Contractile Shortening Response of Ventricular Cells to Extracellular Acidosis
Evidence for Two-Site Control of H⁺-Ca²⁺ Interaction

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The effect of extracellular acidosis on contraction of single isolated ventricular cells from rabbit was measured in a system in which pH₀ could be changed in <200 msec. The contractile response to acidic levels was complete within 25 seconds. The response was measured 30 seconds after pH₀ was decreased to 7.0, 6.5, 6.0, and 5.5 at each of 8 [Ca]₀ levels (0.125–4.0 mM). Cell shortening versus [Ca]₀ was plotted to construct a curve for each pH₀ level, with each point relative to shortening at pH 7.5, [Ca]₀=1 mM (100% value). Calcium current (1 mM [Ca]₀) was also measured 30 seconds after pH₀ was decreased from 7.5 to 6.5 with single-cell patch clamp technique. The contractile response to extracellular acidosis is accurately predicted by assuming two (probably sarcolemmal) sites at which H⁺ ions affect calcium binding and/or flux:

\[
\text{Contractile shortening} = \frac{1}{[\text{antilog} (pK-pH)]+1} \times \frac{\text{shortening}_{\text{max}} \cdot [\text{Ca}]_{0}}{K_d + [\text{Ca}]_{0}}
\]

The first factor represents a set of sites that are proposed to control access, dependent on the degree of their ionization, to sites represented by the second factor. The latter sites are proposed to accept calcium according to mass-action law. The response of calcium channel current to extracellular acidosis was also complete and reversible within 25 seconds. The current response indicates that the two-site model could be predictive for the effect of extracellular acidosis on calcium current in ventricular cells. (Circulation Research 1989;65:894–902)

A prior study showed that calcium binding to isolated sarcolemma was greatly increased as pH was raised from 5.5 to 8.5 at [Ca]₀=1 mM. In this study, using cultured cells from neonatal rat and membranes isolated from them, the apparent pK of the calcium-binding sites at 1.0 mM [Ca]₀ was found to be between 6.6 and 7.2. This is three orders of magnitude higher than the pK of the phosphate or carboxyl groups of the anionic or zwitterionic phospholipids that account for most of the sarcolemmal calcium binding. If the binding were directly to the phospholipid sites, these sites would be fully ionized at pH 5.5, and an increase in pH above this value would have no further effect on the quantity of calcium bound. The experimental results on isolated sarcolemma, on the other hand, show a greater than 100% increase in calcium binding as pH increases from 5.5 to 8.5. It is necessary, therefore, to propose that two sarcolemmal sites interact to regulate calcium binding. Recent studies that use calcium-induced aggregation behavior of sarcolemmal vesicles to study binding clearly indicate that at least two interacting membrane sites are involved in regulation of the binding.

The "two-site model" proposes an ionizable site with a relatively high pK that shields, intermolecularly or intramolecularly, the negative phospholipid phosphate and/or carboxyl groups and prevents calcium binding. The degree of dissociation (α), at various pH levels, of this shielding site is given by a form of the Henderson-Hasselbach equation

\[
\alpha = \frac{1}{[\text{antilog} (pK-pH)]+1}
\]

As pH increases relative to the pK, the site alters its ionization and calcium gains increasing access to the primary binding sites. The latter sites would
be expected to bind calcium having access to them according to the mass-action (Michaelis-Menten [M-M]) relation. The binding would, therefore, be predicted by

\[
\text{Ca binding} = \frac{1}{[\text{antilog (pK-pH)}]+1} \times \frac{[\text{Ca}]_{\text{binding max}}}{K_d + [\text{Ca}]} 
\]

where \([\text{Ca}]_{\text{binding max}}\) represents the concentration of calcium at which the sites are full, and \(K_d\) represents the dissociation constant of the sites.

The two-site model has been proposed to describe sarcolemmal calcium binding and the effect on this binding of changing pH. \(^1\) It is now of interest to test whether the effects of pH alteration on contraction can be described by the same model. The model describes binding, as affected by pH, at the sarcolemma, and it is required, therefore, that changes in pH be limited to the sarcolemmal-extracellular interface of the cell. It is necessary that little or no change of intracellular pH occur at the time contractile measurements are made. A system for perfusion of a single isolated cardiac cell has been developed that meets this requirement. \(^2\) We use this ultrarapid perfusion system to test the ability of the equation above to predict contractile force at pH values of 7.0 and below. Fry and Poole-Wilson \(^6\) have shown that the M-M relation is sufficient to predict contractile force at higher pH values but state that "more complex events" occur at lower values. Krafte and Kass \(^7\) also note that a mechanism in addition to calcium channel gating shifts must contribute to modification of current amplitude at low pH levels. The present study focuses, therefore, on acidosis as it affects contractile amplitude.

**Materials and Methods**

**Cell Preparation**

The technique for isolation of calcium-tolerant, contractile single cells from rabbit hearts is a modification of that described by Claycomb and Palazzo \(^8\) and has been previously described in detail. \(^5,9\) Male New Zealand rabbits (4–5 lbs) were injected intravenously with an overdose of sodium pentobarbital (60 mg/kg) with heparin (200 units/kg). The heart was rapidly removed and attached by the aorta to a cannula in a still hood for retrograde perfusion at 25 ml/min (37° C). The heart was perfused for 6 minutes with a calcium-free MEM/Joklik's modified solution (Irvine Scientific, Santa Ana, California), additionally augmented buffer (isolation buffer #1, IB-1) containing (g/l) MEM/Joklik's modification 11.35, taurine 7.50, carnitine 0.40, adenosine 0.31, L-glutamic acid 1.58, MgCl\(_2\)6H\(_2\)O 0.23, NaHCO\(_3\) 2.00, dextrose 2.70. pH was adjusted to 7.40. At 6 minutes, the perfusate was changed to a recirculated isolation buffer #2 (IB-2) containing IB-1, 50 μM CaCl\(_2\), 1 mg/ml collagenase (Class II, Worthington Biochemical Corp., Freehold, New Jersey), and 0.6 mg/ml hyaluronidase (Type I-S, Sigma Chemical, St. Louis, Missouri) for 30–35 minutes, at which time the hearts demonstrated significant softening in consistency. The ventricle was removed from the cannula and placed in a 100-mm culture dish (Falcon 1005, Becton-Dickinson and Co., Lincoln Park, New Jersey) with 30 ml isolation buffer #3 (IB-3) that contained IB-1 plus 50 μM CaCl\(_2\). Cells were harvested by gently teasing the tissue with forceps and transferring the resultant cell suspension to 50-ml polystyrene centrifuge tubes (Corning Glass Works, Corning, New York). Additional IB-3 buffer was added to the residual ventricle, and the harvesting process was repeated three times. The volume in the four tubes was brought to 50 ml, and the cells were allowed to settle at 1g.

The supernatant was removed by suction, and the pelleted cells were washed again in IB-3 and allowed to settle. After the supernatant was removed, the cells in each tube were resuspended in 15 ml IB-3, transferred to a 100-mm Falcon 1005 dish, and incubated for 1 hour at 37° C. Five milliliters MEM (Earle's salts, Irvine Scientific) were added to each dish, bringing the calcium concentration to 0.5 mM, and the cells were incubated for an additional 15 minutes. Cells were then transferred to 50-ml centrifuge tubes and allowed to settle to a pellet at 1g. The supernatant was removed, the cells were washed with MEM (1.8 mM calcium concentration) and allowed to settle, and the pellet was suspended in MEM with 5% fetal calf serum (MEM/FCS, Hyclone Laboratories, Logan, Utah). The cells were then plated in MEM/FCS onto 60-mm (Falcon 3002) dishes previously incubated with MEM/FCS for at least 4 hours. The cylindrical, calcium-tolerant cells attached to the dish within 5 minutes; any rounded, contracted cells remained suspended in the supernatant. The supernatant was removed, 3 ml fresh MEM/FCS was added, and the cells were incubated overnight at 37° C in MEM/FCS to allow further healing and stabilization of the membrane. The cylindrical, quiescent cells remained strongly adherent to the dish and gave a vigorous contractile response to electrical stimulation. These cells were used for the experimentation either 1 or 2 days after the isolation procedure.

**Quantification of Contraction Amplitude**

The video technique used has been described and illustrated previously. \(^3\) A culture dish with adherent cells was attached to the movable stage of an inverted phase-interference microscope. A cell was selected that, though adherent at various surface sites, showed significant shortening at one or both ends. The image of the cell was passed through an inverted Nikon ×40 variable focal length objective lens (Nikon Inc. Instrument Group, Garden City, New York) to an interlaced EIA video camera.
The method of single-cell voltage clamp was used to measure transsarcolemmal currents in the rabbit cells. The cell chamber was mounted on the stage of a Nikon Diaphot inverted phase-contrast microscope, and the magnified image was displayed on a video screen. For whole-cell clamping, pipettes of tip diameter 3–5 μm, resistance 0.8–1.5 MΩ were pulled from capillary tubing and used without fire polishing. Pipettes were placed in an electrode holder and mounted on the head stage of an Axopatch-1B patch-clamp amplifier driven by a World Precision Instruments (New Haven, Connecticut) programmable stimulator. Current and voltage output were monitored on a Tektronix 1111 storage oscilloscope (Beaverton, Oregon) and were digitized at up to 10 kHz by an A-D converter for storage and analysis in an ARC-286 personal computer (American Research Corp., City of Industry, California). The patch pipette was filled with solution of the following composition (mM): EGTA 14.0, NaCl 10.0, MgATP 10.0, CaCl₂ 1.0, HEPES 20.0, KCl 93, and KOH 53 (pH=7.15). Gentle suction was applied to form a gigaseal >10 GΩ, adequate for whole-cell clamping, and then additional suction was applied to rupture the patch. Cells were studied in two modes: 1) Action potential mode: The patch-clamp amplifier was set to the current clamp mode with zero holding current, and action potentials were stimulated by constant current pulses at desired frequency. Changes in resting membrane potential, action potential configuration, and action potential duration can be recorded over time. 2) Voltage clamp mode: Calcium current was isolated with an external solution in which sodium and potassium were replaced by 146 mM CsCl with addition of 10⁻⁷ M TTX, and pH was adjusted with CsOH (6.67 M). Otherwise, the solution was the same as control perfusate. Cs⁺ also replaced K⁺ in the internal patch-electrode solution. Clamp pulses at fixed duration (100 msec) to various potentials were applied. Changes in holding current, peak inward current, and final current were monitored. In both modes, data were digitized and stored in the computer for later analysis. Relevant traces were photographed directly from the oscilloscope with a Polaroid camera (Polaroid Corp., Cambridge, Massachusetts). The cell chamber used for voltage clamp studies was designed to exchange completely in less than 1.0 second, so that action potential and current responses could be recorded within the first 30
FIGURE 1. The contractile response of a single rabbit cell to pH 6.0 at various [Ca\textsubscript{o}] levels. In each case, the sequence is control solution (pH 7.5, [Ca\textsubscript{o}] 1.0), switch to pH 6.0 and indicated [Ca\textsubscript{o}] (complete within 200 msec) for 30 seconds, and return to control solution. Note that after switch to pH 6.0, the contractile amplitude has reached essential steady state within 20–25 seconds and that the amplitude in control solution has not changed significantly from prior to the first sequence ([Ca\textsubscript{o}] 0.125) to prior to the last sequence ([Ca\textsubscript{o}] 4.0).

seconds of a pH change. Although the primary purpose of the study was to monitor contractile response with respect to [pH\textsubscript{o}], interpretation of the response required that calcium currents be measured over the same time course.

Results

State of Isolated Cells

Although isolated cells remain cylindrical and quiescent in standard perfusion medium containing 1 mM [Ca\textsubscript{o}], we found that this is not a sufficient criterion to rule out small, but significant, increase in sarcolemmal permeability. We, therefore, routinely added 1 mM lanthanum chloride (La) to an aliquot of cells before using other cells from the same preparation for experimentation. La uncouples excitation from contraction and does not, in normal cells, penetrate intracellularly. Its application to a cell with nonleaky sarcolemma produces a relaxed, nonbeating response. Cells that begin to twitch in La and progress into contracture (La can activate contraction at the myofilaments and is not pumped by the sarcoplasmic reticulum) are leaky. A preparation that showed this response was discarded.

Contractile Response

The typical experimental sequence used is illustrated in Figure 1. The cell was continuously perfused with control solution of [Ca\textsubscript{o}]=1 mM, pH 7.5. It was then exposed to a series of different [Ca\textsubscript{o}] (0.125–4.0 mM) and pH (7.0, 6.5, 6.0, and 5.5) solutions for 30-second intervals, followed by return to control solution and function before the next change in [Ca\textsubscript{o}] and pH. Figure 1 shows the response of a cell to [Ca\textsubscript{o}] from 0.125 mM to 4.0 mM at pH 6.0 for 30-second periods. The cell was continuously stimulated at 0.2 Hz, and the solution change was complete within 200 msec. Note that the contractile response does not change significantly after the fourth or fifth beat (20–25 seconds) in the new solution. The contractile response was measured, therefore, at the quasi steady-state sixth beat just prior to return of the perfusate to [Ca\textsubscript{o}]=1.0 mM, pH 7.5. The experimental sequence was performed with a series of cells (n=8–25) at each pH from 7.0 to 5.5. The [Ca\textsubscript{o}] was restricted to the 0.125–4.0 mM range because above 4.0 mM, the cells frequently showed small changes in diastolic level between beats that could affect the magnitude of the systolic response. Multiple exposures to pH below 5.5 were not well tolerated and were, therefore, not performed.

Operative pK and K\textsubscript{d} Measurement

The experimentally determined pK (operative pK) at any [Ca\textsubscript{o}] level can be derived from the half-maximal contractile response to a pH titration at that [Ca\textsubscript{o}] level. The contractile response at pH 8.0 at the particular [Ca\textsubscript{o}], was used as the maximal value. Specifically, the pH value at which contractile amplitude fell to 50% of that at pH 8.0 and the indicated [Ca\textsubscript{o}] between 0.125 and 4.0 mM was taken as the "operative pK" value. This empirically derived pK is not the absolute value for 50% of the true maximum point, but it is appropriate for use in the experimental sequences where pH 7.5, [Ca\textsubscript{o}]=1.0 was consistently used for normalization of contractile response. This apparent or operative
pK would be expected to vary with \([Ca]_o\) on the basis of the Boltzmann relation

\[
\frac{[H^+],}{[H^+]} = e^{-\psi_0/FRT}
\]

where \([H^+]\), and \([H^+]\) represent surface and bulk solution proton concentration, respectively, and \(\psi_0\) is the surface potential. As \([Ca]_o\) increases, surface potential will decrease as fixed negative charge is neutralized, and apparent pK will decrease. This occurs in a nonlinear manner, as indicated in Figure 2, for the range of \([Ca]_o\) (0.125–4.0 mM) used in the experiments. The operative pK values are listed in Table 1.

In addition, the operative \(K_d\) of the putative sarcolemmal calcium-binding sites can be altered by changing pH. Fry and Poole-Wilson\(^6\) show clear evidence with Lineweaver-Burk analysis for \(H^+\)-Ca\(^{2+}\) competition at sites important for control of force development. Similar to the procedure used to empirically determine pK, the experimentally determined \(K_d\) of the putative binding sites can be derived from the half-maximal contractile response to a \([Ca]_o\) titration at a given pH. The contractile response at \([Ca]_o=4\) mM at the particular pH was used as the maximal value. Specifically, the \([Ca]_o\) value at which contractile amplitude fell to 50% of that at \([Ca]_o; 4\) mM at a particular pH was taken as the operative \(K_d\) value. This empirically derived \(K_d\) value is similarly not the absolute value for 50% of a true maximum point, but it serves as the appropriate constant for insertion in the M-M portion of the model for reasons as noted above for pK (see “Discussion”). The \(K_d\) decreases in a nonlinear manner as pH increases, as shown in Figure 3. The \(K_d\) values are listed in Table 2.

**Table 1.** pK Values Derived From pH Titrations at Various [Ca] Levels

<table>
<thead>
<tr>
<th>[Ca]_o (mM)</th>
<th>pK</th>
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<tbody>
<tr>
<td>0.125</td>
<td>6.58</td>
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<td>0.50</td>
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</tr>
<tr>
<td>4.0</td>
<td>5.72</td>
</tr>
</tbody>
</table>

**Table 2.** \(K_d\) Values Derived From [Ca]_o Titrations at Various pH Levels

<table>
<thead>
<tr>
<th>pH</th>
<th>(K_d)</th>
</tr>
</thead>
<tbody>
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</tr>
<tr>
<td>7.5</td>
<td>0.67</td>
</tr>
<tr>
<td>7.0</td>
<td>0.65</td>
</tr>
<tr>
<td>6.5</td>
<td>0.73</td>
</tr>
<tr>
<td>6.0</td>
<td>1.03</td>
</tr>
<tr>
<td>5.5</td>
<td>1.85</td>
</tr>
</tbody>
</table>

**Figure 2.** pH values at which contractile amplitude was at 50% of that at pH 7.5 and indicated \([Ca]_o\) was from 0.125 to 4.0 mM. This is a plot of the operative pK values listed in Table 1.

**Figure 3.** \([Ca]_o\) values at which contractile amplitude was at 50% of that at \([Ca]_o; 1.0\), pH 7.5, over a pH\(_o\) range from 5.5 to 8.0. This is a plot of the operative \(K_d\) values listed in Table 2.
cells, there was no discernible current at $-30$ mV or more negative clamp levels. At pH 6.5, peak current declined at 30 seconds by 32% and recovered to within 9% of control after 30 seconds' return to pH 7.5. Figure 5 is a current-voltage plot illustrating the response of another cell to clamp pulses from $-40$ to $+50$ mV, in which peak current occurred at $+10$ mV, decreased by 35% at pH 6.5, and recovered to 10% above control within 30 seconds. In four cells, pH 6.5 caused a $41.5\pm6.9\%$ (mean±SEM) decline in peak current, with recovery to within $95.0\pm14.6\%$ of control after 30 seconds' return to pH 7.5.

**Discussion**

Previous results indicated that calcium binding to myocardial sarcolemma is affected by $[H^+]$ alteration could not be explained by binding to a single set of sites. It was proposed that binding was controlled by two separate sites. One site was visualized as the primary binding site with $pK < 4.0$ that would, therefore, be expected to be nearly fully ionized at pH 5.5 or greater. Binding at this site would be according to mass action and predicted by the M-M relation:

$$\text{Binding} = \frac{[\text{Ca}^{\text{binding max}} \cdot [\text{Ca}_o]}{K_d + [\text{Ca}_o]}$$  \hspace{1cm} (1)

In the present study, contractile shortening is considered to represent calcium binding to force-controlling sites, thus

$$\text{Shortening} = \frac{\text{shortening}_{\text{max}} \cdot [\text{Ca}_o]}{K_d + [\text{Ca}_o]}$$  \hspace{1cm} (2)

The role of this site is therefore determined by $[\text{Ca}_o]$ and the $K_d$, with pH affecting shortening (binding) only as it alters the $K_d$ for calcium.

The second set of sites was visualized to control calcium access to the primary binding sites. It is modeled as an ionizable site with a relatively high $pK$ that "shields," intermolecularly or intramolecularly, the primary binding site and, dependent on its state of ionization, allows access of calcium to the primary site. The degree of ionization ($\alpha$) is given by a form of the Henderson-Hasselbach equation:

$$\alpha = \frac{1}{[\text{antilog (pK-pH)}]+1}$$  \hspace{1cm} (3)
As pH increases relative to pK, \( \alpha \) approaches 1.0, the unionized state, and this would permit free access of calcium to the primary site. As conditions become more acidic, \( \alpha \) decreases; the site is more ionized, thus limiting access. As noted in Table 1, the operative pK increases as \([Ca]\) decreases from 4.0 to 0.125 mM. Therefore, \( \alpha \) is lowest at low \([Ca]\) and low pH. The critical test, then, of the effect of the postulated access site is with low levels of \([Ca]\) and acidotic conditions. This is illustrated in Table 3. At pH 7.5, \( \alpha \) ranges between 0.89 and 0.98 and would have relatively little effect on the product \( \alpha \times \text{Equation 2 (} \alpha \times \text{M-M)} \). At pH 5.5, however, \( \alpha \) ranges between 0.077 and 0.38 and would be expected to have a marked effect on the \( \alpha \times \text{M-M} \) product.

It is this \( \alpha \times \text{M-M} \) product that we propose as a predictor of force development over a range of \([Ca]\) from 0.125 to 4.0 mM and of pH from 7.0 to 5.5. Figures 6A, B, C, and D each depict an experimental curve, a curve predicted using only Equation 2 (M-M), and the curve predicted using the \( \alpha \times \text{M-M} \) product. The pK and \( K_d \) values (see Tables 1 and 2) appropriate to each level of pH and \([Ca]\) are used to construct each theoretical curve. As seen from Figure 6A, at pH 7.0 there is no statistical difference between the ability of the \( \alpha \times \text{M-M} \) product and the simple M-M relation to predict the experimental curve (\( F = 1.26, p > 0.05 \)). The \( \alpha \times \text{M-M} \) and M-M curves are tested for fit to the experimental curve by using nonlinear regression as described by Motulsky and Ransnas:

\[
F = \frac{(SS_1 - SS_2)/(df_1 - df_2)}{SS_2/df_2}
\]

where \( SS \) refers to the sum of the squares and \( df \) refers to the number of degrees of freedom. The subscript 1 refers to the M-M model and subscript 2 to the \( \alpha \times \text{M-M} \) model. The \( p \) value is derived from \( F \) from a standard table. The lack of difference between the one-site and two-site models to predict the experimental curve at pH 7.0 is expected with \( \alpha \) at 0.9 or higher at \([Ca]\) above 0.75 mM (Table 3). At 6.5 and below, however (Figures 6B, C, and D), the ability of the \( \alpha \times \text{M-M} \) relation to predict the experimental data becomes very significantly better than

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**Figure 6.** Experimental and predicted contractile responses to changes in \([Ca]\) (0.125–4.0 mM) at pH 7.0 (Panel A), 6.5 (Panel B), 6.0 (Panel C), and 5.5 (Panel D). In each case, the experimental response with \( \pm \text{SEM} \) is indicated (○). The response predicted by the Michaelis-Menten (M-M) relation alone (©) is compared with that predicted by use of the \( \alpha \times \text{M-M} \) product (△). At pH 7.0 there is no significant difference in the ability of the M-M relation and the \( \alpha \times \text{M-M} \) product to predict the contractile response. At pH 6.5 and lower, however, the two-site system is significantly better. (See text for discussion.)
the M-M relation ($F >90$ in each case, $p<0.01$). Note also that at pH 6.0 and 5.5, the experimental curve begins to show sigmoidicity, indicating the influence of a factor in addition to the hyperbolic curve. The ($\alpha\times$M-M) relation predicts this sigmoidicity.

It is clear from Figure 6 that $[\text{Ca}]_{o}$ 4.0 mM does not produce maximal shortening and does not, therefore, represent true $[\text{Ca}]_{\text{binding max}}$. $[\text{Ca}]_{o}$ greater than 4.0 mM did not permit absolutely stable diastolic levels, and, therefore, the cells were not subjected to levels above this concentration. If the $[\text{Ca}]_{o}$ could have been raised to achieve maximum shortening, it is possible that the operative $K_d$ as measured would increase at any pH$_o$ level (Table 2, Figure 3). Such an increase might conceivably lower each of the "M-M" curves in Figure 6 closer to the experimental curve and improve the ability of the single-factor M-M curve to predict the data. This is, however, very unlikely. For example, in Figure 6D (in which shortening at $[\text{Ca}]_{o}$ 4.0 mM is farthest from maximal of any pH level), an extrapolation of the curve to $[\text{Ca}]_{o}$ 8 mM indicates a shortening increase of 25% with a corresponding increase in $K_d$. This should produce a maximum decrease in the M-M curve of 6%, which is insignificant.

The specific cellular sites at which protons affect calcium binding and/or movement are unknown. The results of the present study, which demonstrate a complete mechanical response to acidosis in <30 seconds and a significant calcium current response within the same time, indicate that the sites are at the sarcolemma in rapid equilibrium with the extracellular medium. This differs from the conclusion of Irisawa and Sato, who examined single guinea pig ventricular cells with a protocol different from ours. They buffered the internal cellular solution through the ruptured patch with either 5 or 50 mM HEPES and found that "the amplitude of peak $I_{\text{ca}}$ began to decrease within 30 seconds" after changing the external solution from pH 7.4 to pH 6.0. When the internal solution was buffered with 50 mM HEPES, $I_{\text{ca}}$ did not begin to decline until 2 minutes after pH$_o$ was reduced to 6.0. Their conclusion was, therefore, that the decline in $I_{\text{ca}}$ was largely due to protons entering the cell. We buffered our internal pipette solution with 20 mM HEPES and obtained a current response upon pH$_o$ change from 7.5 to 6.5 that was complete within 20-25 seconds and recovered to within 95% control within 30 seconds. In addition, the force response of intact cells, not measured by Irisawa and Sato, was as rapid as the $I_{\text{ca}}$ response. Therefore, with these results from our system (which is capable of introducing rapid turnover in the extracellular medium), we propose that the proton-calcium interaction is, primarily, at the sarcolemmal membrane at sites sensitive to pH$_o$.

The need to propose the interaction of two sites, one an ionizable access site of pH~6.0 at $[\text{Ca}]_{o}$=1.0 mM and another that binds the calcium, to explain the cells' response to acidosis is consistent with the study of Fry and Poole-Wilson. They found difficulty in fitting a simple single-binding-site model to the experimental force responses below a pH$_o$ of 6.8. We differ, however, with their conclusion that an intracellular pH change is responsible. Their use of a papillary muscle preparation would introduce diffusional delays that would make separation of effects at sarcolemmal and intracellular sites difficult.

In their recent extensive study on the effect of pH$_o$ on calcium current in single rat ventricular cells, Krafte and Kass found that gating shifts caused by the binding of protons to fixed sites on the membrane surface was not sufficient to explain the complete abolition of $I_{\text{ca}}$ at pH <4.8. The existence of an ionizable "access" site (pK~6.0 at 1.0 mM Ca [Table 1]) would give an $\alpha$ factor of 0.059 at pH 4.8. Thus, if this site were modeled to control access to the channel, $I_{\text{ca}}$ would be <6% maximum at pH <4.8 and would fall rapidly at lower pH levels, for example, 3% at pH 4.5. The large increases in force and $I_{\text{ca}}$ that occur in the alkaline range are consistent with the preponderant effect of the M-M relation in this range as the operative $K_d$ for calcium falls with rising pH (Table 2). Although the response of the calcium current to a single pH change at a single $[\text{Ca}]_{o}$ in the present study is consistent with the proposed two-site model

### Table 3. Calculated $\alpha$ Values Using Appropriate pK Values at Each $[\text{Ca}]_{o}$

<table>
<thead>
<tr>
<th>$[\text{Ca}]_{o}$</th>
<th>7.5</th>
<th>7.0</th>
<th>6.5</th>
<th>6.0</th>
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<td>0.9804</td>
<td>0.9406</td>
<td>0.8337</td>
<td>0.6131</td>
<td>0.3339</td>
</tr>
<tr>
<td>4.0</td>
<td>0.9837</td>
<td>0.9501</td>
<td>0.8577</td>
<td>0.6558</td>
<td>0.3760</td>
</tr>
</tbody>
</table>
for control of calcium influx, further study of current response is required over a range of pH and [Ca]o, as was done for contractile shortening. The rapid response to extracellular acidosis of both contraction and calcium current as demonstrated in the present study does, however, suggest the presence of a superficial cellular site important in the control of calcium entry.

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References


KEY WORDS • extracellular acidosis • calcium binding
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