Effect of Ischemic Preconditioning on Interstitial Purine Metabolite and Lactate Accumulation During Myocardial Ischemia

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Abstract  The purpose of this study was to determine the effect of ischemic preconditioning on the changes in interstitial fluid (ISF) purine metabolites and lactate during prolonged regional myocardial ischemia. The study consisted of two groups of anesthetized dogs: a control group (n=13) that was exposed to 60 minutes of regional myocardial ischemia and a preconditioned group (n=10). In the preconditioned group, regional myocardial ischemia was induced for two 5-minute episodes, each followed by 10 minutes of reperfusion. These preconditioning episodes were followed by 60 minutes of sustained regional ischemia. Cardiac ISF was sampled by microdialysis probes implanted in the left ventricular myocardium; dialysate levels served as indices of ISF concentrations. In the preconditioned group, dialysate concentrations of adenosine, inosine, hypoxanthine, total purines, and lactate increased during each of the 5-minute ischemia episodes, with further increases occurring during the first 5 minutes of reperfusion. However, the increases in dialysate purine metabolites during the first period of ischemia/reperfusion were greater than those that occurred during the second ischemia/reperfusion. In addition, preconditioning reduced the rate of ISF purine metabolite and lactate accumulation during the prolonged ischemia when compared with nonpreconditioned animals. These data suggest that preconditioning reduces the energy imbalance that occurs during subsequent myocardial ischemia and thereby diminishes the rate of net adenine nucleotide degradation and cellular production and release of purine metabolites and lactate. In addition, the increase in ISF adenosine seen during the preconditioning episode is consistent with the notion that preconditioning-induced cardioprotection is mediated in part by the action of adenosine at extracellular A₁ adenosine receptors. (Circulation. 1994;89:2283-2289.)

Key Words  ischemia  adenosine  preconditioning

Exposure of the myocardium to a brief episode of ischemia followed by reperfusion has been shown to minimize or delay the ischemic damage associated with a subsequent period of prolonged ischemia, a phenomenon referred to as ischemic preconditioning.¹ This has been demonstrated with both intermittent periods of brief coronary artery occlusion as well as by a single occlusion followed by reperfusion.²,³ One of the characteristic features of preconditioning is that brief ischemia/reperfusion appears to reduce myocardial energy demands during the subsequent prolonged ischemia, leading to a preservation of high-energy phosphates and a reduced accumulation of tissue purine metabolites and lactate.⁴⁻⁹

Our laboratory has previously described the changes in cardiac interstitial fluid (ISF) purine metabolites and lactate during regional myocardial ischemia, using the cardiac microdialysis technique to sample cardiac ISF.¹⁰⁻¹² One advantage of this technique is that serial samples of myocardial ISF can be obtained in the intact heart, thereby allowing delineation of the temporal profile of metabolite accumulation during ischemia. We have used this approach to suggest that assessment of the profile of ISF purine metabolite accumulation provides a useful index of ATP depletion during ischemia.¹² The purpose of this study was to determine the profile of ISF purine metabolites and lactate during regional myocardial ischemia in preconditioned myocardium, testing the hypothesis that the rate of ISF purine metabolite and lactate accumulation during ischemia will be reduced in preconditioned myocardium, indicative of a reduced energy demand induced by preconditioning.

Methods

Animal Preparation

All experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 86-23) and were approved by the Institutional Animal Care and Use Committee at the State University of New York at Buffalo. Adult mongrel dogs of either sex weighing 20 to 30 kg were premedicated with 3 mg/kg morphine and then anesthetized with 20 mg/kg IV thiamylal, followed by 70 mg/kg α-d-chloralose and 200 mg/kg IV urethane. The animals were intubated and ventilated (model 613 dog respirator, Harvard Apparatus) with a mixture of 100% oxygen and air. Tidal volume, respiratory rate, and percent oxygen in the inspired air were adjusted to maintain normal blood gas values (model 170 pH/blood gas analyzer, Ciba-Corning Diagnostics Corp.). Core body temperature was monitored with an esophageal temperature probe and maintained with a heating pad at ≈38°C. The right femoral artery was cannulated for the measurement of arterial blood pressure and the determination of arterial blood gases. The right femoral vein was cannulated for infusion of fluids and anesthetic supplement.

A thoracotomy was performed through the left fifth intercostal space, and the pericardium was incised to expose the left ventricle. In preparation for the induction of regional myocardial ischemia, a short length of the left anterior descending coronary artery (LAD) was dissected distal to the first diago-
nal branch, and a silk ligature was passed around the vessel. All visible epicardial collaterals between the branches of the left circumflex coronary artery and the LAD were ligated.

The cardiac microdialysis technique has been described in detail previously, including diagrams of the microdialysis probe and a discussion of the benefits and limitations of microdialysis.10,12 For each animal, three or four microdialysis probes, with a 1-cm window for diffusion and a platinum wire glued to the outside of the probe, were placed approximately midwall within the LAD perfused myocardium. After insertion of the microdialysis probes, the inflow silica tubes were connected to separate gas-tight glass syringes filled with Krebs-Henseleit buffer. Each probe was perfused at 2.0 μL/min; this allowed for diffusion to occur between the fluid within the fiber and the ISF surrounding the fiber as the fluid passed through the dialysis fiber. The effluent concentration from the microdialysis probe, referred to as the dialysate, is therefore representative of intramyocardial ISF concentration. The dialysate was collected from the outflow silica tube in plastic tubes and frozen at −80°C until later analysis.

Local myocardial blood flow was measured in the myocardium surrounding each microdialysis probe by the hydrogen clearance method10,13 using the platinum wire within the microdialysis probe to monitor tissue hydrogen levels. Hydrogen was introduced to the animal in the inspired air (7% to 10%) for 2 minutes, after which the hydrogen was discontinued and its washout from the tissue monitored. Local myocardial blood flow was calculated from the time required for the hydrogen levels to fall from 90% maximum to 40% maximum using the formula

\[ \text{Myocardial blood flow (mL/min per 100 g)} = \frac{-\ln(40/90)}{\text{time}} \times 100 \]

Arterial blood pressure, heart rate, and hydrogen washout curves were recorded on an eight-channel recorder (model 3800, Gould Inc).

**Protocol**

A minimum of 2 hours was allowed for recovery after microdialysis probe implantation. The study consisted of two groups of dogs: a control group of animals (n=13) and a preconditioned group (n=10). After the collection of baseline dialysate samples, animals in the control group were exposed to 60 minutes of regional ischemia followed by reperfusion. Throughout ischemia and the first 60 minutes of reperfusion, dialysate samples were collected for durations ranging from 5 to 20 minutes. In the preconditioned group, the myocardium was preconditioned by two successive periods of 5-minute LAD occlusion followed by 10 minutes of reperfusion. A 60-minute period of regional ischemia then was maintained, followed by reperfusion. Each nonpreconditioned animal and seven preconditioned animals, reperfusion was maintained for a total of 3 hours so that infarct size could be determined.

Local myocardial blood flow in the tissue surrounding each probe was determined during the baseline period, once during the prolonged ischemia, and at 10, 30, and 60 minutes of reperfusion. Immediately before the 60-minute LAD occlusion, hydrogen was administered to the animals so that the myocardium was loaded with hydrogen before ischemia. Approximately 3 minutes after the induction of ischemia, hydrogen was discontinued, thus allowing a single determination of myocardial blood flow from each probe in the ischemic region during ischemia. Subsequent measurements of blood flow were not consistently obtainable during ischemia because of the inability to provide adequate hydrogen to the tissue.

**Infarct Size Determination**

In animals in which infarct size was determined, the LAD was reocluded after 3 hours of reperfusion and 2% Evans blue dye was injected into the left atrium. Immediately upon delineation of the area at risk, the heart was removed. The left ventricle was cut into five to seven slices, and, after weighing each slice, the ischemic zone (area not stained by the Evans blue) and nonischemic zone (area stained by the Evans blue) on each side of the slice were traced on clear acetate. Each slice was subsequently placed in a 1% triphenyl tetrazolium chloride solution (made up in 0.2 mol/L Tris buffer, pH 7.4) for determination of necrotic tissue; upon delineation of the infarct, the infarcted area was traced on the clear acetate. The area of myocardium at risk, the infarcted area, and the percent of the left ventricle at risk were determined knowing the weight of each slice and by cutting and weighing the acetate sheets to estimate the respective areas. Infarct size was reported as a percent of the area at risk (volume of infarcted tissue divided by volume of area at risk) x 100.

**High-Performance Liquid Chromatography**

Before chromatographic analysis, all samples were diluted with 0.01% sodium azide to prevent bacterial degradation. A portion of the dialysate was used for the determination of adenosine, inosine, hypoxanthine, xanthine, and uric acid levels. These compounds were separated using a reverse-phase column (Supelco LC-18S, Supelco, Inc) and a 1% (pH 5.3) to 25% (pH 5.58) methanol in 100 mmol/L KH2PO4, gradient (model 600E system controller, 717 WISP, 466 detector; Maxima 820 Chromatography Software, Waters, Division of Millipore). Uric acid was detected by absorbance changes at 293 nm, and all other peaks were detected at 254 nm. The peaks of interest in the dialysate were identified and quantified by comparing retention times and peak areas to known standards.

The remainder of the dialysate sample was used for lactate analysis, using a modified enzymatic fluorometric assay and a high-performance liquid chromatography system to accurately inject and deliver small volumes of sample to a fluorescence detector.12 Each sample was incubated with 50 μL of a glycine-hydrizine buffer (600 mmol/L glycine, 500 mmol/L hydrazine, pH 9.5), 30 μL of 10 mmol/L NAD+ solution, and 5 μL of lactate dehydrogenase (from porcine muscle, 550 U/mg) and injected into a mobile phase of distilled water. Lactate was determined by fluorometric detection of NADH (excitation wavelength, 340 nm; emission wavelength, 450 nm) and quantified by comparison to identically treated lactate standards.

**Statistical Analysis**

In most animals (17 of 23), dialysate and blood flow data from all the probes at a given time point were averaged, thereby generating a single profile for each animal. In some animals (6 of 23), the hydrogen clearance curve from the most proximal probe indicated that the probe was placed in the border zone of the ischemic region; in these cases, data from that probe were excluded and data from the other probes were averaged. Mean values and standard errors of the means were calculated for the nonpreconditioned and preconditioned groups. For comparison of values obtained during and after LAD occlusion with preocclusion values, differences between mean values were determined by randomized one-way ANOVA followed by Dunnett’s multiple range test for repeated comparisons to control values. For comparison of values in the nonpreconditioned group to those in the preconditioned group at corresponding times, an unpaired r test was used. In the preconditioned animals, comparisons of values obtained during the first preconditioning ischemic period, the second preconditioning ischemic period, and the initial 5 minutes of the prolonged ischemia as well as comparisons of values obtained during the first and second reperfusion periods after the preconditioning episodes were compared by a
Systemic and Cardiac Hemodynamic Data

<table>
<thead>
<tr>
<th>Condition</th>
<th>Mean Arterial Pressure, mm Hg</th>
<th>Heart Rate, beats per minute</th>
<th>Myocardial Blood Flow, mL/min per 100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-preconditioned</td>
<td>Pre-conditioned</td>
<td>Non-preconditioned</td>
</tr>
<tr>
<td>Preischemia</td>
<td>110±4</td>
<td>104±4</td>
<td>127±9</td>
</tr>
<tr>
<td>Precondition 1</td>
<td>104±5</td>
<td>152±8</td>
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<tr>
<td>Reperfusion 1</td>
<td>105±5</td>
<td>143±8</td>
<td></td>
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<tr>
<td>Precondition 2</td>
<td>103±5</td>
<td>153±10</td>
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</tr>
<tr>
<td>Reperfusion 2</td>
<td>103±5</td>
<td>143±10</td>
<td></td>
</tr>
<tr>
<td>10-Minute ischemia</td>
<td>105±4</td>
<td>106±5</td>
<td>136±8</td>
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<tr>
<td>30-Minute ischemia</td>
<td>104±4</td>
<td>102±4</td>
<td>142±6</td>
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<tr>
<td>60-Minute ischemia</td>
<td>101±4</td>
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<td>143±7</td>
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<tr>
<td>10-Minute reperfusion</td>
<td>98±4</td>
<td>97±4</td>
<td>149±5*</td>
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<td>30-Minute reperfusion</td>
<td>94±4</td>
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</tr>
<tr>
<td>60-Minute reperfusion</td>
<td>96±4</td>
<td>92±6</td>
<td>146±7</td>
</tr>
</tbody>
</table>

Data are mean±SEM. *P<.05 vs preischemia.

Results

The systemic and cardiac hemodynamic data are shown in the Table. Arterial blood pressure and heart rate were similar in both groups. Arterial pressure was relatively stable throughout the protocol in both groups, whereas heart rate tended to increase in both groups during ischemia and reperfusion. Local myocardial blood flow in the LAD perfused region before, during, and after the prolonged ischemia was similar in both groups.

The changes in dialysate purine metabolites and lactate are shown in Figs 1 through 8. The initial baseline dialysate values of all measured metabolites were not different between the control group (adenosine, 0.27±0.04 μmol/L; inosine, 0.28±0.07 μmol/L; hypoxanthine, 2.20±0.62 μmol/L; xanthine, 0.90±0.16 μmol/L; uric acid, 4.42±0.29 μmol/L; lactate, 0.39±0.04 mmol/L) and the preconditioned group (adenosine, 0.28±0.04 μmol/L; inosine, 0.37±0.12 μmol/L; hypoxanthine, 2.45±1.39 μmol/L; xanthine, 1.22±0.46 μmol/L; uric acid, 3.92±0.33 μmol/L; lactate, 0.38±0.05 mmol/L).

The changes in dialysate adenosine are shown in Fig 1. In nonpreconditioned animals, dialysate adenosine increased transiently during ischemia, reaching maximum levels at ~20 minutes of ischemia. Upon reperfusion, there was a second transient increase in adenosine. In the preconditioned group, dialysate adenosine increased 6.6-fold during the initial 5-minute LAD occlusion. During the subsequent reperfusion, adenosine increased further during the initial 5 minutes of reperfusion and then declined. The increase in dialysate adenosine during the second 5-minute ischemic episode was not as great as that seen during the first period of ischemia, nor did adenosine increase as much during the second reperfusion period. During the prolonged...
ischemia, hearts that had been preconditioned had an attenuated increase in adenosine during the first 20 to 30 minutes of ischemia compared with nonpreconditioned animals, after which there were no differences between preconditioned and nonpreconditioned animals. Adenosine levels were similar in both groups during reperfusion.

Fig 2 shows the changes in dialysate inosine. Inosine was the predominant purine metabolite that accumulated in the ISF during ischemia both in nonpreconditioned and in preconditioned animals. In nonpreconditioned animals, dialysate inosine increased over 200-fold during ischemia and then decreased during reperfusion. In preconditioned animals, the increase in dialysate inosine during the second brief period of ischemia as well as the increase upon subsequent reperfusion was attenuated as compared with that seen during the first ischemia/reperfusion episode. During the prolonged ischemia, the increase in inosine was delayed and the rate of increase was diminished in preconditioned animals, although by the end of the 60 minutes of ischemia, inosine values were not different between the two groups. There were no differences between the two groups during reperfusion.

The changes in dialysate hypoxanthine are shown in Fig 3. In nonpreconditioned animals, dialysate hypoxanthine increased most rapidly in the first 30 minutes of ischemia, after which the rate of increase was diminished. As with inosine, preconditioning diminished the increase in hypoxanthine resulting from a second ischemia/reperfusion episode and delayed the increase in hypoxanthine seen during the prolonged period of ischemia.

Unlike inosine and hypoxanthine, dialysate xanthine was greater during the second 5-minute period of ischemia than during the first 5-minute period of ischemia in preconditioned animals (Fig 4). This probably was a reflection of the remaining high xanthine levels as a result of the first period of ischemia and reperfusion. Also unlike inosine and hypoxanthine, there were only
modest differences between nonpreconditioned and preconditioned animals during the prolonged ischemia, as statistically significant differences were observed only at 7.5 and 12.5 minutes of ischemia.

The preconditioning protocol resulted in increased uric acid during each period of ischemia and further increases upon reperfusion, but there was no difference between the two groups during the prolonged ischemia; in both groups, uric acid increased transiently during the ischemic period and again during reperfusion (Fig 5).

The changes in dialysate levels of the sum of adenosine, inosine, hypoxanthine, xanthine, and uric acid, plotted in Fig 6 as total purines, are intended to summarize the changes in purine metabolites. Preconditioning not only reduced the increase in total purines during the second ischemia/reperfusion episode but also delayed the accumulation of purine metabolites during the prolonged ischemia. Total purines in the preconditioned animals were 45% and 75% of that seen in the nonpreconditioned animals at 10 and 30 minutes of ischemia, respectively, but by 60 minutes of ischemia, the total purine levels were similar in the two groups. Dialysate total purines were similar in both groups during reperfusion.

The changes in dialysate lactate are shown in Fig 7. In nonpreconditioned animals, lactate increased most rapidly during the first 30 minutes of ischemia, after which the rate of increase diminished. In preconditioned animals, similar increases in dialysate lactate were observed during each of the two preconditioning periods of ischemia/reperfusion, although the increase in lactate during the prolonged ischemia was delayed compared with nonpreconditioned animals. In both groups, lactate levels decreased during reperfusion.

In the animals in which infarct size was determined, the infarct size in preconditioned animals (16.1±4.3% of the area at risk) was significantly less than that of the infarct size in nonpreconditioned animals (40.9±3.3% of the area at risk). Myocardial blood flow during the prolonged ischemia was similar in these two subgroups of dogs (nonpreconditioned, 4.5±0.4 mL/min per 100 g; preconditioned, 5.5±0.4 mL/min per 100 g), as was the percent of the left ventricle at risk (nonpreconditioned, 31.4±2.4%; preconditioned, 30.3±1.9%).

**Discussion**

The major finding of this study, which used cardiac microdialysis to determine the profile of ISF purine metabolites and lactate in the preconditioned myocardium during 60 minutes of regional myocardial ischemia, was that preconditioning attenuated the increase in dialysate adenosine that occurred during the first 20 minutes of the prolonged ischemia and delayed the accumulation of inosine, hypoxanthine, and lactate. Assuming that dialysate concentrations are representative of ISF levels, these data suggest that the rate of cellular production and release of purine metabolites, the degradation products of high-energy phosphates, and lactate, a by-product of glycolysis, are diminished during ischemia in the preconditioned myocardium.

Since the first report of ischemic preconditioning in 1986 by Murry et al,5 preconditioning has become an active area of investigation. Several reviews and editorials have been published recently that describe the salient features of ischemic preconditioning, particularly regarding the timing characteristics of preconditioning.6,7,8,9,10,11,12,13,14,15,16,17,18,19,20 In canine models of regional ischemia, preconditioning reduces infarct size if the prolonged ischemia is 60 minutes but not if the ischemic interval is extended to 90 minutes.20 Our data showing that ISF purine metabolites and lactate levels are similar in the two groups by 60 minutes of ischemia are consistent with the inability of preconditioning to sustain a reduction in infarct size as the duration of ischemia increases and with the notion that preconditioning provides a 20-minute delay in the pathological events associated with ischemia-induced cell death.4

Currently, much of the investigative work regarding preconditioning is aimed at elucidating the underlying mechanisms responsible for preconditioning-induced cardioprotection. Based on measurements of tissue high-energy phosphate content, several investigators have demonstrated that preconditioning reduces the rate of high-energy phosphate utilization during a subsequent ischemic episode, suggesting that the energy demands during ischemia are reduced in preconditioned myocardium.5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20 Consistent with this notion, Murry et al5 found that preconditioning attenuated purine metabolite accumulation in the tissue. Compared with nonpreconditioned myocardium, tissue levels of adenosine were lower in preconditioned myocardium during the first 20 minutes of ischemia; however, at 40 minutes of ischemia, tissue adenosine was similar in preconditioned and nonpreconditioned animals. Tissue inosine levels were significantly less in preconditioned myocardium at 5, 10, 20, and 40 minutes of ischemia, as was the sum of nucleosides and bases. Our data documenting the temporal changes in ISF purine metabolites during ischemia are consistent with these observations, adding further support to the idea that the rate of high-energy phosphate utilization during prolonged ischemia is reduced as a result of preconditioning.

Although our results are consistent with measurements of tissue adenosine levels during ischemia in preconditioned myocardium,5 they are at variance with measurements of venous adenosine release after prolonged ischemia. A recent study by Kitakaze et al,21
implicating enhanced 5'-nucleotidase activity in preconditioned myocardium, demonstrated that coronary venous adenosine levels were actually greater after 40 minutes of regional ischemia in dogs that had been preconditioned by repeated brief ischemia/reperfusion episodes than in nonpreconditioned animals. This phenomenon, in which coronary venous adenosine levels were slightly more than double that seen in nonpreconditioned animals, was sustained for up to 30 minutes of reperfusion. Furthermore, coronary venous adenosine levels were progressively increased during each of the reperfusion phases after the brief periods of ischemia. This is different from our data, which demonstrated attenuated ISF adenosine accumulation during and after the second brief ischemia/reperfusion episode, less ISF adenosine accumulation early during the prolonged ischemia, and no differences in ISF adenosine during reperfusion in preconditioned versus nonpreconditioned animals. Two factors may explain this difference. First, it is now well recognized that the vascular endothelium is not only an effective metabolic barrier for the movement of adenosine between the ISF and the vascular space but also that coronary venous adenosine levels may be determined in part by endothelial cell rather than myocyte adenosine production. Thus, it is possible that one component of the cardioprotection afforded by ischemic preconditioning relates to the selective enhancement of adenosine release by endothelial cells. As such, this would be beneficial by reducing platelet aggregation and minimizing neutrophil adherence, activation, and free radical production. If 5'-nucleotidase is localized primarily in endothelial cells, as has been suggested by Nees and Dendorfer, then the enhanced 5'-nucleotidase activity and elevated coronary venous adenosine levels observed by Kitakaze et al may be a reflection of adenosine production in endothelial cells, whereas the reduction in ISF adenosine and other purine metabolites that we observed may reflect the ATP-sparing effect of preconditioning on the myocyte. Second, it is also possible that by reducing subendocardial infarction, preconditioning minimizes the no-reflow phenomenon in the subendocardium, thereby allowing greater adenosine removal from the endocardium, which has been shown to accumulate greater amounts of adenosine during ischemia than the epicardium.

Our data describing the effect of preconditioning on the temporal profile of ISF lactate levels during ischemia suggest that glycolysis is reduced during ischemia in preconditioned myocardium and are consistent with previous reports of changes in tissue lactate and tissue pH. There are now several reports in a variety of animal models that describe a reduction in the accumulation of lactate and other glycolytic intermediates in the tissue during prolonged ischemia in preconditioned myocardium, along with a minimization of ischemia-induced acidosis. Since preconditioned animals enter the prolonged ischemic period with reduced tissue glycogen, part of the reduced glycolysis may be due to reduced glycogenolysis. Wolfe et al recently demonstrated that the loss of the infarct size—reducing capabilities of preconditioning associated with increasing durations of reperfusion before the prolonged ischemia could be correlated with the recovery of tissue glycogen and the inability to minimize ischemia-induced intracellular acidosis. This suggests that the reduction in lactate or H+ accumulation may be a contributing factor to preconditioning-induced cardioprotection.

Downey and colleagues have advanced the hypothesis that adenosine released into the ISF during the initial occlusion interacts with extracellular adenosine A1 receptors, providing protection against subsequent ischemia through a series of steps involving G proteins, phospholipase C activation, and protein kinase C translocation/activation. There is now evidence from several additional laboratories that supports a key role for adenosine in ischemic preconditioning, although the ischemia-induced release of norepinephrine, acting through the α1-adrenergic receptor, appears to be involved in ischemic preconditioning as well. Despite clear and important species differences, it appears as if the activation of ATP-sensitive K⁺ channels, which are known to be linked to the adenine A1 receptor, may be one of the important effector mechanisms involved in adenosine-mediated protection induced by preconditioning. Activation of ATP-sensitive K⁺ channels and the resulting hyperpolarization, shortening of the action potential, and delay of electrical uncoupling would reduce the energy requirements in the ischemic heart and could therefore be the link between adenosine receptor activation and reduced energy demands in preconditioned myocardium. Although our data demonstrating an increase in ISF adenosine with ischemic preconditioning are not surprising, they nevertheless provide the first description of the magnitude of the increase in ISF adenosine during and after an ischemic preconditioning stimulus and, as such, are consistent with a role for adenosine acting at extracellular adenosine receptors in the induction of preconditioning.

It is of interest that while there were clear differences in adenosine, inosine, and hypoxanthine in preconditioned myocardium, there were only minimal differences in xanthine and uric acid. This suggests that the degradation of hypoxanthine to xanthine and then to uric acid, enzymatic steps that both utilize the xanthine oxidase/reductase system, is limited during ischemia by the absence of oxygen or NAD⁺ as electron acceptors rather than by the availability of hypoxanthine. It also suggests that there is no difference between nonpreconditioned and preconditioned animals in the amount of oxygen free radicals produced as a result of the xanthine oxidase-catalyzed degradation of hypoxanthine and xanthine.

In sum, these data provide a temporal description of the changes in ISF purine metabolites and lactate in the preconditioned myocardium, providing information that delineates the time course of the reduced adenine nucleotide degradation and lactate accumulation during prolonged ischemia in preconditioned myocardium and describes the magnitude of the increase in ISF adenosine during a preconditioning ischemia/reperfusion stimulus in the dog.

Acknowledgments

This work was presented at the Experimental Biology '93 meeting (March 29 to April 1, New Orleans, La) and was supported by a grant from the National Institutes of Health (HL-46027). This work was done during the tenure of an Established Investigatorship of the American Heart Associa-
tion. The expert technical assistance of Elaine Granica is gratefully acknowledged.

References
21. Li Y, Kloner RA. The cardioprotective effects of ischemic 'pre-conditioning' are not mediated by adenosine receptors in rat hearts. Circulation. 1993;87:1642-1648.
Effect of ischemic preconditioning on interstitial purine metabolite and lactate accumulation
during myocardial ischemia.

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Circulation. 1994;89:2283-2289
doi: 10.1161/01.CIR.89.5.2283

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