Coronary Cyclic Flow Variations
"Precondition" Ischemic Myocardium

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Background. Repeated brief episodes of myocardial ischemia performed by mechanical clamping of a coronary artery "precondition" the heart and reduce infarct size after a subsequent sustained ischemia. It is not known, however, whether spontaneous episodes of transient ischemia caused by formation of platelet thrombi, which may occur in unstable angina, have a similar cardioprotective effect.

Methods and Results. Therefore, our objective was to determine whether brief spontaneous thrombotic episodes of ischemia/reperfusion could limit infarct size and preserve contractile function following 60 minutes (protocol 1) or 90 minutes (protocol 2) of sustained ischemia and 4-4.5 hours of reperfusion in the canine model. Before the sustained coronary occlusion, dogs underwent a 30-minute "treatment" period consisting of: no intervention (control group), four repeated episodes of 3-minute mechanical occlusion plus 5-minute reperfusion (preconditioned group), or coronary artery stenosis and endothelial injury, resulting in a mean of four spontaneous episodes of cyclic flow variations (CFV group) caused by formation and dislodgment of platelet thrombi. In protocol 1 (60-minute sustained ischemia plus 4.5-hour reperfusion), infarct size was significantly smaller in both the preconditioned and CFV groups compared with controls (3.5 ± 1.4%, * 3.4 ± 2.1%, * and 9.9 ± 2.7% of the myocardium at risk, respectively; *p < 0.05 versus control). In contrast, neither preconditioning nor CFV preserved contractile function: Segment shortening during sustained occlusion was equally depressed at -15% to -20% of baseline values among the three groups and equally stunned at +12% to +18% of baseline during the 4.5 hours of reflow. In protocol 2 (90-minute sustained ischemia plus 4-hour reperfusion), only CFV continued to exert a cardioprotective effect: Infarct size averaged 15.0 ± 4.1%, 7.4 ± 2.5%, * and 16.5 ± 4.4% of the region at risk in the preconditioned, CFV, and control groups, respectively (*p < 0.05 versus control). Contractile function, however, was similar among all three groups both during 90 minutes of sustained occlusion and throughout 4 hours of reperfusion.

Conclusions. We therefore conclude that repeated coronary thrombus formation preconditions the ischemic myocardium: In fact, in contrast to mechanical preconditioning, cardioprotection provided by CFV persisted following 90 minutes of sustained coronary occlusion. However, preconditioning by thrombotic or mechanical occlusion neither preserved myocardial contractile function during sustained coronary occlusion nor prevented stunning after reperfusion. These data raise the possibility that clinical episodes of unstable angina prior to acute myocardial infarction may precondition the ischemic myocardium. (Circulation 1992;85:779-789)

One or more brief episodes of ischemia increase myocardial tolerance to a subsequent sustained ischemic insult: This phenomenon, termed "preconditioning," has now been described in various animal species.1-4 Furthermore, recent clinical evidence suggests that preconditioning may be induced by balloon inflation during angioplasty.5 All of these experimental and clinical reports, however, have involved abrupt mechanical occlusion and reperfusion of the coronary artery using a vascular clamp or angioplasty balloon. It is not known whether spontaneous episodes of transient ischemia caused by the formation and disaggregation of platelet thrombi have a similar cardioprotective effect. As clinical instances of acute myocardial infarction are often preceded by unstable angina, this raises the possibility that human myocardial infarcts might occur within tissue that has been preconditioned by recurrent brief episodes of ischemia.
Extrapolation of the concept of preconditioning to the clinical setting of spontaneous occlusion/reperfusion is, however, confounded by the fact that the progressive occlusion of a stenosed coronary artery by an active thrombus formed upon damaged endothelium is quite different from the sudden mechanical occlusion followed by abrupt reperfusion commonly used in the experimental setting. Activated platelets that adhere to the injured subendothelial layers and aggregate to build up a thrombus release numerous deleterious metabolites (e.g., thromboxane A2, serotonin, platelet activating factor, and leukotrienes) that may exacerbate myocardial injury and worsen myocardial function.6-9 Platelet thrombi may decrease regional myocardial blood flow (RMBF) by repeated embolizations, modify the balance between oxygen supply and demand by coronary vasoconstriction,10,11 activate polymorphonuclear neutrophils by production of 12-hydroperoxy-icosatetraenoic acid,12,13 or stimulate the arachidonic acid pathway leading to the generation of oxygen-derived free radicals that contribute to ischemia/reperfusion injury.14,15 Moreover, a severe coronary stenosis might limit the restoration of flow and reduce the washout of toxic metabolites accumulated in the ischemic myocardium. Therefore, these deleterious effects could negate the protective effect of preconditioning.

Using the anesthetized canine model, our objective was to determine whether brief spontaneous thrombotic episodes of ischemia/reperfusion exert a cardioprotective effect similar to that seen in myocardium preconditioned by mechanical coronary occlusion/reperfusion. We used the model first described by Folts et al16 in which a severe coronary stenosis with endothelial damage results in repeated formation and disaggregation of platelet thrombi. This preparation reproduces some aspects of the pathophysiology of unstable angina such as coronary stenosis, endothelial damage, thrombus formation, and vasoconstriction.16-18 Specifically, we sought to determine whether these spontaneous cyclic flow variations (CFV) could render the ischemic myocardium more tolerant to a subsequent 60- or 90-minute sustained coronary occlusion and preserve myocardial contractile function during sustained occlusion and/or attenuate postischemic dysfunction or "stunning" following reperfusion.

Methods

This study conformed to the American Heart Association position on research animal use.19 followed the University of Southern California Code of Ethics for the Humane Treatment of Animals, and was approved by the Institutional Animal Care and Use Committee of the Hospital of the Good Samaritan.

Surgical Preparation

One hundred six mongrel dogs of either sex, weighing 13-27 kg, were sedated with morphine sulphate (15 mg s.c.), anesthetized with sodium pentobarbital (30 mg/kg i.v.), intubated, and ventilated with room air. Cannulas were inserted into the left jugular vein (for administration of drugs and fluids) and the left carotid artery (for measurement of heart rate and blood pressure and for withdrawal of reference samples for measurement of myocardial blood flow). A thoracotomy was performed in the fourth left intercostal space, and the heart was suspended in a pericardial cradle. A 5F micro-tipped pressure transducer (Millar) was positioned within the left ventricular (LV) cavity by way of the left atrial appendage for measurement of LV pressure and its first derivative, dP/dt. Through the same incision, a cannula was inserted into the left atrium for later injection of radiolabeled microspheres for measurement of RMBF.

One pair of ultrasonic crystals, used to assess regional contractile function, was positioned in the center of the soon-to-be ischemic left anterior descending coronary artery (LAD) bed, as previously described.15 Crystals were inserted by way of small scalp incisions into the midmyocardium at separations of 6-12 mm and oriented parallel to the minor axis of the heart. Regional contractile function, LV dP/dt, arterial, and LV pressures were monitored continuously throughout the experiment on a Gould recorder (Gould Inc., Cleveland, Ohio).

After the crystals were positioned, a segment of the LAD was isolated, usually just distal to its first major diagonal branch. An ultrasonic flow probe was placed around the LAD for continuous measurement of mean coronary blood flow (CBF). The animals were allowed 15 minutes after these surgical procedures to reach steady state.

Experimental Design

Two separate protocols were performed in this study (Figure 1).

Protocol 1 (60-minute sustained occlusion). Fifty-six dogs were randomly assigned into one of three groups: cyclic flow variation (CFV), mechanical preconditioning, and control.

Using the model first described by Folts et al16 for the cyclic flow variation (CFV) group, endothelial injury was induced by gently squeezing the isolated LAD segment with vascular forceps. A plastic micrometric constrictor was then placed around the site of endothelial injury and tightened so that CBF was reduced to approximately 50% of its baseline value. This scenario of coronary artery stenosis with endothelial damage then results in well-documented variations in CBF because of the repeated accumulation and sloughing of platelet aggregates at the stenotic site and dynamic coronary artery vasoconstriction.17,18,20,21 In the current protocol, the stenosis was maintained for 30 minutes, and the number of CFV cycles during this time was recorded. Specifically, a CFV cycle was defined before the onset of the study as a slow decrescendo followed by an abrupt increase in CBF with an amplitude (defined as the difference between the maximum and minimum of CBF) reaching at least 25% of the poststenotic CBF value.21 If spontaneous restoration of flow
did not occur, the constrictor was gently tapped to dislodge the thrombus without altering the degree of stenosis. At 30 minutes after placement of the stenosis, the LAD underwent sustained occlusion regardless of the CBF value at that time. At the time of sustained occlusion, the micrometric constrictor was removed.

Dogs in the mechanical preconditioning group underwent four repeated 3-minute episodes of LAD occlusion (using vascular clamps), each separated by 5 minutes of reperfusion. This regimen was chosen to match the rate of CFV previously observed to occur within a 30-minute period.11,21

Control animals received no intervention during the 30 minutes before the sustained ischemia.

Immediately after the 30-minute treatment period, dogs in all three groups received an intravenous bolus of lidocaine (1.5 mg/kg), and the LAD was abruptly occluded with vascular clamps. All dogs in protocol 1 underwent 60 minutes of sustained ischemia. The LAD was then reperfused for 4.5 hours by removal of the vascular clamps.

Protocol 2 (90-minute sustained occlusion). In this protocol, 50 dogs were randomized to undergo 30 minutes of CFV, mechanical preconditioning, or control (no intervention) as described above. This 30-minute treatment period was then followed by 90 minutes of sustained LAD occlusion and 4 hours of reperfusion.

At the end of each experiment in both protocols, the LAD was reoccluded, and 0.5 mg/kg Unisperse Blue B-PI pigment (CIBA-GEIGY, Hawthorne, N.Y.) was injected into the coronary vasculature by way of the left atrial appendage to delineate the in vivo area at risk. With this technique, the previously nonischemic myocardium appears blue, whereas the previously ischemic myocardium (area at risk) remains unstained. Under deep anesthesia, the hearts were stopped by intracardiac injection of potassium chloride (40 meq) and excised for further analysis.

**Hemodynamics and Contractile Function**

Measurements (heart rate, systolic, diastolic, mean arterial pressure, and segment shortening [SS]) were made at baseline (i.e., before treatment) and immediately before the sustained occlusion in all treatment groups. In the CFV groups, hemodynamics and SS were also measured at the nadir (minimum) of a CFV and just after restoration of flow following dislodgment of the thrombus. In the preconditioned groups, hemodynamics and SS were measured at 1.5 minutes into each brief occlusion and 2.5 minutes into each brief reperfusion period. In all groups, hemodynamics and SS were then monitored throughout the sustained occlusion and at frequent intervals after reperfusion.

**Measurement of Regional Myocardial Blood Flow**

RMBF was assessed by injection of microspheres labeled with either 141Ce, 95Nb, or 103Ru (New England Nuclear). In all dogs RMBF was measured at 30 minutes into the sustained occlusion and at 3 hours after reperfusion. In the CFV groups, myocardial blood flow was measured at the nadir of the second or third CFV. When the CBF assessed by the flow probe did not reach zero at the nadir of the CFV, no attempt was made to measure the RMBF with microspheres. The persistence of a zero CBF during the entire microsphere injection procedure was carefully monitored. In the preconditioned groups, RMBF was assessed during the third brief occlusion of the treatment period.

**Analysis**

**Area at risk and area of necrosis.** After excision, all hearts were cut into five to seven transverse slices, parallel to the atroventricular groove. After right ventricular tissue had been removed, the heart slices were weighed. The proximal surface of each slice was photographed for later measurement of the area at risk. Then, each slice was incubated for 10 minutes in a 1% solution of triphenyltetrazolium chloride at 37°C. This method has been shown to reliably identify necrotic myocardium (which appears pale) from viable myocardium (which stains brick red).22 The slices were then rephotographed. Enlarged projections of these slides were traced for determination of the boundaries of the area at risk and area of
necrosis. Extents of the area at risk and the area of necrosis were quantified by computerized planimetry and corrected for the weight of the tissue slice. Total weights of the area at risk and the area of necrosis were then calculated and expressed as percentages of the total LV weight.

Regional myocardial blood flow. Tissue blocks were cut from the center of the LAD bed and the circumflex bed and subdivided into endocardial, midmyocardial, and epicardial segments. RMBF was then determined by the technique of Domenech et al.21

Hemodynamics and segment shortening. Heart rate and arterial blood pressure were measured and averaged over five continuous cardiac cycles in sinus rhythm for each sample period. LV dP/dt was used to define the timing of the cardiac cycle for segment length measurements with ultrasonic crystals: End-diastolic lengths (EDL) were measured at the onset of the rapid increase in LV dP/dt, whereas end-systolic lengths (ESL) were measured at peak negative LV dP/dt. EDL and ESL were obtained from three well-separated cardiac cycles in each sample period, averaged, and used to compute SS, an index of regional systolic contractile function, defined as SS = [(mean EDL - mean ESL)/mean EDL] × 100%.24 SS during each sample period was normalized and expressed as percentage of the respective baseline values.

Exclusion criteria. Dogs with high collateral blood flow during coronary occlusion and/or a small area at risk were excluded from the final analysis. Specifically, our standard exclusion criteria, established before the onset of the protocol, were values of RMBF in the ischemic endocardium more than 0.20 ml/min/g during sustained LAD occlusion, and/or an area at risk occupying less than 10% of the left ventricle. In the CFV groups, loosening the stenosis was not permitted. In addition, no attempt was made to resuscitate dogs that developed ventricular fibrillation at any time of the experiment.

Statistics

All measurements are expressed as group mean±SEM values. In both protocols, comparisons of RMBF and infarct size among groups were performed by analysis of variance. If significant F ratios were obtained, comparisons between control and treatment groups were then made by two-tailed Dunnett’s test.25 Analysis of covariance, with infarct size (as percentage of the area at risk) as dependent variable and transmural myocardial blood flow as covariate, was used to analyze differences between control and preconditioned or CFV group regression lines. For comparisons of hemodynamics and SS measurements, we used analysis of variance, and paired t tests for comparisons within groups. The incidence of ventricular fibrillation was assessed by Fisher’s exact test. A p value ≤0.05 was considered indicative of a statistically significant difference.

Results

Mortality and Exclusions

Of the 106 dogs entered into both limbs of the study, five were excluded because they did not develop CFV before the sustained occlusion (two in protocol 1 and three in protocol 2). Sixteen dogs were excluded because they had subendocardial blood flow of more than 0.20 ml/min/g during the sustained occlusion (eight in each protocol).

Thirty-four dogs in the study presented ventricular fibrillation and were not resuscitated: six died during CFV or preconditioning, 20 died during the initial 35 minutes of sustained occlusion, and eight died at the onset of reperfusion. When protocols 1 and 2 were considered separately, there was no significant difference in the incidence of ventricular fibrillation among CFV, preconditioned, and control groups. However, when data from both protocols were pooled, the incidence of ventricular fibrillation during sustained occlusion was less in the CFV group (p<0.01) than in the control group. There was no difference among groups in the incidence of ventricular fibrillation upon
reperfusion in either protocol 1 or 2 or in the pooled results from both protocols (Table 1).

Characteristics of Cyclic Flow Variations

As expected, dogs randomized to receive endothelial injury and stenosis during the 30-minute treatment period experienced a mean of four episodes of CFV (range of three to five, and three to six, in protocols 1 and 2, respectively). The stenosis reduced the CBF to an average of 55% (range, 38–68%) and 47% (range, 30–62%) of baseline in protocols 1 and 2, respectively. Although the baseline CBF was slightly higher in protocol 1, the mean amplitude of the CFV was similar in both protocols (72% and 82% in protocols 1 and 2, respectively). As evidenced in Table 2, coronary flow (as measured by flow probe) did not go to zero during each of the four cycles in each dog. However, myocardial blood flow (as measured by microspheres) was only measured at nadir of zero flow cycles (i.e.,, in five of seven dogs in protocol 1 and six of seven dogs in protocol 2; Table 4).

Arterial and LV pressures did not vary during CFV, whereas the reduction in flow was clearly accompanied by a dilatation of the ischemic segment (increased EDL and ESL) and a slight decrease in LV dP/dt (Figure 2). Furthermore, restoration of flow resulted in a rapid resolution of this regional dilatation and return of LV dP/dt to former values (Table 2 and Figure 2).

Results in Protocol 1

Twenty-eight dogs are included in the final analysis of protocol 1: 12 controls, nine preconditioned, and seven that underwent CFV.

Hemodynamics. Heart rate and mean arterial pressure did not differ among groups at any time point of the experiment (Table 3).

Regional myocardial blood flow. In the ischemic LAD bed, no difference in RMBF existed among groups at any time of the experiment.

In the nonischemic circumflex bed, myocardial blood flow was significantly higher in the preconditioned group than in the control group, during both the sustained occlusion ($p<0.01$) and following reperfusion ($p<0.01$). There was no difference in circumflex blood flow, however, between the CFV and control groups (Table 4).

Infarct size. The area at risk (as percentage of the LV weight) was not different among groups: 21.0±1.1% in controls, 21.1±1.5% in CFV, and 19.8±0.7% in preconditioned dogs (Figure 3).

Infarct size in control dogs averaged 9.9±2.7% of the area at risk (Figure 3). As expected, mechanically preconditioned dogs experienced a significant reduction in infarct size: Area of necrosis was reduced to 3.5±1.4% of the risk region ($p<0.05$ versus control). Dogs in the CFV group exhibited a similar reduction in infarct size, to 3.4±2.1% of the risk region ($p<0.05$ versus control).

Figure 4 shows a regression plot of infarct size versus transmural myocardial blood flow for all three

<table>
<thead>
<tr>
<th>TABLE 2. Cyclic Flow Variations</th>
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<tbody>
<tr>
<td>Frequency (30 min⁻¹)</td>
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<tr>
<td>------------------------</td>
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<tr>
<td>Protocol 1: 60-min occlusion</td>
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<tr>
<td>Protocol 2: 90-min occlusion</td>
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</table>

Peak, maximum coronary blood flow after thrombus dislodgment; Nadir, minimum coronary blood flow during thrombus formation. Values are given as mean±SEM.
groups. Data from the control animals exhibited the expected inverse relation between collateral blood flow and infarct size. Both CFV and preconditioned group regression lines were shifted downward compared with the control group, indicating that CFV and mechanical preconditioning limited infarct size irrespective of the collateral blood flow. However, the difference did not reach statistical significance (p=0.16 and p=0.09 versus control, for preconditioned and CFV groups, respectively), probably due to the considerable scatter in the data. However, when data from both treated groups were pooled (as they were both preconditioned and had a similar reduction in infarct size), the difference became significant (F=4.78; p=0.04 versus control).

**Regional contractile function.** All CFV and preconditioned dogs exhibited dyskinesis in the LAD bed during brief episodes of thrombotic or mechanical coronary occlusion: SS averaged \(-17.0\pm6.1\%\) of baseline in the CFV group and \(-16.2\pm3.3\%\) of baseline in the preconditioned group. In addition, both the CFV and preconditioned groups were stunned by the brief episodes of ischemia. Immediately before the sustained occlusion, SS was reduced to \(79.2\pm7.6\%\) of baseline in the preconditioned group (p<0.01 versus controls) and \(32.7\pm15.5\%\) of baseline in the CFV group (p<0.01 versus controls).

All groups were equally dyskinetic throughout the sustained coronary occlusion (Figure 5A), with SS averaging \(-15\%\) to \(-20\%\) of baseline values among the three groups. Furthermore, CFV and preconditioned animals demonstrated the same degree of contractile dysfunction during both the brief ischemic episodes and the sustained occlusion.

In addition, all groups were equally stunned throughout reperfusion (Figure 5A): At 4 hours after reflow, SS averaged only \(18.2\pm15.4\%, 18.5\pm6.4\%,\) and \(12.4\pm13.8\%\) of baseline values in the control, preconditioned, and CFV groups, respectively.

**Results in Protocol 2**

Twenty-three dogs successfully completed this protocol: eight controls, eight preconditioned, and seven that underwent CFV.

**Hemodynamics.** Heart rate and mean arterial pressure did not differ among groups at any time point of the experiment (Table 3).

**Regional myocardial blood flow.** In the ischemic LAD bed, there was no difference among groups at any time of the experiment (Table 4).

**Infarct size.** The area at risk (as percentage of the LV weight) was not significantly different among the groups: \(20.0\pm1.6\%\) in controls, \(21.4\pm1.5\%\) in CFV, and \(19.4\pm1.8\%\) in preconditioned dogs.

### Table 3. Hemodynamics

<table>
<thead>
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<tr>
<td></td>
<td>Baseline</td>
<td>Preocclusion</td>
<td>Occlusion</td>
<td>Reperfusion</td>
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<tr>
<td>60-Min occlusion</td>
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<tr>
<td>Heart rate (bpm)</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Control</td>
<td>154±8</td>
<td>153±6</td>
<td>138±5</td>
<td>122±6</td>
</tr>
<tr>
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<td>162±8</td>
<td>150±6</td>
<td>156±8</td>
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<tr>
<td>CFV</td>
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<td>Mean arterial pressure (mm Hg)</td>
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<td>Occlusion</td>
<td>Reperfusion</td>
</tr>
<tr>
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<tr>
<td>Heart rate (bpm)</td>
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<tr>
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<td>145±7</td>
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<tr>
<td>CFV</td>
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<tr>
<td>Mean arterial pressure (mm Hg)</td>
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<tr>
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<td>110±4</td>
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<td>116±7</td>
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<tr>
<td>CFV</td>
<td>131±9</td>
<td>136±6</td>
<td>132±7</td>
<td>125±5</td>
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</table>

bpm, Beats per minute; CFV, cyclic flow variation group.
No significant difference among groups by analysis of variance. Values are given as mean±SEM.
**Table 4. Regional Myocardial Blood Flow (ml/min/g)**

<table>
<thead>
<tr>
<th>Protocol 1</th>
<th>Brief occlusions</th>
<th>Sustained occlusion</th>
<th>Reperfusion</th>
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<tbody>
<tr>
<td></td>
<td>ENDO</td>
<td>MID</td>
<td>EPI</td>
</tr>
<tr>
<td>60-Min occlusion</td>
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<tr>
<td>Ischemic left anterior descending coronary artery bed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
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<tr>
<td>Preconditioned</td>
<td>0.06±0.01</td>
<td>0.12±0.02</td>
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<tr>
<td>CFV</td>
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<td>0.16±0.04</td>
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<td>Nonischemic circumflex bed</td>
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<tr>
<td>Control</td>
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<td></td>
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<tr>
<td>Preconditioned</td>
<td>1.63±0.13</td>
<td>1.42±0.10</td>
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<tr>
<td>CFV</td>
<td>1.26±0.17</td>
<td>1.06±0.13</td>
<td>0.95±0.09</td>
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</table>

**Protocol 2**

| 90-Min occlusion | Brief occlusions | Sustained occlusion | Reperfusion |
|                 | ENDO | MID | EPI | TRANS | ENDO | MID | EPI | TRANS | ENDO | MID | EPI | TRANS |
| Ischemic left anterior descending coronary artery bed |     |     |     |       |     |     |     |       |     |     |     |       |
| Control |     |     |     |       | 0.08±0.03 | 0.13±0.03 | 0.24±0.06 | 0.16±0.04 | 0.73±0.08 | 0.61±0.04 | 0.92±0.10 | 0.77±0.05 |
| Preconditioned | 0.03±0.02 | 0.07±0.02 | 0.15±0.04 | 0.09±0.02 | 0.06±0.02 | 0.09±0.03 | 0.19±0.05 | 0.12±0.04 | 0.80±0.12 | 0.65±0.09 | 0.79±0.13 | 0.76±0.11 |
| CFV | 0.09±0.04 | 0.18±0.06 | 0.41±0.17 | 0.24±0.10 | 0.08±0.03 | 0.15±0.04 | 0.33±0.08 | 0.19±0.05 | 1.05±0.20 | 0.90±0.14 | 1.37±0.28 | 1.13±0.18 |
| Nonischemic circumflex bed |     |     |     |       |     |     |     |       |     |     |     |       |
| Control |     |     |     |       | 1.67±0.18 | 1.52±0.24 | 1.45±0.15 | 1.55±0.19 | 1.19±0.12 | 1.06±0.10 | 1.17±0.13 | 1.14±0.11 |
| Preconditioned | 1.27±0.13 | 1.10±0.14 | 1.02±0.11 | 1.13±0.12 | 1.41±0.18 | 1.20±0.16 | 1.10±0.15† | 1.23±0.16 | 1.02±0.10 | 0.87±0.09 | 0.89±0.10 | 0.93±0.10 |
| CFV | 1.33±0.19 | 1.06±0.15 | 1.03±0.09 | 1.13±0.13 | 1.25±0.13† | 0.95±0.10* | 0.96±0.13* | 1.04±0.11* | 1.34±0.17 | 1.08±0.12 | 1.10±0.14 | 1.16±0.14 |

ENDO, subendocardial blood flow; MID, midmyocardial blood flow; EPI, subepicardial blood flow; TRANS, transmural blood flow; CFV, cyclic flow variation group.

In the cyclic flow variation groups, myocardial blood flow was measured in five of seven dogs in the 60-minute occlusion protocol and in six of seven dogs in the 90-minute protocol. Values are mean±SEM.

*p<0.01 vs. control, †p<0.05 vs. control.
Infarct size in control dogs averaged 16.5±4.4% of the area at risk. In contrast to protocol 1, infarct size in preconditioned dogs that then underwent 90 minutes of sustained occlusion did not differ from that observed in the control group (15.0±4.1% versus 16.5±4.4% in controls, p=NS). Area of necrosis in the CFV group was, however, significantly reduced compared with the control group (7.4±2.5% versus 16.5±4.4%, p<0.05; Figure 3).

The regression plot of infarct size versus collateral blood flow confirmed that CFV reduced infarct size caused by a 90-minute sustained occlusion: The CFV regression line was shifted downward compared with the control group. However, this difference did not reach statistical significance by analysis of covariance (p=0.18 versus control). Thus, CFV but not mechanical preconditioning still limited infarct size and favorably altered the relation between myocardial necrosis and collateral blood flow when coronary occlusion was maintained for 90 minutes (Figures 3 and 4).

Regional contractile function. In protocol 2, the alterations in myocardial function throughout the experiment were similar to those observed in protocol 1.

All CFV and preconditioned dogs exhibited dyskinesia in the LAD bed during the brief episodes of thrombotic or mechanical coronary occlusion: SS averaged −22.3±7.7% of baseline in the CFV group and −32.8±8.0% of baseline in the preconditioned group (p=NS). Immediately before the sustained occlusion, SS was reduced to 83.1±11.5% of baseline in the preconditioned group (p=NS versus control) and 41.9±15.7% of baseline in the CFV group (p<0.01 versus control).

All groups were equally dyskinetic throughout the sustained coronary occlusion (Figure 5B), with SS averaging −16% to −27% of baseline values among the three groups. Furthermore, CFV as well as preconditioned animals demonstrated the same degree of contractile dysfunction both during the brief ischemic episodes and during the sustained occlusion. As in protocol 1, all groups were equally stunned throughout reperfusion (Figure 5B): At 4 hours after reflow, SS averaged only 12.5±10.8%, 5.2±11.4%, and 21.1±14.3% of baseline values in the control, preconditioned, and CFV groups, respectively (p=NS).

Discussion

In the present study, we observed that CFV reduced infarct size produced by 60 and even 90 minutes of sustained ischemia. In contrast, mechanical preconditioning only protected the myocardium for 60 minutes, but not 90 minutes, of subsequent coronary occlusion. Second, we found that the protective effect of CFV or preconditioning was not associated with improved contractile function. Brief
mechanical or thrombotic occlusions neither preserved function during subsequent 60- or 90-minute sustained ischemia nor attenuated stunning during the first 4 hours of reperfusion.

**Incidence of Ventricular Fibrillation**

Preconditioning has been shown to limit the incidence of arrhythmias in the rat model. Mechanical preconditioning did not, however, provide protection from ventricular fibrillation in our study compared with control, which confirms previous studies in this canine model. In contrast, CFV dogs appeared to exhibit significantly less ventricular fibrillation than control dogs during the sustained occlusion (but not during reperfusion). The effect of preconditioning on arrhythmias in large animal models remains uncertain, because, in part, of the limited sample sizes and the fact that these studies have not been designed to monitor or assess arrhythmias. Although our study suggests some protective effect against ventricular fibrillation in CFV dogs, further specifically designed studies are needed to confirm any beneficial effect of preconditioning on lethal ventricular arrhythmias in large animals.

**Infarct Size Limitation**

One major finding in this study is that CFV protect, or precondition, the ischemic myocardium. CFV dogs experienced a significant reduction in infarct size after the 60 minute sustained occlusion that could not be explained by differences in area at risk, collateral blood flow, or hemodynamics. Blood flow in the nonischemic circumflex bed probably did not mediate this protective effect as, in protocol 1, both CFV and preconditioned groups demonstrated a reduction in infarct size, whereas only preconditioned nonischemic myocardial blood flow was different from control.

It is especially interesting to note that CFV limited infarct size despite three potentially deleterious elements inherent in this scenario: thrombus formation, coronary stenosis, and endothelial injury. Coronary vasoconstriction and obstruction of part of the coronary microvasculature by platelet emboli are known to occur in this model. These factors, as well as the deleterious metabolic sequelae known to be activated during the thrombotic process (i.e., arachidonic acid metabolism and serotonin production) did not alter or counteract the protective effect of ischemic preconditioning.

Neither did the presence of a severe coronary stenosis during the brief ischemia/reperfusion prevent the reduction in infarct size. The limited blood flow through the ischemic myocardium was likely sufficient to prevent any accumulation of toxic metabolites (lactate, H+ ions). In fact, the persistence of the protective effect in the presence of a severe coronary stenosis raises a further question: Is severe ischemia (i.e., total coronary occlusion) mandatory to cause preconditioning? It is possible that a partial coronary stenosis may be sufficient to induce tolerance to a subsequent sustained ischemic insult. Although RMBF was not measured just after the creation of the coronary stenosis, data from other studies demonstrate that a 50% reduction in CBF results in moderate ischemia. This is supported by the observation that all CFV dogs demonstrated a significant increase in EDL and ESL following placement of the stenosis.

Finally, the role of the endothelial injury must also be considered. The endothelium was damaged at the site of coronary stenosis to trigger adhesion of platelets to subendothelial layers and initiate thrombus
formation. Any possible reduction in endothelium-derived vasoactive substances due to endothelial injury was likely too limited to interfere with preconditioning, because the vessel wall damage was limited to the site of coronary stenosis whereas the remaining coronary vasculature was intact.

Thus, in spite of potential deleterious consequences, the existence of an active thrombotic process at the site of severe coronary artery stenosis with local endothelial damage did not negate the protective effect of preconditioning. This raises the possibility that unstable angina may provide some tolerance to subsequent myocardial ischemia.

An unexpected finding of this study was that myocyte death was limited following 90 minutes of sustained occlusion in animals that underwent CFV, whereas it was not in mechanically preconditioned dogs. Our results in the preconditioned group confirm a report by Nao et al, demonstrating that mechanical preconditioning reduces infarct size after 60 minutes but not 90 minutes of sustained ischemia. However, the reasons for the persistence of the protective effect after 90 minutes of sustained occlusion in the CFV group are not clear.

CFV dogs may have been less ischemic during the treatment period. Myocardial blood flow at the nadir of the CFV tended to be higher than myocardial blood flow during brief occlusions in the preconditioned group, although this trend did not reach statistical significance. Moreover, the average duration of zero CBF ischemia in CFV dogs was approximately 2 minutes 30 seconds versus 12 minutes in the preconditioned dogs. However, previous studies have demonstrated that different durations of brief occlusions provided the same final beneficial effect in terms of reduction in infarct size. Also, the persistence of a protective effect of CFV after 90 minutes of ischemia may merely reflect a slight biological variability in the rate of dissipation of the preconditioning effect. As the mechanism of the protection afforded by CFV was not specifically addressed in this study, resolution of this issue awaits further investigation.

**Regional Contractile Function**

Although numerous studies have confirmed that brief episodes of transient ischemia limit infarct size produced by subsequent prolonged occlusion, the effects of preconditioning on contractile function are unknown.

Despite a significant reduction in infarct size, the previously ischemic but still viable myocardium (where ultrasonic crystals were inserted) of both CFV and preconditioned dogs demonstrated the same dysfunction during the sustained occlusion and the same stunning after the following 4-hour reperfusion as control dogs. This suggests that the mechanism that acutely limits myocyte death does not preserve contractile function of the remaining salvaged myocytes either during the sustained occlusion or during the early phase of reperfusion. However, the fact that we did not observe any difference among groups during the first hours of reflow does not preclude the possibility that function in the CFV or preconditioned hearts might recover at a faster rate in the following days or weeks.

Murry et al have demonstrated that preconditioning is likely not a consequence of myocardial stunning because the cardioprotective effect is largely attenuated when the time delay between brief and sustained occlusions is extended to 2 hours, even though severe stunning persisted. Schott et al recently demonstrated in the swine myocardium that the ischemic tolerance is independent of modifications in coronary oxygen demand associated with stunning. Finally, Iwamoto et al reported that the beneficial effect of preconditioning was not attenuated by oxygen-derived free radicals scavengers, which have consistently been shown to improve stunned myocardium. Our study further supports the concept that the mechanisms of stunning and preconditioning are likely not related.

**Conclusion**

This study demonstrates that repeated constitution and dislodgment of a thrombus upon a severely stenosed and endothelially injured coronary artery effectively precondition the ischemic myocardium. Although the mechanism for this protection is unclear, we found that the tolerance to ischemia provided by coronary CFV appears even more prolonged than that induced by classic mechanical preconditioning.

While brief occlusion/reperfusion in both the CFV and preconditioned groups could limit infarct size, this was not accompanied by preservation of myocardial function during occlusion or attenuation of stunning during the first 4 hours of reperfusion. This suggests that the mechanisms of impairment of contractile function and of the preservation of myocyte viability may be unrelated.

Although any extrapolation of these data to clinical instances of ischemia/reperfusion must be made with caution, this study suggests that unstable angina may precondition the ischemic myocardium but may not protect myocardial function following subsequent sustained ischemia.

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