Preconditioning Causes Improved Wall Motion as Well as Smaller Infarcts After Transient Coronary Occlusion in Rabbits

Michael V. Cohen, MD; Guang S. Liu, PhD; and James M. Downey, PhD

Background. A brief coronary occlusion before a more prolonged occlusion results in less myocardial infarction than the longer occlusion alone. However, the effects of this preconditioning on recovery of systolic function after coronary occlusion have not been determined.

Methods and Results. Ultrasonic crystals implanted in rabbit myocardium measured segment length in the distribution of a branch of the left coronary artery that was fitted with a snare occluder. Rabbits were randomly allocated to either nonpreconditioned or preconditioned groups. Rabbits in the latter group underwent preconditioning with a 5-minute coronary occlusion followed by 10 minutes of reperfusion. Then the coronary artery was occluded for 20 minutes in all rabbits, after which it was allowed to reperfuse for 90 minutes. The hearts were then excised, and infarct size was measured by staining with triphenyltetrazolium chloride. During coronary occlusion, all hearts except one demonstrated either akinesis or paradoxical bulging. Five minutes after release of the 20-minute occlusion, active shortening had returned in the preconditioned rabbits and averaged 27.9±16.6% of baseline shortening. At the same time, paradoxical lengthening persisted in nonpreconditioned rabbits (−15.5±19.8% of baseline). By the end of the 90-minute reperfusion period, segment shortening averaged 40.1±8.4% of baseline in preconditioned rabbits and only 6.2±12.0% in nonpreconditioned rabbits (p<0.05). Infarct size as a percentage of risk area was significantly smaller in preconditioned rabbits as well (3.0±1.6% versus 28.8±7.0%, p<0.002) and likely accounted for the improved shortening.

Conclusions. We conclude that a brief coronary occlusion before a more prolonged occlusion results in not only reduced infarct size but also significantly better recovery of systolic function. (Circulation 1991;84:341–349)

Coronary ligation and resulting myocardial ischemia cause deterioration of myocardial function, followed by cellular necrosis. Furthermore, the extent of injury is proportional to the duration of the ischemia. Therefore, it seems antithetical to find that a brief coronary occlusion before a more prolonged occlusion will actually result in salvage of myocardial tissue rather than extension of necrosis. Murry et al1 demonstrated that dogs subjected to a 40-minute coronary occlusion followed by reperfusion have infarcts only one fourth as large if those hearts first experienced four cycles of 5-minute occlusion/5-minute reperfusion. Others have demonstrated that brief coronary occlusions, termed preconditioning, before longer interruptions of coronary flow can protect the heart against the appearance of subsequent ventricular arrhythmias in ischemic tissue,2,3 electrocardiographic evidence of ischemia,2 accumulation of H+ in tissue during coronary occlusion,4 and ultrastructural changes of ischemia.5 To date, however, no one has tested whether preconditioning can actually preserve wall motion in reperfused hearts, the ultimate goal of any salvage intervention. The likely reason for this obvious omission is that a single 5-minute occlusion, the minimum time needed to precondition the heart,6,7 itself causes an appreciable contractile deficit in the dog heart, which might mask any beneficial effects. Although the rabbit heart is more prone to infarction than the dog heart, it demonstrates a smaller contractile deficit after a brief occlusion.8,9 Therefore, the rabbit heart should be a more appropriate model for study of the preconditioning phenomenon.

Methods

Experimental Preparation

Healthy New Zealand White rabbits weighing between 2 and 2.5 kg were supplied by a single breeder.
Sodium pentobarbital was administered into an ear vein until the rabbit was flaccid. Then, after injecting 1% xylocaine into the tissues overlying the trachea, a tracheotomy was performed, and a large bore polyethylene cannula was inserted into the trachea and connected to a positive pressure respirator. The rabbits were ventilated with 100% oxygen. The respiratory rate and inspiratory/expiratory phase ratio were adjusted to maintain a normal arterial pH and Pco₂. After initiation of mechanical ventilation, additional doses of sodium pentobarbital were administered to abolish the corneal reflex. The carotid artery was cannulated for measurement of arterial pressure. The heart was exposed through a left thoracotomy in the third intercostal space. The pericardium was incised, and a pericardial cradle was created. The left atrial appendage was retracted, and the superficial branches of the left coronary artery were identified. A 3-0 silk suture on a small curved needle was passed through the myocardium beneath the proximal segment of the large arterial branch coursing down the middle of the anterior surface of the left ventricle. Attempts were made to include only the artery in the snare. However, because of proximity it was sometimes not possible to exclude the accompanying vein. Two myocardial sites were then chosen, one on each side of the selected artery at a distance approximately 3–5 mm from it, midway between the snare and the cardiac apex. After inserting the point of an 18-gauge needle into the myocardium at each site, a 0.5-mm 10-MHz ultrasonic crystal (Triton Technology, Inc., San Diego, Calif.) was inserted into the needle tract and buried approximately 2 mm beneath the surface. Myocardial segment lengths were measured with a sonomicrometer (Triton Technology). A 5F solid-state, catheter-tipped transducer (Millar Instruments, Inc., Houston, Tex.) was introduced into the left ventricular cavity through a small incision in the left atrial appendage for measurement of left ventricular pressure. The latter signal was electronically differentiated to derive left ventricular dP/dt.

**Experimental Protocol**

After completion of the instrumentation, the preparation was permitted to stabilize for at least 15 minutes. Thereafter, control hemodynamic measurements of the intramyocardial electrogram from the implanted crystals, aortic pressure, normal and high gain left ventricular pressure, left ventricular dP/dt, and myocardial segment length were recorded by a multichannel oscillograph. All recordings were made at end expiration by temporarily clamping the oxygen inlet line to the respirator. Rabbits were assigned to either a nonpreconditioned group or the experimental group to be preconditioned. In the latter group, the snare was tightened to occlude the branch of the left coronary artery. The appearance of cyanosis and bulging of the anterior left ventricle documented success of the coronary occlusion. After 5 minutes, hemodynamic variables and changes in segment length were again recorded, and then the occlusion was released. Reperfusion was signaled by disappearance of cyanosis and return of myocardial contraction. Reperfusion continued for 10 minutes before hemodynamics were again recorded. In nonpreconditioned rabbits, the hearts were observed for 15 minutes without any occlusion or reperfusion. Thereafter, the coronary artery was occluded for 20 minutes in all rabbits. During this prolonged occlusion, some hearts developed ventricular fibrillation but typically defibrillated spontaneously within 1 minute and usually within 20–30 seconds. Immediately before release of the snare, hemodynamic measurements were made again. Recordings were made at 5 and 10 minutes after reperfusion, and then every 10 minutes for a total of 90 minutes. All instrumentation was then removed, and the heart was excised by transecting the aorta near the arch. In a third group of rabbits, the snare was never tightened, and hence, the coronary artery was never occluded. Hemodynamic measurements were made for 125 minutes, an interval equivalent to that needed for completion of the protocol in the first two groups.

**Myocardial Preparation**

The excised heart was mounted on a modified Langendorff perfusion apparatus. The coronary arteries were flushed with saline delivered at a constant pressure of 100 mm Hg through a cannula tied into the aortic root. Thereafter, the snare was again tightened to occlude the coronary artery branch. To identify the risk region formerly perfused by the occluded branch, a suspension of 1–10-μm yellow-fluorescing zinc-cadmium sulfide particles (Duke Scientific Corp., Palo Alto, Calif.) was injected into the aortic root and flushed into the patent coronary arteries with saline. The heart was then frozen and cut transversely into four or five parallel slices. Each slice was incubated at 38°C in 1% buffered triphenyltetrazolium chloride to demarcate the necrotic myocardium. The slices were then compressed between two plastic plates held apart by 2-mm blocks at each corner. When illuminated with a long-wave ultraviolet lamp, the perfused myocardium displayed yellow fluorescence. The nonfluorescent perfusion defect was considered to be the region at risk. These areas were traced on clear overlays. When illuminated normally, the infarcted regions, which were also traced on the overlays, appeared pale, whereas viable tissue was stained brick red.

The feasibility of staining ischemic myocardium with triphenyltetrazolium chloride after short coronary occlusion and reperfusion periods has been demonstrated by others. Reperfusion for periods of 90–120 minutes facilitates diagnostic postmortem staining and delineation of necrotic myocardium by allowing washout of cellular enzymes and cofactors. Although the size of infarcted myocardium after occlusions of less than 30 minutes may be underestimated by staining techniques when com-
pared with histological assessments, the correlation between the two methods is excellent.12

Data Analysis

Regional myocardial function was assessed as systolic shortening of the myocardium between the paired ultrasonic crystals. The beginning of systole was determined by the onset of the rapid upstroke of left ventricular pressure recorded by the micromanometer-tipped catheter; a point 20 msec before the maximally negative left ventricular dP/dt was defined as the end of systole. Systolic segment shortening was calculated as the difference between end-diastolic and end-systolic measurements. A negative value indicates paradoxical systolic lengthening. For purposes of comparisons between rabbits, all shortening measurements were normalized by dividing by the end-diastolic length. For each time point, measurements were made for three consecutive cycles and averaged.

Tracings of the infarct and risk regions were digitized with an ultrasonic digitizing tablet (Science Accessories Corp., Stratford, Conn.) interfaced with a personal computer. The total volumes of the region at risk and the infarcted tissue were calculated. Infarct size is presented as a percentage of the area at risk.

All results are expressed as mean±SEM. The significance of differences was determined by analysis of variance with repeated measures and by Student’s t tests.13,14 The relation between infarct size and segment length shortening was analyzed with a linear regression model. All statistical calculations were performed using computer software (SOLO, BMDP Statistical Software, Inc., Los Angeles). A value of p<0.05 was considered to be significant.

Results

Twenty-one rabbits were initially instrumented for this study. Three rabbits, two from the group with a prior preconditioning occlusion and one from the nonpreconditioned group, developed ventricular fibrillation during the 20-minute occlusion that neither reverted spontaneously to sinus rhythm nor converted after electrical shocks. These three animals were excluded and not considered further. There were four sham rabbits that were instrumented but did not have any coronary occlusions. In one of these, wall motion could not be monitored after 75 minutes because of accidental dislodgment of the ultrasonic crystals. Eight rabbits were in the preconditioned group; six rabbits were in the nonpreconditioned group. Three preconditioned and two nonpreconditioned rabbits developed ventricular fibrillation. Fibrillation converted spontaneously after 20, 30, and 60 seconds in preconditioned rabbits and after 12 seconds in one nonpreconditioned rabbit. A second nonpreconditioned rabbit developed ventricular fibrillation during the 20-minute ischemic period that did not convert spontaneously. Manual compression of the heart initiated two minutes following the onset of fibrillation and then several electrical shocks resulted in successful defibrillation after approximately 7 minutes. Despite this duration of arrhythmia, this nonpreconditioned rabbit had the smallest infarct size of the group and the second greatest recovery of segmental shortening. Elimination of this rabbit does not significantly alter the significance of the conclusions. Other rabbits had isolated ventricular premature beats without further sequelae.

In the four sham rabbits without coronary occlusion, there were no significant hemodynamic changes during the observation period except for a tendency for left ventricular systolic pressure and maximum dP/dt to decrease. As depicted in Figure 1, myocardial segment length shortening also tended to deteriorate during the 2-hour interval such that the magnitude of shortening at the end averaged 79% of that at the beginning. However, this change over time was not significant.

In nonpreconditioned rabbits without a preliminary 5-minute occlusion, coronary ligation for 20 minutes produced paradoxical bulging of the ischemic region in all the rabbits of this group and was associated with a rise in heart rate, fall in blood pressure, and increase in left ventricular end-diastolic pressure. The recording from a representative rabbit is reproduced in Figure 2, and group data are included in Table 1. After release of the occlusion, most hemodynamic variables did not change appreciably except for a noticeable decrease in end-diastolic pressure. For the entire observation period, however, there were no statistically significant changes. End-diastolic segment length changed very little, whereas end-systolic length increased strikingly during occlusion, with minimal recovery during the reperfusion period. Consequently, the degree of systolic shortening deteriorated markedly and significantly during the occlusion period (p<0.001). After release of the occlusion, active shortening did not return for almost 20 minutes and, at this point, averaged only 16.8% of baseline shortening (p<0.001 compared with the preischemic value) (Figure 1). During the remainder of the reperfusion period, there was further deterioration of shortening until at 90 minutes the monitored segment was nearly akinetic, with active shortening averaging only 6.2% of baseline shortening (p<0.001) (Figure 1).

Changes in hemodynamics after the 20-minute coronary occlusion in preconditioned rabbits were similar to those in nonpreconditioned rabbits (Table 1), and there were no statistically significant differences between the two groups for any of the hemodynamic variables for the duration of the observation period. Only left ventricular end-diastolic pressure significantly changed during the course of the protocol in preconditioned rabbits. On the other hand, posts ischemic segment shortening was distinctly different from that observed in nonpreconditioned rabbits (Figure 3 and Table 1). Although both baseline end-diastolic and end-systolic lengths tended to be greater in preconditioned than in nonpreconditioned
rabbits, these differences were not significant. During the initial 5-minute occlusion, paradoxical bulging was observed in five of the rabbits (Figure 3), akiness was observed in two, and marked hypokinesis was observed in one. After release of the occlusion, however, active shortening returned promptly. After 10 minutes of reperfusion, shortening recovered to 65.8% of baseline (p<0.001) (Figure 1). During the prolonged 20-minute occlusion, similar regional contraction abnormalities were again observed, but in contrast to observations in nonpreconditioned rabbits, active shortening returned almost immediately after reperfusion (Figure 3) and by 5 minutes averaged 27.9% of baseline (Figure 1). During the remaining 65 minutes of reperfusion, recovery gradually progressed, until at 70 minutes shortening reached 46.2% of baseline (p<0.001). By the end of the reperfusion period, segment shortening was 40.1±8.4% of baseline (p<0.001) (Figure 1). Differences in segment shortening between nonpreconditioned and preconditioned groups became noticeable immediately after release of the 20-minute occlusion. Segment shortening in the postsischemic interval was significantly better in the preconditioned rabbits (p<0.05).

Infarct size, quantitated by staining with triphenyltetrazolium chloride, was calculated as a percentage of the myocardial area at risk. In four of six nonpreconditioned rabbits, infarct size exceeded 34% of the area at risk and averaged 28.8±7.0% for the entire group. In contrast, the preconditioned hearts had infarcts that ranged from 0% to 12% of the area at risk, and in four of these hearts there was no detectable necrosis. Average infarct size in the preconditioned rabbits was only 3.0±1.6%. The difference between the two groups is highly significant (p<0.002).

To better appreciate the differences between nonpreconditioned and preconditioned rabbits, individual data for infarct size have been plotted against recovery of segment shortening presented as a percentage of baseline after 90 minutes of reperfusion.
(Figure 4). In nonpreconditioned rabbits, most of the data points are clustered along the abscissa, whereas points in the preconditioned rabbits are grouped near the ordinate. The linear regression equation for the nonpreconditioned rabbits is \( y = -1.25x + 42.24 \), where \( y \) is segment shortening and \( x \) is infarct size. The correlation coefficient is -0.80. In the preconditioned rabbits, the equation is \( y = -3.59x + 50.89 \) with a correlation coefficient of -0.70. The slopes of these two regression lines are not different. Thus, segment shortening was inversely related to infarct size, and infarct size reduction alone could conceivably account for the improvement in wall motion in the preconditioned group.

**Discussion**

These data demonstrate that a short period of ischemia induces changes in the heart that make it more resistant to subsequent ischemic exposures, as is clearly reflected in the postischemic function of the heart. The observation by Murry et al\(^1\) that brief occlusions would precondition the heart and substantially reduce the size of the infarct after a 40-minute occlusion led some investigators to question whether other sequelae of myocardial ischemia could also be attenuated. Indeed, others have demonstrated fewer ventricular arrhythmias,\(^2,3\) including ventricular tachycardia and fibrillation,\(^3\) less ST segment elevation,\(^2\) less evidence of epicardial and endocardial electrocardiographic abnormalities,\(^2\) minimal changes in effective refractory periods,\(^1,3\) less accumulation of hydrogen ions,\(^4\) and fewer myocardial ultrastructural abnormalities\(^5\) in preconditioned ischemic hearts.

However, the success of preconditioning at attenuation of the loss of contractile function after a transient period of ischemia has not been adequately addressed by these other investigations.\(^1-5,15\) Hoffmeister et al\(^16\) and Cohen and Downey\(^9\) have demonstrated that, in dogs with multiple brief coronary occlusion/reperfusion cycles, the greatest deterioration in systolic function is observed after the first occlusion. The decrement in function after release of the second occlusion was significantly smaller, and after several occlusions no further depression of systolic function was apparent. It is suggested that the first occlusion preconditioned the heart against ischemic consequences of subsequent occlusions. The data from the current study support this hypothesis.

In the present study, only one 5-minute occlusion was used to precondition the heart. Whereas Murry et al\(^1\) originally used four cycles of 5-minute occlusions, Li et al\(^6\) found that only one 5-minute occlusion cycle was enough to protect the canine heart. This observation has also been confirmed in rabbit hearts, although a sequence of two cycles of 2-minute occlusions is too brief to confer protection.\(^7\) Rabbit hearts were chosen for this study because they show much less stunning than dog hearts after a preconditioning stimulus. Systolic shortening averaged 66% of the preocclusion value after one 5-minute occlusion in the rabbit hearts in this investigation, but it was found to be only 29% after an identical occlusion in dog hearts.\(^8\) Obviously, it would be impossible to determine if preconditioning preserved wall motion if this initial brief coronary occlusion itself rendered the myocardium akinetic. On the other hand, use of the rabbit model is complicated by the fact that

![Figure 2](http://circ.ahajournals.org/)

**Figure 2.** Representative recording from nonpreconditioned rabbit. As depicted by vertical lines, the onset of upstrokes of the first derivative of the left ventricular pressure (LVP) tracing (LV dP/dt) was selected as the onset of systole, and a point 20 msec before the nadir of the dP/dt tracing was chosen as the functional termination of systole. Coronary occlusion (Occl) produces striking paradoxical bulging of the ischemic segment. Reperfusion (Reperf) results in rapid lessening of the bulging and gradual return of effective shortening, although this improvement is short-lived. By the end of the 90-minute reperfusion period, the instrumented myocardial segment is nearly akinetic. Segment shortening as a percent of the baseline value is depicted at the bottom for each observation point. In this rabbit, 34.2% of the area at risk was infarcted.
rabbit myocardium infarcts easily. Miura et al.\textsuperscript{17} found that occlusions as short as 10 minutes induce infarction. Thus, in the rabbit heart both stunning and necrosis are likely to contribute to wall motion abnormalities in unknown proportions. It is not known what the relative effects and importance of stunning and necrosis are in the human heart.

The mechanism of this preconditioning effect is unclear. Coronary collateral flow was not measured in the present study, but it is unlikely that differences existed between the two groups. Others\textsuperscript{11,18} have demonstrated that collateral flow is uniformly negligible in rabbits. In dogs the variable degree of collateral development before coronary occlusion mandates that collateral flow be quantitated because of the important effect of the magnitude of this flow on ischemic myocardium, whereas in rabbits quantitation is not needed. Although collateral flow is no less a significant determinant of infarct size in this species, the anticipated uniformly minimal flow in all rabbits implies that the effect of this flow would be nearly identical in all experimental preparations. Hence, when rabbits are selected for the study of ischemic myocardium, it is not necessary to measure collateral flow. Additionally, there is no reason to suspect that collateral flow would have changed in the preconditioned rabbits after the initial 5-minute coronary occlusion. In dogs with known collaterals, multiple 5-minute occlusions do not alter collateral flow.\textsuperscript{2,19,20} Furthermore, the degree of dyskinesis was identical in the preconditioned rabbits after both the initial 5-minute and subsequent 20-minute coronary occlusions (Figure 1). Despite these considerations, it is acknowledged that of the eight rabbits in the preconditioned group two demonstrated akinesis and one demonstrated severe hypokinesis after coronary occlusion. One cannot completely exclude the possibility of slightly higher collateral flows in these three hearts, although crystal placement with possible inclusion of islands of normally perfused myocardium is a more likely explanation. Monitored hemodynamics and risk areas were not different in these three animals. Nonetheless, the preconditioned hearts with marked paradoxical bulging during coronary occlusion demonstrated the same recovery of function during the prolonged reperfusion period as did these three hearts. Exclusion of the latter does not alter the significance of the observation.

It has been proposed that myocardial stunning, the temporary contractile dysfunction that follows a transient coronary occlusion, might itself account for the protection. If metabolism of the stunned tissue were diminished, then energy requirements might be lower during a subsequent ischemic insult. However, overall myocardial oxygen consumption of reperfused, stunned myocardium, and therefore metabolic demand, has been found not to be different\textsuperscript{21} or even increased\textsuperscript{22} over that of normal tissue. Nonetheless, oxygen requirements of myocardium do vary with loading conditions, and bulging, ischemic myocar-

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**Table 1. Hemodynamics and Segment Length Shortening in Control and Preconditioned Rabbits During Coronary Occlusion and Reperfusion**

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline</th>
<th>Preconditioning period</th>
<th>Prolonged OCC</th>
<th>REP after prolonged OCC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>5-min OCC</td>
<td>10-min REP</td>
<td>20-min</td>
</tr>
<tr>
<td>Nonpreconditioned</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>303.5±12.8</td>
<td>...</td>
<td>...</td>
<td>312.8±16.8</td>
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<tr>
<td>SBP (mm Hg)</td>
<td>79.3±4.9</td>
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<td>...</td>
<td>73.7±3.6</td>
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<tr>
<td>DBP (mm Hg)</td>
<td>64.0±3.2</td>
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<td>...</td>
<td>57.5±2.8</td>
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<tr>
<td>LVEDP (mm Hg)</td>
<td>6.6±1.0</td>
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<td>...</td>
<td>11.8±3.3</td>
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<tr>
<td>LV dP/dt (mm Hg/sec)</td>
<td>2,842±141</td>
<td>...</td>
<td>...</td>
<td>2,452±219</td>
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<tr>
<td>EDL (mm)</td>
<td>6.95±0.31</td>
<td>...</td>
<td>...</td>
<td>7.04±0.39</td>
</tr>
<tr>
<td>ESL (mm)</td>
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<td>...</td>
<td>...</td>
<td>7.22±0.40</td>
</tr>
<tr>
<td>Δ (%)</td>
<td>13.5±1.2</td>
<td>...</td>
<td>...</td>
<td>−2.5±0.6*</td>
</tr>
</tbody>
</table>

| Preconditioned      |          |           |            |        |       |       |
| HR (beats/min)      | 271.8±11.1 | 272.0±11.6 | 279.1±13.0 | 297.2±15.2 | 286.1±16.4 | 287.4±15.9 |
| SBP (mm Hg)         | 86.2±4.2 | 78.0±2.6 | 85.9±4.0 | 77.8±3.8 | 78.5±3.7 | 77.8±3.5 |
| DBP (mm Hg)         | 70.8±4.9 | 62.2±3.4 | 71.0±4.9 | 63.5±4.3 | 63.1±4.3 | 62.6±3.9 |
| LVEDP (mm Hg)       | 8.6±1.1 | 15.8±2.5† | 11.6±1.1‡ | 17.2±2.0* | 13.0±1.5† | 11.4±1.4§ |
| LV dP/dt (mm Hg/sec)| 3,344±268 | 2,769±122 | 3,032±231 | 2,843±198 | 2,769±164 | 2,859±241 |
| EDL (mm)            | 8.16±0.46 | 8.34±0.46 | 8.43±0.51 | 8.28±0.46 | 8.14±0.41 | 8.34±0.45 |
| ESL (mm)            | 6.95±0.42 | 8.36±0.47 | 7.58±0.49 | 8.34±0.46 | 7.76±0.47 | 7.98±0.44 |
| Δ (%)               | 14.9±1.1 | 0.2±0.9* | −10.1±1.4* | −0.7±0.6* | 4.9±2.2* | 4.2±1.4* |

Values are mean±SEM. OCC, occlusion; REP, reperfusion; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; LVEDP, left ventricular end-diastolic pressure; LV dP/dt, rate of change of left ventricular pressure; EDL, end-diastolic length; ESL, end-systolic length; Δ, segment shortening.

\textsuperscript{*}p<0.001, \textsuperscript{†}p<0.005, \textsuperscript{‡}p<0.025, \textsuperscript{§}p<0.05 vs. corresponding within-group baseline value; \textsuperscript{∥} p<0.025 vs. corresponding value for nonpreconditioned group.
diom may have a greater metabolic demand than akinetic tissue.23 Perhaps altered oxygen consumption during the ischemic phase of the preconditioning stimulus may be important. Schröder et al24 demonstrated faster appearance of dyskinesia in dogs during a brief 30-second coronary occlusion if it had been preceded 30 minutes earlier by a 5-minute occlusion, thus decreasing the duration of metabolic support necessary for myocardial contraction. Recent evidence also documents that preconditioning results in slowing of the rate of ATP depletion during a subsequent sustained ischemic insult, suggesting a reduction of myocardial energy demand during ischemia.25 In the face of diminished demand, coronary occlusion would be expected to produce fewer detrimental effects. Therefore, an initial coronary occlusion could result in myocardial stunning, and the stunned but still viable tissue with its diminished metabolic requirements could better withstand a prolonged ischemic insult than normally functioning tissue with its high metabolic rate.

However, a second report by Murry and his colleagues26 proved that stunning is not the mechanism of preconditioning. Whereas a 15-minute coronary occlusion followed by a 5-minute reperfusion period before a sustained 40-minute occlusion significantly limited infarct size, much less salvage was realized if the reperfusion period between the brief and sustained occlusions was 120 minutes. Although protection was diminished in the hearts undergoing 120 minutes of reperfusion before the prolonged occlusion, stunning during this reperfusion interval was similar in magnitude to that in hearts with only 5 minutes of reperfusion. Whereas the phenomena of stunning and preconditioning are both observed in reversibly injured myocardium, they can be temporally dissociated, thus excluding a simple cause and effect relation.

![Figure 3](http://circ.ahajournals.org/)

**Figure 3.** Representative recording from preconditioned rabbit. As noted for Figure 2, vertical lines indicate timing of beginning and end of systole when segment length was measured. Although the initial brief occlusion (Occl) causes paradoxical bulging of the ischemic segment, reperfusion (Reperf) results in nearly complete return of function with little persistent stunning. After release of the prolonged 20-minute occlusion during which the instrumented segment is akinetic, there is a marked rebound during which function is nearly completely restored. However, function deteriorates quickly thereafter, and by 10 minutes, shortening as a percent of the control value (indicated at bottom) is only 22%. But during the remaining 80 minutes of reperfusion, segment shortening gradually improves and is 71% of the baseline value at the end of the observation period. In this rabbit, no infarction was detected. LVP, left ventricular pressure; LV dP/dt, first derivative of LVP; AoP, aortic pressure.
As shown in Figure 1, segment shortening after the initial 5-minute occlusion/10-minute reperfusion cycle averaged 65.8% of baseline values, indicating that some stunning did occur in these hearts. However, no correlation was found between the magnitude of stunning after this 5-minute occlusion and either the ultimate functional recovery after 90 minutes of reperfusion after the prolonged 20-minute coronary occlusion or the size of the infarct. If stunning had been responsible for the protective effect of the 5-minute occlusion, one might have expected that the hearts with the greatest stunning would have experienced the most protection.

Changes in end-diastolic length may influence the performance of myocardial segments by a local Starling effect. Vinten-Johansen et al.27 demonstrated that a paradoxical postischemic segment may demonstrate renewed active shortening after a simple increase in its end-diastolic length. In the present study, the end-diastolic length of the instrumented myocardial segment increased in preconditioned rabbits and shortened in those without the initial 5-minute occlusion. However, the changes were small and not significant.

Perhaps the preconditioning coronary occlusion results in the release of myoprotective agents such as adenosine, endothelial-dependent relaxing factor, or heat shock protein. Olafsson and colleagues28 previously demonstrated that intracoronary adenosine administered to dogs after a 90-minute coronary occlusion significantly attenuated infarct size. A recent investigation from this laboratory29 confirms a role for adenosine in the limitation of infarct size in rabbits after a preconditioning stimulus. On the other hand, the preconditioning effect is not diminished when protein synthesis is blocked,30 thus effectively eliminating heat shock protein as a likely causative agent.

In preconditioned rabbits, both preservation of systolic function and salvage of myocardial tissue were observed and were correlated with each other. As noted in Figure 4, rabbits with the smallest infarcts also appear to have had the greatest return of systolic segment shortening. It is interesting to speculate whether the preservation of function was solely the result of infarct size limitation or if preconditioning protects against stunning as well. A specific effect against stunning would result in a shift of the plot in Figure 4 upward such that function would be greater for any given infarct size. Unfortunately, it is not possible to determine from this study if stunning was attenuated, since the data are clustered in distinct groups at either end of the plot such that the relation is poorly described. Larger group sizes might have revealed a significant difference.

Preconditioning may have significant clinical implications. Individuals with angina before myocardial infarction may indeed have smaller infarcts and fewer arrhythmias than those with abrupt occlusion and infarction. Additionally, the first brief balloon inflation during coronary angioplasty might protect the myocardium against subsequent prolonged inflations and actually increase the chance of a successful result. A recent report by Heibig et al.31 describes an individual undergoing coronary angioplasty in whom the initial 2-minute balloon inflation resulted in marked ST segment elevation and chest pain. However, subsequent inflations lasting from 1 to 30 minutes caused no chest pain or electrocardiographic changes.

More important, there is the hope that either the mediators or the mechanism of preconditioning can be elucidated such that preconditioning's protection can be conferred pharmacologically to the ischemic patient. The present results reveal that such therapy would not only act to further limit the tissue loss after ischemia/reperfusion but would also cause an immediate and significant improvement in the postischemic function of those hearts as well.

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References


KEY WORDS • myocardial ischemia • occlusion • reperfusion • segment shortening
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