Regional Ischemic ‘Preconditioning’ Protects Remote Virgin Myocardium From Subsequent Sustained Coronary Occlusion

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Background. One or more brief episodes of coronary artery occlusion protect or “precondition” the myocardium perfused by that artery from a subsequent episode of sustained ischemia. We sought to determine whether ischemic preconditioning protects only those myocytes subjected to brief coronary occlusion or whether brief occlusions in one vascular bed also limit infarct size and/or attenuate contractile dysfunction in remote virgin myocardium subjected to subsequent sustained coronary occlusion.

Methods and Results. In the preliminary limb of the study, six anesthetized dogs underwent four episodes of 5-minute circumflex branch occlusion plus 5-minute reperfusion, followed by 1 hour of sustained left anterior descending coronary artery occlusion and 4.5 hours of reflow. Subendocardial blood flow during left anterior descending coronary artery occlusion (measured by injection of radioabeled microspheres) was 0.07±0.03 mL·min⁻¹·g tissue⁻¹, similar to the value of 0.07±0.02 mL·min⁻¹·g⁻¹ observed in a group of eight concurrent control dogs. However, infarct size (assessed by triphenyltetrazolium staining) in the circumflex preconditioned group averaged 4±1% of the myocardium at risk, significantly less (p<0.05) than the value of 13±4% observed in the concurrent controls. An additional 18 dogs were then randomized to undergo either four episodes of circumflex branch occlusion (n=8) or no intervention (n=10) before 1 hour of left anterior descending coronary artery occlusion and 4.5 hours of reflow. Subendocardial blood flow averaged 0.08±0.02 versus 0.08±0.03 mL·min⁻¹·g⁻¹ in the control versus circumflex preconditioned groups, yet infarct size was significantly smaller in circumflex preconditioned dogs than in the controls (6±2% versus 16±5% of the risk region; p<0.05). At 4.5 hours following reperfusion, segment shortening in the left anterior descending coronary artery bed (assessed by sonomicrometry) averaged −21±19% of baseline in control animals versus 13±12% of baseline in the preconditioned group (p=NS). Circumflex preconditioning did not, however, have an independent beneficial effect on contractile function: Regression analysis revealed that the trend toward improved function in circumflex preconditioned dogs reflected the smaller infarct sizes in this group.

Conclusions. Brief episodes of ischemia in one vascular bed protect remote, virgin myocardium from subsequent sustained coronary artery occlusion in this canine model. These data imply that preconditioning may be mediated by factor(s) activated, produced, or transported throughout the heart during brief ischemia/reperfusion. (Circulation 1993;87:893–899)

Key Words • myocardial ischemia • reperfusion • myocardial infarction • contractility

One or more brief episodes of coronary artery occlusion protect or “precondition” the myocardium perfused by that artery from a subsequent episode of sustained ischemia. Several laboratories have now reported that brief occlusions of the circumflex coronary artery limit infarct size caused by subsequent sustained circumflex occlusion; similarly, brief occlusions of the left anterior descending coronary artery (LAD) protect the LAD bed from subsequent prolonged ischemia.5,6 We sought to determine, using the anesthetized canine model, whether ischemic preconditioning protects only those myocytes subjected to brief coronary occlusion or whether brief occlusions in one vascular bed also limit infarct size and/or attenuate contractile dysfunction in remote virgin myocardium subjected to subsequent sustained coronary occlusion.

Methods

This study was approved by the Institutional Care and Use Committee of the Hospital of the Good Samaritan (an AAALAC-accredited institution) and conforms with the position of the American Heart Association on research animal use (Circulation 1985;71:849).

Preliminary Protocol

Six mongrel dogs weighing 18±2 kg were lightly sedated with morphine sulphate (15 mg s.c.), anesthetized with sodium pentobarbital (30 mg/kg i.v.), i.n. -
bated, and ventilated with room air. After the left jugular vein and the left carotid artery were cannulated, the heart was exposed through a left lateral thoracotomy and suspended in a pericardial cradle. A fluid-filled catheter was inserted into the left atrium for later injection of radiolabeled microspheres ($^{111}$Ce, $^{103}$Ru, or $^{99}$Nb) for measurement of regional myocardial blood flow (RMBF). A segment of the main LAD was then isolated, usually just distal to its first major diagonal branch. In addition, the first and/or second anterior marginal branch(es) of the circumflex artery was also isolated near its origin from the main vessel. Specifically, if the first branch (i.e., the branch closest to the origin of the main circumflex) was small and had no bifurcations, the second branch was isolated; if both branches were small and had no bifurcations, then both branches were isolated. This was a subjective decision made at the time of surgical preparation in an effort to ensure that sufficient tissue would be available to measure RMBF and confirm ischemia during circumflex branch occlusion.

All dogs in the preliminary study underwent repeated brief occlusions of the circumflex branch(es) followed by sustained LAD occlusion. Specifically, the circumflex branch was occluded four times for 5 minutes, each occlusion separated by 5 minutes of reperfusion. After administration of lidocaine (1.5 mg/kg i.v. to control the incidence of lethal arrhythmias), the LAD was occluded for 1 hour and reperfused for 4.5 hours. RMBF was assessed during the third circumflex branch occlusion and at 30 minutes into the sustained LAD occlusion.

After 4.5 hours of reflow, the LAD was ligated at the site of previous occlusion, and Unisperse Blue pigment (0.25–0.5 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. With the dog under deep pentobarbital anesthesia, cardiac arrest was produced by intracardiac injection of KCl. The hearts were rapidly excised and cut into five to seven transverse slices parallel to the atrioventricular groove. The basal surface of each heart slice was photographed for later measurement of area at risk. 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 repographed for later determination of the area of necrosis and stored in formalin. Infarct size was then compared with data obtained from eight control dogs that underwent 1 hour of LAD occlusion and 4.5 hours of reperfusion as part of a concurrent protocol in our laboratory.

**Randomized Protocol**

An additional 18 dogs weighing 19±2 kg were sedated, anesthetized, and instrumented as described in the preliminary protocol. As before, the decision to isolate the first and/or second circumflex branch(es) was made for each dog at the time of surgical preparation. Each dog was then further instrumented with a microtipped pressure transducer positioned within the left ventricular cavity via the left atrial appendage for measurement of left ventricular pressure and left ventricular dP/dt. In addition, regional contractile function was monitored in the center of the LAD bed: One pair of ultrasonic crystals was positioned at a depth of 2–4 mm and aligned parallel to the minor axis of the heart for measurement of segment shortening.

After obtaining baseline measurements of hemodynamics and regional wall motion, each dog was randomly assigned to undergo either circumflex preconditioning (four repeated episodes of 5 minutes of circumflex branch occlusion plus 5 minutes of reperfusion as described previously; n=8) or no intervention (control group; n=10). Each dog then received lidocaine (1.5 mg/kg i.v.) and underwent 1 hour of LAD occlusion and 4.5 hours of reperfusion.

Hemodynamics and segment shortening were assessed during each circumflex occlusion/reperfusion sequence (in preconditioned dogs only), immediately before and during LAD occlusion, and throughout reperfusion. RMBF was measured both during the third brief circumflex occlusion (or at 30 minutes into the “no intervention” period in control animals) and at 30 minutes into the sustained LAD occlusion. At 4.5 hours after reperfusion, the animals were killed, and area at risk and area of necrosis were delineated as described in the preliminary protocol.

**Analysis**

**Area at risk and area of necrosis (both protocols).** After fixation, right ventricular tissue was trimmed off each heart slice, and the slices were weighed. Photographic slides of the heart slices were projected and traced. Extents of the area at risk and area of necrosis in each slice were then quantified by computerized planimetry, corrected for the weight of the tissue slice, and summed for each heart. For the randomized limb of the study, area of necrosis and area of risk were measured for all dogs at the completion of the protocol, without knowledge of the treatment group.

**Regional myocardial blood flow (both protocols).** Blocks of tissue weighing 2–3 g were cut both from the center of the LAD bed and immediately distal to the site of circumflex branch occlusion (i.e., at the base of the heart), and subdivided into subendocardial, midmyocardial, and subepicardial segments. RMBF was then quantified using the standard technique of Domenech et al.³

**Hemodynamics (randomized protocol).** Heart rate, mean arterial pressure, and peak positive left ventricular dP/dt were measured and averaged over five continuous cardiac cycles in sinus rhythm for each sample period.

**Segment shortening (randomized protocol).** Correct placement of the ultrasonic crystals (in the center of the LAD bed and within the salvaged subepicardium) was confirmed for each dog. Segment length or separation of the crystals at end diastole (EDL measured at the onset of the rapid rise of left ventricular dP/dt) and end systole (ESL measured at peak negative dP/dt) were averaged from three well-separated cardiac cycles in each sample period. Segment shortening, defined routinely as [(mean EDL–mean ESL)/mean EDL]×100%,⁶ was then calculated for each sample period and expressed as a percentage of the normal baseline value measured before randomization.

**Statistical Analysis**

For the randomized limb of the study, t tests were used to compare area of necrosis, area at risk, and
RMBF between the circumflex preconditioned and control groups. In addition, differences in the relation between infarct size and collateral blood flow for the two groups were evaluated by ANCOVA. Hemodynamic parameters and segment shortening were compared by t tests at baseline, before LAD occlusion, at 60 minutes into LAD occlusion, and at 30 and 270 minutes after reperfusion, and Bonferroni’s correction for multiple comparisons was applied to the probability values. Segment shortening in the circumflex preconditioned group during the fourth circumflex occlusion and fourth brief reperfusion was compared with baseline values by paired t test followed by Bonferroni’s correction.10

Infarct size and RMBF data for the nonrandomized preconditioned dogs were compared by t test with the cohort of concurrent controls, but, with the exception of a pooled ANCOVA calculation, the nonrandomized data are not included with the randomized data in the statistical analysis.

Results

Preliminary Protocol

All six dogs in the preliminary limb of the study experienced ischemia in the circumflex bed during circumflex branch occlusion. Specifically, RMBF in the circumflex bed during the third circumflex branch occlusion averaged 0.20±0.06, 0.20±0.06, and 0.42±0.05 mL·min⁻¹·g tissue⁻¹ in the subendocardial, midmyocardial, and subepicardial layers, respectively. The LAD bed, however, remained normally perfused during circumflex branch occlusion, with RMBF averaging 0.95±0.08, 0.95±0.07, and 1.06±0.12 mL·min⁻¹·g⁻¹ in the subendocardial, midmyocardial, and subepicardial layers, respectively.

All six dogs exhibited ischemia in the LAD bed during LAD occlusion, i.e., mean subendocardial RMBF was 0.07±0.03 mL·min⁻¹·g tissue⁻¹. This was similar to the subendocardial flow of 0.07±0.02 mL·min⁻¹·g⁻¹ in the eight concurrent controls.8 However, infarct size in the six circumflex preconditioned dogs averaged only 4±1% of the myocardium at risk, significantly less (p<0.05) than the value of 13±4% observed in the concurrent control group.8 Based on these observations, we proceeded with the randomized protocol.

Randomized Protocol

Heart rate, mean arterial pressure, and left ventricular dP/dt were similar in the circumflex preconditioned and control groups throughout the protocol (Table 1).

Dogs in the circumflex preconditioned group exhibited ischemia in the circumflex bed during circumflex branch occlusion: Subendocardial RMBF distal to the circumflex branch occlusion was 0.23±0.06 mL·min⁻¹·g⁻¹. This value (and the value of subendocardial RMBF obtained during circumflex branch occlusion in the preliminary protocol) is higher than might be expected with occlusion of the main circumflex artery: As the volume of tissue rendered ischemic by circumflex branch occlusion was small and not delineated by dye injection, it is possible in some cases that samples obtained for measurement of RMBF may have included adjacent nonischemic or border zone tissue. In any case, this value of 0.23±0.06 mL·min⁻¹·g⁻¹ in the circumflex preconditioned group was significantly lower than the value of 1.12±0.07 mL·min⁻¹·g⁻¹ observed in the subendocardium of the circumflex bed in the control animals (p<0.0001; Table 2). Neither group experienced ischemia in the LAD bed during circumflex branch occlusion. Importantly, both groups were equally ischemic during the subsequent LAD occlusion, with subendocardial RMBF in the LAD bed during LAD occlusion averaging 0.08±0.02 and 0.08±0.03 mL·min⁻¹·g⁻¹ tissue in control and preconditioned dogs, respectively (p=NS; Table 2).

The extent of the LAD risk region averaged 23±2% and 19±2% of the left ventricular in the control and preconditioned groups, respectively (p=NS). In control animals, 16±5% of the myocardium at risk became infarcted. In contrast, infarct size in the circumflex preconditioned group was significantly smaller, averaging 6±2% of the risk region (p<0.05; Figure 1A). When area of necrosis divided by area at risk was plotted as a function of subendocardial blood flow in the LAD bed during LAD occlusion (Figure 1B), both groups exhibit the expected inverse relation between infarct size and collateral blood flow. However, ANCOVA revealed that the regression line for the circumflex preconditioned animals was shifted downward with respect to the control line (p=0.055 for the randomized protocol alone; p<0.01 for the randomized protocol plus six nonrandomized circumflex preconditioned dogs), with the greatest reduction in infarct size observed in the
circumflex preconditioned dogs that were the most severely ischemic during LAD occlusion.

As expected, segment shortening in the LAD bed of control dogs remained unchanged at 101±3% of baseline following the 40-minute "no intervention" period (Figure 2A and Table 3). In contrast, dogs in the preconditioned group exhibited modest but significant dilatation and hyperkinesis in the LAD bed during the circumflex branch occlusions, which is in agreement with previous reports of hyperkinesis in remote nonischemic tissue during coronary artery occlusion. Specifically, segment shortening increased to 112±2% during the fourth circumflex occlusion (p<0.004 versus baseline) but was restored to 106±5% (p=NS versus baseline) during the fourth circumflex reperfusion immediately before LAD occlusion (Figure 2A and Table 3).

Both groups exhibited dyskinesis in the LAD bed during LAD occlusion: At 1 hour after occlusion, segment shortening averaged −40±16% and −28±10% of baseline in the control and preconditioned groups, respectively (p=NS; Figure 2A). At 30 minutes after relief of ischemia, there was a trend toward an early enhancement in function in the circumflex preconditioned animals: Segment shortening was 40±9% versus −11±19% in preconditioned versus control groups. This acute trend toward a difference in contractile function did not, however, achieve statistical signifi-
cance ($p=0.11$ by $t$ test before correction for multiple comparisons), and the magnitude of the difference in segment shortening between the groups deteriorated during the subsequent 4 hours of reflow.

Nonetheless, to determine whether this trend was an indicator that preconditioning might have a modest beneficial effect on acute recovery of wall motion, segment shortening at 4.5 hours after reperfusion was plotted as a function of infarct size for all dogs in the randomized protocol (Figure 2B). Not surprisingly, the poorest function was, for the most part, associated with the largest infarcts: This was the case for both control and preconditioned groups. In fact, data points for the preconditioned group fell along the same line as those for the control animals but were clustered toward the small infarct end of the regression relation. That is, the acute trend toward better recovery of function in the circumflex preconditioned dogs reflected the smaller infarct sizes in this group (perhaps resulting in less of a "tethering" influence of the necrosis at the site of the crystals) rather than a direct beneficial effect of circumflex preconditioning on stunned but viable tissue in the LAD bed.

### Discussion

In the present study, we report that brief episodes of ischemia in the circumflex bed of the anesthetized open-chest dog protect or precondition the LAD bed from a subsequent 1-hour episode of sustained LAD occlusion. In fact, infarct size in circumflex preconditioned dogs was reduced to approximately 35% of that observed in control animals, which is similar in magnitude to the reduction in infarct size observed in previous canine studies of circumflex preconditioning followed by circumflex occlusion$^{11,14}$ and LAD preconditioning followed by LAD occlusion.$^{5,6}$

The obvious question is, How can this difference in infarct size between circumflex preconditioned and control dogs be explained? Both groups suffered a similar insult in the LAD bed during LAD occlusion; thus, a difference in collateral perfusion—the major determinant of infarct size in the canine model$^{12}$—was not responsible for the difference in infarct size. Heart rate and arterial pressure (indexes of myocardial oxygen consumption) were also comparable in the control and preconditioned groups. Size of the area at risk may also play a role in determining the extent of infarction,$^{1,12}$ particularly in conscious dogs subjected to permanent coronary occlusion.$^{12}$ To determine whether our data were confounded by the slightly smaller LAD risk regions observed in the preconditioned group, we plotted the relation between the mass of necrosis and the mass of the risk region.$^{13}$ Regression analysis revealed in our anesthetized dogs subjected to ischemia/reperfusion, that area at risk was only a weak predictor of area of necrosis for both control and preconditioned groups ($r=0.27$ and 0.42). Most important, however, data points for the preconditioned dogs consistently fell below those obtained for control animals over the entire range of area at risk values obtained in the study. Thus, the reduction in infarct size with circumflex preconditioning was not due to differences in collateral blood flow, hemodynamic parameters, or area at risk.

There is no question that the canine model is characterized by collateral connections between the LAD and circumflex beds. These connections are present both between large epicardial vessels and between arterioles and capillaries: Approximately 20% of the capillaries at the junction of the LAD and circumflex beds are perfused by both arteries.$^{13}$ Does this suggest that the results of the present study could be explained by simple "overlap" of the preconditioned circumflex bed into the center of the LAD bed? The lateral extent of overlap between the two beds in the in situ, normally perfused, beating canine heart has been shown to be extremely narrow (i.e., 3,000–5,000 μm).$^{14}$ Moreover, acute coronary occlusion does not alter the number of interconnecting capillaries$^{13}$ and does not appreciably increase the extent of overlap between the two beds.$^{15}$ These data strongly suggest that our results cannot be explained by overlap of the circumflex bed into the center of the LAD bed.

Was reduction in infarct size in the circumflex preconditioned group dependent on our subjective decision to occlude the first and/or second circumflex branch(es)? Of the 14 circumflex preconditioned dogs included in the preliminary and randomized protocols, infarct size averaged 5.3±2.0%, 5.6±5.0%, and 4.3±1.7% of the myocardium at risk in dogs that underwent preconditioning occlusions of the first circumflex branch ($n=5$), the second branch ($n=3$), and both branches ($n=6$), respectively. This suggests that comparable protection was conferred on the LAD bed irrespective of whether the first and/or second circumflex branch(es) were occluded. Whether brief occlusions of a posterior circumflex branch—or brief occlusions of the main circumflex artery—would provide similar protection to that observed with anterior circumflex branch occlusion awaits further study.

Previous studies of circumflex preconditioning followed by sustained circumflex occlusion or LAD preconditioning followed by LAD occlusion (or comparable regimens in the rat and rabbit models) have resulted in several potential explanations for the increased tolerance to ischemia induced by preconditioning. Theories currently under intensive investigation include a slowing in the rate of ATP depletion during the initial minutes of sustained ischemia,$^{2}$ perhaps due to inhibition of energy-wasting mitochondrial ATPase$^{16}$; stimulation of cardiac $\beta_1$-receptors by adenosine released.

### Table 3. Segment Shortening in the Left Anterior Descending Coronary Artery Bed During Circumflex Branch Preconditioning: Randomized Protocol

<table>
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<th>Baseline</th>
<th>1st Cx CO</th>
<th>Reperfusion</th>
<th>2nd Cx CO</th>
<th>Reperfusion</th>
<th>3rd Cx CO</th>
<th>Reperfusion</th>
<th>4th Cx CO</th>
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<td>...</td>
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<td>...</td>
<td>102±3</td>
<td>...</td>
<td>105±3</td>
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<tr>
<td>Preconditioned</td>
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<td>109±3</td>
<td>105±3</td>
<td>112±2*</td>
<td>106±5</td>
</tr>
</tbody>
</table>

Cx CO, circumflex branch occlusion. No significant difference between groups during 4th Cx reperfusion: i.e., immediately before left anterior descending coronary artery occlusion.

*p<0.004 vs. baseline by paired t test.
during brief preconditioning ischemia\textsuperscript{17}; and activation or opening of ATP-sensitive potassium (K\textsubscript{ATP}) channels.\textsuperscript{4} Synthesis of a protective protein (such as heat shock protein stimulated by the brief episodes of ischemia)\textsuperscript{18,19} has also been proposed to play a role in infarct size reduction with preconditioning. However, two recent lines of evidence have argued against this hypothesis: Production of heat shock protein failed to limit infarct size in rabbits subjected to 45 minutes of coronary occlusion and 3 hours of reperfusion,\textsuperscript{20} and inhibition of protein synthesis did not attenuate the protective effects of preconditioning in the rabbit model.\textsuperscript{21}

To reconcile one or more of these hypotheses with data obtained in the present study, evidence of altered energy metabolism, activation of A\textsubscript{1}-receptors, and/or opening of K\textsubscript{ATP} channels in virgin, nonischemic myocardium following brief preconditioning ischemia would be required. Furthermore, these changes must be manifest within the 40-minute time frame of the preconditioning regimen. In this regard, Simkhovich et al\textsuperscript{22} have found evidence of altered fatty acid metabolism following coronary occlusion in both ischemic and remote, nonischemic myocardium, whereas Dorheim et al\textsuperscript{23} have documented small increases in adenosine concentration in the interstitial fluid of the circumflex bed during brief LAD occlusions. These theories and observations do not, however, preclude the involvement of other potential mechanisms. For example, regional ischemia precipitates the global release of catecholamines from the sympathetic nervous system: There is, in fact, preliminary evidence that suggests that release of norepinephrine may be a mediator of the preconditioning response in the isolated rat heart.\textsuperscript{24} Collateral connections between the circumflex and LAD beds at the arterial and arteriolar levels may in theory provide a conduit for transport of a "protective substance" (be it adenosine or some other mediator) from one region of the heart to another during the brief episodes of circumflex reperfusion, whereas capillary or postcapillary anastomoses may permit diffusion of the "substance" to at least some myocytes in the LAD bed. Coupling of myocytes via gap junctions may provide an additional means of transport from one region of the heart to another. Finally, it is possible that the dilatation and hyperkinesis induced in the LAD bed during the brief circumflex occlusions may in some way initiate a protective response. Further studies are obviously required to pursue these theories.

The second observation in this study is that preconditioning in the circumflex bed did not have a direct beneficial effect on the acute recovery of contractile function of the viable peri-infarct tissue following sustained occlusion/reperfusion in the LAD territory. This supports previous findings from our laboratory, in which preconditioning of the LAD bed did not improve recovery of function of peri-infarct tissue after 1 hour of LAD occlusion.\textsuperscript{6} In addition, we have recently reported that 2.5 minutes of preconditioning ischemia—which did not compromise wall motion per se—did not enhance the acute recovery of segment shortening in canine myocardium subsequently stunned by 15 minutes of coronary occlusion (i.e., a model not confounded by necrosis).\textsuperscript{25} Data from these three studies indicate that preconditioning does not exert an independent, beneficial effect on recovery of contractile function during the early hours following relief of sustained coronary occlusion in the anesthetized open-chest dog.

In summary, we conclude that benefits of preconditioning are not limited to those myocytes that were subjected to brief ischemia. Rather, our data indicate that in the anesthetized canine model, brief ischemia in one vascular bed also protects remote, virgin myocardium from subsequent sustained coronary artery occlusion. The mechanism(s) responsible for this cardioprotective effect are, at present, unknown, but our observations raise the intriguing possibility that preconditioning may be mediated by factor(s) activated, produced, or transported throughout the heart during brief ischemia/reperfusion.

References

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