Does Myocardial Stunning Contribute to Infarct Size Limitation by Ischemic Preconditioning?

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Background. The mechanism through which ischemic preconditioning causes cardioprotection is unknown. The present study investigated the role of stunning in preconditioning.

Methods and Results. We studied three different protocols of preconditioning: two cycles of 2-minute ischemia separated by 5-minute reperfusion (2'PC), one cycle of 5-minute ischemia by 5-minute reperfusion (5'PC1), and two cycles of 5-minute ischemia separated by 5-minute reperfusion (5'PC2). In the first series of experiments, the stunning associated with 2'PC, 5'PC1, or 5'PC2 was assessed using an epicardial Doppler transducer in anesthetized open-chest rabbits. The thickening fraction (percent baseline) of the preconditioned region was 76.8±7.2% (mean±SEM) after 2'PC but 31.4±9.2% and 34.3±9.7% after 5'PC1 and 5'PC2, respectively, which were significantly lower, thus indicating more severe stunning than that after 2'PC. In the second series of experiments, a branch of the left circumflex artery was occluded for 30 minutes and then reperfused for 72 hours in four groups of rabbits. One group was not preconditioned and three groups were preconditioned with 2'PC, 5'PC1, or 5'PC2 protocols before the 30-minute ischemia. In contrast to the differences observed in the stunning in the first series of experiments, histological infarct size was similar in the three preconditioned groups (21.1±3.0% of area at risk after 2'PC, 20.1±3.4% after 5'PC1, 16.4±4.2% after 5'PC2), all of which were significantly smaller than that in the unpreconditioned group (43.9±5.0%). The third series of experiments examined the degree of stunning by 2'PC, 5'PC1, or 5'PC2 and the size of infarct (tetrozolium staining) in the same animal after 30-minute ischemia/3-hour reperfusion; again, the results showed no significant correlation between degree of stunning and infarct size.

Conclusions. The myocardial infarct size–limiting effect of preconditioning did not correlate with the degree of myocardial stunning accompanying preconditioning. Thus, it is unlikely that myocardial stunning contributes to the cardioprotective effect of ischemic preconditioning. (Circulation 1991;84:2504–2512)

Recent studies have revealed two important consequences of a brief period of myocardial ischemia: post–ischemic myocardial dysfunction (myocardial stunning) and ischemic preconditioning. The stunned heart has contractile dysfunction despite full restoration of coronary flow and absence of necrosis. Complete recovery of contractile function will occur but requires a day or more, depending on the duration of ischemia and the collateral blood flow level.1 During this same period of ischemia, the heart also becomes preconditioned and actually obtains increased tolerance against an infarction of a subsequent ischemic insult.2,3 However, the interrelation between the two phenomena, stunning and preconditioning, remains unclear.

Myocardial stunning could directly contribute to the preconditioning effect by reducing ATP utilization and catabolite accumulation as a result of the depressed contractile state. Indeed, a reduced rate of ATP utilization has been reported for the preconditioned heart,4 suggesting that the protection afforded by preconditioning could be a direct result of stun-
ning. To test that hypothesis in the present study, we used three different preconditioning protocols, each giving a different degree of stunning. We then analyzed the relation between the infarct size-limiting effect and the degree of myocardial stunning accompanying each protocol.

**Methods**

We first assessed the effects of three preconditioning protocols on regional contractile function and on histological infarct size in experiments 1 and 2, respectively. To confirm the findings obtained by these experiments, another group of rabbits was used in experiment 3, in which the degree of stunning and infarct size determined by tetrazolium staining were analyzed in the same animal.

**Experiment 1: Assessment of Myocardial Stunning Associated With Preconditioning Procedures**

**Surgical preparation.** Male rabbits (Japanese White) weighing 2.2–2.9 kg were anesthetized with intravenous sodium pentobarbital (40 mg/kg). The rabbit was tracheostomized, intubated, and mechanically ventilated with room air and supplemented oxygen. The respirator (model 683, Harvard Apparatus, South Natick, Mass.) and oxygen supplement were adjusted to maintain arterial Po2 and pH within the physiological range. A catheter was inserted into the carotid artery and connected to a Nihon-Kohden SCK-580 pressure transducer to measure systemic blood pressure. Precordial electrocardiography (ECG) was monitored using bipolar leads across the chest. A left thoracotomy was performed and the heart was exposed. A catheter-tipped manometer (CTD-096N, Nihon-Kohden, Japan) was introduced through the left atrial appendage and passed into the left ventricle (LV) to record LV pressure. The first derivative of LV pressure (LV dP/dt) was obtained by electronic differentiation using an EQ-601G unit (Nihon-Kohden, Tokyo, Japan). Silk thread (4-0) was passed around a branch of the left circumflex artery with a taper needle, and the ends of the silk tie were threaded through a small vinyl tube to make a snare. The coronary branch was occluded by pulling the snare, which was then fixed by clamping the tube with a mosquito clamp. Myocardial ischemia was confirmed by the ST segment elevation of ECG and regional cyanosis of the myocardial surface. Regional myocardial function was assessed using a pulsed Doppler epicardial probe and Pulsed Doppler Dimension System VF-1 (Crystal Biotec, Hopkinton, Minn.). The epicardial Doppler probe consisted of a 3-mm ultrasonic crystal bonded to a 6-mm-diameter fabric disk impregnated with Silastic. The probe was secured to the epicardium, which was to be rendered ischemic, with four or five 6-0 prolene stitches penetrating approximately 0.3 mm-into the myocardium, thus producing minimal trauma. Regional wall thickening, systemic blood pressure, LV pressure, LV dP/dt, and ECG were recorded on an eight-channel, direct-writing polygraph (WT-687G, Nihon-Kohden) at paper speeds of 50 and 100 mm/sec. The onset of systole was determined as the initial rise in systolic LV pressure and the end of systole was considered to be coincident with the peak negative dP/dt of the LV pressure. For each sample period, thickening fraction was calculated for at least five beats and then averaged.

**Experimental protocol.** We aimed to assess the degree of myocardial stunning caused by three preconditioning protocols: two cycles of 2-minute ischemia separated by 5-minute reperfusion, one cycle of 5-minute ischemia and 5-minute reperfusion, and two cycles of 5-minute ischemia separated by 5-minute reperfusion. To determine regional thickening fraction after the preconditioning protocols, rabbits were randomly divided into two groups. One group (2-minute ischemia group) received two episodes of 2-minute coronary occlusion/5-minute reperfusion. Another group of rabbits (5-minute ischemia group) was subjected to two episodes of 5-minute coronary occlusion/5-minute reperfusion. This group was used to assess both the stunning by one cycle and two cycles of 5-minute ischemic preconditioning. Arterial blood gas and hematocrit were examined before the baseline recording and at the end of the experiment. After the last measurements of myocardial thickening and systemic hemodynamics were obtained, each rabbit was heparinized with 3,000 units of intravenous heparin and given a pentobarbital overdose. The heart was then removed with the coronary snare in place and processed for postmortem analysis.

**Postmortem analysis of the stunned heart.** The heart was mounted on a Langendorff apparatus, perfused with saline at 80 mm Hg, and monastral blue dye was then injected to confirm reperfusion of the occluded circumflex branch. The coronary artery was reocluded using the remaining tie around the branch, and a saline suspension of fluorescent particles (3-30 μm in diameter, Duke Scientific Co., Palo Alto, Calif.) was then infused into the perfusion line to negatively mark the area at risk (i.e., vascular bed of the occluded coronary branch). Under ultraviolet light, the positional relation between the Doppler probe and the area at risk was examined, and when the probe was found to be over the border between the normal zone and the area at risk or the distance between the probe and the border was less than 2 mm, the rabbit was excluded from the data analysis. The heart was taken off the Langendorff apparatus, frozen, and sectioned into approximately 3-mm slices. The heart slices were mounted in a glass press that compressed the slices into uniform 3-mm thicknesses. A clear acetate sheet was laid over the glass plate and the area at risk was traced on the sheets under ultraviolet light. The traces of the risk region were enlarged by two times and the areas were measured by PIAS II, a computer-assisted image analysis system (PIAS Co., Osaka, Japan).
Experiment 2: Myocardial Infarct Size Study

Surgical preparation. Surgical preparation was essentially the same as in our previous studies. Male rabbits were anesthetized and mechanically ventilated as in experiment 1. Systemic blood pressure was measured by a Nihon-Kohden SCK-580 pressure transducer connected to a catheter in the carotid artery. Precordial ECG was monitored by bipolar leads across the chest. The heart was exposed via left thoracotomy, and a coronary snare around a branch of the left circumflex artery was prepared as in experiment 1.

After the preconditioning protocol (see “Experimental Groups”), the coronary artery was occluded for 30 minutes and reperfused by releasing the snare. Myocardial ischemia was confirmed by the ST segment elevation of ECG and regional cyanosis of the myocardial surface. Reperfusion was indicated by color change (cyanosis to hyperemia) over the ventricular surface. The vinyl tube was then removed, and the ends of the silicon thread were tied together to make a loop that was left in the thorax. The surgical wounds were repaired and the rabbit was returned to its cage for recovery.

These surgical procedures were performed under sterile conditions, and a combination of 50 mg ampicillin and 50 mg cloxacillin was injected intramuscularly for the prophylaxis of infection. Seventy-two hours after surgery, each rabbit was heparinized with 2,000 units of intravenous heparin and given a pentobarbital overdose. The heart was removed for postmortem analysis.

Analysis of infarct size and area at risk. The heart was mounted on a Langendorff apparatus and perfused with saline at 80 mm Hg to wash out the remaining blood. The coronary branch was reocluded by ligating the silk tie left around the branch; fluorescent particles were then injected into the perfusion line to subsequently determine the area at risk. After the atria were removed, the heart was weighed, fixed in 20% buffered formalin for 24 hours, and stored in 10% formalin.

The fixed heart was sectioned into 3-mm slices from the apex to the base by using a tissue slicer; sections were then embedded in paraffin. Two 10-μm sections were cut from each paraffin block and one of the two was stained with hematoxylin-eosin and the other with Mallory's connective tissue stain modified by Heidenhain. By illuminating the paraffin block with ultraviolet light, the area at risk was visualized as the area deficient of fluorescent particles. Corresponding histology slides were laid over the paraffin block and the area at risk were traced on the slide itself. The slides were magnified ×7, and the infarct and area at risk were traced on paper. The infarcted region was identified as the areas of coagulation necrosis, contraction band necrosis, and granulation tissue surrounding the necrotic myocytes. When the infarct being traced from the slide stained with Mallory's connective tissue staining had an ambiguous region, the slide stained with hematoxylin-eosin was used as a reference. The areas of the infarct and the risk region traces were determined using the PIA II image analysis system. In our previous study, we found that the heart slices shrank during the process of fixation and embedding in the paraffin. The mean ratio of the area after embedding to before fixation was 0.72.6 To correct this volume change during tissue preparation and to calculate the original volumes of infarct and area at risk, we divided the areas, which were obtained by tracing, by 0.724 and then multiplied by the sample thickness (i.e., 3 mm).

Experimental groups. After the coronary snare was placed and hemodynamic parameters had been stabilized for 5 minutes, rabbits were randomly divided into four groups (Figure 1). Group 1 (n=11) did not receive preconditioning and underwent simple 30-minute coronary occlusion and 72-hour reperfusion. Group 2 (n=12) was preconditioned with two cycles of 2-minute ischemia separated by 5-minute reperfusion. Group 3 (n=11) and group 4 (n=11) were preconditioned with one and two cycles of 5-minute ischemia, respectively, separated by 5-minute reperfusion as shown in Figure 1.

Experiment 3: Correlation Between Myocardial Stunning and Infarct Size After Preconditioning

Twelve rabbits were used to examine the relation between myocardial stunning caused by the preconditioning procedure and the infarct size-limiting effect of preconditioning in individual animals. Surgical preparation was the same as in experiment 1 and regional thickening fraction was measured by the epicardial Doppler probe. All rabbits underwent 30-minute coronary artery occlusion and 3-hour reperfusion. Control rabbits were unpreconditioned (n=4) and seven rabbits were preconditioned as in experiment 2: two cycles of 2-minute ischemia separated by 5-minute reperfusion (n=2), one cycle of 5-minute ischemia and 5-minute reperfusion (n=3), or two cycles of 5-minute ischemia separated by

FIGURE 1. Graph showing experimental protocol. Group 1 rabbits underwent 30-minute coronary occlusion without preconditioning. Preconditioning was performed with two cycles of 2-minute ischemia separated by 5-minute reperfusion in group 2, one cycle of 5-minute ischemia and 5-minute reperfusion in group 3, and two cycles of 5-minute ischemia separated by 5-minute reperfusion in group 4.
5-minute reperfusion \((n=2)\). One thousand units of heparin sodium was injected intravenously every 60 minutes starting 1 hour after the 30-minute ischemia. At 3 hours after reperfusion, the rabbits were killed by pentobarbital overdose, the heart was excised, and the fluorescent particles were injected into the coronary arteries as in experiment 1 to identify the area at risk. The heart was then frozen and sliced into approximately 3.5-mm sections. The heart slices were incubated in 1% triphenyl tetrazolium solution in phosphate buffer \((100 \text{ mmol/l, pH 7.4})\) at 37°C for 20 minutes to visualize the infarct.\(^8\) The heart slices were mounted in a glass press that compressed the slices into uniform 3-mm thicknesses. A clear acetate sheet was laid over the glass plate and the tetrazolium-negative region and the area at risk were traced under room light and under ultraviolet light, respectively. The traces were enlarged by two times and the infarcted area and area at risk were measured by the PIA2 system; the areas were then multiplied by slice thickness \((3 \text{ mm})\) to calculate each volume.

Statistical Analysis

Differences in the data among the groups were tested by one-way analysis of variance. If the \(F\) test showed an overall difference, comparison of the two groups was performed with the \(t\) test using Bonferroni correction. The differences in parameters of a group for different time periods of measurement were tested by repeated-measures analysis of variance.\(^9\) The \(\chi^2\) test was used for analysis of mortality differences.

This study conformed to the guidelines of Sapporo Medical College on animal usage and was conducted in accordance with the position of the American Heart Association on research animal use.

Results

Experiment 1: Myocardial Stunning During Preconditioning

Of 20 rabbits entered in experiment 1, four were excluded because the Doppler transducer was found to be over the border between the nonischemic and ischemic regions in the postmortem analysis. The remaining 16 rabbits contributed to the following analysis.

The 2-minute ischemia group and the 5-minute ischemia group were equivalent in the size of area at risk \((14.9\pm1.9\% \text{ of the heart versus } 19.1\pm2.8\%, \text{ } p=NS)\). There were also no significant differences between the two groups in hematocrit at baseline condition \((38\pm3\% \text{ versus } 42\pm2\%)\) and at the end of the experiment \((42\pm3\% \text{ versus } 43\pm3\%)\). Data of hemodynamic parameters and regional function are summarized in Table 1. Under baseline conditions, both 2-minute ischemia and 5-minute ischemia groups were comparable in systemic blood pressure, heart rate, LV end-diastolic pressure \((\text{LVEDP})\), LV \(dP/dt\), and systolic thickening fraction.

Repetitive coronary occlusion and reperfusion did not produce significant changes in heart rate or systemic blood pressure during the experiments. Slight elevation of LVEDP was noted during the first and the second coronary occlusions in both the 2-minute ischemia and 5-minute ischemia groups. No significant alteration in LV \(dP/dt\) compared with the preclosure value in either group was observed, although a slight decrease in LV \(dP/dt\) during the first reperfusion and a slight increase during LV \(dP/dt\) during the first reperfusion were noted in the 2-minute ischemia group and the 5-minute ischemia group, respectively (Table 1).

In the 2-minute ischemia group, coronary occlusion reduced regional systolic thickening fraction from the baseline value of \(13.2\pm1.4\%\) to \(-3.6\pm0.9\%\), suggesting paradoxical systolic thinning. However, the thickening fraction returned to \(10.0\pm1.2\%\) at 5 minutes after reperfusion, which was not statistically different from the baseline value. Similar alteration of the thickening fraction was observed during the second cycle of 2-minute ischemia and 5-minute reperfusion (Table 1). On the other hand, in the 5-minute ischemia group, thickening fraction after 5-minute reperfusion was \(7.3\pm3.1\%\), which was significantly lower than its baseline value of \(19.6\pm4.0\%\). After the second 5-minute ischemia, the recovery of the thickening fraction after 5-minute reperfusion \((7.6\pm3.4\%)\) was similar to that after the first cycle of ischemia (Table 1).

To minimize the influence of individual variability in the baseline thickening fraction, we normalized the thickening fraction as a percentage of each baseline value and plotted its time course in Figures 2 and 3. In the 2-minute ischemia group (Figure 2), the thickening fraction recovered to \(78.3\pm7.9\%\) and \(76.8\pm7.2\%\) of baseline at 5 minutes after reperfusion after the first and the second ischemia, respectively. This finding indicates that myocardial stunning was very modest after 2-minute ischemia in the rabbit and that stunning was not exacerbated by the repetition of 2-minute coronary occlusion.

However, myocardial stunning was more severe after 5-minute ischemia, as shown in Figure 3. The thickening fraction after the first cycle of 5-minute ischemia/5-minute reperfusion was \(31.4\pm9.2\%\) of baseline and after the second cycle it was \(34.3\pm11.0\%\), both of which were significantly lower than the thickening fractions at the corresponding time points in the 2-minute ischemia group. These results suggest that ischemic preconditioning with two cycles of 2-minute ischemia/5-minute reperfusion is accompanied by significantly less stunning than preconditioning with one or two cycles of 5-minute ischemia/5-minute reperfusion in the rabbit heart.

Experiment 2: Infarct Size Study

Mortality of rabbits. Table 2 summarizes the total number of rabbits operated on and their mortality. No rabbits died during preconditioning procedures. Four rabbits (two rabbits in group 2 and two in group...
Data are 3 minutes after coronary baseline values.

2-minute coronary occlusion

3) died from ventricular fibrillation during sustained coronary occlusion. Mortality appears lower in group 1 (unpreconditioned controls) and group 4, but the difference was not statistically significant. Actually, the mortality rates of groups 2 and 3 were very similar to those of the untreated control rabbits (20–27%) in our previous studies.3,6

**Hemodynamic parameters.** The hemodynamic variables in the four groups are summarized in Table 3. Heart rate, systemic blood pressure, and rate-pressure product were significantly lower in the 5-minute ischemia group than in the 2-minute ischemia group. The left ventricular end-diastolic pressure (LVEDP) increased significantly in both 2- and 5-minute ischemia groups compared to the preocclusion values. The systolic and diastolic blood pressures decreased significantly in the 2- and 5-minute ischemia groups compared to the preocclusion values. The heart rate, rate-pressure product, and left ventricular end-diastolic pressure increased significantly during reperfusion compared to ischemic values in all groups. The thickening fraction also decreased significantly during ischemia and increased significantly during reperfusion in all groups.

**Table 1. Hemodynamic Parameters and Cardiac Function During Two Episodes of Myocardial Ischemia and Reperfusion**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>1st Oc</th>
<th>1st Reperfusion</th>
<th>2nd Oc</th>
<th>2nd Reperfusion</th>
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<tr>
<td>Heart rate (bpm)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>2-Min ischemia group</td>
<td>253±11</td>
<td>256±10</td>
<td>254±9</td>
<td>255±10</td>
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<td>5-Min ischemia group</td>
<td>273±11</td>
<td>274±11</td>
<td>273±12</td>
<td>276±13</td>
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<tr>
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<td>90±3</td>
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<td>91±6</td>
<td>92±6</td>
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<tr>
<td>5-Min ischemia group</td>
<td>98±3</td>
<td>97±4</td>
<td>100±5</td>
<td>96±5</td>
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<td>Diastolic blood pressure (mm Hg)</td>
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<tr>
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<td>75±5</td>
<td>71±4</td>
<td>73±6</td>
<td>76±5</td>
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<td>76±6</td>
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<tr>
<td>LVEDP (mm Hg)</td>
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<td>2-Min ischemia group</td>
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<td>3,468±239†</td>
<td>3,929±294‡</td>
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<td>5-Min ischemia group</td>
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<td>3,500±327</td>
<td>4,143±378§</td>
<td>3,150±230†</td>
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<tr>
<td>Thickening fraction (%)</td>
<td></td>
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<tr>
<td>2-Min ischemia group</td>
<td>13.2±1.4</td>
<td>-3.6±0.9*</td>
<td>11.7±1.6</td>
<td>10.0±1.2</td>
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<tr>
<td>5-Min ischemia group</td>
<td>19.6±4.0</td>
<td>-2.5±1.1†</td>
<td>14.9±3.2</td>
<td>7.3±3.1†</td>
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</table>

Pre-Oc, preocclusion of coronary artery; 1st Oc, first coronary occlusion; 1st Reperfusion, first coronary reperfusion; 2nd Oc, second coronary occlusion; 2nd Reperfusion, second coronary reperfusion; bpm, beats per minute; LVEDP, left ventricular end-diastolic pressure, LV dp/dt, left ventricular pressure.

2-Min ischemia group, n=9; 5-min ischemia group, n=7.

†p<0.05 vs. value at Pre-Oc in 2-minute ischemia group (by repeated-measures analysis of variance), ‡p<0.05 vs. value at Pre-Oc in 5-minute ischemia group, §p<0.05 each other, §p<0.05 each other.

**Figure 2.** Time course plot of systolic thickening in the 2-minute ischemia group, which received two episodes of 2-minute coronary occlusion separated by 5-minute reperfusion. Thickening fraction was expressed as percentage of baseline values. Pre-O, before coronary occlusion; O, 1 minute after coronary occlusion; R1’, 1 minute after reperfusion; R3’, 3 minutes after reperfusion; R5’, 5 minutes after reperfusion. Data are mean±SEM.

**Figure 3.** Time course plot of systolic thickening in the 5-minute ischemia group, which received two episodes of 5-minute coronary occlusion separated by 5-minute reperfusion. Thickening fraction was expressed as percentage of baseline values. Pre-O, before coronary occlusion; O, 1 minute after coronary occlusion; R1’, 1 minute after reperfusion; R3’, 3 minutes after reperfusion; R5’, 5 minutes after reperfusion. Data are mean±SEM.
sure products did not differ significantly among the four groups before coronary occlusion or during occlusion and reperfusion.

Infarct size data. The myocardial infarct size of the four experimental groups are presented in Table 4 along with the heart weight and the size of area at risk. Absolute infarct size was significantly smaller in groups 3 and 4 compared with group 1. That the infarct size difference between groups 1 and 2 (5.2±0.8 versus 3.0±0.7% of heart weight) did not reach a significant level was probably due to a slightly larger area at risk in group 2. Actually, when the infarct size was expressed as the percentage of area at risk (%I/AAR), a more sensitive index of infarct size, a marked limitation of infarct size became evident in all three preconditioned groups; %I/AAR was 21.1±3.0 in group 2, 20.1±3.4 in group 3, and 16.4±4.2% in group 4, all of which were significantly smaller than the %I/AAR of group 1 (43.9±5.0%). The differences in %I/AAR among groups 2, 3, and 4 were not statistically significant. These findings indicate that the three different preconditioning protocols used in the present study were equivalent regarding infarct size–limiting effect.

Figure 4 illustrates the comparison of the three different preconditioning protocols in terms of the accompanying myocardial stunning and the effects on myocardial infarct size. These results show that the degree of myocardial stunning was lower after preconditioning with 2-minute ischemia than after one or two episodes of 5-minute ischemia (panel A); however, the three protocols of preconditioning limited infarct size to a similar extent (panel B).

Experiment 3: Myocardial Stunning and Preconditioning Effect in Individual Animals

Of the 12 rabbits in this experiment, one rabbit was excluded because of technical failure of the surgical preparation and one unpreconditioned rabbit died from heart failure after coronary occlusion. The remaining 10 rabbits were analyzed.

Heart rates and systemic blood pressure were 219±6 beats per minute and 85±3/73±3 mm Hg at baseline and 215±6 beats per minute and 86±3/69±3 mm Hg during coronary artery occlusion, respectively. Baseline thickening fraction was 13.5±1.5% and the size of the area at risk was 9.1±1.0% of the heart. Figure 5 illustrates the plot of infarct size of each animal against the thickening fraction immediately before the 30-minute coronary occlusion. The size of infarcts determined by tetrazolium staining was widely scattered in unpreconditioned animals, whereas the histological infarct size in unpreconditioned hearts (group 1) averaged 43.9±5.0% in experiment 2. Nevertheless, there was no significant correlation between the infarct size and the degree of myocardial stunning caused by preconditioning (r=0.386, p=NS) as shown in Figure 5, which is consistent with the dissociation between the

<table>
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<tr>
<th>Table 3. Hemodynamic Data From the Infarct Size Study</th>
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<td><strong>Group</strong></td>
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<tr>
<td>Heart rate (beats/min)</td>
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<td>Group 1</td>
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<td>Group 2</td>
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<td>Group 3</td>
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<td>Group 4</td>
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<td>Systolic blood pressure (mm Hg)</td>
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<td>Group 1</td>
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<td>Group 4</td>
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<td>Diastolic blood pressure (mm Hg)</td>
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<td>Group 1</td>
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<td>Group 2</td>
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<td>Group 3</td>
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<td>Group 2</td>
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<td>Group 3</td>
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<td>Group 4</td>
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with preconditioning farct size-limiting effect. ±SEM. Data reperfusion. at 2'PCx2, reperfusion; preconditioning-induced stunning indicated cycle of stunning ischemia/5-minute Myocardial infarct B: FIGURE 2510 Circulation = W U) z C 0 z I < .-O 1-O In the 0-1 U/) z5-minute ischemia/5-minute reperfusion; closed triangles, rabbits preconditioned by two cycles of 2-minute ischemia/5-minute reperfusion; closed circles, rabbits preconditioned by one cycle of 5-minute ischemia/5-minute reperfusion; closed squares, rabbits preconditioned by two cycles of 5-minute ischemia/5-minute reperfusion. Correlation is not significant (r=0.386, p=NS). Mean infarct size of unpreconditioned hearts with histological technique was 43.9±5.0% (see Table 4).

preconditioning-induced stunning and infarct size limitation indicated by experiments 1 and 2 (Figure 4).

Discussion
In the present study, two cycles of 2-minute ischemia/5-minute reperfusion caused less myocardial stunning than either one or two cycles of 5-minute ischemia/5-minute reperfusion. However, the infarct size–limiting effect of 2-minute ischemic preconditioning were not different from that of the 5-minute ischemic protocols. Moreover, no significant correlation was detected when both the degree of stunning by preconditioning and infarct size after preconditioning were examined in the same animals. These findings did not support the hypothesis that myocardial stunning contributes to the cardioprotective effect of ischemic preconditioning.

Different groups of rabbits were used to study the effect of preconditioning on stunning (experiment 1) and on infarct size (experiment 2). We took this approach because we aimed to accurately determine infarct size by histology, the gold standard method, at 72 hours after ischemia, and that long protocol was unlikely to be tolerated by rabbits receiving surgical preparations for the study of myocardial stunning. In addition, to confirm the findings in experiments 1 and 2, infarct size (visualized by tetrazolium staining) and myocardial stunning were analyzed in the same animals in experiment 3, even though tetrazolium stain-

![Figure 4](image-url)

**Figure 4.** Graphs show comparison of the three preconditioning protocols accompanying myocardial stunning vs. infarct size–limiting effect. Panel A: Systolic thickening fraction at 5 minutes after reperfusion following preconditioning. Panel B: Myocardial infarct size–limiting effect of preconditioning. 2'PC×2, Preconditioning with two cycles of 2-minute ischemia/5-minute reperfusion; 5'PC×1, preconditioning with one cycle of 5-minute ischemia/5-minute reperfusion; 5'PC×2, preconditioning with two cycles of 5-minute ischemia/5-minute reperfusion. Data of stunning for 5'PC×1 and 5'PC×2 were sequentially collected from the same animals. Data are mean±SEM.

![Figure 5](image-url)

**Figure 5.** Scatterplot shows relation between regional systolic thickening immediately before 30-minute ischemia and infarct size (tetrazolium staining) after 30-minute ischemia and 3-hour reperfusion. Open circles, unpreconditioned rabbits; closed triangles, rabbits preconditioned by two cycles of 2-minute ischemia/5-minute reperfusion; closed circles, rabbits preconditioned by one cycle of 5-minute ischemia/5-minute reperfusion; closed squares, rabbits preconditioned by two cycles of 5-minute ischemia/5-minute reperfusion. Correlation is not significant (r=0.386, p=NS). Mean infarct size of unpreconditioned hearts with histological technique was 43.9±5.0% (see Table 4).

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Heart weight (g)</th>
<th>Area at risk (% heart weight)</th>
<th>Infarct heart weight (% heart weight)</th>
<th>Infarct/area at risk (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>11</td>
<td>8.3±0.3</td>
<td>11.9±1.1</td>
<td>5.2±0.8</td>
<td>43.9±5.0</td>
</tr>
<tr>
<td>Group 2</td>
<td>10</td>
<td>7.3±0.2</td>
<td>13.1±1.5</td>
<td>3.0±0.7</td>
<td>21.1±3.0*</td>
</tr>
<tr>
<td>Group 3</td>
<td>9</td>
<td>8.2±0.4</td>
<td>8.8±1.2</td>
<td>1.7±0.4*</td>
<td>20.1±3.4*</td>
</tr>
<tr>
<td>Group 4</td>
<td>11</td>
<td>8.1±0.2</td>
<td>10.4±1.2</td>
<td>1.6±0.4*</td>
<td>16.4±4.2*</td>
</tr>
</tbody>
</table>

Values are presented as mean±SEM.
*p<0.05 vs. group 1.
ing potentially underestimates the infarct size and has poor resolution compared with the histological method.10

The infarct size of three un preconditioned rabbits in experiment 3 appears widely scattered (20.3–58.6%), as shown in Figure 5. That scattering of infarct size could partly be due to the infarct size variation in the rabbit population that was also observed in the un preconditioned group (group 1) of experiment 2, in which infarcts ranged from 28.4% to 65.2% of area at risk and averaged 43.9±5.0%. It is also possible that tetrazolium staining may have underestimated infarcts in some hearts because of the relatively short reperfusion period (i.e., 3 hours), causing apparently large variations in infarct size. Nevertheless, the infarct size did not significantly correlate with myocardial stunning in experiment 3 (Figure 5), which confirmed the dissociation between the preconditioning-induced stunning and infarct size–limiting effect shown by experiments 1 and 2.

The cardioprotective effect of preconditioning was the same despite widely differing levels of myocardial stunning for the three protocols (Figure 4). The present observation is further supported by a recent study by Murry et al.11 In their study, myocardial stunning by preconditioning with 15-minute ischemia persisted for 2 hours, but the infarct size–limiting effect of the preconditioning was undetectable at that time. We also found that preconditioning protection was lost after only 30 minutes of reperfusion in the rabbit,12 a time insufficient for recovery from myocardial stunning. Although the Murry et al study11 was in the dog, which has a variable level of collateral blood flow and a higher level of myocardial xanthine oxidase than the rabbit, both studies failed to observe any correlation between the degree of myocardial stunning accompanying preconditioning and infarct size limitation, which indicates that myocardial stunning does not directly mediate cardioprotective effects.

The mechanism for stunning is similarly obscure. Because myocardial stunning did not correlate with the cardioprotective effects of preconditioning, it is likely that unrelated mechanisms are responsible for each. The evidence is strong, however, that oxygen-derived free radicals contribute to myocardial stunning.1,13–15 On the other hand, a recent study from this laboratory demonstrated that free radical scavengers (superoxide dismutase and catalase) did not block the infarct size–limiting effect of ischemic preconditioning3 even though they are known to suppress stunning.1,15

Another important fact is that the reduction of the contractile function of stunned myocardium is not parallel with oxygen consumption. Although two studies16,17 have suggested that oxygen consumption might be modestly reduced in stunned myocardium, regionally stunned myocardium in the heart in situ18 and globally stunned myocardium in an isolated preparation19 were both found to have the same level of oxygen consumption as in normal myocardium. Accordingly, oxygen consumption relative to contractile function appears paradoxically higher than normal in those preparations. Furthermore, it is interesting to note that the time course of oxygen consumption change in stunned myocardium may even parallel the preconditioning effect. In a study by Renstrom et al.,17 the oxygen consumption of myocardium that received a 60% reduction of coronary flow for 45 minutes was modestly decreased and then recovered quickly to its preischemic level while a marked reduction of regional systolic shortening persisted. That pattern of dissociation between oxygen consumption and function has a time course similar to that observed between preconditioning effect and stunning in the study by Murry et al.11 Such a hypothesis is speculative, however, because no study has examined the correlation between oxygen consumption of preconditioned myocardium and its ischemic tolerance in the same experimental model.

The mechanism of increased oxygen consumption of stunned myocardium relative to its contractile function appears to be related to excitation-contraction coupling, because the basal oxygen consumption of stunned myocardium is not greater than that of normal myocardium.19,20 Rather, an increased oxygen cost of excitation-contraction coupling appears to explain the dissociation between contractility and myocardial oxygen consumption.20 If there is any relation between the increase in oxygen consumption and the preconditioning effect, it is more likely to be on the excitation side of the excitation-contraction coupling, such as ion pumps consuming more ATP.

We found that two cycles of 2-minute ischemia separated by 5 minutes of reperfusion still preconditioned the heart quite well. However, Downey et al21 reported that two ischemic cycles separated by 10 minutes of reperfusion did not offer protection. The explanation for the discrepancy is not clear because rabbits were used for both studies. One possible explanation could be that 5 minutes between ischemic periods does not allow complete recovery of some ischemia-induced metabolic changes as opposed to 10 minutes. Most recently, adenosine accumulation during the ischemic period has been proposed to be the trigger for preconditioning.22 If that is the case, 5 minutes might be too short a period to normalize adenosine production. The other possibility is that subtle differences might exist between the strains of rabbits used, Japanese White in the present study and New Zealand White in the study by Downey et al.21

A dissociation between preconditioning protection and myocardial stunning was shown in this study. We found a full cardioprotective effect even when the preconditioning ischemia was so short that little stunning accompanied it. This result leads us to conclude that the two phenomena are unrelated.

References
3. Iwamoto T, Miura T, Adachi T, Noto T, Ogawa T, Tsuchida A, Iimura O: Myocardial infarct size-limiting effect of ischemia preconditioning was not attenuated by oxygen free radical scavengers in the rabbit. Circulation 1991;83:1015-1022
18. Dean EN, Shlafer M, Nicklas JM: The oxygen consumption paradox of "stunned myocardium" in dogs. Basic Res Cardiol 1990;85:120-131

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