Acidic Preconditioning Inhibits Na\(^+\)/H\(^+\) and Na\(^+\)/Ca\(^{2+}\) Exchanger Interaction via PKC\(\varepsilon\) in Guinea-Pig Ventricular Myocytes

Libing Li\(^{1,\#2}\), Yasuhide Watanabe\(^{1,\#3}\), Isao Matsuoka\(^{1,\#4}\), and Junko Kimura\(^{1,*}\)

\(^{1}\)Department of Pharmacology, School of Medicine, Fukushima Medical University, Fukushima 960-1295, Japan

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Abstract. An interaction between the Na\(^+\)/Ca\(^{2+}\) exchanger (NCX) and the Na\(^+\)/H\(^+\) exchanger (NHE) induces reperfusion injury. We investigated the effect of brief repetitive acidosis as acidic preconditioning on NCX and NHE interaction during recovery from acidosis. NCX current with the reversal potential was measured in guinea-pig ventricular myocytes using the whole-cell voltage clamp. The cells were exposed to 5 min of acidosis preceded by two episodes of brief acidosis as acidic preconditioning. Acidosis inhibited NCX current and upon recovery shifted its reversal potential in the negative direction. The shift was prevented by cariporide, but was augmented by a high concentration of phorbol 13-myristate acetate (PMA). Acidic preconditioning prevented the shift, but not in the presence of a selective PKC\(\varepsilon\) inhibitor. A low concentration of PMA, which activates PKC\(\varepsilon\) selectively, prevented the shift, but together with PKC\(\varepsilon\) inhibitor (\(\varepsilon\)V1-2) restored the shift during recovery. 5-Hydroxydecanoate inhibited the effects of acidic preconditioning and those of both low and high concentrations of PMA. The negative shift of NCX reversal potential during recovery from acidosis may be due to [Na\(^+\)] accumulation by the NHE. Acidic preconditioning prevented the shift most likely by activating PKC\(\varepsilon\), which in turn inhibited the NHE. The NHE–NCX interaction may be one of the important end-effectors of preconditioning.

Keywords: acidosis, preconditioning, Na\(^+\)/Ca\(^{2+}\) exchanger, Na\(^+\)/H\(^+\) exchanger, guinea-pig ventricular myocyte

Introduction

Ischemic reperfusion injury is one of the major cardiac pathologies. The following cascade of events has been proposed to cause the injury (1): Ischemic hearts develop intracellular acidosis. Intracellular protons are extruded by the Na\(^+\)/H\(^+\) exchanger (NHE) resulting in [Na\(^+\)] increase. Increased [Na\(^+\)] limits Ca\(^{2+}\) extrusion and even promotes Ca\(^{2+}\) entry by the Na\(^+\)/Ca\(^{2+}\) exchanger (NCX). The functional coupling of the two transporters, NHE and NCX, is responsible for Ca\(^{2+}\) overload during ischemia and reperfusion.

Preconditioning consists of brief episodes of myocardial ischemia before prolonged ischemia (2). The protective effect of ischemic preconditioning on myocardial reperfusion injury is well established, but the underlying mechanism is not fully uncovered (3). Brief ischemia during preconditioning results in brief acidosis in the isolated heart (4 – 7). Brief acidosis alone without concomitant ischemia has been demonstrated to have protective effects against ischemic reperfusion injury (6, 8, 9). Therefore, brief repetitive acidosis induces the effect of preconditioning. In single cardiac myocytes, the effects of preconditioning have been evaluated by cell morphology, enzyme activity, and velocity of shortening (10), but not by membrane currents. We hypothesized that if the NHE–NCX interaction is a cause of ischemic reperfusion injury, it could be a target effector of preconditioning.

The NHE–NCX interaction could be demonstrated with the whole cell voltage clamp because we found a
negative shift of the reversal potential of the NCX current ($E_{\text{NCX}}$) upon recovery from acidosis, most likely due to $[\text{Na}^+]$, increase by the NHE, reflecting the coupling of the NCX and the NHE. In the present study, we investigated the effect of brief repetitive acidosis as acidic preconditioning on the interaction of the NHE and the NCX in single guinea-pig ventricular cells. We also found that repetitive acidosis before prolonged acidosis prevented the $E_{\text{NCX}}$ shift. This effect was inhibited by agents known to inhibit the effects of ischemic preconditioning. We explored the underlying mechanism of the effect of acidic preconditioning on $E_{\text{NCX}}$ during recovery from acidosis.

Materials and Methods

Cell isolation
All experiments were performed in accordance with the regulations of the Animal Research Committee of Fukushima Medical University. The method of isolating the cells was described previously (11). Briefly, male guinea pigs (250–400 g) were anesthetized with intraperitoneal injection of sodium pentobarbital. The heart was removed, mounted on a Langendorff apparatus, and perfused for about 20 min with Ca$^{2+}$-free Tyrode solution containing collagenase (5 mg/50 ml; Wako, Tokyo) and protease (0.1 mg/50 ml; Nagase, Tokyo). The ventricles were removed and the cells were dissociated in a low Cl$^-$-high K$^+$ solution. The cells were kept in this solution at 4°C until use.

Solutions

The Na$^+$-external solution contained 140 mM NaCl, 1 mM CaCl$_2$, 1 mM MgCl$_2$, 0.02 mM ouabain, 0.01 mM ryanodine, 0.01 mM D600, and 2 mM HEPES (adjusted to pH 7.4 or HCl, respectively). For acidosis, pH 6.0 or pH 4.9 were used. pH 4.9 was employed because in a preliminary version of this work, bubbling the external solution with CO$_2$ resulted in a pH of 4.9. Instead of using CO$_2$, the present experiments were performed by adjusting the pH of the external solution to 4.9 with HCl. We confirmed that pH 6.0 produced similar results in $E_{\text{NCX}}$ to those obtained at pH 4.9 with a longer period (up to 8 min) of acidosis.

The pipette solution contained: 20 mM NaCl, 120 mM CsOH, 50 mM aspartic acid, 5 mM MgATP, 20 mM BAPTA (10 mM CaCl$_2$, 226 nM free Ca$^{2+}$), and 2 mM HEPES (adjusted to pH 7.2 with CsOH). For inducing the transient inward current, 0.1 mM EGTA was used instead of 20 mM BAPTA in the pipette solution, ryanodine and ouabain were omitted from the external solution, and pH 6.0 was used instead of pH 4.9 for the fear that the cells would quickly induce rigor at pH 4.9 during reperfusion.

Current recording

The whole-cell voltage clamp was performed using pClamp8 software (Axon Instruments, Foster City, CA, USA). The protocol for isolating $I_{\text{NCX}}$ was similar to that described previously (11). Ramp pulses were given at 10-s intervals at a speed of 698 mV/s. The shape of the voltage pulse is shown in Fig. 7. The I-V curve of $I_{\text{NCX}}$ was obtained from the descending limb of the ramp without capacitance compensation.

The value of $E_{\text{NCX}}$ was determined by the voltage point where the difference I-V curve between the control and the test I-V curves of the NCX current crosses with the voltage axis (see Fig. 1: C and D).

Drugs

Cariporide (4-isopropyl-3-methylsulfonylbenzoylguanidine methane-sulfonate) was a gift from Hoechst Marion Roussel (Tokyo). Phorbol 12-myristate 13-acetate (PMA), 5-hydroxydecanoate (5HD), εV1-2, ouabain, and D600 were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Data analysis

The data, expressed as means ± S.E.M. (number of cells), were analyzed by Student’s $t$-tests. A difference with $P<0.05$ was considered significant.

Results

Effect of acidosis and acidic preconditioning on membrane current at a high concentration of Ca$^{2+}$ chelator in the pipette solution

Figure 1A shows typical current traces in response to lowering pH$_i$ 7.4 to 4.9 for 5 min and then returning to pH$_i$ 7.4. At each pH, 5 mM Ni$^{2+}$ was added to confirm $I_{\text{NCX}}$ as a Ni$^{2+}$-sensitive current. Figure 1B shows I-V curves corresponding to the labels in Fig. 1A. $I_{\text{NCX}}$ was completely inhibited at pH 4.9. Figure 1C and 1D show net I-V curves of the Ni$^{2+}$- and acid-sensitive currents obtained by subtraction, respectively. The reversal potentials of the acid-sensitive currents, which are the voltages at which each current (Fig. 1D: c – d or h – g) crosses with the voltage axis, were similar to those of the Ni$^{2+}$-sensitive current before acidosis (Fig. 1C: a – b) and during recovery (Fig. 1C: i – j), respectively, indicating that the acid-sensitive currents were $I_{\text{NCX}}$. $E_{\text{NCX}}$ shifted in the negative potential during recovery compared to the control before acidosis. The maximum shift of $E_{\text{NCX}}$ was $-13 ± 1.7$ mV ($n = 7$) for acid-sensitive $I_{\text{NCX}}$ and $-11 ± 0.7$ mV ($n = 7$) for Ni$^{2+}$-sensitive $E_{\text{NCX}}$. $E_{\text{NCX}}$ during recovery shifted with time and gradually
returned to the control after about 2 min in this cell (Fig. 1E). The average values of $E_{\text{NCX}}$ are summarized in Table 1.

**Effect of cariporide on $E_{\text{NCX}}$ shift**

We repeated the protocol in Fig. 1 in the presence of cariporide, a selective inhibitor of NHE1 (12). Cariporide at 1 $\mu$M, which is supposed to inhibit NHE1 completely, did not alter the control current (Fig. 2A). The net I-V curves of $I_{\text{NCX}}$ were obtained by subtraction before acidosis (a – b) and during recovery (d – c) (Fig. 2D). There was no notable shift in $E_{\text{NCX}}$ during recovery in the presence of cariporide (Fig. 2D).

NHE is activated by intracellular acid (13), while it is inhibited by extracellular acid (14). Therefore, in our experiments, Na⁺ entry via NHE might have occurred during the recovery period. To test this possibility, we applied cariporide only during recovery (Fig. 2: C and D). No apparent shift of $E_{\text{NCX}}$ was observed during the recovery, indicating that NHE was inhibited during exposure to pH 4.9 and operated only during the recovery.

**Effect of a high concentration of PMA on $E_{\text{NCX}}$**

If NHE is involved in the shift of $E_{\text{NCX}}$, acceleration of NHE by protein kinase C (PKC) (15) should enhance the

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**Table 1. Effects of acidosis on $E_{\text{NCX}}$ under various conditions**

<table>
<thead>
<tr>
<th>Conditions</th>
<th>pH 7.4/4.9 (mV)</th>
<th>pH 4.9/7.4 (mV)</th>
<th>$\Delta E$ (mV)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>−60 ± 2.1</td>
<td>−74 ± 2.3</td>
<td>−13 ± 1.7</td>
<td>7</td>
</tr>
<tr>
<td>Cariporide</td>
<td>−61 ± 2.2</td>
<td>−60 ± 1.5</td>
<td>0.4 ± 1.5*</td>
<td>7</td>
</tr>
<tr>
<td>800 nM PMA</td>
<td>−62 ± 1.4</td>
<td>−84 ± 1.5</td>
<td>−22 ± 1.5*</td>
<td>6</td>
</tr>
<tr>
<td>800 nM PMA + Carip</td>
<td>−60 ± 0.9</td>
<td>−66 ± 3.3</td>
<td>−6 ± 2.5*</td>
<td>3</td>
</tr>
<tr>
<td>APC</td>
<td>−60 ± 2.2</td>
<td>−64 ± 2.7</td>
<td>−4 ± 0.8*</td>
<td>5</td>
</tr>
<tr>
<td>APC + εV1-2</td>
<td>−64 ± 2.2</td>
<td>−75 ± 2.5</td>
<td>−11 ± 1.0</td>
<td>8</td>
</tr>
<tr>
<td>10 nM PMA</td>
<td>−63 ± 1.4</td>
<td>−65 ± 1.1</td>
<td>−2 ± 1.6*</td>
<td>7</td>
</tr>
<tr>
<td>10 nM PMA + εV1-2</td>
<td>−63 ± 1.6</td>
<td>−72 ± 1.0</td>
<td>−9 ± 1.3</td>
<td>8</td>
</tr>
<tr>
<td>5-HD</td>
<td>−63 ± 1.5</td>
<td>−76 ± 1.8</td>
<td>−12 ± 1.2</td>
<td>3</td>
</tr>
<tr>
<td>APC + 5-HD</td>
<td>−62 ± 1.0</td>
<td>−73 ± 1.4</td>
<td>−12 ± 0.8</td>
<td>8</td>
</tr>
<tr>
<td>800 nM PMA + 5HD</td>
<td>−61 ± 0.9</td>
<td>−69 ± 1.7</td>
<td>−9 ± 1.1</td>
<td>6</td>
</tr>
<tr>
<td>10 nM PMA + 5HD</td>
<td>−62 ± 0.9</td>
<td>−72 ± 1.5</td>
<td>−10 ± 1.9</td>
<td>5</td>
</tr>
</tbody>
</table>

$E_{\text{NCX}}$ values are expressed as means ± S.E.M. (mV). $\Delta E$ indicates difference between $E_{\text{NCX}}$ values of acid-sensitive $I_{\text{NCX}}$ at the onset (pH 7.4/4.9) of and during recovery (pH 4.9/7.4) from acidification. n indicates number of cells. *$P<0.05$.

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**Fig. 1.** Effect of acidosis on $I_{\text{NCX}}$. A: Chart recording of the current. The line above the current indicates the pH change of the external solution. Ni²⁺ (5 mM) was added briefly to the external solution at each pH. B: I-V curves of the corresponding labels in panel A. C: Difference I-V curves of the Ni²⁺-sensitive current in the control (a-b) and during recovery (i-j) from panel B. $E_{\text{NCX}}$ of the control is indicated by a filled arrow and that during recovery, by an open arrow. D: Difference I-V curves of the acid-sensitive currents in the control (c-d) and during recovery (h-g) from panel B. Filled and open arrows indicate $E_{\text{NCX}}$ of the control and that during recovery, respectively. E: Changes of $E_{\text{NCX}}$ of each pulse shown in panel A during recovery from acidosis. $E_{\text{NCX}}$ of acid-sensitive $I_{\text{NCX}}$ were measured. “C” indicates $E_{\text{NCX}}$ of the control before acidosis.
shift of $E_{\text{NCX}}$. We tested this using a high concentration of PMA (0.8 μM), which activates conventional and novel types of PKC isoforms (cPKC and nPKC, respectively) (Fig. 3A). Addition of PMA did not change the control current. The $E_{\text{NCX}}$ shift during recovery was enhanced to $-22 \pm 1.5$ mV ($n = 6$) by PMA (Fig. 3B, Table 1). This effect of PMA was also inhibited by the presence of 1 μM cariporide (Table 1), confirming that NHE was involved in the $E_{\text{NCX}}$ shift.

**Effect of acidic preconditioning on $E_{\text{NCX}}$**

Xiao and Allen (7) suggested that NHE is inhibited during reperfusion after ischemia in preconditioned hearts. We tested whether acidic preconditioning would affect the $E_{\text{NCX}}$ shift during recovery from acidosis. The myocytes were subjected to two 1-min episodes of acidosis before 5 min of acidosis (Fig. 4: A and B). $I_{\text{NCX}}$ was reversibly blocked by each acidic preconditioning, but $E_{\text{NCX}}$ before and after each acidic preconditioning were not changed. After 5 min of acidosis, the $E_{\text{NCX}}$ shift was $-4 \pm 0.8$ mV ($n = 5$) with acidic preconditioning, which was significantly smaller than that of the control without acidic preconditioning (Table 1). This result is similar to that with cariporide, suggesting that acidic preconditioning inhibits NHE.

It has been suggested that preconditioning activates PKCε, which plays a protective role (11). To test for PKCε activation, acidic preconditioning was performed in the presence of a specific PKCε inhibitor, εV1-2, in the pipette solution (16). In these experiments, $E_{\text{NCX}}$ shifted again during recovery and the acidic preconditioning effect was abolished (Fig. 4: C and D), indicating that PKCε was activated by acidic preconditioning.

**Effect of a low concentration of PMA on $E_{\text{NCX}}$**

We next tested whether activation of PKCε inhibits NHE with a low concentration of PMA (10 nM), which selectively activates PKCε (15). In the presence of 10 nM PMA, pH 4.9 for 5 min did not shift $E_{\text{NCX}}$ during the recovery (Fig. 5: A and B, Table 1). However, when the pipette solution contained εV1-2, the shift of $E_{\text{NCX}}$ was restored in the presence of 10 nM PMA (Fig. 5: C and D, Table 1). These results led us to hypothesize that acidic preconditioning activates PKCε, which in turn inhibits NHE and prevents Na⁺ accumulation and $E_{\text{NCX}}$ shift during recovery from acidosis (see also Fig. 8).
Effect of 5HD on E$_{\text{NCX}}$ shift

5HD is an inhibitor of mitochondrial K$_{\text{ATP}}$ channels (17) and is known to inhibit the effect of ischemic preconditioning (18). We tested the effect of 5HD on APC by performing acidic preconditioning in the presence of 50 μM 5HD (Fig. 6A). During the recovery from acidosis, E$_{\text{NCX}}$ shifted significantly (Fig. 6B, Table 1), indicating that 5HD inhibited the effect of acidic preconditioning. 5HD also inhibited the effect of both a low (Table 1) and a high (Fig. 6: C and D) concentration of PMA, but did not affect the E$_{\text{NCX}}$ shift during recovery from acidosis without acidic preconditioning (Fig. 6E). We also observed that 50 μM 5HD inhibited the effect of a low concentration of PMA (10 nM). Since K$^+$ was not in the external and pipette solutions, it is unlikely that 5HD exerted its effect by inhibiting K$_{\text{ATP}}$ channels. All the numerical results are summarized in Table 1.

Effect of acidic preconditioning on transient inward current

We tested the functional aspect of the acidic preconditioning on NCX current during reperfusion at a low concentration of Ca$^{2+}$ chelator (0.1 mM EGTA) in the pipette solution. Figure 7A shows typical current traces in response to changing pH, 7.4 to 6.0 for 5 min and then returning to pH, 7.4 at 0.1 mM EGTA in the pipette solution. During the recovery from pH 6.0, oscillatory transient inward current developed. The top panel of Fig. 7A shows a continuous recording of the current. The current traces obtained at each label are shown below. Each of the patterns (a – d) shows a current trace in response to a ramp voltage pulse and subsequent holding potential, which is illustrated in the inset at the bottom of A. The ramp pulse was given every 10 s. As illustrated in Figure 7B, when brief (1 min) external acidification to pH 6.0 was applied twice before prolonged (5 min) acidosis, the development of transient inward current was significantly suppressed during recovery from acidosis (Fig. 7B: g and h). The transient inward current was suppressed by a specific NCX inhibitor, SEA0400 (Figure not shown), confirming that it was comprised of NCX current. We propose that the above mentioned mechanism (see Fig. 8) is involved in the suppression of arrhythmogenic oscillatory NCX current by the acidic preconditioning.

Discussion

In the present study, acidosis at pH 4.9 for 5 min shifted E$_{\text{NCX}}$ maximally by $-13$ mV during the recovery. The negative shift of E$_{\text{NCX}}$ accelerates Ca$^{2+}$ entry and
decelerates Ca\(^{2+}\) efflux and could result in Ca\(^{2+}\) overload. The shift of E\(_{\text{NCX}}\) was most likely caused by [Na\(^{+}\)]\(_i\) accumulation due to the NHE because it was abolished by cariporide and enhanced by PMA, which is an activator of PKC and known to accelerate NHE (13).

At 3Na\(^{+}\):1Ca\(^{2+}\) stoichiometry (19), the amount of [Na\(^{+}\)]\(_i\) accumulation can be calculated by using the equation \(E_{\text{NCX}} = 3E_{\text{Na}} - 2E_{\text{Ca}}\), where \(E_{\text{Na}}\) and \(E_{\text{Ca}}\) are the equilibrium potentials of Na\(^{+}\) and Ca\(^{2+}\), respectively. If [Na\(^{+}\)]\(_i\) is increased, while [Na\(^{+}\)]\(_o\), [Ca\(^{2+}\)]\(_o\) and [Ca\(^{2+}\)]\(_i\) are constant, the maximum shift of \(E_{\text{NCX}}\) of -13 mV gives a [Na\(^{+}\)]\(_i\) increase up to 3.55 mM during the recovery from pH 6.9. In the presence of PMA, the maximum shift of -22 mV yields an increase of 6.32 mM [Na\(^{+}\)]\(_i\). In the whole heart, [Na\(^{+}\)]\(_i\) increase was reported to be 6.2 ± 3.5 mM during 30 min of ischemia at pH 6.08 (7) and 12.4 mM during 20 min of metabolic inhibition at pH 6.90 (20). Therefore, the total [Na\(^{+}\)] accumulated was similar to those at severe acidosis for a short period of time and mild acidosis for a longer period of time. If [Ca\(^{2+}\)]\(_i\) had also accumulated, the calculated value of increased [Na\(^{+}\)]\(_i\) would be underestimates because a [Ca\(^{2+}\)]\(_i\) increase would shift \(E_{\text{NCX}}\) in a positive direction, while a [Na\(^{+}\)]\(_i\) increase would shift \(E_{\text{NCX}}\) in a negative direction. In addition, the pipette solution continuously perfused the cell interior under the whole-cell clamp and this could also cause an underestimation of [Na\(^{+}\)]\(_i\).

In the present study, acidic preconditioning abolished the \(E_{\text{NCX}}\) shift during recovery from acidosis. This effect of acidic preconditioning was mimicked by a low concentration (10 nM) of PMA, which mainly activates PKC\(_\varepsilon\) (15, 16). In addition, the effects of acidic preconditioning and 10 nM PMA were both inhibited by \(\varepsilon V 1-2\), a specific PKC\(_\varepsilon\) inhibitor. Our result is consistent with the previous reports that preconditioning activates PKC\(_\varepsilon\) in the whole heart (16, 21). Thus acidic preconditioning and ischemic preconditioning seem to share a common underlying mechanism of PKC\(_\varepsilon\) activation.

Xiao and Allen (7) observed that pH\(_i\) recovery during reperfusion after ischemia was slowed by preconditioning and proposed that preconditioning inhibits the NHE. In this study, the result that acidic preconditioning abolished the \(E_{\text{NCX}}\) shift during recovery from acidosis was similar to the result with cariporide, supporting the view that acidic preconditioning inhibits the NHE (Fig. 6). However, there is a controversial report that constitutively active PKC\(_\varepsilon\) did not improve post-ischemic pH\(_i\) recovery, although cardiac function was
improved (22). We do not know whether or not constitutively active PKCε could maintain a persistent inhibitory effect on the NHE or whether other proton transporters compensated for the function of NHE. Another controversial result (4) was that upon NH4 removal in preconditioned heart, pH1 was more acidic, and pH recovery was faster than that of the control. In this case, pH1 was sensitive to inhibitors of the NHE as well as the Na⁺-K⁺-2Cl⁻ cotransporter, indicating that transporters other than Na⁺-K⁺-2Cl⁻ might have been involved, thus causing the discrepancy.

PMA appears to have two opposite effects on the NHE in a concentration-dependent manner. In this study, a low concentration (10 nM) of PMA activated PKCε and appeared to inhibit the NHE. In contrast, a high concentration (800 nM) of PMA activated the NHE and enhanced E_{NCX} during recovery from acidosis. PMA was originally reported to activate the NHE (10) at a concentration that activates classical PKCs. Recently, it was shown that PKCat but not PKCε, PKCζ, or PKCδ, activates the NHE (23). Inagaki et al. (24) showed that a PKCβ-inhibitor prevents repercussion injury, while a PKCε-activator mimics ischemic preconditioning and these effects were additive. Various PKC isoforms but not PKCε might activate the NHE. As illustrated in Fig. 8, since the NHE molecule has several possible phosphorylation sites in its C-terminus (13), we propose that PKCε inhibits the NHE but other PKC isoforms activate NHE possibly by phosphorylating distinct sites.

PMA has been reported to stimulate the NCX1 indirectly (25), but we did not see any change in control I_{NCX} caused by PMA. Even if PMA had activated I_{NCX}, E_{NCX} would not change unless [Na⁺] and/or [Ca²⁺] were changed.

5HD is known to inhibit the beneficial effects of ischemic preconditioning (18), possibly by inhibiting mitochondrial K_{ATP} channels (17). In the present study, 5HD abolishes the effect of acidic preconditioning on the E_{NCX} shift during recovery, even though K⁺ was replaced by Cs⁺ in the external and pipette solutions. Thus the effect of 5HD could not have been due to inhibition of K_{ATP} channels. Recently, various targets of 5HD other than mitochondrial K_{ATP} channels have been reported (26–28). Five-HD inhibited the effect of both high and low concentrations of PMA and yet 5HD per se did not affect the E_{NCX} shift during recovery from the control acidosis (Fig. 6, Table 1). Since 5HD resembles an acyl residue of PMA or diacylglyceride (DG), we speculate that 5HD might have interfered with PMA or DG and prevented activation of PKCs.

Hinata et al. (29) have recently shown in guinea-pig ventricular cells that hydrogen peroxide increased NCX current without a change in E_{NCX} and this effect was inhibited by cariporide. We tentatively hypothesized that hydrogen peroxide activated NHE and alkalized the cell interior near NCX and thus augmented the NCX activity without significantly changing [Na⁺] and [Ca²⁺] (29), but we do not have any evidence for this hypothesis yet. We mention this because it is interesting that both the effects of acidification and hydrogen peroxide on NCX were sensitive to cariporide, but yet the former resulted in inhibition of NCX with a change in E_{NCX} during recovery and the latter caused augmentation of NCX without an E_{NCX} change. There still is a mystery in the way of interaction between NCX and NHE.

Fujioka et al. (30) and Dostanic et al. (31) suggested the functional interaction of NCX and Na⁺/K⁺-ATPase via [Na⁺], in a restricted space of ventricular myocytes. In the present study, we examined the E_{NCX} during recovery from acidosis in the presence of ouabain, an inhibitor of Na⁺/K⁺-ATPase. What would happen if ouabain was omitted from the external solution? We performed the experiment of acidification in the absence of ouabain and observed no shift in the E_{NCX} during reperfusion. This indicates that with the pipette solution containing 5 mM ATP, the Na⁺-K⁺ pump was active and thereby prevented intracellular accumulation of Na⁺.

Under the ischemic condition where ATP decreases, the Na⁺-K⁺ ATPase activity decreases and the accumulation of Na⁺ could occur. We presume that NCX and NHE may also be spatially coupled, although we could not find any direct evidence in the literature. If so, the three transporters: NCX, NHE, and Na⁺/K⁺-ATPase are closely localized in the cardiac membrane, and the changes in [Na⁺] will occur in a restricted interactive space.

As shown in Fig. 7, with a low concentration of a Ca²⁺ chelator in the pipette solution, arrhythmogenic transient inward current develops during recovery from acidosis. This is compatible with the negative E_{NCX} shift, which accelerates Ca²⁺ entry and decelerates Ca²⁺ efflux. The repetitive brief acidification prevents the development of this current and cell contracture, verifying that this procedure is indeed ‘preconditioning’. A similar phenomenon likely occurs with ischemic preconditioning and we propose that the NCX-NHE interaction is one of the target effectors of preconditioning.

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