Preconditioning Improves Energy Metabolism During Reperfusion but Does Not Attenuate Myocardial Stunning in Porcine Hearts

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Background. It has been reported that a brief period of coronary occlusion and reperfusion slows the rate of ATP depletion during subsequent sustained ischemia as well as limiting infarct size. However, it has not yet been determined whether ischemic preconditioning also has an effect on the functional and metabolic recovery of stunned myocardium. Our study was designed to address this problem.

Methods and Results. Farm pigs were anesthetized with halothane and randomly assigned to either a control group or a preconditioned group. The control group (n=15) underwent 10 minutes of coronary occlusion followed by 120 minutes of reperfusion. The preconditioned group (n=14) underwent two episodes of 5-minute occlusion and 5-minute reperfusion followed by 15 minutes of occlusion and 120 minutes of reperfusion. This protocol was designed to exclude the stunning effect of the preconditioning procedure itself as much as possible besides preconditioning the heart. A pair of ultrasonic crystals was implanted in the area at risk perfused by the left anterior descending coronary artery. 31P-nuclear magnetic resonance spectroscopy and sonomicrometry were performed alternately. Regional myocardial blood flow (RMBF) was determined with labeled microspheres. At 15 minutes of sustained ischemia, phosphocreatine (Pcr), ATP, and intracellular pH were significantly better preserved in the preconditioned group (Pcr: control/preconditioned, 1±1%/14±1%; ATP: control/preconditioned, 66±2%/74±2%; pH: control/preconditioned, 6.32±0.07/6.52±0.05; P<.05). After reperfusion, ATP increased progressively and was almost normalized at 120 minutes of reperfusion in the preconditioned group (control/preconditioned, 73±4%/95±3%; P<.05). Overshoot of Pcr (which indicates that the energy generating system is operating better than energy utilizing system) persisted in preconditioned hearts but disappeared rapidly in controls (control/preconditioned, 104±3%/130±3% after 120 minutes of reperfusion). There was no significant difference in percent segment shortening (SS), RMBF, and hemodynamics between the two groups throughout the experiment (%SS: control/preconditioned, 29.8±5.9%/28.8±6.3% of baseline after 120 minutes of reperfusion).

Conclusions. Preconditioning improves energy metabolism during reperfusion, although it does not attenuate myocardial stunning for at least 2 hours after reperfusion. (Circulation 1993;88:223-234)

KEY WORDS • myocardial ischemia • 31P-NMR spectroscopy • high energy phosphates • phosphocreatine overshoot

Since the first report by Murry et al,1 a brief episode of ischemia and reperfusion termed ‘‘ischemic preconditioning’’ has been established as rendering hearts resistant to irreversible damage during a subsequent prolonged ischemic insult. The adenosine A1 receptor appears to be one of the determinants in limiting infarct size.2-4 In addition, ischemic preconditioning has been reported to reduce the incidence of ventricular arrhythmias during subsequent ischemia and reperfusion.5,6 Recently, Cohen et al7 showed that ischemic preconditioning results in not only a limitation of infarct size but also in the significantly better recovery of systolic function in rabbit hearts. However, it was not determined whether this improved recovery was due to a smaller infarct size and/or an amelioration of myocardial stunning. To explore this question, a study needs to be performed in a model without infarction.

It has been reported that during sustained ischemia, ischemic preconditioning preserves ATP8,9 and phosphocreatine (Pcr) levels and delays the decrease of intracellular pH,9 probably because of reduction of energy consumption. However, the changes in the energy metabolism of preconditioned hearts during reperfusion after sustained ischemia have not been elucidated yet. Thus, the purpose of the present study was to assess the effects of ischemic preconditioning on the functional and metabolic recovery of stunned myocardium in pig hearts without collateral circulation, using paired ultrasonic crystals and 31P-nuclear magnetic resonance (NMR) spectroscopy.9-15
Methods

Animal Preparation

This experiment conformed to the guiding principles of the American Physiological Society regarding the use of laboratory animals.

Thirty-six farm pigs weighing 13 to 16 kg were sedated with ketamine (15 mg/kg), anesthetized with sodium pentobarbital (15 mg/kg), and randomly assigned to a control group (n=19) or a preconditioned group (n=17). After tracheotomy, they were mechanically ventilated at 18 ventilations per minute, and the tidal volume was adjusted from 13 to 17 mL/kg using a mixture of oxygen, nitrogen, and carbon dioxide (0.24:0.75:0.01) to keep arterial blood gas within physiological range. Anesthesia was maintained with fentanyl (0.5% to 1.5%). An 8.5F introducer sheath was inserted into the ascending aorta via the left carotid artery to record the aortic pressure and heart rate on a polygraph system (Nihon Kohden Inc, Tokyo). Through the sheath, a 7F catheter-tipped micromanometer (Catheter Tip Pressure Sensor, Nihon Kohden Inc) was advanced to the left ventricle (LV) for the measurement of LV pressure and LV dP/dt. The left external jugular vein was cannulated for fluid infusion. The left atrium was cannulated for the injection of colored microspheres, and the left femoral artery was cannulated to allow the withdrawal of reference blood samples during measurement of the regional myocardial blood flow (RMBF).

The heart was exposed through a midline thoracotomy and suspended in a pericardial cradle. A 3-mm segment of the distal third of the left anterior descending coronary artery (LAD) was dissected free from the surrounding tissue, and an air occluder was positioned around it. A brief test occlusion was done for a few seconds by inflating the occluder, and the area at risk was confirmed by the cyanotic color. The average area at risk was approximately 35x35 mm. A 17-mm diameter surface coil tuned to 32.7 MHz was positioned in the center of the area at risk. At the end of the experiment, we reconfirmed the position of the surface coil to see whether it was within the area at risk by reoccluding the artery. If the surface coil was out of the area at risk, the case was excluded from analysis. For the measurement of segmental shortening (%SS) of the ischemic myocardium, a pair of ultrasonic crystals was implanted 8 to 12 mm apart and approximately 3 mm beneath the surface just apical to the surface coil and oriented parallel to the short axis of the heart. Pigs were allowed to rest for 30 minutes to reach the steady state.

Experimental Protocol

The experimental protocol is shown in Fig 1. After the resting period, initial baseline measurements of segmental length, hemodynamics, and magnetic resonance spectroscopy (MRS) were performed. In the control group, pigs underwent 15 minutes of LAD occlusion and 120 minutes of reperfusion; in the preconditioned group, pigs underwent two 5-minute cycles of LAD occlusion separated by a 5-minute reflow period followed by 15 minutes of LAD occlusion (sustained ischemia) and 120 minutes of reperfusion.

In our original study,9 four episodes of 5-minute occlusion and 5-minute reperfusion were used for preconditioning. However, in the present study, two episodes of 5-minute occlusion and 5-minute reperfusion were used to precondition the pig hearts. It is well
known that the number of ischemic episodes is related positively to the magnitude of stunning. In our preliminary study, percent segment shortening normalized by the baseline value was 74.8±7.5% after two episodes of 5-minute occlusion and 5-minute reperfusion (n=4 pigs) and 45.2±6.8% after four episodes of 5-minute occlusion and 5-minute reperfusion (n=4 pigs). Thus, the preconditioning procedure of four episodes of 5-minute occlusion and 5-minute reperfusion induced considerable stunning, which might make it difficult to evaluate the effect of preconditioning on the functional recovery of stunned myocardium after sustained ischemia. To exclude this as much as possible, two episodes of 5-minute occlusion and 5-minute reperfusion were used for preconditioning in the present study.

Measurement of Segment Shortening

Hemodynamic measurements using the micromanometer did not affect 31P-MRS data. However, the measurement of segment length using a sonomicrometer caused unacceptable noise in 31P-NMR spectra. Therefore, after the measurement of segment length, the 31P-NMR spectra were interrupted for 5 minutes, as indicated in Fig 1. The end-diastolic segment length (EDL) was measured at the onset of positive LV dP/dt, and the end-systolic segment length (ESL) was measured at peak negative LV dP/dt. The %SS was calculated as 100×(EDL−ESL)/EDL and was represented by the average of at least five beats. Time course change of %SS was evaluated after normalization by the baseline value. Measurements were obtained before occlusion, during occlusion, and at 30, 60, 90, and 120 minutes after reperfusion.

31P-NMR Spectroscopy

31P-NMR spectra were obtained using a BEM-250/80 in vivo spectrometer (Otsuka Electronics Inc, Philadelphia) with a 1.9-T, 31-cm bore superconducting magnet. The spectrometer was interfaced with the surface coil. The homogeneity of the magnetic field in the region of the surface coil was optimized by shimming on the proton signal using a 12-channel shim supply to make the width smaller than 0.5 ppm. Respiration- and arterial pressure–gated spectra were obtained at end inspiration and peak systole, accumulating 90 free induction decays (FIDs) for each spectrum over 5 minutes. The pulse was 90 broad band (15 μsec) with a cycle time of 3.3±0.2 seconds and sweep width of 3 kHz. The FID was multiplied by an exponential to 10 Hz line broadening to improve the signal-to-noise ratio. Tissue levels of ATP, Pcr, and inorganic phosphate (Pi) were estimated by integrating the areas under the individual peaks using a computer program (MEAS1, Graphtec Co, Tokyo) and a digitizer. Their time course of changes after ischemia and reperfusion were expressed as percentages relative to the baseline values. We used a convolution difference algorithm for baseline correction. Intracellular pH was calculated from the chemical shift of the major Pi peak using the Flaherty equation. Pcr was used as a reference for the chemical shift of Pi. After disappearance of Pcr, a small glass tube containing hexamethylphosphoric triamide set in the center of the surface coil was used as a reference; this is also used as a standard to correct the changes in spectrometer characteristics.

The levels of Pcr and ATP are known to change during the cardiac cycle. In the present study, blood pressure gating eliminated such cardiac cyclic variations, and respiration gating kept the position of the heart constant in the magnetic field, which enhanced the accuracy of the metabolic information.

Postmortem Analysis

After the pigs were killed, the hearts were removed and both the left and right coronary arteries were cannulated. Keeping the air occluder inflated, 1% monastral blue dye (Sigma) was injected into the coronary artery at a pressure of 90 mm Hg. The monastral blue staining allowed visualization of the unstained ischemic area at risk that served as a guide for sampling tissues for regional blood flow. After fixation in 10% formalin, the hearts were cut into 5-mm serial slices in a plane parallel to the atrioventricular groove. The right ventricle, the atria, and the valvular structures were removed. The slices of the isolated left ventricle were weighed, and their apical surfaces were photographed. The area at risk was identified, traced from enlarged projections (×10) of the photographic slide of each ventricular slice, and quantified with a digitizer. The percent area at risk (area at risk divided by the slice area) was calculated for each slice and multiplied by the slice weight to obtain the weight of area at risk. The weights of each area at risk were summed and divided by the LV weight to yield the percentage of the LV at risk. The second slice from the apex was examined histologically with Masson’s trichrome staining and hematoxylin and eosin staining and was examined immunohistologically with antimyoglobin antibody.

Measurement of RMBF

Measurements of RMBF were made with 15-μm colored polystyrene microspheres (E-Z Trac, Los Angeles, Calif). Approximately 107 microspheres (red, yellow, blue, and black) were injected into the left atrium before occlusion, at 5 minutes after occlusion, and at 60 and 120 minutes after reperfusion. Reference blood samples were withdrawn at a rate of 5 mL/min from the femoral artery starting 10 seconds before and continuing for 2 minutes after each injection.

Tissue samples for RMBF measurements were taken transmurally from the central ischemic area and the remote nonischemic area. Each sample was divided into endocardial and epicardial halves, each of which usually weighed 1.5 to 2.0 g. The extraction of microspheres from the blood and tissue samples was performed as described by Hale et al. RMBF was calculated from the formula RMBF=CM×QR/CR, where CM is the microsphere count per gram of tissue, QR is the withdrawal rate of the reference blood sample (5 mL/min), and CR is the microsphere count in the reference blood sample.

Additional Experiments

To determine how the myocardium is stunned by the preconditioning procedure itself, only segment shortening was recorded and 31P-MRS was not performed in three subgroups (five pigs in each). The first group underwent two episodes of 5-minute occlusion and 5-minute reperfusion followed by 15 minutes of occlusion and 120-minute reperfusion (5-minute precondi-
Mortality

Thirty-six pigs were initially entered into this study. Two of 19 pigs in the control group and 2 of 17 pigs in the preconditioned group died during sustained ischemia. Two of 19 pigs in the control group and 1 of 17 pigs in the preconditioned group died immediately after reperfusion of sustained ischemia. The cause of death was ventricular fibrillation in all cases. Thus, 15 pigs in the control group and 14 pigs in the preconditioned group were used for analysis. There was no significant difference in the mortality rate between the two groups. Eighteen pigs were entered into the additional experiment, and 3 of these pigs died immediately after reperfusion of sustained ischemia.

Area at Risk

There was no significant difference in the area at risk between the control group (19.1±1.7% of the LV) and the preconditioned group (18.2±1.1% of the LV).

Histological examination with Masson’s trichrome and hematoxylin and eosin staining and immunohistological examination with antimyoglobin antibody revealed no necrosis in the area at risk in any of the pigs.

Hemodynamic Changes

Hemodynamic parameters are summarized in Table 1. Heart rate (HR), systolic arterial pressure (SAP), and diastolic arterial pressure (DAP) were expressed by the mean values for each 5-minute period. In both the preconditioned and the control groups, SAP and DAP decreased during occlusion but recovered promptly after reperfusion. There was no significant difference in HR, SAP, DAP, LV dp/dt (mm Hg/sec), and LVEDP between the two groups throughout the experiment. Similar results were obtained in the subgroups of the additional experiment.

Regional Myocardial Blood Flow

RMBF values are summarized in Table 2. The area at risk was not stained by the monastral blue dye, indicating that there was no significant collateral circulation. Indeed, RMBF in the area at risk was below 0.03 mL·g⁻¹·min⁻¹ during ischemia in both groups. RMBF did not differ significantly between the two groups before occlusion, during occlusion, and during reperfusion in both the subendocardium and the subepicardium.

Percent Segment Shortening

The time course change of %SS in the area at risk is presented in Fig 2. Baseline %SS was 17.1±1.0% in the control group and 16.0±1.0% in the preconditioned group (P=NS). We normalized %SS by the baseline value to minimize variability among individual animals. During 15 minutes of sustained ischemia, %SS was

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**Table 1. Hemodynamic Parameters and Cardiac Function in Control and Preconditioned Pigs During Coronary Occlusion and Reperfusion**

<table>
<thead>
<tr>
<th>Group</th>
<th>Preconditioning period</th>
<th>Prolonged OCC</th>
<th>Reperfusion</th>
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<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>O1</td>
<td>R1</td>
</tr>
<tr>
<td>Control (n=15)</td>
<td>HR (bpm)</td>
<td>100±4</td>
<td>98±5</td>
</tr>
<tr>
<td></td>
<td>SBP (mm Hg)</td>
<td>96±3</td>
<td>89±2</td>
</tr>
<tr>
<td></td>
<td>DBP (mm Hg)</td>
<td>55±2</td>
<td>52±2</td>
</tr>
<tr>
<td></td>
<td>LVEDP (mm Hg)</td>
<td>4±1</td>
<td>5±1</td>
</tr>
<tr>
<td></td>
<td>LV dp/dt  (mm Hg/sec)</td>
<td>1022±50</td>
<td>1022±50</td>
</tr>
<tr>
<td></td>
<td>%SS</td>
<td>17.1±1.0</td>
<td></td>
</tr>
<tr>
<td>Preconditioned (n=14)</td>
<td>HR (bpm)</td>
<td>104±3</td>
<td>102±3</td>
</tr>
<tr>
<td></td>
<td>SBP (mm Hg)</td>
<td>102±4</td>
<td>94±4</td>
</tr>
<tr>
<td></td>
<td>DBP (mm Hg)</td>
<td>60±2</td>
<td>56±2</td>
</tr>
<tr>
<td></td>
<td>LVEDP (mm Hg)</td>
<td>5±1</td>
<td>6±1</td>
</tr>
<tr>
<td></td>
<td>LV dp/dt  (mm Hg/sec)</td>
<td>1013±53</td>
<td>1013±53</td>
</tr>
<tr>
<td></td>
<td>%SS</td>
<td>16.0±1.0</td>
<td></td>
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</tbody>
</table>

There were no significant differences in hemodynamics between the two groups. Values are mean±SEM. O1, first occlusion; R1, first reperfusion; O2, second occlusion; R2, second reperfusion; OCC, 15-minute occlusion; HR, heart rate; bpm, beats per minute; SBP, systolic blood pressure; DBP, diastolic blood pressure; LVEDP, left ventricular end-diastolic pressure; LV dp/dt, rate of change of left ventricular pressure; %SS, percent segment shortening.

Statistical Analysis

For comparison of each metabolite, intracellular pH, %SS, and RMBF across time between preconditioned and control groups, a two-factor ANOVA for repeated measures was used. When the ANOVA was significant, comparisons between the two groups were made by Student’s t test. A value of P<.05 was considered to be statistically significant, and all results were expressed as mean±SEM.

Results

**Table 1. Hemodynamic Parameters and Cardiac Function in Control and Preconditioned Pigs During Coronary Occlusion and Reperfusion**

<table>
<thead>
<tr>
<th>Group</th>
<th>Preconditioning period</th>
<th>Prolonged OCC</th>
<th>Reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>O1</td>
<td>R1</td>
</tr>
<tr>
<td>Control (n=15)</td>
<td>HR (bpm)</td>
<td>100±4</td>
<td>98±5</td>
</tr>
<tr>
<td></td>
<td>SBP (mm Hg)</td>
<td>96±3</td>
<td>89±2</td>
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<td></td>
<td>DBP (mm Hg)</td>
<td>55±2</td>
<td>52±2</td>
</tr>
<tr>
<td></td>
<td>LVEDP (mm Hg)</td>
<td>4±1</td>
<td>5±1</td>
</tr>
<tr>
<td></td>
<td>LV dp/dt  (mm Hg/sec)</td>
<td>1022±50</td>
<td>1022±50</td>
</tr>
<tr>
<td></td>
<td>%SS</td>
<td>17.1±1.0</td>
<td></td>
</tr>
<tr>
<td>Preconditioned (n=14)</td>
<td>HR (bpm)</td>
<td>104±3</td>
<td>102±3</td>
</tr>
<tr>
<td></td>
<td>SBP (mm Hg)</td>
<td>102±4</td>
<td>94±4</td>
</tr>
<tr>
<td></td>
<td>DBP (mm Hg)</td>
<td>60±2</td>
<td>56±2</td>
</tr>
<tr>
<td></td>
<td>LVEDP (mm Hg)</td>
<td>5±1</td>
<td>6±1</td>
</tr>
<tr>
<td></td>
<td>LV dp/dt  (mm Hg/sec)</td>
<td>1013±53</td>
<td>1013±53</td>
</tr>
<tr>
<td></td>
<td>%SS</td>
<td>16.0±1.0</td>
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TABLE 2. Regional Myocardial Blood Flow

<table>
<thead>
<tr>
<th></th>
<th>Ischemic zone (mL·g⁻¹·min⁻¹)</th>
<th>Nonischemic zone (mL·g⁻¹·min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>END</td>
<td>EPI</td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.40±0.08</td>
<td>1.23±0.11</td>
</tr>
<tr>
<td>Preconditioned</td>
<td>1.22±0.06</td>
<td>1.09±0.06</td>
</tr>
<tr>
<td>Occlusion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.01±0.00</td>
<td>0.01±0.00</td>
</tr>
<tr>
<td>Preconditioned</td>
<td>0.03±0.01</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>Reperfusion (60 min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.02±0.13</td>
<td>0.82±0.07</td>
</tr>
<tr>
<td>Preconditioned</td>
<td>0.88±0.08</td>
<td>0.73±0.07</td>
</tr>
<tr>
<td>Reperfusion (120 min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.88±0.11</td>
<td>0.75±0.08</td>
</tr>
<tr>
<td>Preconditioned</td>
<td>0.91±0.06</td>
<td>0.77±0.06</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

END, subendocardium; EPI, subepicardium.

reduced to −40.9±11.0% of baseline in the control group and −30.6±4.9% of baseline in the preconditioned group (P=NS). Although active shortening returned after reperfusion in both groups, regional function remained depressed throughout the reperfusion period (control/preconditioned, 32.2±6.9%/18.1±5.1%, 31.6±5.5%/22.5±5.2%, 35.1±5.6%/24.4±5.2%, 29.8±5.9%/28.8±6.3% of baseline at 30, 60, 90, and 120 minutes after reperfusion, respectively). There was no significant difference in %SS between the two groups throughout the reperfusion period. In the additional experiment, %SS was reduced to 74.7±6.5% of baseline after preconditioning in the first group preconditioned with two episodes of 5-minute

**FIG 2.** Time course of changes in segment shortening in the area at risk normalized for baseline values are plotted. During preconditioning period, segment shortening could not be measured because the sonomicrometer caused unacceptable noise in 31P-nuclear magnetic resonance spectra. During 15 minutes of sustained ischemia, paradoxical bulging was observed in all pigs. Although active shortening returned after reperfusion, regional function remained depressed throughout the reperfusion period in both groups. By the end of the reperfusion period, segment shortening was 29.8±5.9% in the control group and 28.8±6.3% in the preconditioned group. There was no significant difference in %SS between the two groups. O1, first occlusion; R1, first reperfusion; O2, second occlusion; R2, second reperfusion; OCC, 15 minutes of sustained ischemia.
occlusion and 5-minute reperfusion, whereas it returned to baseline level (103.1±6.9% of baseline) after preconditioning in the second group preconditioned with a single episode of 2-minute occlusion and 5-minute reperfusion. There was no significant difference in the degree of paradoxical contraction during 15 minutes of ischemia among the three groups: After 120 minutes of reperfusion, it was 21.8±10.6% in the first group, 22.2±7.8% in the second group, and 28.5±11.3% in the control group without preconditioning. There was no significant difference in %SS among the groups (Fig 3).

**3P-NMR Spectroscopy**

During baseline measurements, Pcr, ATP, P, and intracellular pH did not change significantly. Accordingly, the time course of changes in Pcr, ATP, and P, were expressed as percentages relative to the baseline values determined just before the first ischemic episode. Time course changes of the 3P-NMR spectrum are shown in Fig 4.

Pcr decreased to 20±2% at 5 minutes of sustained ischemia and almost disappeared at the end of ischemia in the control group. In the preconditioned group, Pcr decreased to 22±4% and 31±5% after the first and second 5-minute preconditioning ischemia, respectively, and returned above baseline value during each 5-minute reperfusion period (111±6% and 119±11%), then decreased to 34±4% and 14±1% at 5 and 15 minutes of sustained ischemia, respectively. At 5 and 15 minutes of sustained ischemia, Pcr was significantly higher in the preconditioned group than in the control group. After 20 minutes of reperfusion, Pcr increased to 110±3% in the control group and 124±3% in the preconditioned group (the "overshoot" phenomenon of Pcr). This phenomenon disappeared after 30 minutes of reperfusion in the control group, whereas it persisted in the preconditioned group. By the end of the reperfusion period, Pcr increased markedly in the preconditioned group (control/preconditioned, 104±3%/130±3%; P<.05). Pcr was significantly higher in the preconditioned group than in the control group throughout the reperfusion period (Fig 5).

In the control group, ATP decreased to 89±4% and 66±2% at 5 and 15 minutes of sustained ischemia, respectively. In the preconditioned group, ATP decreased to 89±4% after preconditioning, then decreased to 87±4% and 74±2% at 5 and 15 minutes of sustained ischemia, respectively. Thus, during sustained ischemia, ATP decreased more slowly in the preconditioned group than in the control group. ATP became significantly higher at 15 minutes of sustained ischemia in the preconditioned group than in the control group. After 10 minutes of reperfusion, there was little recov-
ery of ATP in both groups (control/preconditioned, 66±2%/75±4%). In the control group, ATP did not increase during the remaining reperfusion period, whereas ATP showed good recovery and increased to 86±3% after 60 minutes of reperfusion and to 95±3% after 120 minutes of reperfusion in the preconditioned group. ATP was significantly higher in the preconditioned group than in the control group throughout the reperfusion period (Fig 6).

In the preconditioned group, P, increased rapidly and reached 176±10% and 190±10% after the first and second 5-minute preconditioning ischemia, respectively, but returned quickly to the baseline level during each 5-minute reperfusion period. After 15 minutes of sustained ischemia, P, increased about 2.5 times of baseline level in both groups. After reperfusion, P, varied between 120% and 140% of baseline level toward the end of the reperfusion period, and no significant difference in P, was observed between the two groups.

In the control group, intracellular pH was 7.28±0.03 before ischemia and fell to 6.82±0.05 and 6.32±0.07 at 5 and 15 minutes of ischemia, respectively. In the preconditioned group, intracellular pH was 7.30±0.03 before ischemia (P=NS) and fell to 6.78±0.04 and 6.86±0.03 after the first and second 5-minute preconditioning ischemia, respectively, with a return to the baseline level during each 5-minute reperfusion period. Subsequently, it fell to 6.85±0.05 and 6.52±0.05 at 5 and 15 minutes of sustained ischemia, respectively. Intracellular pH became significantly higher at 15 minutes of sustained ischemia in the preconditioned group than in the control group (P<.05). After reperfusion, intracellular pH returned to baseline level in both groups (Fig 7).

Discussion

Our data demonstrated that ischemic preconditioning caused a better recovery of ATP level and persistent overshoot phenomenon of Pcr. However, ischemic preconditioning did not improve the recovery of regional LV contractility during reperfusion after 15 minutes of sustained ischemia.

Methodological Considerations

Previous in vivo studies on myocardial stunning and energy metabolism have been performed by obtaining biopsy samples from the region of interest. This method might cause injury to the remaining tissue and thus affect its structure, energy metabolism, blood flow, and particularly its regional contractile function. In addition, these studies measured energy metabolism at only a few time points. 31P-MRS can overcome these problems by supplying metabolic information in a serial and noninvasive manner.

The radius of our surface coil was 8.5 mm. Thus, our surface coil collects signals only from the 8.5-mm radius.
hemisphere under the surface coil. We set the surface coil in the center of the area at risk (average size, 35 x 35 mm). Therefore, we believe that contamination of non-ischemic myocardium within the area of the surface coil would be minimal. However, contamination from blood in the LV cavity might occur in the P1 peak area, especially during ischemia with bulging and reflow with stunning. Furthermore, P1 is fused with other phosphate compounds, and it is difficult to differentiate them precisely. Therefore, P1 level by 31P-NMR spectroscopy is not as precise as ATP and Pcr levels. This may explain the lack of recovery to the baseline level in P1 during a reflow period. In the present study, however, segment shortening during ischemia and reflow periods was similar for the two groups with and without ischemic preconditioning. This suggests that the degree of contamination from blood in the LV cavity and the amount of myocardial mass within the region of the surface coil are also similar for the two groups.

It was impossible to measure both segment shortening and high energy phosphates simultaneously because of radiofrequency noise. Therefore, 31P-MRS and sonomicrometry were performed alternatively throughout the experiment to allow repeated measurements of energy metabolism and cardiac function. However, during ischemic preconditioning, only 31P-MRS was performed because of the short ischemic duration of 5 minutes. To explore segment shortening during ischemic preconditioning, subgroups of pigs underwent sonomicrometry only.

It has been established that regional myocardial blood flow assessed by the nonradioactive microsphere method was related closely with that assessed by the radioactive microsphere technique. 27 In the present study, regional myocardial blood flow during occlusion was 0.90 to 1.02 mL g⁻¹ min⁻¹ in nonischemic tissue and was transmurally almost zero (0 to 0.03 mL g⁻¹ min⁻¹) in ischemic tissue, as shown in Table 2. These results are consistent with those in pig hearts obtained by the radioactive microsphere analysis in our previous study. 28 Therefore, we believe that the nonradioactive microsphere technique used in the present study is a reliable method.

Effect of Two Episodes of 5-Minute Occlusion and 5-Minute Reperfusion on Myocardial Metabolism During Sustained Ischemia

In the first report by Murry et al, 4 four episodes of 5-minute occlusion and 5-minute reperfusion were used to precondition the heart. However, it has also been reported that a single 5-minute occlusion was sufficient to precondition canine 29 and rabbit hearts. 27 According to Schott et al, 30 ischemic preconditioning can also be induced in pigs by two episodes of 10-minute occlusion and 30-minute reperfusion. In a recent study, 9 we showed that four episodes of 5-minute occlusion and 5-minute reperfusion preserved the ATP, Pcr levels,
FIG 6. Time course of changes in ATP are plotted. In the control group, ATP decreased to 89±4% and 66±2% at 5 and 15 minutes of sustained ischemia, respectively. In the preconditioned group, ATP decreased to 89±4% after preconditioning, then decreased to 87±4% and 74±2% at 5 and 15 minutes of sustained ischemia, respectively. During sustained ischemia, ATP decreased more slowly in the preconditioned group than in the control group. ATP became significantly higher at 15 minutes of sustained ischemia in the preconditioned group than in the control group. After reperfusion, there was a rapid recovery of ATP in the preconditioned group, and it returned to 95±3% after 120 minutes of reperfusion. ATP was significantly higher in the preconditioned group than in the control group throughout the reperfusion period. O1, first occlusion; R1, first reperfusion; O2, second occlusion; R2, second reperfusion; OCC, 15 minutes of sustained occlusion.

and intracellular pH during subsequent sustained ischemia in pigs. The present study has shown that two episodes of 5-minute occlusion and 5-minute reperfusion appear to provide effective cardioprotection in pigs.

In contrast to our 31P-MRS data, McDonough et al demonstrated more rapid depletion of ATP during ischemia in porcine myocardium, using high-performance liquid chromatography. In their study, pigs were
paced at 160 beats per minute, and SAP was over 100 mm Hg during ischemia. However, in our study, HR was about 100 beats per minute and SAP was about 90 mm Hg. Thus, the rate-pressure products in their study and in ours were greater than 16,000 mm Hg/min and about 9,000 mm Hg/min, respectively. The slower depletion of ATP during sustained ischemia noted in the present study may thus be explained by the lower rate-pressure product. Indeed, our previous 31P-MRS data obtained under pentobarbital anesthesia showed a higher rate-pressure product and more rapid ATP depletion than the present data obtained under fluothane anesthesia.

*Effect of Ischemic Preconditioning on Myocardial Stunning*

In the present study, hearts preconditioned with two episodes of 5-minute occlusion and 5-minute reperfusion did not recover their regional systolic function during 120 minutes of reperfusion after 15 minutes of sustained ischemia. Cohen et al. have shown that ischemic preconditioning results in a better recovery of systolic function and a limitation of infarct size in rabbit hearts with subendocardial infarcts. However, a recent study by Ovize et al. revealed that ischemic preconditioning did not attenuate myocardial stunning in a canine model with collateral circulation and without myocardial infarction. It was reported that in the pig heart without collateral circulation, infarct does not occur when reperfusion is provided within 20 minutes of ischemia. Therefore, we used a 15-minute ischemia protocol in the present study, and histological and immunohistochemical examinations showed no myocardial necrosis in any of the pigs with or without preconditioning. There was no significant difference in the HR, SAP, DAP, LV dP/dt, and LVEDP between the preconditioned and control groups throughout the experiment. In addition, the RMBF did not show any significant difference between the two groups. Thus, the determinants of myocardial function such as infarct size, hemodynamic parameters, and myocardial blood flow were comparable between the two groups. Therefore, ischemic preconditioning with two episodes of 5-minute occlusion and 5-minute reperfusion also had no beneficial effect on stunning in pig hearts.

Because the preconditioning procedure (two episodes of 5-minute occlusion and 5-minute reperfusion) itself depressed the segment shortening to 74.7±6.5% of baseline, myocardial stunning after sustained ischemia could be due to preconditioning in addition to sustained ischemia. This might confound the evaluation of the present study regarding the effect of ischemic preconditioning on stunning. To solve this problem, an additional preconditioning protocol with a single episode of 2-minute occlusion and 5-minute reperfusion, which by itself did not stun the myocardium, was also performed. However, this preconditioning protocol also failed to improve the segment shortening after sustained ischemia. Thus, ischemic preconditioning did not attenuate myocardial stunning for at least 2 hours after reperfusion.

*Energy Metabolism During Reperfusion in Preconditioned Hearts*

The present study revealed that during 120 minutes of reperfusion after 15 minutes of ischemia, ATP was almost normalized and overshoot phenomenon of Pcr persisted in the preconditioned hearts. In the previous study, ATP recovered slightly during a early reperfusion period after 15 minutes of ischemia but remained depressed for at least 24 hours in dog hearts without preconditioning. In the present study, ATP recovered slightly from 66±2% to 73±4% during 120 minutes of reperfusion in the control group. However, the difference did not reach statistical significance. According to the previous study using 31P-NMR spectroscopy, Pcr overshoot is observed during an early reperfusion period after a short episode of ischemia, as was seen in our control hearts. Despite random animal selection, each pig in the preconditioned group showed a higher Pcr level than any control pigs and a definite Pcr overshoot throughout the reperfusion period. This difference in ATP recovery and Pcr overshoot between the two groups could not be explained by the difference in the filling factor (amount of myocardial mass within the region of the surface coil) for the following reasons. Because segment shortening during a reflow period was similar for the two groups, the seating of the surface coil over the myocardium was comparable between them. Furthermore, contamination of nonischemic myocardium within the area of the surface coil also could not account for these results because we excluded it, as described above.

Three possible mechanisms are considered for the explanation of improved recovery of high energy phosphates during reperfusion in the preconditioned heart. One is preservation of high energy phosphates during ischemia. According to a previous study in pigs, injection of carteolol (β-blocker) before occlusion preserved ATP during 20 minutes of ischemia and allowed it to be almost normalized after 120 minutes of reperfusion. Therefore, both in preconditioned hearts and hearts treated with β-blocker, rapid recovery of ATP during reperfusion may be related to an energy sparing effect during sustained ischemia. The second mechanism is persistent reduced energy utilization in cytoplasm during reperfusion. It has been established that ischemic preconditioning reduces energy utilization during sustained ischemia. The same effect may continue during reperfusion. Although the magnitude of stunning during a reperfusion period was comparable between the two groups, ATP consumption does not always parallel contractility. Even if ATP production were similar for the two groups, persistent reduced energy utilization results in rapid recovery of ATP. The third mechanism is an acceleration of energy production in mitochondria. Generally, the overshoot phenomenon of Pcr indicates that mitochondrial aerobic energy production is greater than energy utilization in the cytosol, i.e., the energy generating system is operating better than the energy utilizing system. Thus, we cannot define the specific pathway for rapid recovery of high energy phosphates in the present 31P-NMR study. Further investigations are warranted.

Recently, Ovize et al. demonstrated that preconditioning has no beneficial effect on energy metabolism during reperfusion using a biochemical technique in a canine model. According to their data, Pcr overshoot was seen even after 3 hours of reperfusion in both subepicardium and subendocardium in the control group. Moreover, it was even more striking than that
observed in the preconditioned group. In contrast, Farber et al38 previously demonstrated that Pcr overshoot measured by the biochemical method was observed in the subendocardium but not in the subepicardium at 3 hours of reperfusion after 15 minutes of ischemia in dogs. Thus, Pcr overshoot during a reperfusion period after a short period of ischemia is controversial despite the fact that measurement of Pcr and experimental protocol were nearly identical between these studies. In the present porcine study, whereas Pcr overshoot disappeared after 30 minutes of reperfusion in the control group, it persisted throughout the reperfusion period in the preconditioned group. We measured high energy phosphates by 31P-NMR spectroscopy, which collects signals transmurally. Although the reasons for the discrepancy between their data and ours remain unclear, it may be partly explained by the differences in method for determination of high energy phosphates and animal species.

Clinical Implications

This study has several clinical implications. Patients with coronary artery disease may have multiple episodes of angina pectoris. Similarly, intermittent cross-clamping of the aorta is performed in open heart surgery. Under these situations, ischemic preconditioning may play a role in myocardial salvage. Our results suggested that ischemic preconditioning has beneficial effects on metabolism but not on the function of stunned myocardium. Some interventions would be necessary to lead beneficial effects on metabolism by ischemic preconditioning to an improvement in the functional recovery of stunned myocardium. Further investigations are warranted.

Conclusions

Ischemic preconditioning improves energy metabolism of stunned myocardium. However, it does not attenuate myocardial stunning.

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