidly ex-
cised and cut into 5 to 7 transverse slices parallel to the atrioventricular groove. The proximal surface of each heart slice was photographed for later measurement of AR. The heart slices were then incubated for 10 minutes in a 1% solution of triphenyltetrazolium chloride at 37°C to distinguish necrotic from viable myocardium. The slices were rephotographed for later determination of the area of necrosis (AN) and then stored in formalin.

Protocol 2: Vasodilators Administered Only After Sustained Occlusion

A recent preliminary report has suggested that administration of acetylcholine before sustained occlusion limits infarct size and essentially mimics the protective effects of ischemic preconditioning on the myocytes by activation of the G protein-coupled to the adenosine A1 and muscarinic cholinergic receptors. Thus, to avoid any confounding "preconditioning" effect of the vaso-active agents per se, we performed the second limb of the study, in which acetylcholine and nitroglycerin were injected only at 30 minutes and 4 hours after reperfusion (ie, not before occlusion).

Twenty-three dogs in protocol 2 were randomized to undergo the same preconditioning or control regimens as described for protocol 1. All dogs again underwent 1 hour of sustained LAD occlusion and 4 hours of reflow. Measurements of hemodynamics, RMBF, AR, and infarct size were performed as described in protocol 1. The flow probe was positioned on the LAD after reperfusion in this second protocol (ie, just before the first challenge with acetylcholine and nitroglycerin); thus, measurements of CBF were not obtained before coronary occlusion.

Protocol 3: Effect of Brief Repeated Coronary Occlusions

To determine whether the preconditioning regimen per se resulted in low reflow or a loss in vasodilator reserve, six dogs were instrumented as described for protocol 1. Each dog then underwent four episodes of 5 minutes of LAD occlusion interrupted by 5 minutes of reflow, followed by 4 hours of sustained reperfusion. The response to acetylcholine and nitroglycerin was assessed at baseline (ie, before brief repeated ischemia) and at 30 minutes and 4 hours after reperfusion. RMBF was measured during the fourth brief occlusion and at 30 minutes and 4 hours after reflow. AR and AN were delineated as described in protocol 1.

Protocol 4: Sham-Operated Controls

To determine whether changes in RMBF or vasodilator reserve were caused in part by prolonged anesthesia or deterioration of the open-chest preparation, three dogs underwent the same instrumentation and regimen as described for the control dogs in protocol 1. In this case, however, the LAD was not occluded. "AR" was not assessed in these animals, but the hearts were incubated in triphenyltetrazolium chloride to confirm the absence of necrosis.

Analysis

Hemodynamics and CBF. Heart rate and arterial pressures (systolic, diastolic, and mean) were measured and averaged over five continuous cardiac cycles in sinus rhythm for each sample period. Mean CBF was also recorded during these same five cardiac cycles.

Vasodilator reserve. The response to acetylcholine and nitroglycerin during each sample period was quantified by measuring the maximum mean CBF (in mL/min) after drug injection. In addition, mean arterial pressure during vasodilator challenge (ie, at maximum CBF) was also recorded.

Area at risk and infarct size. After fixation, right ventricular tissue was trimmed from each heart slice, and the remaining left ventricular tissue was weighed. Photographic images of the heart slices were projected and traced at magnifications of approximately ×2 to ×4. Extent of the AR and AN in each heart slice were quantified by computerized planimetry, corrected for the weight of the tissue slice, and summed for each heart. AN was then expressed as a percentage of the AR.

Regional myocardial blood flow. After the left ventricular weight had been obtained, tissue blocks were cut from the center of the previously ischemic LAD bed and remote, normally perfused circumflex bed and subendocardial, midmyocardial, and subepicardial segments. RMBF was then quantified by the method of Domenech et al. Histology (protocols 1 and 2 only). Additional tissue blocks were cut from the center of the LAD bed and the remote circumflex region. After routine histological processing, 5-μm sections were cut and stained with hematoxylin and eosin and viewed at magnifications of ×20 to ×40. The severity of hemorrhage, intracellular edema, and intermyofibrillar edema in each LAD sample was graded in a blinded fashion by one observer (R.A.K.), and each parameter was assigned a score of 0 (absent), 1 (present but moderate), or 2 (present and severe).

Statistics

Statistical analysis was performed separately for each protocol in the study, and data are presented as group mean±SEM.

Comparisons within groups. For each protocol, the presence of low reflow was assessed within each group by use of the paired t statistic to compare RMBF at 30 minutes vs 4 hours after reperfusion. Similarly, loss of vasodilator reserve within each group was assessed by comparing CBF in response to acetylcholine and nitroglycerin at 30 minutes vs 4 hours after reflow by paired t tests. To determine whether low reflow or loss in vasodilator reserve was caused by hemodynamic alterations, changes in heart rate and mean arterial pressure (both before and during vasodilator challenge) were also assessed within each group at 30 minutes vs 4 hours after reperfusion by paired t tests.

Comparisons between groups. For discrete variables in each protocol (ie, risk region, infarct size), comparisons between the control and preconditioned groups were made by unpaired t tests. For variables measured repeatedly throughout the protocol (response to acetylcholine and nitroglycerin, mean arterial pressure, etc), comparisons between the groups were made at baseline and 30 minutes and 4 hours after reperfusion, and Bonferroni's correction for multiple comparisons was applied to the P values. ANCOVA was used to determine whether the relationship between infarct size and
collateral blood flow differed between control and preconditioned groups. Semiquantitative histological scores were compared by the Mann-Whitney nonparametric test.55

**Results**

**Protocol 1: Dilators Administered Before and After Occlusion**

Of the 18 dogs entered into protocol 1, 3 died of ventricular fibrillation within the first 30 minutes of sustained LAD occlusion (1 control, 2 preconditioned), and 1 preconditioned animal died on reperfusion. Thus, a total of 14 dogs—7 preconditioned and 7 controls—successfully completed protocol 1.

**Hemodynamics.** Heart rate and mean arterial pressure did not differ between the two groups at any time during the protocol (Table 1). Both variables decreased as a function of time during the experiment. However, heart rate did not differ between 30 minutes and 4 hours after reperfusion—that is, the time during which the vascular perturbations were expected to occur. A trend toward a reduction in mean arterial pressure was noted during this time in the control group, but no change in pressure occurred after reperfusion in the preconditioned animals.

**Regional Myocardial Blood Flow and Low Reflow.** Regional myocardial blood flow data were obtained in 11 of the 14 dogs in protocol 1 (Figs 1 and 2). Subendocardial blood flow in the LAD bed during LAD occlusion was similar in control and preconditioned groups: 0.14±0.05 and 0.08±0.03 mL·min⁻¹·g tissue⁻¹, respectively (P=NS; Fig 1, A). Subepicardial RMBF during LAD occlusion, however, was higher in the control group than in preconditioned dogs (P<.05; Fig 1, B).
At 30 minutes after reperfusion, subendocardial RMBF was restored to 0.91±0.20 mL·min⁻¹·g⁻¹ in the control group and 1.03±0.25 mL·min⁻¹·g⁻¹ in the preconditioned animals (P=NS). Blood flow then deteriorated in both groups: at 4 hours after reperfusion, subendocardial RMBF was reduced to 0.40±0.03 and 0.35±0.02 mL·min⁻¹·g⁻¹ in the control and preconditioned animals, respectively (P=.05 and P<.05 vs the corresponding values measured at 30 minutes after reflow by paired t tests; P=NS between control and preconditioned groups; Fig 1, A). Low reflow (albeit less pronounced) was also observed in the previously ischemic subepicardium (Fig 1, B) and did not differ between the control and preconditioned groups. In contrast, RMBF in the normally perfused circumflex bed remained unchanged between 30 minutes and 4 hours of reperfusion in both control and preconditioned animals (Fig 2, A and B).

Low reflow in the LAD bed was also reflected in the measurement of mean CBF (Table 1). CBF did not differ between the two groups at any time during the protocol. However, a significant decrease in CBF was observed in both the control and preconditioned animals between 30 minutes and 4 hours after reperfusion.

Vasodilator reserve. Fig 3 illustrates the progressive decrease in vasodilator reserve after sustained isch-
emia/reperfusion observed in one control animal in the study. Before randomization, injection of acetylcholine increased CBF from a baseline value of 9.5 mL/min to 25.5 mL/min, and challenge with nitroglycerin increased CBF to 15.5 mL/min. At 30 minutes after reflow, the responses to acetylcholine and nitroglycerin remained essentially unchanged. However, at 4 hours after reperfusion, CBF increased to only 17.0 mL/min and 9.6 mL/min in response to acetylcholine and nitroglycerin, respectively.

Table 2 summarizes the mean vasodilator response to acetylcholine for all dogs in protocol 1. The increase in CBF in response to acetylcholine was similar between control and preconditioned groups before randomization and remained unchanged at 30 minutes after sustained reperfusion. A decrease in endothelium-dependent vasodilatation, however, was observed at 4 hours after reflow: maximum CBF in response to acetylcholine averaged 18.2±2.1 vs 15.4±1.7 mL/min at 30 minutes vs 4 hours after reperfusion in the control group (P<.05) and 23.6±5.3 vs 15.6±3.5 mL/min in the preconditioned animals (P<.01). There were no significant differences in the response to acetylcholine between the control and preconditioned groups either before randomization, at 30 minutes after reperfusion, or at 4 hours after reflow.

As expected, intravenous injection of acetylcholine also resulted in systemic vasodilation and a subsequent transient decrease (on the order of 20 mm Hg) in mean arterial pressure. Importantly, however, mean arterial pressure during acetylcholine challenge did not differ between 30 minutes and 4 hours after reperfusion in either the control or preconditioned groups.

Changes in the vasodilator response to nitroglycerin challenge followed a time course similar to those observed with acetylcholine (Table 2). That is, whereas there was no change in endothelium-independent vasodilation at 30 minutes after reperfusion, the response to nitroglycerin decreased during the subsequent 3.5 hours of reflow; specifically, maximum CBF was 15.3±1.8 vs 12.6±1.6 mL/min at 30 minutes vs 4 hours after reflow in control animals (P<.05) and 17.8±4.1 vs 13.6±3.1 mL/min in the preconditioned group (P<.05). The response to nitroglycerin did not differ between the two groups at any time during the protocol.

As anticipated, intravenous injection of nitroglycerin also resulted in transient systemic vasodilation. Mean arterial pressure during nitroglycerin challenge did not differ between 30 minutes vs 4 hours after reflow in control animals but decreased slightly in the preconditioned group (85±5 vs 79±6 mm Hg; P<.05).

Histology. Only one dog in protocol 1 exhibited moderate subendocardial hemorrhage (Table 3). In contrast, edema was evident in 13 of the 14 animals, with no significant difference in either intracellular or interstitial edema between the control and preconditioned groups.

Area at risk and infarct size. AR averaged 17±1% of the left ventricle (or 14.7±1.4 g) in control animals and 18±2% of the left ventricle (or 15.3±3.1 g) in preconditioned dogs and did not differ between the two groups (Figure 4).

To confirm that our preconditioning regimen truly protected the myocytes from subsequent ischemia, infarct size was assessed in all dogs. Surprisingly, however, all dogs in protocol 1 had negligible infarcts, with AN averaging only 3±1% and ≤1% of the AR in the control and preconditioned dogs, respectively (P=NS). Furthermore, when infarct size was plotted as a function of collateral blood flow during sustained LAD occlusion for the 11 dogs in which paired data were obtained (Fig 4, A), the expected inverse relation between these two variables was not seen in the control group; even the most severely ischemic control animals exhibited virtually no necrosis. Infarct size in all dogs was similar to data reported for preconditioned groups in previous studies1,3,4 and considerably

### Table 2. Vasodilator Reserve: Protocol 1 (Dilators Given Before Occlusion)

<table>
<thead>
<tr>
<th>Response to vasodilators</th>
<th>Maximum response to acetylcholine</th>
<th>Maximum response to nitroglycerin</th>
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<tr>
<td></td>
<td>CBF (mL/min)</td>
<td>MAP (mm Hg)</td>
</tr>
<tr>
<td>Control</td>
<td>Before drug injection</td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>10.1±1.3</td>
<td>102±6</td>
</tr>
<tr>
<td>30 Minutes after R</td>
<td>11.3±1.9</td>
<td>96±6</td>
</tr>
<tr>
<td>4 Hours after R</td>
<td>9.3±1.3*</td>
<td>88±4†</td>
</tr>
<tr>
<td>Preconditioned</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>12.0±2.9</td>
<td>106±10</td>
</tr>
<tr>
<td>30 Minutes after R</td>
<td>10.4±1.7</td>
<td>97±8</td>
</tr>
<tr>
<td>4 Hours after R</td>
<td>8.0±1.5‡</td>
<td>97±10</td>
</tr>
</tbody>
</table>

CBF, mean coronary blood flow; MAP, mean arterial pressure; R, reperfusion.

*P<.05; †P<.05; ‡P<.01 vs corresponding values at 30 minutes after reperfusion. No significant difference between control and preconditioned groups at any time during the protocol.

### Table 3. Histological Analysis: Protocol 1 (Dilators Given Before Occlusion)

<table>
<thead>
<tr>
<th></th>
<th>Hemorrhage</th>
<th>Intracellular edema</th>
<th>Interstitial edema</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.14±0.14</td>
<td>1.57±0.20</td>
<td>1.00±0.31</td>
</tr>
<tr>
<td>Preconditioned</td>
<td>0.00±0.00</td>
<td>1.14±0.26</td>
<td>0.57±0.30</td>
</tr>
</tbody>
</table>

0, Absent; 1, present but moderate; 2, present and severe. No significant difference between control and preconditioned groups.
less than the mean AN/AR values of 10% to 20% previously obtained in our laboratory for control dogs subjected to 1 hour of LAD occlusion.4,36,37 This suggests that both our preconditioned and control groups may have been “preconditioned” in protocol 1. Importantly, the similarity in low reflow and vasodilator reserve between the two groups in protocol 1 may reflect this fact.

Protocol 2: Dilators Administered Only After Occlusion

Of the 23 dogs entered into protocol 2, three animals (two control, one preconditioned) died of ventricular fibrillation during sustained LAD occlusion. In addition, one preconditioned animal died during the first brief occlusion, and one preconditioned dog fibrillated at the time of reperfusion. Thus, a total of 18 dogs—7 control and 11 preconditioned—are included in the analysis of protocol 2.

Hemodynamics. Heart rate and mean arterial pressure did not differ between the two groups at any time during protocol 2 (Table 4). As in protocol 1, both variables decreased as a function of time during the experiment. However, in contrast to protocol 1, both groups exhibited a decrease in mean arterial pressure between 30 minutes and 4 hours after reperfusion (P=.05 for the control group and P<.05 for the preconditioned dogs; Table 4).

Regional myocardial blood flow and low reflow. Regional myocardial blood flow data were obtained for all dogs in protocol 2. Control animals in this second limb of the study were severely ischemic during sustained LAD occlusion: subendocardial RMBF was reduced to 0.08±0.03 mL min⁻¹ g⁻¹ tissue⁻¹. In contrast, subendocardial blood flow in the preconditioned group was 0.26±0.08 mL min⁻¹ g⁻¹. Although these two values did not differ statistically (P=.10), 5 of 11 dogs in the preconditioned group (vs 1 of 7 in the control group) had extensive collateral perfusion during sustained LAD occlusion, defined routinely in our laboratory as endocardial RMBF during “ischemia” >0.20 mL min⁻¹ g⁻¹ (Fig 1, C).

When all dogs in protocol 2 were considered, subendocardial RMBF at 30 minutes after reperfusion was restored to 1.84±0.50 mL min⁻¹ g⁻¹ in control animals vs 0.74±0.08 mL min⁻¹ g⁻¹ in the preconditioned group (P<.05). Furthermore, this significant difference in blood flow during early reperfusion persisted when all six dogs with high collateral flow were excluded from analysis (2.05±0.53 vs 0.70±1.2 mL min⁻¹ g⁻¹ in the six control vs six preconditioned dogs that exhibited severe ischemia; P<.05). Thus, in all dogs, subendocardial blood flow measured early after reperfusion was significantly greater in control animals than in those preconditioned before sustained ischemia (Fig 1, C). This difference, however, was limited to the subendoocardium: subepicardial RMBF was similar in both control and preconditioned dogs at 30 minutes after reperfusion (Fig 1, D).

As in protocol 1, all dogs in the second limb of the study exhibited low reflow. Specifically, subendocardial RMBF deteriorated to 0.55±0.11 and 0.50±0.06 mL min⁻¹ g⁻¹ during the subsequent 3.5 hours of reflow in the control and preconditioned groups, respectively (P<.05 vs corresponding value at 30 minutes after reflow for both groups; P=NS between groups). This deterioration in flow was not mediated by the degree of collateral perfusion: when dogs with high collateral flow were excluded, subendocardial RMBF at 4 hours after reperfusion was 0.57±0.13 and 0.53±0.06 mL min⁻¹ g⁻¹ in the control and preconditioned groups, respectively. Thus, despite a difference in flow at 30 minutes after reperfusion, RMBF decreased in all dogs between 30 minutes and 4 hours after reflow and decreased to the same values at 4 hours after reflow in both the control and preconditioned groups.

As in protocol 1, evidence of low reflow was also apparent from the significant decrease in CBF observed in both groups between 30 minutes and 4 hours after reperfusion (Table 4).
Vasodilator reserve. At 30 minutes after reperfusion, CBF in response to acetylcholine injection increased from a predrug value of 15.5±3.4 mL/min to 27.1±5.3 mL/min in control animals and from 16.9±1.9 to 32.6±3.2 mL/min in the preconditioned group (P=NS between groups). However, at 4 hours after reperfusion, maximum CBF in response to acetylcholine was only 20.3±2.3 mL/min in the control group (P<.05 vs 30 minutes after reflow) and 23.9±2.3 mL/min in preconditioned dogs (P<.01 vs 30 minutes after reperfusion; P=NS between groups; Table 5). As was the case with low reflow, the loss in endothelium-dependent vasodilator reserve was not mediated by the severity of ischemia during LAD occlusion: for the 12 dogs with low collateral flow, CBF in response to acetylcholine at 30 minutes vs 4 hours after reperfusion was 27.2±6.3 vs 20.5±2.8 mL/min in control animals and 31.6±2.6 vs 23.7±1.8 mL/min in the preconditioned group.

Similar results were obtained in response to nitroglycerin challenge (Table 5). When all dogs in protocol 2 were considered, the response to nitroglycerin at 30 minutes vs 4 hours after reflow averaged 21.1±3.6 vs 18.2±2.4 mL/min in the control group (P=.07) and 25.7±2.6 vs 21.5±2.4 mL/min in the preconditioned group (P<.01). These results were not altered by exclusion of dogs with high collateral blood flow.

As in protocol 1, injection of acetylcholine and nitroglycerin resulted in transient systemic vasodilation. In control animals, mean arterial pressure during drug injection did not differ significantly for either agent between 30 minutes and 4 hours after reperfusion. However, in the preconditioned group, mean arterial pressure during drug injections decreased slightly but significantly (P<.05) between 30 minutes and 4 hours after reflow.

Most importantly, there were no significant differences in the response to acetylcholine or nitroglycerin between control and preconditioned groups either at 30 minutes after reperfusion or at 4 hours after reflow (Table 5).

Histology. None of the dogs in protocol 2 exhibited evidence of hemorrhage (Table 6). Edema was evident in 17 of 18 hearts, with no significant difference in intracellular or interstitial edema between the two groups.

Area at risk and infarct size. When all dogs in protocol 2 were considered, AN averaged 2±1% of the risk region in the preconditioned group, significantly smaller than the value of 11±3% observed in control animals (P<.01). However, interpretation of these infarct size data is confounded by the high collateral blood flow and smaller risk regions in the preconditioned group vs the

### Table 4. Hemodynamics: Protocol 2 (Dilators Given After Occlusion) (All Dogs)

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Pre-CO</th>
<th>After reperfusion</th>
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<tbody>
<tr>
<td></td>
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<tr>
<td>Heart rate (bpm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>140±6</td>
<td>115±5</td>
<td>110±7</td>
</tr>
<tr>
<td>Preconditioned</td>
<td>157±4</td>
<td>154±6</td>
<td>134±7</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>117±4</td>
<td>123±7</td>
<td>111±8</td>
</tr>
<tr>
<td>Preconditioned</td>
<td>108±5</td>
<td>113±6</td>
<td>104±4</td>
</tr>
<tr>
<td>Mean CBF (mL/min)</td>
<td></td>
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</tr>
<tr>
<td>Control</td>
<td>...</td>
<td>...</td>
<td>15.5±3.4</td>
</tr>
<tr>
<td>Preconditioned</td>
<td>...</td>
<td>...</td>
<td>16.9±1.9</td>
</tr>
</tbody>
</table>

CO, coronary artery occlusion; bpm, beats per minute; ... data not obtained.

*P<.05; †P=.05; ‡P<.01 vs corresponding values at 30 minutes after reperfusion. No significant difference between control and preconditioned groups at any time during the protocol.

### Table 5. Vasodilator Reserve: Protocol 2 (Dilators Given After Occlusion) (All Dogs)

<table>
<thead>
<tr>
<th></th>
<th>Before drug injection</th>
<th>Response to vasodilators</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CBF (mL/min)</td>
<td>MAP (mm Hg)</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>...</td>
<td>117±4</td>
</tr>
<tr>
<td>30 Minutes after R</td>
<td>15.5±3.4</td>
<td>111±8</td>
</tr>
<tr>
<td>4 Hours after R</td>
<td>10.3±1.7†</td>
<td>100±8†</td>
</tr>
<tr>
<td>Preconditioned</td>
<td>...</td>
<td>108±5</td>
</tr>
<tr>
<td>Baseline</td>
<td>...</td>
<td>104±4</td>
</tr>
<tr>
<td>4 Hours after R</td>
<td>12.6±1.1†</td>
<td>93±4*</td>
</tr>
</tbody>
</table>

CBF, mean coronary blood flow; MAP, mean arterial pressure; R, reperfusion; ... data not obtained.

*P<.05; †P=.05; ‡P=.07; §P=.10; ††P<.01 vs corresponding values at 30 minutes after reperfusion. No significant difference between control and preconditioned groups at any time during the protocol.
controls; ie, AR was 15±1% of the left ventricle (or 16.7±1.3 g) vs 21±2% of the left ventricle (or 22.1±2.1 g), respectively; P<.05.

Not surprisingly, all six dogs with high collateral blood flow during LAD occlusion had virtually no necrosis. When these dogs with high collateral flow were excluded from analysis, AR in the remaining 12 dogs was comparable, averaging 21±2% of the left ventricle (or 21.3±2.3 g) and 19±2% of the left ventricle (or 19.2±1.2 g) in the control and preconditioned subgroups, respectively (P=NS). However, as predicted from previous studies, infarct size in the six severely ischemic preconditioned dogs was significantly smaller than that observed in the six severely ischemic controls (3±2% and 13±3% of the risk region, respectively; P<.05). When infarct size was plotted as a function of endocardial blood flow during occlusion for these 12 animals, the expected inverse relationship between infarct size and collateral blood flow was observed for the control group (Fig 4, B). Furthermore, the regression line for the preconditioned animals fell below that for the control group, indicating that, for any level of collateral blood flow, infarct size was smaller in preconditioned animals than in controls. In fact, ANCOVA revealed a significant difference in this relation for control vs preconditioned groups (F=9.39 and P<.05). Thus, in contrast to protocol 1, infarct size in control animals was similar to values previously reported for 1 hour of LAD occlusion in this model,1,36,37 and the preconditioning regimen, as expected, effectively limited infarct size.

Protocol 3: Effect of Brief Repeated Coronary Occlusions

Of the six dogs entered into protocol 3, one died of ventricular fibrillation at the onset of the first brief reperfusion. Thus, data are presented for the remaining 5 dogs (Table 7).

Mean arterial pressure was 116±5 mm Hg at baseline and remained unchanged at 121±5 and 119±4 mm Hg at 30 minutes and 4 hours after reperfusion, respectively.

CBF remained stable throughout the protocol, averaging 16.9±3.4, 17.2±2.9, and 16.5±2.6 mL/min at baseline and 30 minutes and 4 hours after reperfusion, respectively. There was a trend toward a modest decrease in RMBF after sustained reperfusion (ie, subendocardial blood flow in the LAD bed was 0.91±0.04 vs 0.75±0.04 mL·min⁻¹·g tissue⁻¹ at 30 minutes vs 4 hours after reflow, respectively), but this trend was not statistically significant (P<.10).

There was, however, no evidence for a loss in vasodilator reserve after brief repeated occlusion. Injection of acetylcholine increased CBF from a baseline value of 16.9±3.4 mL/min to 29.3±5.6, 29.7±4.8, and 29.5±4.2 mL/min before brief occlusions and 30 minutes and 4 hours after reflow, respectively (P=NS). Similarly, the response to nitroglycerin remained constant throughout the protocol. In addition, mean arterial pressure during injection of acetylcholine and nitroglycerin did not differ between 30 minutes and 4 hours after reperfusion.

AR averaged 20±1% of the left ventricle (or 20.1±1.5 g) in these five dogs. As expected, four episodes of 5 minutes of coronary occlusion did not result in myocyte necrosis.

Protocol 4: Sham-Operated Controls

One sham-operated control was excluded from analysis because of respirator failure (and hypoxia) during the protocol; thus, results are presented from the remaining two animals (Table 8).

Mean arterial pressure in the two sham-operated controls decreased during the protocol, averaging 120±13, 121±4, and 107±0 mm Hg at baseline and 30 minutes and 4 hours after “reperfusion,” respectively.

### Table 6. Histological Analysis: Protocol 2 (Dilators Given After Occlusion) (All Dogs)

<table>
<thead>
<tr>
<th>Hemorrhage</th>
<th>Intracellular edema</th>
<th>Interstitial edema</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.43±0.20</td>
<td>1.71±0.18</td>
</tr>
<tr>
<td>Preconditioned</td>
<td>1.10±0.18</td>
<td>1.00±0.30*</td>
</tr>
</tbody>
</table>

0, Absent; 1, present but moderate; 2, present and severe. No significant difference between control and preconditioned groups (*P=15 vs control).

### Table 7. Vasodilator Reserve and Low Reflow: Protocol 3 (Brief Repeated Coronary Occlusions)

<table>
<thead>
<tr>
<th>Before drug injection</th>
<th>CBF (mL/min)</th>
<th>MAP (mm Hg)</th>
<th>Maximum response to acetylcholine</th>
<th>CBF (mL/min)</th>
<th>MAP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>16.9±3.4</td>
<td>116±5</td>
<td>29.3±5.6</td>
<td>75±2</td>
<td>25.7±4.9</td>
</tr>
<tr>
<td>30 Minutes after R</td>
<td>17.2±2.9</td>
<td>121±5</td>
<td>29.7±4.8</td>
<td>73±2</td>
<td>25.6±4.1</td>
</tr>
<tr>
<td>4 Hours after R</td>
<td>16.5±2.6</td>
<td>119±4</td>
<td>29.5±4.2</td>
<td>72±3</td>
<td>25.3±3.2</td>
</tr>
</tbody>
</table>

Regional myocardial blood flow (mL·min⁻¹·g tissue⁻¹)

<table>
<thead>
<tr>
<th>Ischemic/reperfused LAD bed</th>
<th>Endocardium</th>
<th>Epicardium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fourth LAD occlusion</td>
<td>0.05±0.03</td>
<td>0.17±0.09</td>
</tr>
<tr>
<td>30 Minutes after R</td>
<td>0.91±0.04</td>
<td>1.31±0.16</td>
</tr>
<tr>
<td>4 Hours after R</td>
<td>0.75±0.04</td>
<td>1.03±0.07</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Normally perfused circumflex bed</th>
<th>Endocardium</th>
<th>Epicardium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fourth LAD occlusion</td>
<td>1.11±0.15</td>
<td>0.82±0.09</td>
</tr>
<tr>
<td>30 Minutes after R</td>
<td>1.09±0.09</td>
<td>0.82±0.05</td>
</tr>
<tr>
<td>4 Hours after R</td>
<td>1.06±0.07</td>
<td>0.96±0.09</td>
</tr>
</tbody>
</table>

CBF, mean coronary blood flow; MAP, mean arterial pressure; R, reperfusion; LAD, left anterior descending coronary artery. No significant differences between 30 minutes and 4 hours after reperfusion.
Table 8. Vasodilator Reserve and Low Reflow: Protocol 4 (Sham-Operated Controls)

<table>
<thead>
<tr>
<th></th>
<th>Before drug injection</th>
<th>Response to vasodilators</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CBF (mL/min) MAP (mm Hg)</td>
<td>Maximum response to acetylcholine CBF (mL/min) MAP (mm Hg)</td>
<td>Maximum response to nitroglycerin CBF (mL/min) MAP (mm Hg)</td>
</tr>
<tr>
<td>Baseline</td>
<td>12.5±1.5 120±13</td>
<td>31.3±1.3 103±8</td>
<td>30.7±3.2 106±13</td>
</tr>
<tr>
<td>30 Minutes after “R”</td>
<td>11.8±2.0 121±4</td>
<td>28.9±0.9 99±13</td>
<td>30.8±3.0 99±4</td>
</tr>
<tr>
<td>4 Hours after “R”</td>
<td>14.5±2.6 107±0</td>
<td>29.5±0.3 90±3</td>
<td>28.7±1.9 91±3</td>
</tr>
</tbody>
</table>

Regional myocardial blood flow (mL·min⁻¹·g tissue⁻¹)

<table>
<thead>
<tr>
<th>LAD occlusion</th>
<th>Endocardium</th>
<th>Epicardium</th>
<th>Endocardium</th>
<th>Epicardium</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 Minutes after “R”</td>
<td>0.64±0.19</td>
<td>0.67±0.24</td>
<td>0.79±0.26</td>
<td>0.55±0.14</td>
</tr>
<tr>
<td>4 Hours after “R”</td>
<td>0.63±0.19</td>
<td>0.69±0.27</td>
<td>0.77±0.22</td>
<td>0.69±0.22</td>
</tr>
</tbody>
</table>

CBF, mean coronary blood flow; MAP, mean arterial pressure; “R”, “reperfusion”; LAD, left anterior descending coronary artery; . . . , data not obtained.

Sham-operated animals, however, showed no evidence of low reflow. Subendocardial blood flow for the two dogs remained stable at 0.64±0.19 vs 0.63±0.19 mL·min⁻¹·g⁻¹ at 30 minutes vs 4 hours after “reperfusion.” Similarly, resting CBF did not decrease during this time.

In addition, the sham-operated dogs developed no loss in vasodilator reserve. Injection of acetylcholine increased CBF from a baseline value of 12.5±1.5 mL/min to 31.3±1.3, 28.9±0.9, and 29.5±0.3 mL/min before “occlusion” and 30 minutes and 4 hours after “reflow,” respectively. Similarly, the response to nitroglycerin remained essentially unchanged. Mean arterial pressure during the three repeated drug injections did, however, tend to decrease during the protocol for both acetylcholine (103±8, 99±13, and 90±3 mm Hg) and nitroglycerin (106±13, 99±4, and 91±3 mm Hg).

Not surprisingly, neither sham-operated animal showed any evidence of infarction by triphenyltetrazolium chloride staining.

Discussion

In this study, we report that the well-documented cardioprotective effects of ischemic preconditioning on the myocyte do not extend to the coronary vasculature in this canine model. Despite the expected reduction in infarct size with preconditioning observed in the second limb of our study, preconditioning did not attenuate the decrease in resting myocardial blood flow or the deterioration in submaximal vasodilator reserve observed between 30 minutes and 4 hours of reperfusion in the anesthetized open-chest dog.

Coronary Vasodilator Reserve

Sustained ischemia/reperfusion results in a loss in coronary vasodilator reserve; this has been extensively documented both in isolated arterial rings previously exposed to ischemia and in in vivo models of coronary artery occlusion followed by reperfusion. What duration of ischemia is necessary to precipitate these abnormalities in vascular reactivity after reflow? Although most of these previous studies have involved 1 hour or more of sustained coronary occlusion, even a 15-minute episode of sustained ischemia has been shown to impair vascular reactivity during the initial hours after reperfusion. Results obtained after 10 minutes of sustained coronary occlusion, however, are inconclusive: one study has described subtle abnormalities in coronary reactivity, yet others have found that 10 minutes of sustained ischemia did not alter endothelium-dependent or endothelium-independent vasodilator response.

Interestingly, neither 12 repeated episodes of 5 minutes of coronary occlusion plus 10 minutes of reperfusion nor three episodes of 10 minutes of occlusion plus 30 minutes of reperfusion impaired coronary vasodilator reserve during subsequent sustained reperfusion. Similarly, our data from protocol 3 indicate that four episodes of brief 5-minute coronary occlusion (ie, our preconditioning regimen) did not compromise vascular reactivity. These results indicate that repeated short (<10-minute) episodes of transient ischemia interrupted by transient reflow do not impair vasodilator reserve. Rather, it appears that >10 minutes of sustained ischemia is needed to initiate subsequent abnormalities in vasodilator reserve.

Although there is no doubt that 1 hour or more of sustained ischemia results in a loss in coronary vasodilator reserve, the precise time course of this deterioration in vascular reactivity is uncertain. Impaired vasodilator reserve has, in some cases, been observed early (ie, within the initial 2.5 minutes to 1 hour) after relief of sustained ischemia. In contrast, our present study and others found essentially normal vasodilator reserve during the initial 30 minutes after reperfusion, with deterioration during the subsequent hours after reflow. This difference among studies may be explained by the results of Vanhaecke et al: these authors observed that vasodilator reserve in regions of viable myocardium was preserved at the onset of reperfusion but was depressed at 2.5 hours after reflow; in contrast, in necrotic myocardium, the loss in vasodilator reserve was apparent at the time of reperfusion. Thus, the question of whether abnormalities in vascular reactivity are manifest immediately or progressively after reperfusion may depend on the proportions of necrotic...
and viable myocardium in the ischemic/reperfused territory.

In any case, results obtained in the present protocol are in general agreement with previous observations, in that 1 hour of sustained LAD occlusion in our open-chest canine model consistently resulted in a decrease in endothelium-dependent vasodilation, as assessed by in vivo acetylcholine challenge, at 4 hours after relief of ischemia. We also observed a decrease in the response to nitroglycerin; this attenuated response to an endothelium-independent vasodilator may reflect dysfunction of the vascular smooth muscle, reduction in cross-sectional area of the coronary vascular bed by neutrophil plugging, or compression of the vasculature by intracellular and interstitial edema. Although most previous in vivo studies administered the vasodilators directly into the coronary artery, we observed comparable results with an intravenous route of administration.

Repeated assessment of vasodilator reserve in the intact animal, however, can be confounded by the progressive decrease in arterial pressure (and thus coronary perfusion pressure) that often occurs during prolonged anesthesia. In fact, it could be argued that the decrease in mean arterial pressure between 30 minutes and 4 hours after reperfusion observed in protocol 2 may have contributed to the loss of vasodilator reserve in this limb of the study. Regression analysis, however, did not support this concept: there was no significant relationship between the decrease in mean arterial pressure and the decreased response to either acetylcholine ($r = .31$) or nitroglycerin ($r = .05$). In addition, we observed a deterioration in vasodilator reserve in protocol 1 in the absence of any reduction in arterial pressure, and the sham-operated controls in protocol 4 (not subjected to coronary occlusion) showed a decrease in arterial pressure with essentially no change in the response to vasodilator challenge. Similarly, it is possible that differences in mean arterial pressure during drug injections may also have contributed to the decrease in vasodilator reserve. However, systemic vasodilation during drug injections remained unchanged between 30 minutes and 4 hours after reperfusion in control animals in both protocols 1 and 2 and thus cannot explain the loss in vascular reactivity observed in these groups. Moreover, the greatest reductions in mean arterial pressure during drug infusions were observed in sham-operated controls (protocol 4), yet the responses to acetylcholine and nitroglycerin did not decrease in these animals between 30 minutes and 4 hours after “reperfusion.” The fact that our consistent reductions in vascular reactivity were not accompanied by consistent alterations in mean arterial pressure suggests that the small decrease in perfusion pressure observed in some of our experimental groups did not play a crucial role in our measurement of coronary vasodilator reserve.

Most importantly, we found that preconditioning did not protect against the deterioration in coronary vasodilator reserve after sustained ischemia/reperfusion. In both protocols 1 and 2, the response to acetylcholine and nitroglycerin did not differ between the control and preconditioned groups at any time during the experiment. Furthermore, the responses to acetylcholine and nitroglycerin were similar in both protocol 1 (in which all animals appear to have been “preconditioned”) and protocol 2.

Only one previous study has specifically focused on the effects of ischemic preconditioning on coronary vasodilation. Specifically, DeFily and Chilian used intravital microscopy to visualize and measure arteriolar diameters on the epicardial surface in anesthetized open-chest dogs. The authors reported that 1 hour of coronary occlusion followed by 90 minutes of reflow significantly attenuated the increase in arteriolar diameter in response to serotonin, an endothelium-dependent vasoactive agent. Serotonin-mediated dilation was preserved, however, in dogs preconditioned with a single 10-minute brief ischemic episode.

Since coronary vasodilator reserve at any given perfusion pressure is, in principle, directly proportional to the cross-sectional area of the vascular bed, the reasons for the apparent discrepancy between our measurements of vascular reserve with acetylcholine and the arteriolar diameter measurements with serotonin are uncertain. Obvious differences in the preconditioning regimen and the choice of test agent may in part account for the discrepancy between the two studies. In particular, the vasoactive effects of serotonin are complex: serotonin has been shown to act as a dilator in vessels in which the endothelium is intact, as a constrictor in vessels with damaged endothelium, and appears to have a dose-dependent biphasic effect in conscious dogs without endothelial injury. It should also be stressed that we did not assess maximal coronary vasodilator reserve: differences between control and preconditioned animals might have become apparent at maximal vasodilation. However, conclusive resolution of this discrepancy awaits concurrent measurement of both arteriolar diameters and CBF.

Low Reflow

In addition to the progressive deterioration in coronary vasodilator reserve, reperfusion after sustained ischemia also results in a reduction in resting myocardial perfusion with respect to baseline, preclosure values. This deficit in resting perfusion may be a result of two distinct phenomena: “no reflow” and “low reflow.” Low reflow refers to a mild depression in resting myocardial perfusion (to 65% to 80% of normal values) that occurs in viable myocardium salvaged by reperfusion. In contrast, no reflow refers to an anatomic perfusion defect—that is, tissue fails to reperfuse—after removal of the coronary artery occlusion. No reflow is characterized by a marked reduction in flow to ≤35% of normal values and is associated with severe endothelial injury (often accompanied by hemorrhage). Most importantly, however, no reflow is confined within areas of frankly necrotic myocardium, whereas low reflow occurs in regions of viable myocardium.

One previous study has specifically assessed the effects of preconditioning on resting myocardial perfusion after relief of ischemia. Using the rabbit model, Hale and Kloner reported that transmural myocardial blood flow at 3 hours after reperfusion was significantly higher in preconditioned rabbits than in controls. Infarct size was reduced by preconditioning in this rabbit model (53% vs 30% of the risk region in control vs preconditioned groups), but in contrast to our canine...
model of subendocardial necrosis, the infarct in both control and preconditioned rabbits involved the subendocardial, mid, and subepicardial layers. Thus, the deficit in myocardial perfusion in this rabbit model represents no reflow, and the attenuation in no reflow associated with preconditioning was a secondary consequence of infarct size reduction.¹³

All animals in protocols 1 and 2 of the present study demonstrated a decrease in resting myocardial perfusion in the ischemic/reperfused LAD bed between 30 minutes and 4 hours after reflow. In addition, myocardial blood flow at 4 hours after reperfusion was consistently lower in the ischemic/reperfused LAD bed compared with the normally perfused circumflex bed. The deterioration in flow in the ischemic/reperfused subepicardium clearly represents low reflow. Infarct size even in the most severely ischemic control animals in protocol 2 occupied <25% of the risk region; that is, all infarcts were confined to the subendocardium, and the decrease in subepicardial flow involved only viable tissue. Furthermore, the deterioration in resting myocardial perfusion observed in protocols 1 and 2 was not a consequence of prolonged anesthesia or a reduction in coronary perfusion pressure, since myocardial blood flow in the sham-operated controls remained unchanged between 30 minutes and 4 hours after “reflow.”

In contrast, the deterioration in flow in the ischemic/reperfused subendocardium may represent a mixture of both no reflow (ie, an anatomic perfusion defect within the subendocardial necrosis) and low reflow in the remaining viable tissue. However, the contribution of no reflow in this protocol would appear to be minimal. Although we did not assess the ultrastructure of the myocytes or vasculature in this study, only 1 of the 32 dogs in protocols 1 and 2 showed any histological evidence of hemorrhage (an indicator of vascular injury) within the necrosis. This is in agreement with previous reports that have demonstrated that vascular injury and obstruction, even within necrotic myocardium, occurred only after >60 minutes of ischemia in the canine model.⁴⁶ In addition, if no reflow were important, we would anticipate that in protocol 2, endocardial blood flow would be lower in control animals than in the preconditioned dogs, simply because of infarct size reduction. This, however, was not the case: endocardial blood flow at 4 hours of reperfusion averaged 0.55 and 0.50 mL·min⁻¹·g⁻¹ in the control and preconditioned groups, respectively. Whether the deterioration in subendocardial blood flow represents solely low reflow or a mixture of low reflow and no reflow, the overall conclusion remains unchanged: preconditioning did not attenuate the decrease in resting perfusion observed at 4 hours after reflow in our canine model.

The only significant difference between control and preconditioned animals in this study, aside from the expected reduction in infarct size in protocol 2, was the difference in subendocardial blood flow in this second limb of the study at 30 minutes after reperfusion. Specifically, mean resting blood flow in the subendocardium was two to three times higher in control animals than in the preconditioned group (Fig 1, C). There was no difference in endocardial flow between control and preconditioned groups in protocol 1, presumably because all dogs in the first limb of the study had been “preconditioned.”

Although we did not measure myocardial blood flow during the initial minutes after reperfusion, the data at 30 minutes after reflow may indicate that control animals in protocol 2b exhibited a greater magnitude or duration of hyperemia in the subendocardium. Reactive hyperemia has been attributed, at least in part, to accumulation of adenosine during coronary occlusion and subsequent washout of this potent vasodilator on reperfusion.²⁵,²⁶,⁴⁷ Thus, the increased flow in the control group could reflect prolonged washout of enhanced concentrations of adenosine. However, Kitakaze et al⁴⁸ have recently reported the converse: that is, adenosine release was higher in preconditioned dogs than in controls throughout the initial 40 minutes after relief of sustained ischemia. Our results combined with those of Kitakaze et al raise the possibility that the sensitivity of the coronary vasculature to adenosine might be altered with preconditioning, but this hypothesis obviously requires confirmation. In any case, despite the difference in resting subendocardial blood flow at 30 minutes after reperfusion, there was no difference in the deterioration in flow between the control and preconditioned groups during the subsequent 3.5 hours.

Did Preconditioning Alter Blood Flow During Sustained Coronary Occlusion?

Although the differences did not achieve statistical significance, preconditioned dogs in protocol 2 had higher values of RMBF during sustained coronary occlusion than did the controls. This apparently enhanced flow during sustained occlusion could be interpreted as a beneficial effect of preconditioning. In fact, repeated measurement of RMBF in the rabbit indicated that blood flow in preconditioned animals was higher at 35 minutes than at 2.5 minutes of sustained ischemia in this model.¹³ This, however, does not appear to be the case in the anesthetized dog: repeated measurement of RMBF revealed no increase in collateral blood flow during the initial 40 minutes of sustained coronary occlusion in the canine model.² In addition, RMBF measured during the third brief episode of preconditioning ischemia has been shown to be similar to that measured at 30 minutes into the sustained occlusion.⁴ Furthermore, this “beneficial” effect of preconditioning on RMBF during occlusion did not occur in protocol 1. We therefore conclude that the higher values of RMBF during occlusion in preconditioned dogs in the second limb of the study was simply a consequence of the well-documented variability in collateral blood flow in the canine model.

Did the Vasodilators Given Before Sustained Ischemia ‘Precondition’ the Myocardium?

In our hands, 1 hour of LAD occlusion in the anesthetized open-chest dog typically results in infarct sizes ranging from approximately 40% of the AR (in dogs with essentially no collateral blood flow) to 0% of the risk region (in dogs with extensive collateral perfusion), with a mean on the order of 10% to 20% of the myocardium at risk.⁴,³⁶,³⁷ Preconditioning before 1 hour of occlusion in this canine model significantly reduces infarct size for all values of collateral blood flow, to a mean of approximately 5% of the risk region.⁴ These expected results were observed for control and preconditioned animals in protocol 2. However, an unexpected
observation in this study was the uniformly small infarcts observed in protocol 1, even in the most severely ischemic control animals. These data indicate that evaluation of coronary flow reserve with nitroglycerin and/or acetylcholine before sustained ischemia reduced infarct size to an extent similar to that achieved by preconditioning.

Nitroglycerin given before and during coronary occlusion has been reported to reduce infarct size. However, the brief injections of nitroglycerin given before occlusion in protocol 1, combined with the short half-life of this agent (on the order of 3 minutes), suggest that the observed infarct size reduction was probably not a result of a direct treatment effect of nitroglycerin. Injection of both acetylcholine and nitroglycerin induced a transient increase, followed by a modest transient decrease, in CBF (Fig 3). Cyclic variations in CBF (produced by aggregation and dissolution of a platelet thrombus) before sustained ischemia have been shown by our laboratory to precondition the canine myocardium. In this previous study, however, the dogs were rendered severely ischemic during the flow variations, with endocardial blood flow at the nadir being reduced to a mean of 0.05 mL · min⁻¹ · g tissue⁻¹. Whether the smaller amplitude of coronary flow variation produced by acetylcholine and nitroglycerin, which did not result in profound myocardial ischemia, is sufficient to induce a cardioprotective effect is unknown. Alternatively, a recent preliminary report has suggested that acetylcholine administered before sustained ischemia in the rabbit model mimics the protective effect of preconditioning (that is, reduces infarct size) by activation of the G protein coupled to the A₁ and muscarinic cholinergic receptors.

It was not our objective to assess the role of adenosine, the A₁ receptor, or the G protein as the mechanism(s) for infarct size reduction by preconditioning. Our results from protocol 1 indirectly support the concept that acetylcholine-mediated activation of the G protein may play a role in the protective effect of preconditioning on the myocytes. However, even if this pathway were to play a role in our canine model, it does not protect against low flow or the loss in vasodilator reserve after sustained ischemia/reperfusion.

Summary
Ischemic preconditioning protects the myocytes from a subsequent 1-hour episode of sustained coronary artery occlusion by an as yet unknown mechanism. However, results from the present study indicate that the protective effects of this phenomenon do not extend to the coronary vasculature in the anesthetized open-chest dog: preconditioning neither prevented the deterioration in resting myocardial blood flow nor blunted the loss in submaximal vasodilator reserve observed between 30 minutes and 4 hours after reperfusion in this canine model.

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References
Does preconditioning protect the coronary vasculature from subsequent ischemia/reperfusion injury?

B Bauer, B Z Simkhovich, R A Kloner and K Przyklenk

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