Intermittent Perfusion of Ischemic Myocardium
Possible Mechanisms of Protective Effects on Mechanical Function in Isolated Rat Heart

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Intermittent restoration of coronary flow during ischemia reduced myocardial damage and improved recovery of function. The mechanisms of the protective effects of intermittent perfusion were investigated in isolated rat hearts. Ventricular function was assessed as the product of developed pressure (left ventricular systolic pressure minus end-diastolic pressure) and heart rate. Recovery of function was calculated by division of the product at the end of reperfusion by that before ischemia. After 40 minutes of sustained global ischemia, intracellular Na⁺ (Naᵢ) increased from 11 to 74 μmol/g dry wt. During 30 minutes of reperfusion, these hearts took up a large amount of ⁴⁵Ca²⁺ (10 μmol/g dry wt), recovered only 24% of preischemic function, and had an increased left ventricular end-diastolic pressure (48 mm Hg). When the 40-minute period of ischemia was interrupted at 10-minute intervals by intermittent perfusion (three periods of 3 minutes) with either oxygenated or hypoxemic buffer, Naᵢ increased to only 12 or 17 μmol/g dry wt, and reperfusion resulted in much lower ⁴⁵Ca²⁺ uptake (0.5 and 0.5 μmol/g dry wt, respectively). Recovery of function was 100% of the preischemic value. When hypoxemic buffer without glucose was used for intermittent perfusion, Naᵢ increased to 50 μmol/g dry wt, ATP was depleted, and reperfusion resulted in reduced recovery of function (76%) and moderately increased ⁴⁵Ca²⁺ uptake (2.1 μmol/g dry wt). The role of Na⁺-K⁺ pump activity in maintaining low Naᵢ was assessed by removing K⁺ from oxygenated or hypoxemic buffers used during intermittent perfusion. Under these conditions, Naᵢ rose to 64 or 102 μmol/g dry wt, ⁴⁵Ca²⁺ uptake increased to 4.4 or 9.4 μmol/g dry wt, and recovery of function was poor. There was a highly significant correlation between Naᵢ during ischemia and reperfusion Ca²⁺ overload (r=0.87) or impaired recovery of function (r=0.96). These results indicate that prevention of an increase in Naᵢ by maintenance of Na⁺-K⁺ pump activity is associated with a reduction of Ca²⁺ overload through Na⁺/Ca²⁺ exchange. (Circulation 1990;82:536–548)

Recent ischemia of the myocardium is frequently seen in patients with severe stenotic lesions or spasms of the coronary arteries. Moreover, increased use of transluminal coronary angioplasty¹ and thrombolysis² results in repeated brief coronary occlusions during treatment of coronary stenosis and thrombus under conditions where interventions can be used to avoid additional myocardial injury (reperfusion injury). In addition, intermittently interrupted ischemia of the myocardium is inevitable during cardiac surgery. In these circumstances, repeated applications of cardioplegic solution (secondary cardioplegia) are essential to reduce perioperative myocardial damage during prolonged procedures.³

Repeated brief periods of coronary occlusion in canine hearts caused less cumulative deterioration of regional function and high-energy phosphate contents compared with hearts in which coronary blood flow was restored after a single occlusion of the same total duration.⁴–⁷ It is important to understand the mechanisms of this protective effect so procedures can be formulated to enhance the preservation of myocardial function after procedures that involve recurrent ischemia. The possible mechanisms of protection are reestablishment of aerobic metabolism with restoration

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of high-energy phosphates by delivery of oxygen and substrates and washout of harmful metabolic products that accumulated during ischemia.

Although many possible mediators of lethal myocardial injury have been proposed, excessive accumulation of Ca\(^{2+}\) is one of the most likely mediators.\(^{8-11}\) We and others suggested that uptake of extracellular Ca\(^{2+}\) through Na\(^+\)-Ca\(^{2+}\) exchange in response to increased intracellular Na\(^+\) (Na\(_i\)) that accumulated during ischemia is likely to be an important mechanism.\(^{12-14}\) The present study was designed to test the hypothesis that intermittent perfusion can prevent Na\(_i\) accumulation and result in restraint of Ca\(^{2+}\) overload and improvement of functional recovery by resumption of energy metabolism or washout of metabolic products.

**Methods**

Male Sprague-Dawley rats weighing between 300 and 350 g were anesthetized with an intraperitoneal injection of sodium pentobarbital (40 mg/kg). Hearts were rapidly excised and perfused by the Langendorff technique with Krebs-Henseleit bicarbonate buffer. Aortic pressure was 60 mm Hg, and coronary flow was approximately 15 ml/min. Left ventricular pressure was monitored via a plastic, perfuse-filled catheter with a small, perforated ball tip that was inserted into the left ventricle via the mitral valve as described earlier.\(^{15}\) Krebs-Henseleit buffer (mM) NaCl 118, NaHCO\(_3\) 25, KCl 4.7, KH\(_2\)PO\(_4\) 1.2, MgSO\(_4\) 1.2, CaCl\(_2\) 1.75, EDTA 0.5, glucose 11, and pyruvate 5. Hearts were maintained at 37°C during control perfusion and ischemia in the following protocols.

**Experimental Design**

**Sustained global ischemia.** After an initial equilibration period (20 minutes) at normal rates of coronary flow with buffer gassed with a 95% O\(_2\)-5% CO\(_2\) mixture, sustained ischemia (Table 1, protocol 2) was induced by cross-clamping the aortic perfusion tube after recording the preischemic hemodynamic values. When hearts were reperfused after specific periods of ischemia, restoration of coronary flow was accomplished by releasing the clamps on the aortic perfusion tubes and returning the aortic perfusion pressure to 60 mm Hg. Hearts were reperfused for 30 minutes with oxygenated buffer containing \(^{45}\text{Ca}^{2+}\) (ICN Radiochemical Co., Costa Mesa, Calif.), and recovery of ventricular function was calculated from measurements of ventricular pressure and heart rate. Coronary effluent containing \(^{45}\text{Ca}^{2+}\) was collected from each heart to estimate the specific radioactivity of \(^{45}\text{Ca}^{2+}\) in the perfusate. After 30 minutes of recirculation with buffer containing \(^{45}\text{Ca}^{2+}\), hearts were switched to an identical perfusate without \(^{45}\text{Ca}^{2+}\) for 3 minutes to wash away radioactivity trapped in the extracellular space.\(^{10}\) Hearts were quickly frozen with Wollenberger clamps precooled in liquid nitrogen. Some hearts were frozen at the end of specific periods of ischemia before reperfusion for assay of metabolites.

**Intermittent perfusion in globally ischemic hearts.** After an initial 20-minute equilibration period, two, three, or four 10-minute episodes of zero-flow ischemia were interrupted by 3-minute periods of intermittent perfusion (Table 1, protocols 3–7). Several kinds of perfusate were used during intermittent perfusion: 1) oxygen-deficient perfusate gassed with 95% N\(_2\)-5% CO\(_2\), 2) substrate-free perfusate in which glucose and pyruvate were replaced by sucrose, and 3) K\(^+\)-free buffer in which potassium chloride and KH\(_2\)PO\(_4\) were replaced by the same concentrations of choline chloride and NaH\(_2\)PO\(_4\), respectively. To assess the direct effects of oxygen, substrate, or K\(^+\) during intermittent perfusion on function, metabolites, and ion contents, perfusion of hearts for 70 minutes with oxygenated buffer was interrupted by three 3-minute episodes of intermittent perfusion with hypoxemic, hypoxemic no-substrate, or oxygenated no-K\(^+\) buffer at 10-minute intervals during the first 40 minutes of oxygenated perfusion after 20 minutes of the equilibration period (Table 1, protocol 1). The period of intermittent perfusion was followed by 30 minutes of oxygenated perfusion. Reperfusion buffer was Krebs-Henseleit bicarbonate buffer containing glucose and pyruvate in all hearts. In these experimental protocols, hearts were not paced because effects of electrical stimulation on metabolism, ionic homeostasis, or function during intermittent perfusion could be different in control and ischemic hearts.

**Analytical Methods**

Tissue levels of ATP, total adenine nucleotides (ATP plus ADP plus AMP), creatine phosphate (CP), and lactate were determined at the ends of periods of control perfusion, ischemia, intermittent perfusion, or reperfusion using neutralized perchloric acid (6% wt/vol) extracts and standard enzymatic methods.\(^{16}\)

For estimation of \(^{45}\text{Ca}^{2+}\) uptake, aliquots of tissue extracts and coronary effluents were used to determine radioactivity by liquid scintillation spectrometry (model LS3801, Beckman Instruments Inc., Irvine, Calif.). In previous studies,\(^{10,13}\) we observed \(^{45}\text{Ca}^{2+}\) uptake increased rapidly during 10–20 minutes of reperfusion and approached a plateau at 30 minutes. \(^{45}\text{Ca}^{2+}\) uptake at 30 minutes of reperfusion was closely correlated with Na\(_i\) content at the end of ischemia. When hearts were used for measurement of \(^{45}\text{Ca}^{2+}\) uptake during ischemia, perfusion was performed with a buffer containing \(^{45}\text{Ca}^{2+}\) for 20 minutes before ischemia and during intermittent perfusion. Coronary effluent collected just before induction of the final ischemic episode was used for calculation of \(^{45}\text{Ca}^{2+}\) uptake at the end of subsequent ischemic period, based on the assumption that specific radioactivity of \(^{45}\text{Ca}^{2+}\) in the extracellular space did not change during ischemia. \(^{45}\text{Ca}^{2+}\) uptake was calculated by division of radioactivity in tissue extracts by the specific radioactivities of \(^{45}\text{Ca}^{2+}\) in the
### Table 1. Experimental Protocols

1. **Oxygenated perfusion (no ischemia)**
   - a. 70 minutes of perfusion
   - b. 70 minutes of perfusion with three 3-minute episodes of hypoxemic intermittent perfusion
   - c. 70 minutes of perfusion with three 3-minute episodes of hypoxemic, no-substrate intermittent perfusion
   - d. 70 minutes of perfusion with three 3-minute episodes of oxygenated, no-K⁺ intermittent perfusion
   - e. 70 minutes of perfusion with three 3-minute episodes of hypoxemic, no-K⁺ intermittent perfusion

2. **Sustained global ischemia**
   - a. 10 minutes of ischemia
   - b. 20 minutes of ischemia
   - c. 30 minutes of ischemia
   - d. 40 minutes of ischemia
   - e. 30 minutes of ischemia followed by 30 minutes of reperfusion
   - f. 40 minutes of ischemia followed by 30 minutes of reperfusion

3. **Oxygenated intermittent perfusion**
   - a. 10 minutes of ischemia
   - b. 10 minutes of ischemia and one 3-minute episode of oxygenated intermittent perfusion
   - c. Two 10-minute episodes of ischemia and one 3-minute episode of oxygenated intermittent perfusion
   - d. Two 10-minute episodes of ischemia and two 3-minute episodes of oxygenated intermittent perfusion
   - e. Three 10-minute episodes of ischemia and two 3-minute episodes of oxygenated intermittent perfusion
   - f. Three 10-minute episodes of ischemia and three 3-minute episodes of oxygenated intermittent perfusion
   - g. Four 10-minute episodes of ischemia and three 3-minute episodes of oxygenated intermittent perfusion
   - h. Four 10-minute episodes of ischemia and three 3-minute episodes of oxygenated intermittent perfusion followed by 30 minutes of reperfusion

4. **Hypoxemic intermittent perfusion**
   - a. 10 minutes of ischemia
   - b. 10 minutes of ischemia and one 3-minute episode of hypoxemic intermittent perfusion
   - c. Two 10-minute episodes of ischemia and one 3-minute episode of hypoxemic intermittent perfusion
   - d. Two 10-minute episodes of ischemia and two 3-minute episodes of hypoxemic intermittent perfusion
   - e. Three 10-minute episodes of ischemia and two 3-minute episodes of hypoxemic intermittent perfusion
   - f. Three 10-minute episodes of ischemia and three 3-minute episodes of hypoxemic intermittent perfusion
   - g. Four 10-minute episodes of ischemia and three 3-minute episodes of hypoxemic intermittent perfusion
   - h. Four 10-minute episodes of ischemia and three 3-minute episodes of hypoxemic intermittent perfusion followed by 30 minutes of reperfusion

5. **Hypoxemic, no-substrate intermittent perfusion**
   - a. 10 minutes of ischemia
   - b. 10 minutes of ischemia and one 3-minute episode of hypoxemic, no-substrate intermittent perfusion
   - c. Two 10-minute episodes of ischemia and one 3-minute episode of hypoxemic, no-substrate intermittent perfusion
   - d. Two 10-minute episodes of ischemia and two 3-minute episodes of hypoxemic, no-substrate intermittent perfusion
   - e. Three 10-minute episodes of ischemia and two 3-minute episodes of hypoxemic, no-substrate intermittent perfusion
   - f. Three 10-minute episodes of ischemia and three 3-minute episodes of hypoxemic, no-substrate intermittent perfusion
   - g. Four 10-minute episodes of ischemia and three 3-minute episodes of hypoxemic, no-substrate intermittent perfusion
   - h. Three 10-minute episodes of ischemia and two 3-minute episodes of hypoxemic, no-substrate intermittent perfusion followed by 30 minutes of reperfusion
   - i. Four 10-minute episodes of ischemia and three 3-minute episodes of hypoxemic, no-substrate intermittent perfusion followed by 30 minutes of reperfusion

6. **Oxygenated, no-K⁺ intermittent perfusion**
   - a. 10 minutes of ischemia
   - b. 10 minutes of ischemia and one 3-minute episode of oxygenated no-K⁺ intermittent perfusion
   - c. Two 10-minute episodes of ischemia and one 3-minute episode of oxygenated no-K⁺ intermittent perfusion
   - d. Two 10-minute episodes of ischemia and two 3-minute episodes of oxygenated no-K⁺ intermittent perfusion
   - e. Three 10-minute episodes of ischemia and two 3-minute episodes of oxygenated no-K⁺ intermittent perfusion
   - f. Three 10-minute episodes of ischemia and three 3-minute episodes of oxygenated no-K⁺ intermittent perfusion
   - g. Four 10-minute episodes of ischemia and three 3-minute episodes of oxygenated no-K⁺ intermittent perfusion
   - h. Three 10-minute episodes of ischemia and two 3-minute episodes of oxygenated no-K⁺ intermittent perfusion followed by 30 minutes of reperfusion
   - i. Four 10-minute episodes of ischemia and three 3-minute episodes of oxygenated no-K⁺ intermittent perfusion followed by 30 minutes of reperfusion

7. **Hypoxemic, no-K⁺ intermittent perfusion**
   - a. 10 minutes of ischemia
   - b. 10 minutes of ischemia and one 3-minute episode of hypoxemic, no-K⁺ intermittent perfusion
   - c. Two 10-minute episodes of ischemia and one 3-minute episode of hypoxemic, no-K⁺ intermittent perfusion
   - d. Two 10-minute episodes of ischemia and two 3-minute episodes of hypoxemic, no-K⁺ intermittent perfusion
   - e. Three 10-minute episodes of ischemia and two 3-minute episodes of hypoxemic, no-K⁺ intermittent perfusion
   - f. Three 10-minute episodes of ischemia and three 3-minute episodes of hypoxemic, no-K⁺ intermittent perfusion
   - g. Four 10-minute episodes of ischemia and three 3-minute episodes of hypoxemic, no-K⁺ intermittent perfusion
   - h. Three 10-minute episodes of ischemia and two 3-minute episodes of hypoxemic, no-K⁺ intermittent perfusion followed by 30 minutes of reperfusion
   - i. Four 10-minute episodes of ischemia and three 3-minute episodes of hypoxemic, no-K⁺ intermittent perfusion followed by 30 minutes of reperfusion

Six to eight hearts were used for each protocol to analyze energy metabolites, ventricular function, or calcium uptake.

*In these protocols, different hearts were used for measurement of intracellular Na⁺ and K⁺ contents.

†In these hearts, calcium uptake was evaluated with hearts different from those used for measurements of metabolites or intracellular Na⁺ and K⁺ contents.
perfusate and was expressed as micromoles per gram of dry weight.

For estimation of Na\textsubscript{i} and intracellular K\textsuperscript{+} (K\textsubscript{i}), hearts that were different from those used for measurement of function, metabolites, or \textsuperscript{45}Ca\textsuperscript{2+} uptake were perfused for 5 minutes before ischemia with a buffer containing \textsuperscript{14}C-sorbitol (ICN Radiochemical Co.) and washed out for 1 minute with 15 ml of ice-cold 0.35 M sucrose and 5 mM histidine (pH 7.4) at the end of ischemia.\textsuperscript{17} This procedure minimized the contribution of extracellular ions to total tissue ion contents. Hearts were opened and blotted on paper towels. Ventricles were frozen in liquid nitrogen and pulverized. Frozen tissue powder was resuspended in deionized H\textsubscript{2}O (1 ml/g wet tissue) and stirred several times with a vortex mixer for 1 hour at 4\textdegree{}C. After 15 minutes of centrifugation (40,000g), the activities of Na\textsuperscript{+} and K\textsuperscript{+} were measured in the supernatant with an ion selective electrode system (Lytening 1, AMDEV Inc., Danvers, Mass.). Perfuse remaining in the tissue extract was calculated by division of the radioactivity in the extract by the radioactivity of \textsuperscript{14}C-sorbitol in the perfusate. Extracellular ion contents contaminating the extract were estimated based on the assumption that ion concentrations of perfuse remaining in the extract were the same as those of the recirculating buffer.\textsuperscript{18} Na\textsubscript{i} and K\textsubscript{i} were calculated by subtracting the extracellular ion contents from total ion contents.\textsuperscript{18}

Ventricular function was assessed from the product of developed pressure (systolic minus end-diastolic pressure) and heart rate. The percentage recovery of function was calculated by dividing this product at the end of reperfusion by that measured at the end of the 20-minute equilibration period before induction of ischemia.

Statistics

Values are given as mean±SEM of six to eight hearts. Statistical analysis was performed by a paired Student’s \( t \) test with Bonferroni’s correction to assess the significance of differences of hemodynamic data before ischemia and after reperfusion in each group. Analysis of variance (ANOVA) was followed by Neuman-Keul’s multiple-range test to compare differences in each group when the data were obtained from different hearts. ANOVA with Neuman-Keul’s multiple-range test was used to compare differences in groups of hearts with different interventions. To examine the relation between two variables, linear regression lines were calculated by the least-squares method. Differences were considered significant when \( p \) was less than 0.05.

Results

Effects of Oxygenated or Hypoxic Intermittent Perfusion

Ventricular function. Ventricular function was maintained for more than 90 minutes when hearts were perfused with oxygenated buffer containing substrates (Table 1, protocol 1a). When 40 minutes of oxygenated perfusion was interrupted by three 3-minute episodes of hypoxic perfusion (Table 1, protocol 1b), function recovered to control values after 30 minutes of perfusion (data not shown). Ventricular function recovered to only 46\% of the preischemic value after 30 minutes of sustained ischemia, mainly due to an increase in left ventricular end-diastolic pressure and a reduction in developed pressure (Table 1, protocol 2e, and Table 2). A further decrease in functional recovery was observed when ischemia was extended to 40 minutes (Table 1, protocol 2f, and Table 2). On the other hand, function recovered completely and left ventricular end-diastolic pressure was unchanged when 40 minutes of zero-flow ischemia was interrupted by three 3-minute periods of perfusion at 10-minute intervals with oxygenated or hypoxic buffer (Table 1, protocols 3h and 4h, and Tables 3A and 3B).

High-energy phosphates and lactate. In hearts perfused with oxygenated buffer for 70 minutes or hearts with three 3-minute episodes of hypoxic intermittent perfusion during the first 40 minutes of aerobic perfusion (Table 1, protocol 1b), final values of high-energy phosphates and lactate did not differ from those at the end of 70 minutes of oxygenated perfusion (ATP: 20.6±1.2, 20.4±0.9; CP: 33.4±1.8, 32.3±1.2; lactate: 2.7±0.3, 3.3±0.4 \( \mu \)mol/g dry wt, respectively). Tissue ATP decreased progressively during the first 30 minutes of sustained ischemia (Table 1, protocol 2, and Figure 1A). CP levels declined more rapidly (Figure 1A). The level of ATP was less than 20\% of the preischemic value after 30 or 40 minutes of sustained ischemia. In ischemic hearts with intermittent perfusion (Table 1, protocols 3 and 4, and Figures 1B and 1C), ATP was restored during each short perfusion period so its levels were preserved at about 60\% of the preischemic values after a total of 40 minutes of ischemia with either oxygenated (Table 1, protocol 3g, and Figure 1B) or hypoxic (Table 1, protocol 4g, and Figure 1C) buffer. Loss of total adenine nucleotides was also delayed, but restoration of CP was much poorer in hearts with hypoxic intermittent perfusion, and the CP content was the same as in hearts with sustained ischemia. Tissue lactate levels increased gradually and reached more than 200 \( \mu \)mol/g dry wt after 40 minutes of sustained ischemia (Table 1, protocol 2, and Figure 1A). When hearts were intermittently perfused (Table 1, protocols 3 and 4, and Figures 1B and 1C), each short perfusion washed out lactate so its final levels were approximately 100 \( \mu \)mol/g dry wt. Recovery of high-energy phosphates and total adenine nucleotides on reperfusion were also improved in hearts with intermittent perfusion.

Ion contents. Accumulation of Na\textsubscript{i} increased to eightfold its preischemic value in hearts with sustained ischemia (Table 1, protocol 2, and Table 2) but was unchanged in hearts provided intermittent perfusion (Table 1, protocols 3 and 4, and Tables 3A and 3B). K\textsubscript{i}, however, decreased in intermittently
TABLE 2.  Effect of Sustained Ischemia on Recovery of Function, Intracellular Ion Contents, and 
$^{45}$Ca$^+$ Uptake

<table>
<thead>
<tr>
<th>Ventricular function</th>
<th>Recovery of function (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>End of 20-minute equilibration period</td>
</tr>
<tr>
<td>70 minutes of oxygenated perfusion (protocol 1a)</td>
<td>64±5 0.8±0.3 262±3 16.8±0.9</td>
</tr>
<tr>
<td>Total of 30 minutes of ischemia</td>
<td>69±7 1.0±0.2 245±6 16.9±1.0</td>
</tr>
<tr>
<td>Total of 40 minutes of ischemia</td>
<td>68±6 0.8±0.2 256±8 17.4±1.2</td>
</tr>
</tbody>
</table>

Intracellular ion contents and $^{45}$Ca$^+$ uptake (μmol/g dry wt)

<table>
<thead>
<tr>
<th></th>
<th>End of 20-minute equilibration period</th>
<th>End of ischemia-cumulative ischemic time (min)</th>
<th>Total of 30 minutes of ischemia+30 minutes of reperfusion</th>
<th>Total of 40 minutes of ischemia+30 minutes of reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na$^+$</td>
<td>10.6±2.0 13.9±2.1 26.7±2.0* 63.5±2.5*</td>
<td>83.9±2.5*</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>K$^+$</td>
<td>290±8 283±6 286±6 292±10</td>
<td>299±12</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>$^{45}$Ca$^+$ uptake</td>
<td>0.64±0.07 0.64±0.08 0.66±0.10 5.8±0.4*</td>
<td>10.0±0.8*</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; HR, heart rate; DP, developed pressure. Values are given as mean±SEM of six to eight hearts.

* p<0.05 versus preischemic values.

Perfused hearts but remained high in hearts with sustained ischemia. Myocardial $^{45}$Ca$^+$ uptake did not change during either sustained or interrupted ischemia; however, massive Ca$^+$ overload on reperfusion was observed only in hearts with sustained ischemia (Table 1, protocol 2e and 2f, and Table 2).

Effect of Omission of Energy Substrates During Intermittent Perfusion

Substrate-free intermittent perfusion during the first 40 minutes of oxygenated perfusion did not decrease function (Table 1, protocol 1c, and Table 4) or modify metabolite contents (ATP: 18.6±0.8; CP: 29.3±1.3; lactate: 3.4±0.4 μmol/g dry wt) at the end of 70 minutes of nonischemic perfusion. Even when substrates were omitted from the perfusate during hypoxemic intermittent perfusion (Table 1, protocol 5h), ventricular function recovered completely after three 10-minute episodes of ischemia with a slight increase in left ventricular end-diastolic pressure and reperfusion $^{45}$Ca$^+$ uptake (Table 4). The levels of ATP and total adenine nucleotides were preserved and less lactate accumulated in these hearts (Table 1, protocol 5e, and Figures 2A and 2B). However, addition of a third period of intermittent perfusion and a fourth 10-minute episode of ischemia caused an abrupt deterioration of energy metabolism (Table 1, protocol 5g) and a reduction of functional recovery (Table 1, protocol 5i). The reduced tissue levels of high-energy phosphates were probably due to deple-

Effect of Omission of K$^+$ During Intermittent Perfusion

Aside from contraction of myocardium, which consumes 70% of aerobic ATP production, a major energy demand results from sarcolemma ATPases that maintain ion homeostasis.19 Moreover, it has been reported that ATP generated by glycolysis is sufficient to drive the Na$^+$-K$^+$ pump because $K_m$ for ATP is low in sarcolemmal Na$^+$,K$^+$-ATPase.20,21 The role of the Na$^+$-K$^+$ pump in maintenance of low Na$^+$ during intermittent perfusion was examined by removal of extracellular K$^+$ to restrain pump activity (Table 1, protocols 6 and 7). Although three 3-minute periods of oxygenated no-K$^+$ perfusion during the first 40 minutes of oxygenated perfusion did not have any deleterious effects on function (Table 1, protocol 1d), ion contents (Table 5), or metabolites (ATP: 19.3±0.8; CP: 30.3±0.8; lactate: 2.6±0.4 μmol/g dry wt) at the end of 70 minutes of oxygenated perfusion, the omission of K$^+$ during oxygenated intermittent perfusion depressed recovery of function (Table 1, protocols 6h and 6i, and Table 5A) and increased Na$^+$ (Table 1, protocols 6c and 6g, and
Table 3. Effect of Intermittent Perfusion With Oxygenated or Hypoxemic Buffer on Recovery of Function, Intracellular Ion Contents, and \(^{45}\text{Ca}^2+\) Uptake

A. Ischemia with oxygenated intermittent perfusion (protocol 3)

<table>
<thead>
<tr>
<th>Ventricular function</th>
<th>Recovery of function (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before ischemia</td>
</tr>
<tr>
<td></td>
<td>LVSP (mm Hg)</td>
</tr>
<tr>
<td>Total of 40 minutes of ischemia</td>
<td>79±8</td>
</tr>
</tbody>
</table>

Intracellular ion contents and \(^{45}\text{Ca}^2+\) uptake (\(\mu\text{mol/g dry wt}\))

<table>
<thead>
<tr>
<th>End of ischemia - cumulative ischemic time (min)</th>
<th>Total of 40 minutes of ischemia + 30 minutes of reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>...</td>
</tr>
<tr>
<td>20</td>
<td>...</td>
</tr>
<tr>
<td>30</td>
<td>...</td>
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<tr>
<td>40</td>
<td>...</td>
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</table>

B. Ischemia with hypoxemic intermittent perfusion (protocol 4)

<table>
<thead>
<tr>
<th>Ventricular function</th>
<th>Recovery of function (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>End of 20-minute equilibration period</td>
</tr>
<tr>
<td></td>
<td>LVSP (mm Hg)</td>
</tr>
<tr>
<td>Total of 40 minutes of ischemia</td>
<td>68±5</td>
</tr>
</tbody>
</table>

Intracellular ion contents and \(^{45}\text{Ca}^2+\) uptake (\(\mu\text{mol/g dry wt}\))

<table>
<thead>
<tr>
<th>End of 70 minutes of oxygenated perfusion with three 3-minute hypoxemic periods (protocol 1b)</th>
<th>End of ischemia - cumulative ischemic time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Start of perfusion</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>30</td>
</tr>
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<td></td>
<td>40</td>
</tr>
</tbody>
</table>

LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; HR, heart rate; DP, developed pressure. Values are given as mean±SEM of six to eight hearts. Ventricular function was assessed by the product of developed pressure (systolic pressure minus end-diastolic pressure) and heart rate. Recovery of function was calculated by division of the product at the end of 30 minutes of reperfusion by the product before induction of ischemia. Ionic data in intermittently perfused hearts without 30 minutes of reperfusion were obtained at the end of each ischemic episode.

*p<0.05 versus the corresponding values in hearts with sustained ischemia in Table 2.

Table 5A) without further deleterious changes in energy metabolism (Figure 3A). When hypoxemic, no-K\(^+\) buffer was used for three 3-minute periods during the first 40 minutes of oxygenated perfusion (Table 1, protocol 1e), CP decreased (22.1±1.3 \(\mu\text{mol/g dry wt}\)) and \(^{45}\text{Ca}^2+\) uptake increased. However, function, other metabolites, and ion contents did not change significantly. As long as ATP and adenine nucleotide pools were preserved, hearts intermittently perfused with hypoxemic no-K\(^+\) buffer recovered function to the same extent as those perfused with oxygenated no-K\(^+\) buffer (Table 1, protocol 6h and 7h, Tables 5A and 5B, and Figures 3A and 3B). Omission of extracellular K\(^+\) accelerated the decline of high-energy phosphate levels in hearts with hypoxemic intermittent perfusion (Table 1, protocols 4 and 7, and Figures 1C and 3B). The beneficial effects of intermittent perfusion on recovery of energy metabolism and function disappeared in these hearts in association with an increase in Na, and
subsequent aggravated Ca^{2+} overload after four 10-minute episodes of ischemia (Table 1, protocols 6i and 7i, Table 5B and Figure 3B). Myocardial \(^{45}\text{Ca}^{2+}\) uptake increased during recurrent ischemia by 20–40% in association with elevation of Na, regardless of whether the buffer was oxygenated (Tables 5A and 5B), but Ca^{2+} overload was observed only on reperfusion. Examination of the relations among Na, reperfusion myocardial \(^{45}\text{Ca}^{2+}\) uptake, and recovery of function (Figures 4 and 5) revealed that Na correlated closely with reduced recovery of function (Figure 4A) and reperfusion \(^{45}\text{Ca}^{2+}\) uptake (Figure 4B). As expected from these relations, there was a close negative correlation between reperfusion \(^{45}\text{Ca}^{2+}\) uptake and recovery of function (Figure 5A). The regression line was similar to those obtained in hearts with various durations of sustained ischemia\(^{11}\) and with prolonged low-flow anoxia.\(^{22}\) In addition, left ventricular end-diastolic pressure increased as \(^{45}\text{Ca}^{2+}\) uptake increased (Figure 5B).

**Discussion**

Repetitive brief ischemic episodes caused less myocardial damage than did sustained ischemia.
Also, preconditioning with transient periods of ischemia reduced infarct size after subsequent prolonged ischemia.\textsuperscript{23} Vascular washout by intermittent perfusion or reperfusion after transient ischemia can remove harmful metabolic products such as lactate, protons, or inorganic phosphate from the myocardium and reduce cumulative effects of these products during subsequent ischemia. Accumulation of metabolic intermediates causes further disturbances of energy metabolism,\textsuperscript{23,24} ion homeostasis,\textsuperscript{24,25} and intracellular pH (pHi) regulation\textsuperscript{23,26,27} and eventually leads to loss of cellular viability. Even a short period of perfusion prevented the progressive accumulation of lactate in globally ischemic hearts (Table 1, protocols 3–7, and Figures 1B, 1C, 2, 3A, and 3B). Removal of harmful metabolites, such as substrates, for free radical–generating reactions may also be important. If this is the case, multiple transient ischemic episodes would be expected to have cumulative effects; however, a single transient ischemic episode preconditioned the myocardium to the same extent as multiple episodes.\textsuperscript{28} On the contrary, Murry et al suggested that the protective effect of preconditioning might be mediated by free radical–induced myocardial injury in a blood-perfused regional ischemic model.\textsuperscript{29}

### Effects of Energy Preservation on Ion Homeostasis

Preservation of energy stores is essential for many cellular functions and may be another important factor for the protective effect of intermittent perfusion or reperfusion. ATP and total adenine nucleotide levels remained at about 60% and 80%, respectively, of the preischemic values during intermittent perfusion with oxygenated or hypoxic buffer, but ATP and total adenine nucleotide contents decreased progressively to 20% and 60% of preischemic values, respectively, in hearts subjected to sustained ischemia (Table 1, protocols 2 versus 3 and 4, and Figures 1A–1C). The reason for maintenance of ATP and total adenine nucleotides is not clear. Murry et al reported that preconditioning with brief ischemic episodes delayed ATP depletion and attenuated lactate accumulation during subsequent sustained ischemia in a regional ischemic model, although collateral blood flow measured by microspheres was similar regardless of preconditioning.\textsuperscript{23} Brief periods of perfusion may reduce energy demands.

### Table 4. Effect of Omission of Substrates During Hypoxemic Intermittent Perfusion on Recovery of Function, Intracellular Ion Contents, and \( ^{45}\text{Ca}^{2+} \) Uptake (Protocol 5)

<table>
<thead>
<tr>
<th></th>
<th>Ventricular function</th>
<th>Intracellular ion contents and ( ^{45}\text{Ca}^{2+} ) uptake (( \mu \text{mol/g dry wt} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LVSP (mm Hg)</td>
<td>LVEDP (mm Hg)</td>
</tr>
<tr>
<td></td>
<td>LVSP (mm Hg)</td>
<td>LVEDP (mm Hg)</td>
</tr>
<tr>
<td>End of 20-minute equilibration period</td>
<td></td>
<td></td>
</tr>
<tr>
<td>70 minutes of oxygenated perfusion with three 3-minute hypoxic, no-substrate periods (protocol 1c)</td>
<td>56±5</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>Before ischemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total of 30 minutes of ischemia</td>
<td>64±4</td>
<td>0.4±0.4</td>
</tr>
<tr>
<td>Total of 40 minutes of ischemia</td>
<td>62±7</td>
<td>0.6±0.2</td>
</tr>
<tr>
<td>End of ischemic—cumulative ischemic time (min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>End of 40 minutes of ischemia</td>
<td>70±6</td>
<td>1.5±0.8*</td>
</tr>
<tr>
<td>Total of 30 minutes of ischemia</td>
<td>158±8*</td>
<td></td>
</tr>
<tr>
<td>Total of 40 minutes of ischemia</td>
<td>299±19</td>
<td>12.4±1.0*</td>
</tr>
</tbody>
</table>

LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; HR, heart rate; DP, developed pressure.

Values are given as mean±SEM of six to eight hearts.

Ventricular function was assessed by the product of developed pressure (systolic pressure minus end-diastolic pressure) and heart rate. Recovery of function was calculated by division of the product at the end of 30 minutes of reperfusion by the product before induction of ischemia. Ionic data in intermittently perfused hearts without 30 minutes of reperfusion were obtained at the end of each ischemic episode.

\( ^{45}\text{Ca}^{2+} \) uptake (\( \mu \text{mol/g dry wt} \))

\( \text{Na}^{+} \)

19.3±4.2

13.9±2.1

10.1±1.8*

17.5±1.2*

50.1±5.7†

60±1

20: 0.68±0.12

30: 0.83±0.10

40: 1.2±0.1*

\( ^{45}\text{Ca}^{2+} \) uptake

0.63±0.10

\( \ldots \)

\( \ldots \)

0.68±0.12

0.83±0.10

1.2±0.1*

2.1±0.2*†

\( *p<0.05 \) versus the corresponding values in hearts with sustained ischemia in Table 2. \( \uparrow p<0.05 \) versus the corresponding values in hearts with hypoxic intermittent perfusion in Table 3.
omission of energy substrates during intermittent perfusion caused deterioration of metabolic and functional recovery along with increased reperfusion \(^{45}\text{Ca}^{2+}\) uptake. This deterioration was temporally related to depletion of the high-energy phosphate pool (Table 1, protocol 5, Figure 2A, and Table 4). This observation suggested that some energy-dependent system is involved in the protective effect seen in intermittently perfused hearts. Furthermore, the depletion of high-energy phosphates was closely associated with an increase in Na\(_\text{a}\) during ischemia and in \(^{45}\text{Ca}^{2+}\) uptake during reperfusion. These close associations suggested that energy-dependent maintenance of low Na\(_\text{a}\) resulted in restraint of Ca\(^{2+}\) entry, probably through Na\(^{+}\)-Ca\(^{+}\) exchange during reperfusion,\(^13\) although associations between Na\(_\text{a}\), \(^{45}\text{Ca}^{2+}\) uptake, and recovery of function may not necessarily prove causal relations. Although we did not measure free cytosolic ion concentrations, these values have recently been measured by nuclear magnetic resonance in a whole-heart preparation. These measurements were performed, however, with chelating or shift agents or superphysiologically high [Ca\(^{2+}\)] in perfusate. The use of such additives could affect function and metabolism of ischemic-reperfused myocardium and make it very difficult to investigate the relations between ion homeostasis and functional or metabolic recovery.

**Regulation of Na\(_\text{a}\) by Na\(^{+}\)-K\(^{+}\) Exchange and Na\(^{+}\)-K\(^{+}\) Pump**

In cardiac myocytes, the Na\(^{+}\)-H\(^{+}\) exchange system operates to regulate pH\(_i\) when pH\(_i\) is low but not when extracellular pH (pH\(_o\)) is normal.\(^31\) We observed previously that pretreatment with amiloride, a Na\(^{+}\)-H\(^{+}\) exchange inhibitor, diminished the increase in Na\(_\text{a}\) during early reperfusion.\(^13,32\) Na\(^{+}\) influx through Na\(^{+}\)-H\(^{+}\) exchange would be expected to increase when pH\(_i\) returned toward normal during each short period of intermittent perfusion because half readjustment of decreased pH\(_i\) through Na\(^{+}\)-H\(^{+}\) exchange by addition of extracellular Na\(^{+}\) to acid-loaded chick heart cells had a \(t_{1/2}\) of 2.9 minutes.\(^33\) However, we did not detect a substantial rise in Na\(_\text{a}\) as long as high-energy phosphate contents were preserved during either oxygenated or hypoxic intermittent perfusion after transient ischemic episodes (Table 1, protocols 3 and 4, and Tables 3A and 3B). Therefore, the expected increase in Na\(^{+}\) influx was considered to be offset by energy-dependent Na\(^{+}\) efflux through the Na\(^{+}\)-K\(^{+}\) pump that was driven by the ATP produced by anaerobic glycolysis.\(^20\) In addition, correction of pH\(_i\) and washout of metabolites could potentiate the activity of the Na\(^{+}\)-K\(^{+}\) pump and increase energy production. In support of this, omission of extracellular K\(^{+}\) immediately increased Na\(_\text{a}\) during recurrent ischemia (Table 1, protocols 6 and 7, and Tables 5A and 5B). We also observed that cardiac glycogenolysis restrained the decrease in Na\(_\text{a}\) during early reperfusion when cardiac glycose was used during the first 10 minutes of reperfusion after 25 minutes of global ischemia.\(^34\) Furthermore, an additional large increase
### TABLE 5. Effect of Omission of K⁺ During Oxygenated or Hypoxic Intermittent Perfusion on Recovery of Function, Intracellular Ion Contents, and ⁴⁵Ca²⁺ Uptake

**A. Ischemia with oxygenated, no-K⁺ intermittent perfusion (protocol 6)**

<table>
<thead>
<tr>
<th>Ventricular function</th>
<th>Recovery of function (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVSP (mm Hg)</td>
<td>LVEDP (mm Hg)</td>
</tr>
<tr>
<td>End of 20-minute equilibration period</td>
<td>End of 70 minutes of perfusion</td>
</tr>
<tr>
<td>70 minutes of oxygenated perfusion with three 3-minute oxygenated, no-K⁺ periods (protocol 1d)</td>
<td>63±4 0.0±0.0 245±4 15.4±0.7</td>
</tr>
<tr>
<td>Before ischemia</td>
<td>After 30 minutes of reperfusion</td>
</tr>
<tr>
<td>Total of 30 minutes of ischemia</td>
<td>66±6 0.6±0.2 248±12 16.4±1.3</td>
</tr>
<tr>
<td>Total of 40 minutes of ischemia</td>
<td>69±6 0.8±0.2 242±14 16.7±1.4</td>
</tr>
</tbody>
</table>

**Intracellular ion contents and ⁴⁵Ca²⁺ uptake (μmol/g dry wt)**

<table>
<thead>
<tr>
<th>End of 70 minutes of oxygenated perfusion with three 3-minute oxygenated, no-K⁺ periods</th>
<th>End of ischemia—cumulative ischemic time (min)</th>
<th>Total of 30 minutes of ischemia + 30 minutes of reperfusion</th>
<th>Total of 40 minutes of ischemia + 30 minutes of reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺</td>
<td>18.3±4.1</td>
<td>13.9±2.1</td>
<td>41.3±1.8*</td>
</tr>
<tr>
<td>K⁺</td>
<td>288±10</td>
<td>283±6</td>
<td>267±5†</td>
</tr>
<tr>
<td>⁴⁵Ca²⁺ uptake</td>
<td>0.65±0.08</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

**B. Ischemia with hypoxic, no-K⁺ intermittent perfusion (protocol 7)**

<table>
<thead>
<tr>
<th>Ventricular function</th>
<th>Recovery of function (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVSP (mm Hg)</td>
<td>LVEDP (mm Hg)</td>
</tr>
<tr>
<td>End of 20-minute equilibration period</td>
<td>End of 70 minutes of perfusion</td>
</tr>
<tr>
<td>70 minutes of oxygenated perfusion with three 3-minute oxygenated, no-K⁺ periods (protocol 1e)</td>
<td>64±7 0.0±0.0 260±8 16.6±1.2</td>
</tr>
<tr>
<td>Before ischemia</td>
<td>After 30 minutes of reperfusion</td>
</tr>
<tr>
<td>Total of 30 minutes of ischemia</td>
<td>77±8 1.0±0.2 272±10 20.9±1.6</td>
</tr>
<tr>
<td>Total of 40 minutes of ischemia</td>
<td>73±7 0.8±0.2 261±11 19.1±1.0</td>
</tr>
</tbody>
</table>

**Intracellular ion contents and ⁴⁵Ca²⁺ uptake (μmol/g dry wt)**

<table>
<thead>
<tr>
<th>End of 70 minutes of oxygenated perfusion with three 3-minute oxygenated, no-K⁺ periods</th>
<th>End of ischemia—cumulative ischemic time (min)</th>
<th>Total of 30 minutes of ischemia + 30 minutes of reperfusion</th>
<th>Total of 40 minutes of ischemia + 30 minutes of reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺</td>
<td>19.3±4.2</td>
<td>13.9±2.1</td>
<td>34.1±2.2†‡</td>
</tr>
<tr>
<td>K⁺</td>
<td>288±13</td>
<td>283±6</td>
<td>247±4†</td>
</tr>
<tr>
<td>⁴⁵Ca²⁺ uptake</td>
<td>0.63±0.10</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; HR, heart rate; DP, developed pressure. Values are given as mean±SEM of six to eight hearts.

Ventricular function was assessed by the product of developed pressure (systolic pressure minus end-diastolic pressure) and heart rate. Recovery of function was calculated by division of the product at the end of 30 minutes of reperfusion by the product before induction of ischemia. Ionic data in intermittently perfused hearts without 30 minutes of reperfusion were obtained at the end of each ischemic episode.

*p<0.05 versus the corresponding values in hearts with sustained ischemia in Table 2.

†p<0.05 versus hearts with hypoxic intermittent perfusion with normal perfusate K⁺ in Table 3.

‡p<0.05 versus hearts with oxygenated intermittent perfusion with zero K⁺ perfusate.
in Na, was detected when depletion of ATP was associated with omission of extracellular K+ (Figure 3B and Table 5). Additive effects of ATP depletion and K+ omission might be explained in part by an increase in conductance of the sarcolemmal membrane to Na+ ions because accumulation of Na+ during ischemia was attenuated by pretreatment with lidocaine, a Na+ channel blocker.35,36 The cause of accelerated depletion of high-energy phosphates despite the hypothetical inhibition of energy-dependent Na+ -K+ pump is not clear. Murphy et al37 reported that inhibition of the Na+ -K+ pump in normoxically cultured embryonic heart cells resulted in elevation of intracellular [Ca2+] and the subsequent uptake of Ca2+ into mitochondria that resulted in impaired oxidative phosphorylation. Uptake of Ca2+ by mitochondria requires that the mitochondria be energized.38 We observed accelerated depletion of ATP, however, by omission of K+, even in hearts with hypoxemic intermittent perfusion. Less lactate accumulated in hearts subjected to hypoxemic intermittent perfusion, suggesting that flux through anaerobic glycolysis was reduced by no-K+ perfusate.

Possible Importance of Ca2+ Entry Through Na+ -Ca2+ Exchange

Excessive Ca2+ influx occurred, at least in part, through Na+ -Ca2+ exchange in response to increased Na+ in both hypoxic-reoxygenated39,40 and ischemic-reperfused12-14,32 myocardium. Although Na+ increased during ischemia, 45Ca2+ uptake increased to only a small extent or nor at all during ischemia, regardless of the type or its duration (the present study and Reference 13). These observations may be accounted for by the fact that Na+ -Ca2+ exchange is pH dependent in myocytes and that Ca2+ uptake is inhibited under conditions where pH is low.41 In addition, we observed that the increase in 45Ca2+ uptake was not detectable during the first few minutes of reperfusion.13 With a short period of intermittent perfusion, readjustment of pH, through Na+-H+ exchange may not be sufficient to reanimate Na+ -Ca2+ exchange and cause a large increase in Ca2+ influx before reinitiation of ischemia.33 Disturbance of phosphorylation-dephosphorylation interconversion of the Na+-Ca2+ exchanger by depletion of ATP may also suppress Ca2+ entry through this mechanism.42 In addition, the direction of flux of ions through Na+ -Ca2+ exchange depends on the membrane potential. The reversal potential is calculated by the equation:

\[ V_R = \frac{nV_{N_a} - 2V_{Ca}}{n-2} \]

where n is the Na+-Ca2+ coupling ratio, and V_Na and V_Ca are the equilibrium potentials for Na+ and Ca2+, respectively.43 Depolarization to values exceeding the reversal potential is needed to initiate Ca2+ influx. Therefore, a severalfold increase in cytosolic free [Ca2+] can offset the change in reversal potential caused by elevation of Na+. Steenbergen et al44 reported that cytosolic free [Ca2+] increased to 3 μM within 20 minutes of the induction of ischemia. An increase in Ca2+ uptake of less than 0.01 μmol/g dry wt can result in an increase of cytosolic free [Ca2+] to this

![Figure 3. Bar graphs of effect of omission of K+ during oxygenated or hypoxemic intermittent perfusion. Panel A: Intermittent perfusion with oxygenated no-K+ buffer (protocol 6). Panel B: Intermittent perfusion with hypoxemic no-K+ buffer (protocol 7). Metabolites were measured at the end of the ischemic episode, period of intermittent perfusion, or 30 minutes of reperfusion after a total of 30 or 40 minutes of ischemia. CP, creatine phosphate; IP, intermittent perfusion; cross-hatched bars, ATP; open bars, total adenine nucleotides; stippled bars, creatine phosphate; solid bars, lactate. Values are given as mean±SEM of six to eight hearts.](http://circ.ahajournals.org/doi/abs/10.1161/01.CIR.82.8.546?journalCode=circ)
extent if the intracellular Ca\(^{2+}\) sequestration sites, particularly the mitochondria, do not buffer the rise in cytosolic [Ca\(^{2+}\)].

In conclusion, our observations strongly suggest that outward transport of Na\(^{+}\) during intermittent perfusion prevents accumulation of Na\(^{+}\) and subsequently reduces Ca\(^{2+}\) entry through Na\(^{+}\)-Ca\(^{2+}\) exchange. These events may be responsible for improved post-ischemic contractile function.

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References


5. Lange R, Ware J, Kloner RA: Absence of a cumulative deterioration of regional function during three repeated 5 or 15 minute coronary occlusions. Circulation 1984; 69:400–408


13. Tani M, Neely JR: Role of intracellular Na\(^{+}\) in Ca\(^{2+}\) overload and depressed recovery of ventricular function of reperfused ischemic rat hearts: Possible involvement of H\(^+\)-Na\(^+\) and Na\(^+\)-Ca\(^{2+}\) exchange. \textit{Circ Res} 1989;65:1045–1056


23. Murry CE, Reimer KA, Jennings RB: Preconditioning with ischemia delays ATP depletion and limits lactate accumulation in severely ischemic canine myocardium (abstract). \textit{Circulation} 1987;76(suppl IV):I–228


29. Murry CE, Richard VJ, Jennings RB: Preconditioning with ischemia: Is the protective effect mediated by free radical-induced myocardial stunning (abstract)? \textit{Circulation} 1988;78(suppl II):II-77


32. Tani M, Neely JR: Intracellular Na (Na\(^{+}\)), calcium uptake and ventricular function of reperfused ischemic rat hearts (abstract). \textit{Circulation} 1988;78(suppl II):II-642


34. Tani M: Mechanisms of digitalis hypersensitivity in reperfused ischemic rat myocardium. \textit{J Mol Cell Cardiol} 1990;22(suppl) in press


36. Pike MM, Kitakaze M, Marban E: Increase in intracellular free sodium concentration during ischemia revealed by \textit{\textsuperscript{28}Na} NMR in perfused ferret hearts (abstract). \textit{Circulation} 1988;78(suppl II):II-56


**KEY WORDS** \* intracellular Na\(^{+}\) \* Ca\(^{2+}\) overload \* reperfusion injury \* Na\(^+\)-K\(^+\) pump \* preconditioning
Intermittent perfusion of ischemic myocardium. Possible mechanisms of protective effects on mechanical function in isolated rat heart.

M Tani and J R Neely

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