Ca$^{2+}$ Increase and pH Decrease Induced by Hypochlorous Acid in Single Quiescent Myocytes Isolated from Rat Ventricles

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Abstract Cytosolic free calcium concentration ([Ca$^{2+}$]$_i$) and intracellular pH (pH$_i$) were simultaneously measured with a fluorescence technique using fura-2 and BCECF. Hypochlorous acid (HOCI) increased [Ca$^{2+}$]$_i$ and decreased pH$_i$ of single quiescent myocytes isolated from rat ventricles. The HOCI-induced changes in [Ca$^{2+}$]$_i$ and pH$_i$ ($\Delta$[Ca$^{2+}$]$_i$ and $\Delta$pH$_i$) were $129 \pm 18$ nm and $0.18 \pm 0.02$ (mean $\pm$ SD), respectively, with $200 \mu$M HOCI. A positive linear correlation was obtained between $\Delta$[Ca$^{2+}$]$_i$ and $\Delta$pH$_i$ under various extracellular Ca$^{2+}$, pH, and Na$^+$ conditions. Chelation of extracellular Ca$^{2+}$ depressed only one-fourth of $\Delta$[Ca$^{2+}$]$_i$ and Ca$^{2+}$ antagonists (verapamil and nifedipine) did not reduce $\Delta$[Ca$^{2+}$]$_i$, indicating that $\Delta$[Ca$^{2+}$]$_i$ originates mainly from intracellular Ca$^{2+}$ stores. A disulfide-reducing reagent, 1,4-dithiothreitol (DTT), recovered the increased [Ca$^{2+}$]$_i$ and decreased pH$_i$ to the control levels. The simultaneous changes in [Ca$^{2+}$]$_i$ and pH$_i$ induced by HOCI and the simultaneous restoration of the normal [Ca$^{2+}$]$_i$ and pH$_i$ levels by DTT suggest a closely coupled control of [Ca$^{2+}$]$_i$ and pH$_i$ in cardiac myocytes.

Key words: HOCI, rat ventricular myocytes, intracellular Ca$^{2+}$ and pH, fura-2 and BCECF dual loading, 1,4-dithiothreitol.

Recently, several studies have shown that post-ischemic reperfusion of the heart accelerates the infiltration of polymorphonuclear leukocytes [1]. Polymorphonuclear leukocytes induce capillary plugging, causing irreversible microvascular and myocardial cell injuries [2]. In the reperfused heart, activated neutrophils produce H$_2$O$_2$ which is halogenated to hypochlorous acid (HOCI) by myeloperoxidase.
HOCI is a powerful oxidant causing lethal damage to cardiomyocytes with its high reactivity with proteins [3] and amines [4]. The ATPase activity of the sarcoplasmic reticulum (SR) relating Ca²⁺ uptake was shown to be highly sensitive to HOCI [5–7]. Post-ischemic reperfusion of the heart fails to regulate Ca²⁺ homeostasis and results in cell dysfunctions for protecting myocytes from injuries.

In cardiomyocytes, cytosolic free Ca²⁺ concentration ([Ca²⁺]), intracellular pH (pHi) and contractile force are closely correlated to each other. For example, as the pHi of cardiomyocytes was decreased, both the Ca²⁺-activated force development [8] and the Ca²⁺ binding to troponin [9] were inhibited, and the increase in [Ca²⁺], caused the decrease in pHi [10]. Borle and Bender [11] showed that for canine kidney cells, H⁺ extrusion from the cells was stimulated with increasing Na⁺ influx at a high extracellular pH, and at the same time, [Ca²⁺]; was increased because of the interaction between the Na⁺/Ca²⁺ and Na⁺/H⁺ antiporters. Na⁺ retention induced by HOCI [5, 12] may be related to such an interaction between the two antiporters. Experiments with isolated myocytes are useful to evaluate the direct effects of HOCI on the cellular function of regulating [Ca²⁺], and pHi. Eley et al. [13] demonstrated HOCI-induced increases in [Ca²⁺], using isolated rabbit ventricular myocytes. Eley et al. [14] also showed HOCI-induced inactivation of the SR Ca²⁺-ATPase in perfused rat heart and its reversal with 1,4-dithiothreitol (DTT), one of the most potent disulfide-reducing reagents [15]. Protective effects of sulfhydryl compounds on HOCI-induced increases in [Ca²⁺], were also shown by Fukui et al. [16]. The purpose of the present study was to investigate the effects of HOCl on both [Ca²⁺], and pHi measured simultaneously, using single isolated myocytes. Preliminary results on the HOCI-induced increase in [Ca²⁺], were presented in abstract form [17].

Our experiments using a simultaneous measurement of [Ca²⁺], and pHi in single viable myocytes were designed to examine the following problems:

1) How are the HOCI-induced changes in [Ca²⁺], and pHi modified by various extracellular conditions (Ca²⁺, pH, and Na⁺)?

2) What is the correlation exists between the HOCI-induced changes in [Ca²⁺], and pHi?

3) What is the effect of DTT on the HOCI-induced changes in [Ca²⁺], and pHi?

MATERIALS AND METHODS

Cell isolation. Single cardiomyocytes were isolated from rat ventricles by a collagenase digestion. Male Sprague-Dawley rats, weighing from 250 to 350 g, were anesthetized with pentobarbital sodium (5 mg/100 g, i.p.) 5 min before the heart perfusion. Heparin (100 U) was injected into the vena cava inferior. A retrograde perfusion of the heart via the aorta was carried out for 5 min at 37°C with an oxygenated Krebs-Henseleit buffer solution. The perfusate was changed to an oxygenated Joklik minimal modified tissue culture medium (JMM) [18] consisting

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of (mM): NaCl 150, KCl 5.4, CaCl₂ 0.05 or 0.1, MgCl₂ 1.0, NaH₂PO₄ 5.4, glucose 10.0, and HEPES 5.0 at pH 7.30 (37°C). Osmolarity of the JMM was 280 mOsm/kg H₂O. JMM containing 100 U·ml⁻¹ of collagenase (Worthington, CLS II) was circulated for 30 min. The ventricles were cut from the half-digested heart, minced, transferred to fresh medium containing collagenase (250 U·ml⁻¹), and shaken for 25 min at 35°C. Cell isolation was accelerated by gentle pipetting. The suspension was filtered through a nylon mesh (pore size 250 μm) to sediment viable myocytes in JMM containing 1.5% bovine serum albumin (BSA). Then, the myocytes were resuspended in JMM containing 0.1% BSA. The fraction of the rod-shaped myocytes and that of trypan blue-excluding myocytes were comparable with those reported by Uchida et al. [19] (67.3 and 83.1%, respectively).

**Loading of fluorescent dyes.** The number of myocytes was adjusted to about 10⁵ cells·ml⁻¹ before simultaneous loading of acetoxymethyl esters (AM) of fura-2 and BCECF (Dojindo Laboratories). The concentrations of fura-2-AM and BCECF-AM were 5 and 1 μM, respectively. After the loading of dyes for 1 h at 30°C, myocytes were washed twice with dye-free JMM.

**Simultaneous measurements of [Ca²⁺], and pH.** The dye-loaded myocytes were placed in a cuvette regulated at 37±0.2°C and superfused with JMM. The fluorescent dyes were excited with a xenon lamp at wavelengths of 340, 380, 440, and 500 nm, selected with bandpass filters. [Ca²⁺] and pH measurements were repeated every 4 s during the superfusion. Fluorescence emissions from single myocytes passing through dichroic mirrors and interference filters were detected with a random access camera (C3329, Hamamatsu Photonics). We obtained the ratio of the fluorescence intensity for the excitation at the wavelengths of 340 and 380 nm for [Ca²⁺], and that for the excitation at 500 and 440 nm for pH. Details of the method used in the present study are described elsewhere [20]. The ratios were converted to the absolute values of [Ca²⁺] and pH using in vivo calibration curves [20].

HOCl did not alter emissions of fura-2 and BCECF in solutions without myocytes, indicating that these fluorescent dye are not influenced by HOCl. It was confirmed that fura-2 emission is independent of pH and BCECF emission is independent of the Ca²⁺ concentration as shown by Shibuya et al. [20].

**Protocols.** The experimental protocol is shown in Fig. 1. Dye-loaded myocytes were placed in the cuvette and superfused with various solutions for the [Ca²⁺] and pH measurements. Data acquisition was executed if myocytes remained rod-shaped after the superfusion for 20 min. The superfusing solution was JMM with or without HOCl at a concentration of 200 μM. In some experiments, Ca²⁺ and Na⁺ concentrations, or pH of JMM ([Ca²⁺]₀, [Na⁺]₀, or pH₀) were controlled before the superfusion and kept constant during the [Ca²⁺] and pH measurements. To block the Ca²⁺ influx via voltage-gated Ca²⁺ channels, verapamil (10 μM) or nifedipine (10 μM) was added to JMM. HOCl-induced changes in [Ca²⁺] and pH (Δ[Ca²⁺] and ΔpH) were defined as the differences in [Ca²⁺] and pH measured at time 0 and 20 min after application of HOCl (cf., Fig. 2). [Ca²⁺] and pH, did

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not show any significant change during the 20 min when JMM did not contain HOCl. For the experiments of HOCl washout, the superfusing solution was altered from HOCl-containing JMM to standard JMM or JMM with DTT (1 mM). The washout was started following the HOCl treatment for 15 min.

We analyzed only the data derived from the myocytes in which the control values of $[\text{Ca}^{2+}]_i$ and $pH_i$ were between 40 and 100 nm and 6.98 and 7.26, respectively. The shape of the myocytes was always inspected before and after the fluorescence measurements, and the data obtained from the rod-shaped myocytes were used exclusively for the analysis. $[\text{Ca}^{2+}]_i$ and $pH_i$ were alternately measured every 4 s. The flow rate of the superfusion was constant at 2 ml·min$^{-1}$. EGTA (0.5 mM) was used to chelate extracellular Ca$^{2+}$ for [Ca$^{2+}$]$_i$-free experiments. pH$_o$ was regulated by titrating the JMM (HEPES) solution with 5 N HCl or 5 N NaOH. For low and high [Na$^+$]$_o$ experiments, JMM was blended with an appropriate volume of 150 mM N-methyl-D(-)-glucamine (NMDG) or 200 mM NaCl solution. Substitution of NaCl with NMDG caused low Cl-concentration and alkalization of the solution. Increased pH of the NMDG solution was adjusted to 7.30 by titrating the solution with 5 N HCl. The Cl-concentration of the titrated solution was 134 mM. Osmolality of the low and high [Na$^+$]$_o$ solutions was 280 mOsm/kg H$_2$O.

Statistics. All results are expressed as mean±SD. The data were compared using unpaired Student’s t-test.

RESULTS

**HOCl-induced changes in $[\text{Ca}^{2+}]_i$ and $pH_i$ of single cardiomyocytes**

As shown in Fig. 2, $[\text{Ca}^{2+}]_i$ was gradually increased and $pH_i$ decreased, reaching plateau levels after the application of HOCl. The time courses of both changes were similar to each other. In the absence of HOCl, $[\text{Ca}^{2+}]_i$ and $pH_i$ were
Fig. 2. HOCl-induced increase in [Ca$^{2+}$]$_i$ and decrease in pH$_i$ of a single cardiomyocyte. Myocytes were superfused with HOCl (200 $\mu$M)-containing JMM for 20 min (indicated by a bar) at 37°C. $\Delta$[Ca$^{2+}$]$_i$ is defined as the difference in [Ca$^{2+}$]$_i$ between the plateau and the pretreatment levels, and $\Delta$pH$_i$ as the difference in pH$_i$ values between the two levels.

Table 1. Dependence of $\Delta$[Ca$^{2+}$]$_i$ and $\Delta$pH$_i$ (n = 14 for each) on HOCl concentrations.

<table>
<thead>
<tr>
<th>HOCl (µM)</th>
<th>$\Delta$[Ca$^{2+}$]$_i$ (nm)</th>
<th>$\Delta$pH$_i$</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>66±14</td>
<td>0.14±0.03</td>
</tr>
<tr>
<td>100</td>
<td>101±15</td>
<td>0.16±0.03</td>
</tr>
<tr>
<td>200</td>
<td>129±18</td>
<td>0.18±0.02</td>
</tr>
<tr>
<td>500</td>
<td>188±16</td>
<td>0.26±0.05</td>
</tr>
<tr>
<td>1,000</td>
<td>212±15</td>
<td>0.32±0.04</td>
</tr>
</tbody>
</table>

stable for the corresponding time periods. Thus, we defined the HOCl-induced changes in [Ca$^{2+}$]$_i$ and pH$_i$ ($\Delta$[Ca$^{2+}$]$_i$ and $\Delta$pH$_i$) as the differences between the plateau and the pretreatment levels, which usually required a period of ~20 min. The mean and SD of $\Delta$[Ca$^{2+}$]$_i$ and $\Delta$pH$_i$ induced by HOCl were 129±18 nm and 0.18±0.02 (n=14), respectively, for 100 µM [Ca$^{2+}$]$_o$, pH$_o$ 7.30, 150 mM [Na$^+$]$_o$, and 200 µM HOCl. Both $\Delta$[Ca$^{2+}$]$_i$ and $\Delta$pH$_i$ were increased with increasing HOCl-concentrations, and the concentration dependence of $\Delta$[Ca$^{2+}$]$_i$ and $\Delta$pH$_i$ is shown in Table 1. In the present study, we used the HOCl concentration at 200 µM, which is comparable to that produced by activated neutrophils in vivo [21].

Effects of [Ca$^{2+}$]$_o$ on $\Delta$[Ca$^{2+}$]$_i$ and $\Delta$pH$_i$

In the absence of HOCl, [Ca$^{2+}$]$_i$ and pH$_i$ remained unchanged during the superfusion regardless of the [Ca$^{2+}$]$_o$ values tested (0–2 mM). [Ca$^{2+}$]$_o$ dependence of $\Delta$[Ca$^{2+}$]$_i$ and $\Delta$pH$_i$ are shown in Table 2. $\Delta$[Ca$^{2+}$]$_i$ and $\Delta$pH$_i$ were significantly augmented by increasing [Ca$^{2+}$]$_o$ from 0 to 100 µM (p<0.001 for $\Delta$[Ca$^{2+}$]$_i$ and $\Delta$pH$_i$), but they were not affected by [Ca$^{2+}$]$_o$ between 100 µM and 2 mM. Therefore, we chose 100 µM as the standard [Ca$^{2+}$]$_o$ of the perfusing solution throughout the present work.

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Table 2. \([\text{Ca}^{2+}]_o\) dependence of \(\Delta[\text{Ca}^{2+}]_i\) and \(\Delta\text{pH}_i\).

<table>
<thead>
<tr>
<th>([\text{Ca}^{2+}]_o) ((\mu\text{M}))</th>
<th>(n)</th>
<th>(\Delta[\text{Ca}^{2+}]_i) (nM)</th>
<th>(\Delta\text{pH}_i)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>12</td>
<td>93±19</td>
<td>0.16±0.03</td>
</tr>
<tr>
<td>100</td>
<td>14</td>
<td>129±18</td>
<td>0.18±0.02</td>
</tr>
<tr>
<td>200</td>
<td>9</td>
<td>127±10</td>
<td>0.20±0.03</td>
</tr>
<tr>
<td>500</td>
<td>10</td>
<td>126±11</td>
<td>0.20±0.03</td>
</tr>
<tr>
<td>1,000</td>
<td>10</td>
<td>129±14</td>
<td>0.20±0.02</td>
</tr>
<tr>
<td>2,000</td>
<td>9</td>
<td>131±10</td>
<td>0.19±0.04</td>
</tr>
</tbody>
</table>

Fig. 3. \(\text{pH}_o\) dependence of \(\Delta[\text{Ca}^{2+}]_i\) and \(\Delta\text{pH}_i\). Open bars: changes measured at different \(\text{pH}_o\) in the absence of HOCl. Shaded bars: changes measured at different \(\text{pH}_o\) in the presence of 200 \(\mu\text{M}\) HOCl. Numbers of the experiments are shown next to respective columns.

Effects of \(\text{Ca}^{2+}\) antagonists on \(\Delta[\text{Ca}^{2+}]_i\) and \(\Delta\text{pH}_i\)

In the presence of verapamil (10 \(\mu\text{M}, n=5\)) and nifedipine (10 \(\mu\text{M}, n=5\)), HOCl-induced \(\Delta[\text{Ca}^{2+}]_i\) were 118±16 and 120±13 nM, respectively. The corresponding \(\Delta\text{pH}_i\) values were 0.17±0.02 and 0.19±0.02. These \(\Delta[\text{Ca}^{2+}]_i\) and \(\Delta\text{pH}_i\) values were not significantly different from those obtained in the absence of \(\text{Ca}^{2+}\) antagonists (125±14 nM for \(\Delta[\text{Ca}^{2+}]_i\), 0.18±0.02 for \(\Delta\text{pH}_i, n=7\)). \(\text{Ca}^{2+}\) antagonists per se had no effect on \(\Delta[\text{Ca}^{2+}]_i\) and \(\Delta\text{pH}_i\) when tested in the absence of HOCl.

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Effects of pH\textsubscript{o} on $\Delta$[Ca\textsuperscript{2+}]\textsubscript{i} and $\Delta$pH\textsubscript{i}

$\Delta$[Ca\textsuperscript{2+}]\textsubscript{i} and $\Delta$pH\textsubscript{i} were measured under conditions of varied pH\textsubscript{o} (6.80, 7.30, and 7.80). Even in the absence of HOCl, [Ca\textsuperscript{2+}]\textsubscript{i} and pH\textsubscript{i} were slightly changed by the pH\textsubscript{o} variation. [Ca\textsuperscript{2+}]\textsubscript{i} increases caused by a change in pH\textsubscript{o} in the absence of HOCl were all below 20 nM and showed no consistent change among the three pH\textsubscript{o} tested (6.80, 7.30, and 7.80) (Fig. 3). Upon increasing pH\textsubscript{o} from 7.30 to 7.80, pH\textsubscript{i} in the absence of HOCl was increased by 0.02 ± 0.01 (negative $\Delta$pH\textsubscript{i}). On the other hand, both $\Delta$[Ca\textsuperscript{2+}]\textsubscript{i} and $\Delta$pH\textsubscript{i} caused by HOCl were consistently decreased as pH\textsubscript{o} was raised (Fig. 3). To estimate the [Ca\textsuperscript{2+}]\textsubscript{i} and pH\textsubscript{i} changes attributable exclusively to the effect of HOCl, we subtracted pH\textsubscript{o} change-induced $\Delta$[Ca\textsuperscript{2+}]\textsubscript{i} and $\Delta$pH\textsubscript{i} from the corresponding HOCl-induced $\Delta$[Ca\textsuperscript{2+}]\textsubscript{i} and $\Delta$pH\textsubscript{i}. The corrected $\Delta$[Ca\textsuperscript{2+}]\textsubscript{i} at pH\textsubscript{o} 6.80 was significantly higher than that measured at pH\textsubscript{o} 7.30 ($p < 0.001$), while the corrected $\Delta$pH\textsubscript{i} was not different from that measured at pH\textsubscript{o} 7.30. Both corrected $\Delta$[Ca\textsuperscript{2+}]\textsubscript{i} and corrected $\Delta$pH\textsubscript{i} at pH\textsubscript{o} 7.80 were significantly smaller than those measured at pH\textsubscript{o} 7.30 ($p < 0.001$).

![Graph showing [Na\textsuperscript{+}]\textsubscript{o} dependence of $\Delta$[Ca\textsuperscript{2+}]\textsubscript{i} and $\Delta$pH\textsubscript{i}.

Fig. 4. [Na\textsuperscript{+}]\textsubscript{o} dependence of $\Delta$[Ca\textsuperscript{2+}]\textsubscript{i} and $\Delta$pH\textsubscript{i}. Myocytes were superfused with HOCl (200 μM)-containing medium with different [Na\textsuperscript{+}]\textsubscript{o} as indicated. Numbers of the experiments are shown next to respective columns. *$p < 0.05$, **$p < 0.001$. Vol. 45, No. 4, 1995
Effects of $[Na^+]_o$ on $\Delta [Ca^{2+}]_i$ and $\Delta pH_i$

Effects of $[Na^+]_o$ alteration on $\Delta [Ca^{2+}]_i$ and $\Delta pH_i$ are shown in Fig. 4. In contrast to the experiments on pH$_o$ intervention (Fig. 3), pH$_i$ and $[Ca^{2+}]_i$ were not changed in the absence of HOCl when $[Na^+]_o$ was altered at a range of 50-200 mM. Therefore, HOCl-induced changes in $[Ca^{2+}]_i$ and pH$_i$ are shown in Fig. 4 without subtraction. In the presence of HOCl, $\Delta [Ca^{2+}]_i$ and $\Delta pH_i$ were significantly augmented as $[Na^+]_o$ was lowered: at 100 mM $[Na^+]_o$, $\Delta [Ca^{2+}]_i$ and $\Delta pH_i$ were significantly larger than those at 150 mM $[Na^+]_o$ ($p < 0.05$ for $\Delta [Ca^{2+}]_i$, $p < 0.001$ for $\Delta pH_i$), and at 50 mM $[Na^+]_o$, both $\Delta [Ca^{2+}]_i$ and $\Delta pH_i$ were further increased ($p < 0.001$ vs. values at 150 mM $[Na^+]_o$).

![Graph showing effects of DTT on HOCl-induced $[Ca^{2+}]_i$ and pH$_i$ changes.](image)

Fig. 5. Effects of DTT (1 mM) on the HOCl-induced $[Ca^{2+}]_i$ and pH$_i$ changes. The superfusing solution was altered at the point of the arrow to JMM with or without DTT, except for the control (○) and non-washout (●) measurements. The following symbols are used: ○, control observation (30 min) in the absence of HOCl; ●, 200 μM HOCl (30 min) (without washout); ■, 200 μM HOCl (15 min) followed by washout with JMM (15 min); △, 200 μM HOCl (15 min) followed by washout with DTT-containing JMM (15 min); Numbers of the experiments were 6 for each series of experiments. *$p < 0.001$ and NS vs. respective values at 15 min.

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Effects of DTT on the HOCl-induced changes in \([\text{Ca}^{2+}]_i\) and \(pH_i\)

To evaluate whether the HOCl-induced changes in \([\text{Ca}^{2+}]_i\) and \(pH_i\) are reversible or not, we examined the effect of washout of HOCl, using the solution containing DTT. As shown in Fig. 5, \([\text{Ca}^{2+}]_i\) and \(pH_i\) reached plateau levels in 30 min after superfusion with JMM containing 200 \(\mu\text{M}\) HOCl (○). Washout with DTT-containing JMM (started at 15 min after application of HOCl) showed nearly complete recovery (Δ) in both \([\text{Ca}^{2+}]_i\) and \(pH_i\) to the control levels (98 and 92% reduction of \(\Delta[\text{Ca}^{2+}]_i\); \(p < 0.001\) and \(\Delta pH_i\); \(p < 0.001\), respectively) in \(~15\text{ min,}\) while washout with JMM alone (■) reduced \(\Delta[\text{Ca}^{2+}]_i\) and \(\Delta pH_i\) by only 10 and 39%, respectively (NS) (Fig. 5).

**DISCUSSION**

In the present study, we examined the effects of HOCl on both \([\text{Ca}^{2+}]_i\) and \(pH_i\) using single quiescent cardiomyocytes. Introduction of HOCl increased \([\text{Ca}^{2+}]_i\) and decreased \(pH_i\) concomitantly. For the evaluation of the correlation between \(\Delta[\text{Ca}^{2+}]_i\) and \(\Delta pH_i\), we plotted \(\Delta[\text{Ca}^{2+}]_i\) vs. \(\Delta pH_i\) (Fig. 6). A fairly good linear correlation was obtained between \(\Delta pH_i\) and \(\Delta[\text{Ca}^{2+}]_i\) in spite of varied extracellular \(\text{Ca}^{2+}\), \(pH\), and \(\text{Na}^+\) conditions. However, under an increased \(pH_o\) condition (♯8; Fig. 6), application of HOCl tended to produce a relatively small \(pH_i\) decrease with respect to the increase in \([\text{Ca}^{2+}]_i\), (deviated from the regression line). This is possibly due to the activation of \(\text{H}^+\) extrusion via \(\text{Na}^+/\text{H}^+\) antiporter at this higher \(pH_o\) of 7.80 [11]. The accompanying increase in \([\text{Na}^+]_i\) may not be set off

![Graph showing correlation between ΔpH_i and Δ[Ca^{2+}]_i](image)

Fig. 6. Correlation between \(\Delta pH_i\) and \(\Delta[\text{Ca}^{2+}]_i\), with a linear regression line between the mean values. Differences from the composition of JMM were as follows: ♯1, [Na^+]_o 50 mM (5); ♯2, [Na^+]_o 100 mM (7); ♯3, pH_o 6.80 (10); ♯4, [Ca^{2+}]_o 1 mM (9); ♯5, JMM (control) (14); ♯6, [Na^+]_o 200 mM (9); ♯7, [Ca^{2+}]_o-free (9); and ♯8, pH_o 7.80 (12). Numbers of the experiments are presented in parentheses.

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by Na⁺/K⁺-ATPase, because the HOCl concentration used in the present study (200 μM) was comparable to that for the half-maximal inhibition of Na⁺/K⁺-ATPase (100 μM) though lower than that of the Na⁺/Ca⁺⁺ antiporter (1 mM) [5]. In the absence of HOCl, Na⁺/K⁺-ATPase might have contributed more to extrude the increased [Na⁺].

The variations of extracellular Ca⁺⁺, pH, and Na⁺ conditions probably modified sarcolemmal transport of Ca⁺⁺, H⁺, and Na⁺ via the Na⁺/H⁺ and Na⁺/Ca⁺⁺ antiporters. Borle and Bender [11] showed that high pH stimulated the Na⁺/H⁺ antiporter, and that the subsequent rise in [Na⁺] increased Ca⁺⁺ influx via the Na⁺/Ca⁺⁺ antiporter. Because of the good correlation seen between Δ[Ca⁺⁺], and ΔpH under a variety of extracellular ionic conditions (Fig. 6), we postulated that the primary effect of HOCl was not the modification of these antiporters, but the direct inactivation of the Ca⁺⁺ uptake of SR [5-7]. According to Kako [5], the Ca⁺⁺ uptake to SR is most susceptible to HOCl with the concentration of the half-maximal inhibition of 1 μM. As a secondary effect, the increased level of [Ca⁺⁺] may release H⁺ from Ca⁺⁺-binding proteins [10]. The experimental results that the [Ca⁺⁺] free condition decreased only 25% of Δ[Ca⁺⁺], and that Ca⁺⁺ antagonists were not effective to suppress Δ[Ca⁺⁺], also suggest that the HOCl-induced increase in [Ca⁺⁺] may derive from the intracellular source of Ca⁺⁺. It is possible that high [Na⁺] also stimulated the Na⁺/H⁺ antiporter to reduce ΔpH (6) from those of the normal (5) or low [Na⁺]c (1 and 2). We assume that the main source of Δ[Ca⁺⁺] was the increased [Ca⁺⁺] secondary to the HOCl-induced reduction of SR Ca⁺⁺ uptake, and partly due to an enhanced Ca⁺⁺ influx via the Na⁺/Ca⁺⁺ antiporter following an increase in [Na⁺] by the Na⁺/K⁺-pump inhibition [5].

Schraufstätter et al. [22] reported the toxicity of HOCl using the sarcolemmal proteins of cultured tumor cells, and attributed the toxicity to the oxidation of sulfhydryl (SH) groups of the proteins. DTT was shown to increase the developed force and diminish the resting tension in oxidant-injured isolated rat papillary muscles with an increase in the amount of cysteine with an intact SH group [23]. Kako [5], Kaminishi et al. [7], Eley et al. [14], and Fukui et al. [16] showed that the HOCl-induced changes in [Ca⁺⁺] were protected by DTT. We examined the effect of DTT on ΔpH as well as on Δ[Ca⁺⁺]. The addition of DTT to the washout solution decreased both Δ[Ca⁺⁺] and ΔpH to close to the control levels (Fig. 5). Such a simultaneous restoration of [Ca⁺⁺] and pH seen in the presence of DTT may provide further evidence for a coupled control of intracellular Ca⁺⁺ concentration and pH via the Na⁺/H⁺ and Na⁺/Ca⁺⁺ antiporters.

In summary, HOCl increased [Ca⁺⁺], and decreased pH simultaneously in single quiescent cardiomyocytes. A positive linear correlation was obtained between Δ[Ca⁺⁺] and ΔpH under various extracellular Ca⁺⁺, pH, and Na⁺ conditions. DTT is effective to restore the HOCl-induced abnormalities in [Ca⁺⁺], and pH to the control levels.
We thank Dr. Kiyoji Matsuyama and Mr. Kazuhiro Tanaka for their assistance in carrying out the experiments.

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