Role of Na+/H+ Exchanger During Ischemia and Preconditioning in the Isolated Rat Heart

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Abstract—The role of the Na+/H+ exchanger in ischemia, reperfusion, and preconditioning was investigated in isolated perfused rat hearts. Contractile function, [Na+]i, and pH, were measured; ischemic damage was assessed by the recovery of developed pressure (DP) on reperfusion. After 30 minutes of ischemia, DP recovered to only 14±4% of preischemic control. In contrast, after preconditioning (3×5-minute periods of ischemia) followed by 30 minutes of ischemia, DP recovered to 75±4%. Hearts treated with the Na+/H+ exchange inhibitor 5-(N-methyl-N-isobutyl)amiloride (MIA) also showed an enhanced recovery after ischemia (DP 62±9%). Treatment with a low concentration of tetrodotoxin (TTX, 100 nmol/L), which blocks the persistent component of the Na+ current, had a small beneficial effect on recovery (DP 37±8%). Thirty minutes of ischemia caused a small [Na+]i rise (3.2±0.9 mmol/L); reperfusion resulted in a further [Na+]i increase (+11.9±2.5 mmol/L), which partially recovered over 30 minutes. Preconditioning did not change the [Na+]i, rise during ischemia but abolished the large [Na+]i, rise on reperfusion, and [Na+]i instead fell (−3.6±1.3 mmol/L). In the presence of MIA, the [Na+]i rise was unchanged from ischemia only; on reperfusion, [Na+]i fell (−3.7±0.9 mmol/L), similar to the preconditioned hearts. TTX abolished the [Na+]i rise during ischemia (+0.3±0.7 mmol/L), and the increase on reperfusion was similar to ischemia only. We conclude that the rise of [Na+]i during ischemia is caused by Na+ entry through persistent Na+ channels. The rise of [Na+]i on reperfusion is caused by activation of the Na+/H+ exchanger and is blocked by MIA and by preconditioning. It is known that the Na+/H+ exchanger is inhibited during ischemia; the present result suggests that this inhibition is prolonged into the early part of reperfusion by preconditioning. To test this hypothesis, we measured the time course of pH recovery after ischemia and preconditioning. Preconditioning slowed the rate of pH recovery after ischemia, providing further support for the hypothesis that preconditioning inhibits the Na+/H+ exchanger during early reperfusion. This inhibition of the Na+/H+ exchanger during reperfusion prevents Na+ entry, and therefore Ca2+ loading, and is part of the protective pathway involved in preconditioning. (Circ Res. 1999;85:723-730.)

Key Words: Na+/H+ exchanger ■ preconditioning ■ ischemia ■ reperfusion

In 1986, Murry et al1 first demonstrated that the myocardial damage associated with ischemia could be substantially reduced by several preceding short periods of ischemia; a phenomenon they termed preconditioning. This surprising and provocative finding has stimulated great interest in the underlying mechanism, which appears to represent an endogenous protective pathway. Substantial progress has been made in identifying possible triggers to preconditioning, which include adenosine, acetylcholine, α- and β-adrenergic transmitters, bradykinin, and endothelin (for review, see Reference 2). These triggers bind to appropriate receptors that are G protein coupled and cause phospholipase C activation, production of inositol triphosphate and diacylglycerol, and eventually, activation of protein kinase C.3,4 Presumably, phosphorylation of some key proteins then results in protection of the myocardiun from the damaging effects of ischemia and/or reperfusion. This latter part of the mechanism is the least well-defined. The ATP-sensitive K+ channel (KATP) is one of the phosphorylated proteins that provides protection. The initial evidence was that blockers of the KATP channel prevented preconditioning.5 Subsequently, it was shown that protein kinase C causes phosphorylated KATP and contributes to its activation.6 Recently, it has been suggested that it is the mitochondrial KATP channel that is involved,7 although the mechanism of protection remains unclear.

To understand how the protection invoked by preconditioning might occur, it is important to understand the possible mechanisms of myocardial damage during ischemia and/or reperfusion. One of the best established pathways is that protons produced during ischemia leave the myocytes on the Na+/H+ exchanger during reperfusion causing Na+ loading. Subsequently, Ca2+ loading occurs as Na+ leaves the cell on the Na+/Ca2+ exchanger. The resulting rise in [Ca2+]i, is believed to trigger Ca2+ -activated proteases and phospho-
lipases that cause the cellular damage. In support of this theory is the observation that maneuvers which reduce Ca$^{2+}$ entry at the time of reperfusion lead to myocardial protection. Another important finding is that inhibition of the Na$^+$/H$^+$ exchanger reduces myocardial damage on reperfusion.

These findings suggest that coupled activation of the Na$^+$/H$^+$ exchanger and Na$^+$/Ca$^{2+}$ exchanger in response to intracellular acidosis may be part of the damage mechanism during reperfusion. If this is the case, modulation of this pathway might explain the beneficial effects of preconditioning. One version of this hypothesis was supported by Steenbergen et al. They showed that after preconditioning the acidosis in the long period of ischemia was reduced, presumably because glycogen had been partly consumed in the preconditioning ischemias, and therefore the anaerobic lactic acid production was reduced. Consequently, the Na$^+$ accumulation and the Ca$^{2+}$ accumulation were reduced and the myocardial damage was lessened.

It has long been suspected that the Na$^+$/H$^+$ exchanger would be inhibited during ischemia, and recent studies from our laboratory provide strong support for this view, although suggesting a different mechanism. We also established that the Na$^+$/H$^+$ exchanger reactivates rapidly on reperfusion, leading to a rapid rise of [Na$^+$]. If this rise in [Na$^+$] was prevented by an Na$^+$/H$^+$ exchange inhibitor, then the damaging effects of ischemia would be prevented. These observations led us to hypothesize that preconditioning might inhibit the Na$^+$/H$^+$ exchanger during reperfusion and prevent myocardial damage by this pathway. In the present study, we have investigated this possibility by means of measurements of [Na$^+$] and pH during ischemia and reperfusion.

**Materials and Methods**

**Heart Preparation**
The preparation and monitoring of Langendorff-perfused rat hearts have been described in detail previously. Rats were supplied by Gore Hill Research Laboratories (Sydney), and the experiments were approved by the Animal Ethical Committee of the University of Sydney. Briefly, female Sprague-Dawley rats were anesthetized with pentobarbitone, and the heart was rapidly excised. The aorta was cannulated and was retrogradely perfused with a modified Tyrode solution at a flow rate of 12 to 15 mL/min.

**Fluorescence Measurements**
The hearts were placed in a chamber mounted on the stage of an inverted microscope modified for fluorescence measurements. Briefly, after measuring the autofluorescence, the hearts were loaded in the heart and preventing the loss of CO$_2$ from the heart by diffusion. To minimize this effect, we replaced the external solution in the bath with standard Tyrode equilibrated with 70% N$_2$ and 30% CO$_2$ at 10 and 20 minutes. This solution would have the dual effect of preventing any oxidative metabolism in the epicardial cells of the heart and preventing the loss of CO$_2$ from the heart by diffusion. Under these conditions, pH generally decreased smoothly during ischemia.

**Ischemia, Preconditioning, and Drug Treatment**
The ischemia was induced by stopping perfusion inflow to the heart while the heart was maintained at 35°C. The standard period of ischemia was 30 minutes; preconditioning consisted of 3 periods of 5 minutes of ischemia each followed by 5 minutes reperfusion and then followed by the standard 30 minutes of ischemia. 5-(N-methyl-N-isobutyl)amiloride (MIA) and tetrodotoxin (TTX) were applied 5 minutes of ischemia each followed by 5 minutes reperfusion.

**Results**

**DP and Contractures After Ischemia**
We first established that preconditioning produced a substantial improvement in recovery from ischemia as shown in many earlier studies.
Table 1. Mechanical Performance During Ischemia and Reperfusion Under Various Conditions

<table>
<thead>
<tr>
<th>Condition</th>
<th>Preischemic</th>
<th>Reperfusion</th>
<th>IC, mm Hg</th>
<th>RC, mm Hg</th>
<th>Time of Contracture, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ischemia only</td>
<td>100</td>
<td>14±4</td>
<td>33±12</td>
<td>87±13</td>
<td>19±4</td>
</tr>
<tr>
<td>(n=5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preconditioning</td>
<td>100</td>
<td>75±4*</td>
<td>28±7</td>
<td>16±8*</td>
<td>23±3</td>
</tr>
<tr>
<td>(n=5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MIA (n=5)</td>
<td>88±6</td>
<td>62±9*</td>
<td>18±4</td>
<td>34±6*</td>
<td>20±3</td>
</tr>
<tr>
<td>TTX (n=6)</td>
<td>86±10</td>
<td>37±8</td>
<td>31±4</td>
<td>36±8*</td>
<td>19±2</td>
</tr>
<tr>
<td>5 Hz (n=4)</td>
<td>100</td>
<td>14±5</td>
<td>43±8</td>
<td>90±14</td>
<td>7±1*</td>
</tr>
</tbody>
</table>

DP is expressed as a percentage of control. In the MIA and TTX conditions, the preischemic value of DP is that after 5 minutes of exposure to the drug. Time of contracture shows the first appearance of ischemic contracture. *P<0.05 compared with ischemia only (ANOVA).

Note that during ischemia only (Figure 1A), the ischemic contracture (IC) is not prominent; by contrast the reperfusion contracture (RC) is very large and shows little recovery. There was little recovery of DP in this example. In 5 hearts, the IC was 33±12 mm Hg and appeared at 19±4 minutes. The RC was 87±13 mm Hg whereas the recovery of DP was 14±4%.

Figure 1B illustrates the preconditioning protocol. Note that the RC is much smaller than in the ischemia-only record and that DP shows a good recovery. In 5 hearts, there was no significant difference in either the magnitude of the IC (28±7 mm Hg) or the time of first appearance (23±3 minutes) compared with ischemia only. However, the RC was much smaller (16±8 mm Hg) and the recovery of DP was much greater (75±4%).

To identify possible mechanisms of the elevations of [\(\text{Na}^+\)] during ischemia and on reperfusion, we blocked various Na\(^+\) influx pathways. Inhibitors of the Na\(^+\)/H\(^+\) exchanger have been shown to reduce the rise in [\(\text{Na}^+\)] during ischemia,13 to reduce the [\(\text{Na}^+\)], during reperfusion,12,17 and to improve the mechanical recovery after ischemia.11–13 In the experiment shown in Figure 1C, the Na\(^+\)/H\(^+\) exchanger inhibitor MIA (10 \(\mu\)mol/L) was applied 5 minutes before the start of ischemia. Note that there was little or no RC in this experiment and the DP showed a substantial recovery. In 5 experiments, the initial 5 minutes of exposure to MIA caused no significant change in DP (DP after 5 minutes of exposure was 88±6%). The IC (18±4 mm Hg) was not significantly different than ischemia whereas the RC (34±6 mm Hg) was significantly smaller than that during ischemia only. The recovery of DP (62±9%) was much greater than ischemia alone but not significantly different than preconditioned hearts.

Another possible source of Na\(^+\) influx into the cell during ischemia is through Na\(^+\) channels.21,22 We were particularly interested in the possibility that a persistent component of the Na\(^+\) channel (\(I_{\text{Na(P)}}\)) might contribute to Na\(^+\) influx during ischemia. There are several reasons for thinking that this component of Na\(^+\) influx might increase during ischemia. (1) \(I_{\text{Na(P)}}\) is less inactivated by the depolarized conditions that occur in ischemia.23 (2) \(I_{\text{Na(P)}}\) is preferentially increased during hypoxia.24 To test these ideas, we made use of the fact that 100 nmol/L TTX almost completely suppresses \(I_{\text{Na(P)}}\) but has very little effect on the conventional \(I_{\text{Na}}\).23 Figure 1D shows that this concentration of TTX did not prevent regular contractions, confirming that normal \(I_{\text{Na}}\) was little affected. A typical IC developed and a moderately sized RC is apparent, and recovery of DP was intermediate in size between ischemia only and the enhanced recovery observed in preconditioning and MIA. In 6 hearts, DP did not change significantly in the initial 5 minutes of exposure (86±10%), IC was 31±4 mm Hg, RC was 36±8 mm Hg, and DP recovered to 37±8% (not significantly greater than ischemia only but significantly smaller than the preconditioned recovery).

[\(\text{Na}^+\)]\(_i\) During Ischemia and Reperfusion

Having determined that preconditioning improved the functional recovery after ischemia in a manner similar to that produced by inhibition of the Na\(^+\)/H\(^+\) exchanger, we were interested in whether changes in [\(\text{Na}^+\)]\(_i\), were involved in these effects. The changes in [\(\text{Na}^+\)]\(_i\), during ischemia and reperfusion under various conditions are illustrated in Figure 2 and summarized in Table 2. Figure 2A shows a small, slow rise of [\(\text{Na}^+\)]\(_i\), during 30 minutes of ischemia whereas reperfusion caused a large transient increase of [\(\text{Na}^+\)]\(_i\), which partially recovered over 20 minutes. Under control conditions, [\(\text{Na}^+\)]\(_i\), was 7.2±0.3 mmol/L (n=24). In 6 hearts, the ischemic rise was 3.2±0.9 mmol/L; the increase on reperfusion was +11.9±2.5 mmol/L. Over 30 minutes of reperfusion, [\(\text{Na}^+\)]\(_i\), partially recovered to 13.4±3.0 mmol/L.

The rise in [\(\text{Na}^+\)]\(_i\), during ischemia in the present experiments is relatively small, but we showed recently that when the stimulation frequency was higher (5 Hz), the rise of [\(\text{Na}^+\)]\(_i\), during a 10-minute period of ischemia was larger.17 We therefore measured the rise of [\(\text{Na}^+\)]\(_i\), during ischemia in 4 hearts paced at 5 Hz. The [\(\text{Na}^+\)]\(_i\), at 5 Hz was higher (11.5±1.3 mmol/L) than at 2 Hz (7.3±1.2 mmol/L), as expected,25 and after 30 minutes of ischemia, it reached 21.3±3.1 mmol/L, an increase of 9.8±1.6 mmol/L, which...
was larger than hearts stimulated at 2 Hz. The IC occurred at 7±1 minutes, which was much earlier than the 2-Hz group. Another possible reason that the rise in [Na\(^+\)], during ischemia might be smaller in the present experiments is that the extracellular and/or intracellular acidosis might be reduced in myocytes close to the surface (because CO\(_2\), which normally accumulates in the heart during ischemia,\(^1\)) can diffuse into the surrounding perfusate). To prevent this, in 2 experiments we replaced the extracellular solution around the heart during ischemia with one containing 30% CO\(_2\) (pH 6.6). The rise in [Na\(^+\)] in 30 minutes of ischemia in these experiments was 6.2±3.5 mmol/L, which is not significantly different than the control group at 2 Hz.

Figure 2B shows an example of the [Na\(^+\)] record during preconditioning and the subsequent ischemia and reperfusion. Note that the 3 short preconditioning ischemias have no significant effect on [Na\(^+\)]. The increase in [Na\(^+\)], during the subsequent long ischemia remained small, but a striking difference is apparent on reperfusion when the [Na\(^+\)], did not rise as in ischemia only but declined rapidly back to control level. In 5 hearts, the ischemic rise was 4.6±1.0 mmol/L, which is not significantly different than in ischemia only. The [Na\(^+\)], change on reperfusion was a fall (−3.6±1.3 mmol/L) to a level that was not significantly different than the preischemic level.

MIA had no effect on [Na\(^+\)], concentration over 5 minutes in control conditions nor any effect on the [Na\(^+\)], rise caused by the 30-minute periods of ischemia (4.4±0.6 mmol/L). In the presence of MIA, reperfusion caused an immediate decline in [Na\(^+\)], (−3.7±0.9 mmol/L) similar to that observed in the preconditioned heart. This final level was not significantly different than control and did not change thereafter.

TTX treatment (100 nmol/L) had no effect on [Na\(^+\)], concentration during the 5-minute application. However, the rise in [Na\(^+\)], caused by 30 minutes of ischemia was abolished by TTX treatment (+0.3±0.7 mmol/L). Reperfusion resulted in a relatively large transient increase of [Na\(^+\)], (+8.6±3.5 mmol/L), which was not significantly different from that observed during ischemia only. The [Na\(^+\)], level slowly declined so that after 30 minutes of reperfusion, [Na\(^+\)], concentration was 13.0±1.7 mmol/L,

### TABLE 2. Effects of Preconditioning: Treatment With MIA and TTX and 5-Hz Stimulation on the Changes in [Na\(^+\)], During 30 Minutes of Ischemia and Reperfusion

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control [Na(^+)], mmol/L</th>
<th>Ischemia</th>
<th>Reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Preischemia</td>
<td>30 Minutes</td>
<td>5 Minutes</td>
</tr>
<tr>
<td>Ischemia only (n=6)</td>
<td>6.9±0.6 ... 10.0±1.3 3.2±0.9</td>
<td>21.9±3.2 11.9±2.5 13.4±3.0</td>
<td></td>
</tr>
<tr>
<td>Preconditioning (n=5)</td>
<td>7.2±0.8 ... 11.8±1.7 4.6±1.0</td>
<td>8.2±1.1 −3.6±1.3† 7.6±1.0</td>
<td></td>
</tr>
<tr>
<td>MIA (n=5)</td>
<td>7.2±1.2 7.2±1.0 11.6±1.2† 4.4±0.6</td>
<td>7.9±0.6 −3.7±0.9† 6.8±0.9</td>
<td></td>
</tr>
<tr>
<td>TTX (n=7)</td>
<td>7.4±0.3 7.7±0.2 8.0±0.8‡ 0.3±0.7†</td>
<td>16.6±3.0 8.6±3.5* 13.0±1.7‡</td>
<td></td>
</tr>
<tr>
<td>5 Hz (n=4)</td>
<td>7.3±1.2 (2 Hz) 11.5±1.3‡ 21.3±3.1† 9.8±1.6†</td>
<td>28.3±3.4 7.0±3.4* 23.4±1.8‡</td>
<td></td>
</tr>
</tbody>
</table>

\(\Delta I\) and \(\Delta R\) are the changes in [Na\(^+\)], during ischemia and the first 5 minutes of reperfusion, respectively. Preischemia values for MIA and TTX represent [Na\(^+\)], after 5 minutes of exposure to the drug. Control value for 5-Hz data represents [Na\(^+\)], at 2-Hz stimulation rate, whereas preischemia value shows the [Na\(^+\)], at 5 Hz.

*\(P<0.05\) compared with zero (paired \(t\) test); †\(P<0.05\) compared with ischemia only (ANOVA); ‡\(P<0.05\) compared with control (repeated-measures ANOVA).
which was still significantly higher than the pres ischemic [Na⁺], level.

**pHi Measurements During Ischemia and Reperfusion**

The absence of a rise in [Na⁺] after preconditioning suggests that the Na⁺/H⁺ exchanger may be inhibited. If this were the case, we would expect the recovery of pHi after a preconditioned ischemia to be slower than ischemia only.26 Figure 3 illustrates experiments designed to test this possibility. In 12 experiments, the resting pHi was 7.41±0.04. In 5 experiments, 30 minutes of ischemia caused pHi to decline to 6.08±0.17. On reperfusion, pHi recovered rapidly with an approximately exponential time course as shown in Figure 3A. The rate of recovery was characterized by the half-time of recovery, which was 26±5 seconds. In 7 experiments, preconditioned ischemia was examined, and a representative record is shown in Figure 3B. The acidosis in the 5-minute ischemias is smaller and recovers quickly, and the half-time of recovery after the first period of ischemia was 17±2 seconds. In contrast to ischemia only, the acidosis during the preconditioned ischemia was 6.55±0.18, which is significantly smaller than the ischemia only (P<0.05, unpaired t test). Despite the smaller acidosis, the recovery of acidosis is somewhat slower and the half-time was significantly longer, at 46±6 seconds. The rate of proton extrusion, which underlies these pH recoveries, is a function of pHl, because both the extrusion mechanisms and the buffering are pH dependent.27 For this reason, we also measured the rate of pHl recovery at a constant pHl (6.8) and obtained the following values: 5 minutes of ischemia, 0.025±0.002 pHl units/s, 30 minutes of ischemia only, 0.016±0.002 pHl units/s, and preconditioned ischemia, 0.008±0.001 pHl units/s. These values are all significantly different (P<0.001, one-way ANOVA) and confirm the results obtained with the half-times.

**Discussion**

This work contains three novel findings. (1) The rise of [Na⁺], during ischemia is prevented by a very low concentration of TTX. (2) The rise of [Na⁺], observed during reperfusion is inhibited after preconditioning. (3) The rate of recovery of pHi after ischemia is slowed by preconditioning.

**What Causes the [Na⁺] Rise During Ischemia?**

Na⁺ enters the heart by a variety of routes (channels and exchangers) and is extruded by the Na⁺ pump. An increase in [Na⁺], must arise from increased influx and/or decreased efflux. There is evidence for all three possibilities during ischemia.

**Inhibition of the Na⁺ Pump**

The appearance of an ischemic contracture is usually taken to mean that the ATP is very low and the Na⁺ pump might be expected to inhibited. Strong support for this hypothesis is provided by Cross et al.,28 who showed that in a low-flow glucose-free model of ischemia the rise in [Na⁺], occurred only after the Na⁺ pump was inhibited. In our experiments, at 2 Hz, an ischemic contracture is usually not apparent until relatively late (19 minutes), and we attribute this to using a low stimulation rate with improved metabolic status. The Na⁺ pump therefore remains effective for longer, and the [Na⁺], is maintained at a low level for longer in ischemia. Thus, in our present experiments (2 Hz), we believe that inhibition of the Na⁺ pump makes relatively little contribution to the rise of [Na⁺], during ischemia. In contrast, at 5 Hz, the ischemic contracture develops much earlier and the rise in [Na⁺], during ischemia is much larger and presumably reflects inhibition of the Na⁺ pump (for discussion see Reference 17).

**Entry of Na⁺ on the Na⁺/H⁺ Exchanger**

A number of studies have shown that inhibitors of the Na⁺/H⁺ exchanger applied before ischemia reduce the rate of rise of [Na⁺], during ischemia.12,13,29 One interpretation of this observation is that the Na⁺/H⁺ exchanger is active during ischemia. However, there is strong evidence to suggest that the Na⁺/H⁺ exchanger is inhibited during ischemia. (1) [Na⁺], does not rise at the rate one would predict for the degree of acidosis.17 (2) The pHi in ischemia is unaffected by inhibitors of the Na⁺/H⁺ exchanger.30,13,17 (3) Our earlier17 and present
work show that MIA had no effect on the [Na\(^+\)] during ischemia. Obviously, if the Na\(^+/H^+\) exchanger is inhibited, it cannot be the cause of the rise of [Na\(^+\)] during ischemia. An alternative interpretation of the effects of amiloride on [Na\(^+\)], during ischemia is that they arise from other effects of these drugs. Amiloride and even the high-affinity derivatives have many side effects including blocking Na\(^+\) channels. Haigney et al\(^{21}\) have suggested that the reduced [Na\(^+\)], during hypoxia produced by amiloride and its derivatives is caused by blocking Na\(^+\) channels rather than the Na\(^+/H^+\) exchanger.

**Na\(^+\) Entry Through Channels**

Haigney et al\(^{21}\) showed that in rat myocytes the Na\(^+\) rise caused by glucose-free hypoxia could be reduced by Na\(^+\) channel blockers including 60 \(\mu\)mol/L TTX. More recently, Van Emous et al\(^{22}\) showed that the increase in [Na\(^+\)], during ischemia of the rat heart was substantially reduced by 200 \(\mu\)mol/L lidocaine. Note that in both of these studies relatively high concentrations of drugs were used that would block conventional \(I_{Na}\) channels. Our experiments extend these results by using a low concentration of TTX (100 nmol/L), which would not be expected to affect \(I_{Na}\) but instead would inhibit the \(I_{Na-p}\), which remains active at the resting potential and is less inactivated by the depolarization that occurs during ischemia. In addition, \(I_{Na-p}\) is activated by hypoxia so that its contribution to the Na\(^+\) influx would be expected to rise during ischemia.\(^{24}\) Thus, in our experiments, the main cause of the rise of [Na\(^+\)], during ischemia appears to be Na\(^+\) entry through the persistent Na\(^+\) channels. This finding is in agreement with the demonstration that the [Na\(^+\)], rise during anoxia is largely through \(I_{Na-p}.\(^{32}\)

**Why Does [Na\(^+\)], Rise on Reperfusion After Ischemia?**

A number of published studies\(^{14,22,29}\) show that the [Na\(^+\)], falls on reperfusion, whereas in our study and other studies,\(^{12,23}\) the [Na\(^+\)], shows a transient rise followed by a fall. What is the cause of this variability between experiments? We suggest that the Na\(^+\) influx on reperfusion is either partly or completely masked by the activity of the Na\(^+\) pump. If the [Na\(^+\)], during ischemia is very high, then the Na\(^+\) pump will be maximally activated when metabolites return to normal on reperfusion.\(^{35}\) In this case, the Na\(^+\) efflux by the pump will be greater than the Na\(^+\) influx on the exchanger, and the [Na\(^+\)], will fall. Conversely, if the [Na\(^+\)], during ischemia is low, then the Na\(^+\) pump will have a low efflux rate, and the Na\(^+\) influx from the exchanger will be greater than the efflux, and [Na\(^+\)], will transiently rise.\(^{28}\)

**Why Does [Na\(^+\)], Not Rise on Reperfusion in the Preconditioned Heart?**

Our second novel finding is that [Na\(^+\)], does not rise on reperfusion in the preconditioned heart. We have shown that the rise of [Na\(^+\)], after ischemia is caused by the Na\(^+/H^+\) exchanger removing protons that have accumulated during ischemia. Why does [Na\(^+\)], fail to rise during reperfusion in the preconditioned heart? (1) If the preconditioned heart were not acidic, then there should be no rise of [Na\(^+\)], on reperfusion. It is generally accepted that the acidosis is smaller in the preconditioned heart, and this is confirmed by our data.\(^{14,20,36}\) Typically, the acidosis in the preconditioned heart is \(\approx 75\%\) of that in the ischemia-only heart, so we might expect the [Na\(^+\)], rise on reperfusion to be reduced to \(\approx 75\%\) of its previous level, whereas, in fact, it changed from +11.9 to -3.6 mmol/L. Thus, the difference in acidosis during ischemia only versus preconditioned ischemia is not nearly enough to explain the differences in [Na\(^+\)]. (2) An alternative explanation is that the Na\(^+/H^+\) exchanger, which is believed to be inhibited during ischemia,\(^{16,17}\) remains inhibited during reperfusion in the preconditioned heart. This possibility is reinforced by the MIA result, in which the mechanical recovery and [Na\(^+\)], changes are indistinguishable from the preconditioned heart. This interpretation is strongly supported by our third novel finding that the rate of recovery of pH, after ischemia is reduced in the preconditioned heart. Vandenberg et al\(^{36}\) showed that in the CO\(_2\)/HCO\(_3\) perfused heart the Na\(^+/H^+\) exchanger carried \(\approx 18\%\) of the proton efflux, the remainder being carried by the Na\(^+/HCO_3^-\) cotransporter, the lactate transporter, and CO\(_2\). The efflux in our experiments was reduced by \(\approx 50\%\) by preconditioning, suggesting that the Na\(^+/H^+\) exchanger appears to be making a greater contribution in our experiments than theirs. Surprisingly, we observed no increase in [Na\(^+\)], after MIA or preconditioning, whereas from their observations we would predict a residual [Na\(^+\)], rise caused by Na\(^+\) entry on the Na\(^+/HCO_3^-\) cotransporter. (3) Another explanation would be that a different proton extrusion mechanism, which does not cause Na\(^+\) loading, has been activated by preconditioning, such as the vacuolar proton ATPase.\(^{37}\)

We conclude that preconditioning appears to cause inhibition of the Na\(^+/H^+\) exchanger during reperfusion for unknown reasons, and this contributes to protection from ischemic damage. Normally, on reperfusion, the rise of [Na\(^+\)], causes Ca\(^{2+}\) entry, and the elevated Ca\(^{2+}\), then initiates damage, probably by activating proteases and phospholipases and, in addition, by loading mitochondria with Ca\(^{2+}\) and impairing their function. If preconditioning prevents the rise in [Na\(^+\)], this provides a simple explanation for the protection against damage that preconditioning produces.

**What Inhibits the Na\(^+/H^+\) Exchanger?**

It appears from earlier work that the Na\(^+/H^+\) exchanger is inhibited during ischemia. However, the mechanism of this inhibition is debated. Lazdunski et al\(^{16}\) initially suggested that the inhibition arose from the extracellular acidosis, which is widely agreed to inhibit the exchanger.\(^{16,38}\) During ischemia, there is both an intracellular acidosis, which activates the exchanger, and an extracellular acidosis, which inhibits the exchanger. Thus, the net effect depends on the balance of these opposing effects. We have shown that under conditions that simulate both the extracellular and the intracellular acidosis of ischemia the exchanger appears to be active.\(^{18,39}\) Nevertheless, during ischemia, most pH measurements suggest that the exchanger is inhibited,\(^{13,17,30}\) and our own measurement of [Na\(^+\)], confirms this view.\(^{72}\) However, the exchanger is also inhibited by situations in which the metabolic status of the cardiac cell is reduced,\(^{17,40,41}\) but the nature
of the inhibition is unclear. Simplistically, one could propose a phosphorylation site required for activation, but Goss et al.\(^1\) could find no evidence for a phosphorylation site that was dephosphorylated by reduced ATP. Alternatively, one could propose that ATP has a direct effect on the activity of the exchanger, but because ATP does not decline rapidly in ischemia, it is difficult to explain the rapid inhibition of the Na\(^+/\)H\(^-\) exchanger observed during ischemia. Furthermore, the metabolic status of cardiac myocytes improves on reperfusion, and this recovery is equally evident in preconditioned hearts as in ischemic-only heart.

Thus, if the mechanism by which the Na\(^+/\)H\(^-\) exchanger is inhibited during ischemia is low ATP, then one would predict an equally rapid recovery on reperfusion in the preconditioned heart as in the ischemic-only heart.

There is a great deal of evidence that activation of protein kinase C during the long ischemia is essential to the protection that occurs on reperfusion.\(^2,3,4\) Thus, one could postulate that protein kinase C phosphorylates the exchanger causing inhibition and that, crucially, this inhibition does not reverse immediately on reperfusion and provides the observed protection against ischemic damage. Current evidence on the role of protein kinase C on the Na\(^+/\)H\(^-\) exchanger is conflicting.\(^2,4\) Alternatively, protein kinase C might phosphorylate a phosphatase that maintains the exchanger dephosphorylated and inactive, and this effect is only reversed slowly on reperfusion. These and other possibilities, such as changes in the expression of the exchanger\(^4,3\) or changes in the distribution of exchanger between surface membrane and internal sites,\(^4\) require further investigation.

**Significance**

The successful protection against reperfusion damage provided by Na\(^+/\)H\(^-\) exchanger inhibitors shows the critical role of the Na\(^+/\)H\(^-\) exchanger in ischemic damage. Our results suggest that the endogenous protection provided by preconditioning also operates by inhibition of the Na\(^+/\)H\(^-\) exchanger in the crucial early minutes of reperfusion. One prediction of our results is that Na\(^+/\)H\(^-\) exchanger inhibitors will have no effect during ischemia but exert protection during a critical short period in the first few minutes of reperfusion.

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12. Tan M, Neely JR, Robl W. Na\(^+/\)H\(^-\) exchange inhibitors shows the critical role of the inhibition is unclear. Simplistically, one could propose a phosphorylation site required for activation, but Goss et al.\(^1\) could find no evidence for a phosphorylation site that was dephosphorylated by reduced ATP. Alternatively, one could propose that ATP has a direct effect on the activity of the exchanger, but because ATP does not decline rapidly in ischemia, it is difficult to explain the rapid inhibition of the Na\(^+/\)H\(^-\) exchanger observed during ischemia. Furthermore, the metabolic status of cardiac myocytes improves on reperfusion, and this recovery is equally evident in preconditioned hearts as in ischemic-only heart.

Thus, if the mechanism by which the Na\(^+/\)H\(^-\) exchanger is inhibited during ischemia is low ATP, then one would predict an equally rapid recovery on reperfusion in the preconditioned heart as in the ischemic-only heart.

There is a great deal of evidence that activation of protein kinase C during the long ischemia is essential to the protection that occurs on reperfusion.\(^2,3,4\) Thus, one could postulate that protein kinase C phosphorylates the exchanger causing inhibition and that, crucially, this inhibition does not reverse immediately on reperfusion and provides the observed protection against ischemic damage. Current evidence on the role of protein kinase C on the Na\(^+/\)H\(^-\) exchanger is conflicting.\(^2,4\) Alternatively, protein kinase C might phosphorylate a phosphatase that maintains the exchanger dephosphorylated and inactive, and this effect is only reversed slowly on reperfusion. These and other possibilities, such as changes in the expression of the exchanger\(^4,3\) or changes in the distribution of exchanger between surface membrane and internal sites,\(^4\) require further investigation.

**Acknowledgment**

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