Mechanism of effect of extracellular pH on L-type Ca\(^{2+}\) channel currents in human mesenteric arterial cells

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It is well known that voltage-gated Ca\(^{2+}\) channel currents in a variety of tissues are modified by alterations in extracellular pH (pH\(_e\)). Reduction of pH\(_e\) below the control value of 7.2–7.4 (acidification) caused a decrease in the Ca\(^{2+}\) channel current amplitude in tunicate egg cells (20), guinea pig ventricular myocytes (13, 17, 29), and hybridoma cells (11). Alkalization caused the opposite effect. The reduction/enhancement of the Ca\(^{2+}\) channel current during acidification/alkalinization was explained by a decrease/increase in the surface concentration of the charge-carrying ion near the Ca\(^{2+}\) channel mouth because of alterations in surface potential created by the interaction of protons with fixed negative charges on the outer surface of the cell membrane (11, 17, 20). Simultaneous displacements of the voltage dependencies of activation and inactivation were also ascribed to surface potential effects (17, 20). In addition, it has been shown that extracellular protons can have a direct action on Ca\(^{2+}\) channel conductance state (23).

Although it is known that acidosis is associated with vasodilation, the relative roles of intracellular pH (pH\(_i\)) and pH\(_e\) in causing vascular relaxation are controversial (5, 8, 12, 27). Surprisingly, the effects of pH\(_e\) on voltage-gated Ca\(^{2+}\) channel currents in single vascular smooth muscle cells (SMCs) have received limited attention. Decreasing pH\(_e\) caused an inhibition of the Ba\(^{2+}\) current through voltage-gated Ca\(^{2+}\) channels in guinea pig cerebral arterial cells (30). Prominent changes in current kinetics were also described, but significant shifts in the current-voltage (I-V) relationship associated with changing pH\(_e\) were absent. On the other hand, alteration of pH\(_e\) caused changes in the Ca\(^{2+}\) channel current amplitude and in the voltage dependencies of channel activation and inactivation in single bovine pial and porcine coronary arterial myocytes (14). These divergent observations may have reflected differences in the regulation of activity of Ca\(^{2+}\) channels by pH\(_e\) in various vascular SMCs.

Therefore, to investigate the mechanism of the modulation of L-type Ca\(^{2+}\) channels by extracellular protons in SMCs isolated from human mesenteric arteries (HMA), the effect of pH\(_e\) on the amplitude, kinetics, and voltage dependencies of activation and inactivation of the Ca\(^{2+}\) channel current was examined. The current was studied in the presence of physiological (1.5 mM) and high (10 mM) concentrations of Ca\(^{2+}\) and also in the same concentrations of Ba\(^{2+}\). The effect of pH\(_e\) on the nonactivating “window current” (25) measured in the presence of 10 mM Ba\(^{2+}\) was also studied. Our results suggest strongly that the alteration of the Ca\(^{2+}\) current by pH\(_e\) can be explained primarily by changes in the surface concentration of divalent cations in the vicinity of the mouth of the Ca\(^{2+}\) channel in these cells.
MATERIALS AND METHODS

Human mesenteric (omental) arteries were obtained from the fat attached to sections of stomach or small or large bowel removed during routine surgery at St. Thomas's Hospital (London). This procedure was approved by the St. Thomas's Ethical Committee.

Methods of SMC isolation, current recording, and data analysis have been described in detail previously (25, 26). The external solution had the following composition (mM): 130 NaCl, 1 CsCl, 1.2 MgCl₂, 1.5 CaCl₂, 4 tetraethylammonium chloride, 10 HEPES, and 10 glucose. NaOH was used to adjust the pH of the solution to 6.2, 7.2, or 8.2. In some solutions, 1.5 mM Ba²⁺, 10 mM Ca²⁺, or 10 mM Ba²⁺ replaced 1.5 mM Ca²⁺. In 10 mM Ba²⁺- or Ca²⁺-containing solutions, NaCl was reduced to 120 mM. At pH 9.2, 5 mM HEPES was replaced with 5 mM 2-(N-cyclohexylamino)ethanesulfonic acid in the Ba²⁺-containing solution. The pH in this solution fell during experiments to 9.15–9.02, probably because of a slow precipitation of Ba²⁺ by hydroxide ions. This difference was considered negligible. The pipette solution contained (mM) 135 CsCl, 2.5 MgCl₂, 2 Na₂ATP, 10 HEPES, and 10 EGTA, and its pH was adjusted to 7.2 with NaOH. Basic chemicals were purchased from BDH (Poole, UK); HEPES, 2-(N-cyclohexylamino)ethanesulfonic acid, EGTA, and tetraethylammonium chloride were obtained from Sigma Chemical (Poole, UK).

Ca²⁺ and Ba²⁺ currents through L-type Ca²⁺ channels were measured in whole cell voltage-clamp mode at room temperature and analyzed using pClamp 6.0 software (Axon Instruments). We previously described T- and L-type voltage-gated Ca²⁺ channel current in HMA myocytes. The L-type Ca²⁺ channel current, which dominated in these cells, was the main subject of the present investigation. T-type current was eliminated by using a holding potential of −60 mV (25). To compare activation for L-type Ca²⁺ channel currents, a standard voltage protocol was applied. Cells were held at −60 mV, and Ca²⁺ or Ba²⁺ current was recorded using 100-ms depolarizing voltage steps applied at 5-mV increments from −70 to +80 mV. The I-V relationship was initially recorded at pH 7.2 and then repeated at the test pH in the same cell. Peak current amplitude was measured at each membrane potential and converted to current density, which was calculated as the ratio of the current amplitude to the cell membrane capacitance with the assumption of a specific membrane capacitance of 1 μF/cm². The cell membrane capacitance was determined from the area under the capacitive transients elicited by a 10-mV hyperpolarizing pulse of 5-ms duration recorded with 50-kHz filtering.

Values are means ± SE. The significance of an experimental value was determined using Student’s paired or unpaired t-test as appropriate, with P < 0.05 considered to be significant, unless otherwise stated.

RESULTS

Effects of acidification and alkalinization on activation and inactivation of L-type Ca²⁺ currents. Figure 1A shows families of Ca²⁺ currents recorded in the same HMA cell at 6.2, 7.2, and 8.2 pH. A decrease in pH from 7.2 to 6.2 caused a diminution of the Ca²⁺ current from 57 to 33 pA at +10 mV and from 40 to 10 pA at −10 mV. Alkalinization to pH 8.2 produced the opposite effect on the Ca²⁺ current, increasing its amplitude by 1.4 and 1.9 times at +10 and −10 mV, respectively. The effects of altering pH were completely reversible in this and all other external solutions tested (data not shown). Figure 1B shows the mean I-V relationships for the peak of the Ca²⁺ current recorded at each membrane potential and converted to current density, as described in MATERIALS AND METHODS. The maximal peak amplitudes of the Ca²⁺ current was decreased by 50 ± 4% during external acidification to 6.2 and increased by 38 ± 5% when pH was increased to 8.2 in comparison to those measured at pH 7.2 in five and six cells studied, respectively. The nadir of the I-V relationship also occurred at progressively more-negative membrane potentials as the pH was raised, shifting from approximately +15 mV at pH 6.2 to between +5 and 0 mV and between 0 and −5 mV at pH 7.2 and 8.2, respectively (Fig. 1C). This suggested that channel activation was affected by changes in pH (20). To
determine the parameters of activation for Ca\textsuperscript{2+} current at different pH, each I-V curve for Ca\textsuperscript{2+} current was normalized to its peak, as shown in Fig. 1C, and was described using the following equation

\[ I = g \cdot m \cdot (V - E_c) \]  

(1)

where I is the amplitude of the normalized Ca\textsuperscript{2+} current, V and \( E_c \) are the membrane and apparent reversal potentials for Ca\textsuperscript{2+} current, g is a scaling factor, and m is the Boltzmann function for activation described by the following equation

\[ m = \frac{1}{1 + \exp[(V - V_m)/k_m]} \]  

(2)

in which \( V_m \) is the half-activation potential and \( k_m \) is the slope factor. Parameters \( E_c \), \( V_m \), \( k_m \), and g (the latter ranged between 0.02 and 0.06) were varied in each individual cell at each pH to obtain the best fit of the I-V curves. The mean \( V_m \) values at various pH levels are compared in Table 1. This comparison showed that acidification had a significantly larger effect on the half-activation potential for Ca\textsuperscript{2+} current than did alkalinization. Under these conditions, \( k_m \) varied between 6.2 and 10.3 mV in various HMA cells, with mean values of 8.1 ± 0.9 (n = 5), 7.8 ± 0.6 (n = 8), and 7.6 ± 0.6 (n = 6) mV at pH\textsubscript{0} 6.2, 7.2, and 8.2, respectively, which were not significantly different. Similarly, g and \( E_c \) values were also not significantly affected by altering pH\textsubscript{0}.

**Table 1. Effect of pH on L-type Ca\textsuperscript{2+} channel activation**

<table>
<thead>
<tr>
<th>Test pH\textsubscript{0}</th>
<th>Test pH\textsubscript{0}</th>
<th>pH\textsubscript{0} 7.2</th>
<th>( \Delta V_m ), mV</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5 mM Ca\textsuperscript{2+}</td>
<td>6.2</td>
<td>6.3 ± 4.4*</td>
<td>−5.3 ± 4.1</td>
<td>+11.6 ± 1.4</td>
</tr>
<tr>
<td>8.2</td>
<td>−16.1 ± 3.2*</td>
<td>−9.8 ± 2.8</td>
<td>−6.3 ± 0.6</td>
<td>6</td>
</tr>
<tr>
<td>10 mM Ca\textsuperscript{2+}</td>
<td>6.2</td>
<td>+12.1 ± 1.7*</td>
<td>+3.9 ± 1.7</td>
<td>+8.1 ± 0.8</td>
</tr>
<tr>
<td>8.2</td>
<td>+3.6 ± 1.8*</td>
<td>+6.5 ± 1.5</td>
<td>−3.5 ± 1</td>
<td>8</td>
</tr>
<tr>
<td>1.5 mM Ba\textsuperscript{2+}</td>
<td>6.2</td>
<td>14.4 ± 3.2*</td>
<td>−4.3 ± 1.8</td>
<td>+18.8 ± 1.6</td>
</tr>
<tr>
<td>8.2</td>
<td>−7.5 ± 2.4*</td>
<td>−3.6 ± 2.4</td>
<td>−3.9 ± 0.2</td>
<td>7</td>
</tr>
<tr>
<td>10 mM Ba\textsuperscript{2+}</td>
<td>6.2</td>
<td>+10 ± 1*</td>
<td>+4 ± 1.1</td>
<td>+7.5 ± 0.5</td>
</tr>
<tr>
<td>8.2</td>
<td>+2 ± 1.2*</td>
<td>+5 ± 1.4</td>
<td>−2.5 ± 0.4</td>
<td>8</td>
</tr>
<tr>
<td>9.2</td>
<td>−5 ± 0.8*</td>
<td>0 ± 1.1</td>
<td>−3.9 ± 0.5</td>
<td>8</td>
</tr>
<tr>
<td>10 mM Ba\textsuperscript{2+}</td>
<td>6.2</td>
<td>13.7 ± 1.4*</td>
<td>4.5 ± 0.4</td>
<td>+9.2 ± 1.2</td>
</tr>
<tr>
<td>8.2</td>
<td>0.2 ± 1*</td>
<td>3.6 ± 0.7</td>
<td>−3.4 ± 0.8</td>
<td>6</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of cells. Half-activation potential (\( V_m \)) was obtained using Eqs. 1 and 2. \( \Delta V_m \), difference between \( V_m \) measured at test extracellular pH (pH\textsubscript{0}) and control pH\textsubscript{0} 7.2.

*0.02 > P > 0.0001 (paired t-test). †Pipette solution contained 10 mM HEPES unless otherwise stated.

The effect of pH\textsubscript{0} on the steady-state inactivation of the Ca\textsuperscript{2+} current was studied in a separate set of experiments. Cells were placed into an external solution with a given pH\textsubscript{0} and were stimulated with a 30-s conditioning potential applied in the voltage range of −100 to +10 mV in 10-mV increments followed by a short (150-ms) test pulse to +10 mV (pH\textsubscript{0} 6.2) and +20 mV (pH\textsubscript{0} 8.2) after a series of 30-s preconditioning steps to various voltages (\( V_m \)). Cell membrane capacitance was 48 pF. Dashed lines, zero current. Voltage protocol is shown at top. The interpulse interval was 30 ms. B: steady-state inactivation dependencies for \( I_{Ca} \) averaged for 5 (pH\textsubscript{0} 6.2), 13 (pH\textsubscript{0} 7.2), and 9 (pH\textsubscript{0} 8.2) cells. Solid lines, theoretical fit according to the Boltzmann equation with half-inactivation potentials (\( V_{50} \)) equal to −23.1, −32.4, and −36.3 mV and slope factors (\( k_m \)) equal to 11.3, 9.3, and 9.2 mV at pH\textsubscript{0} 6.2, 7.2, and 8.2, respectively. Vertical dashed lines, \( V_m \).

The effects of acidification and alkalinization on the Ca\textsuperscript{2+} channel current in high Ca\textsuperscript{2+}- and Ba\textsuperscript{2+}-containing solutions. Figure 3 presents the effect of varying pH\textsubscript{0} on the I-V curves for the Ca\textsuperscript{2+} channel current recorded in 1.5 mM Ba\textsuperscript{2+}, 10 mM Ca\textsuperscript{2+}, and 10 mM Ba\textsuperscript{2+}. As described in MATERIALS AND METHODS, the I-V relationship was initially recorded at pH\textsubscript{0} 7.2 and then repeated at the test pH\textsubscript{0} in the same cell with the use of a standard voltage protocol. For 1.5 mM Ba\textsuperscript{2+} and 10 mM Ca\textsuperscript{2+}, the pH was altered from the control pH\textsubscript{0} of 7.2 to 6.2 or 8.2; for 10 mM Ba\textsuperscript{2+}, pH\textsubscript{0} was altered from 7.2 to 6.2, 8.2, or 9.2. In Fig. 3, the current recorded at pH\textsubscript{0} 7.2 is shown paired with the current obtained at
one test pHo. Figure 3 illustrates that the effect of changing pHo on the I-V curve was very similar for each of the three external solutions examined and also closely resembled the effect recorded in the solution containing 1.5 mM Ca2+ (Fig. 1).

Alteration of pHo caused no obvious changes in the decay of the current, regardless of the external solution employed. The lack of effect of pHo on current kinetics is apparent in Figs. 1 and 2 for the solution containing 1.5 mM Ca2+ and is further emphasized by the fact that any particular alteration in pHo always had very similar effects on the peak inward current and the current at the end of a 100-ms depolarization. For example, in 10 mM Ba2+ solution, at the nadir of the I-V curve, the current measured at the end of the pulse 0.4 ± 0.02, 1.4 ± 0.1, and 1.6 ± 0.1 times that at pHo 7.2 for 10, 8, and 8 cells studied at pHo 6.2, 8.2, and 9.2, respectively. The peak inward current in these cells was 0.5 ± 0.02, 1.4 ± 0.1, and 1.6 ± 0.1 times that measured at pHo 7.2 at pHo 6.2, 8.2, and 9.2, respectively.

Also the effect of pHo on the Ca2+ channel current was more prominent at negative than at positive membrane voltages (Fig. 3). For example, in 10 mM Ba2+, acidification decreased the density of Ba2+ current by about sixfold, from 0.6 ± 0.1 to 0.1 ± 0.02 μA/cm² (n = 10), at −10 mV but only by twofold, from 1.0 ± 0.1 to 0.5 ± 0.1 μA/cm² (n = 10, P < 0.05), at +40 mV. Similarly, in the group of cells in which pHo was increased from 7.2 to 9.2, Ba2+ current was significantly enhanced from 0.7 ± 0.1 to 1.6 ± 0.2 μA/cm² (n = 8) at −10 mV and showed only a nonsignificant increase from 1.0 ± 0.2 to 1.4 ± 0.3 μA/cm² (n = 8) at +40 mV (Fig. 3C). A similar potential dependency of the effect of pHo on Ca2+ current in 1.5 mM Ca2+ is also apparent in Fig. 1.

The similarity between the effects of pHo on the Ca2+ channel current recorded in each of the four solutions is emphasized in Fig. 4, which compares the extent to which the mean current density at the peak of the I-V curve was altered by acidification and alkalization in each case. The effect of pHo on the current in 10 mM Ba2+, the solution in which the widest range of pHo was examined, was fitted using the equation shown in the legend to Fig. 4. This curve predicted a 60% maximal enhancement of current through L-type Ca2+ channels in HMA cells, compared with the current measured at pHo 7.2, as a result of strong alkalization. The effects of pHo in each of the other three solutions were virtually indistinguishable from those observed in 10 mM Ba2+.

### Table 2. Effect of pHo on L-type Ca2+ channel inactivation

<table>
<thead>
<tr>
<th>External Divalent Cation Concentration</th>
<th>6.2</th>
<th>7.2</th>
<th>8.2</th>
<th>9.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca2+ 1.5 mM</td>
<td>−22.3 ± 2.9(5)</td>
<td>−31.4 ± 2(13)</td>
<td>−36 ± 2.1(9)</td>
<td></td>
</tr>
<tr>
<td>10 mM</td>
<td>−14.2 ± 2.3(4)</td>
<td>−19.1 ± 1.9(5)</td>
<td>−21 ± 1.5(4)</td>
<td></td>
</tr>
<tr>
<td>Ba2+ 1.5 mM</td>
<td>−20.6 ± 2.6(4)</td>
<td>−32 ± 1.6(6)</td>
<td>−35 ± 1.3(6)</td>
<td></td>
</tr>
<tr>
<td>10 mM</td>
<td>−15 ± 1.8(6)</td>
<td>−22 ± 0.9(9)</td>
<td>−23 ± 1.4(10)</td>
<td></td>
</tr>
<tr>
<td>10 mM†</td>
<td>−17.7 ± 1.9(4)</td>
<td>−23 ± 1.6(6)</td>
<td>−26 ± 2(6)</td>
<td></td>
</tr>
</tbody>
</table>

Values (means ± SE) represent the half-inactivation potential (in mV), with the number of cells studied in parentheses. *Significantly different from value at control pHo 7.2. †Pipette solution contained 100 mM HEPES.

Fig. 3. Effect of various pHs on L-type ICa, in the presence of low and high concentrations of divalent cations. A and C: Ba2+ currents (ICa) recorded in the presence of 1.5 and 10 mM Ba2+, respectively; B: ICa measured in 10 mM external Ca2+ with use of the same experimental protocol described in the legend of Fig. 1. Currents are expressed as current densities. Data represent means for 6 and 7 (A), 5 and 4 (B), and 10, 8, and 8 (C) paired human mesenteric arterial (HMA) cells studied at pHo 6.2 and 8.2 (A and B) and 6.2, 8.2, and 9.2 (C), respectively.
Fig. 4. Relationship between pH_o and the peak L-type I_{Ca} measured at the nadir of the I-V curve. I_{P,pH}/I_{P,7.2} represents the ratio of the peaks of I_{Ca} or I_{Ba} at test pH_o to that at pH_o 7.2, respectively, at each divalent cation concentration. The line was drawn according to the following equation: I_{P,pH}/I_{P,7.2} = A_{max}/[1 + 10^{pK_{a} - pH}], where A_{max}, the maximal saturated ratio, was 1.6, the apparent pK_a value was 6.8, and n, the apparent Hill coefficient, was 0.6.

The I-V curves from each of the individual experiments carried out in 10 mM Ca^{2+} and in both concentrations of Ba^{2+} were analyzed as described above for Ca^{2+} current in 1.5 mM Ca^{2+} to determine the effect of pH_o on the half-activation potentials. These are listed in Table 1. The extent to which changing pH_o affected the steady-state inactivation of the Ca^{2+} channel current was also studied in each of these three external solutions by use of the voltage protocol described in Fig. 2, and the data are shown in Table 2. Neither k_m nor k_h (slope factor) values derived from activation and inactivation experiments, respectively, were significantly affected by alterations in pH_o (data not shown). A comparison of the shifts in the half-activation (V_m) and half-inactivation (V_h) potentials for the Ca^{2+} channel current at different pH_o showed in all cases that alkalization to pH_o 8.2 caused smaller shifts in both parameters than did acidification to pH_o 6.2. Although there was some variability in the extent to which changing pH_o altered V_m and V_h in the different solutions, no clear trends that would suggest that the effect of pH_o on gating differed greatly between solutions were apparent.

Effects of changing pH_o on the Ca^{2+} channel window current. Figure 5 depicts the normalized steady-state inactivation dependencies together with the activation curves (generated using the mean values of k_m and V_m at each pH_o) for the Ca^{2+} channel current recorded in 10 mM Ba^{2+} physiological saline solution, the solution in which the greatest range of pH_o was studied. Figure 5 illustrates the extent to which activation and inactivation of the Ca^{2+} channel current were displaced to more-negative voltage ranges as pH_o was increased. The shift in the inactivation curve would be expected to have little effect on the current, since even at pH_o 9.2, Ba^{2+} current is almost completely available at a holding potential of -60 mV. On the other hand, the position of the activation curve is such that its displacement should have an important effect on current amplitude, especially at negative potentials.

The overlap of the activation and inactivation curves was also increased and shifted to more-negative potentials as pH_o was increased. This would predict that the noninactivating window current, which we previously demonstrated in these cells (25), should be progressively suppressed as pH_o is reduced. To evaluate this more directly, the noninactivating window current in the 10 mM Ba^{2+} solution was measured using 30-s membrane depolarizations to -10 and 0 mV from a holding potential of -60 mV at control pH_o 7.2 and at two test pH_o, 6.2 and 8.2. This solution was used because it gave the largest and most stable Ca^{2+} channel current. The result of one such experiment, where Ba^{2+} current was recorded during membrane depolarization to -10 mV, is shown in Fig. 6A. The whole cell inward Ba^{2+} current was blocked by 0.5 mM Cd^{2+} at the end of the experiment, and the window Ba^{2+} current was measured as the Cd^{2+}-inhibitable sustained current (25). The example shown in Fig. 6A demonstrates that alkalinization of the external solution increased the window Ba^{2+} current by 33%, whereas acidification inhibited this current by 57%. The sustained current measured in five cells under these conditions at test potentials of -10 and 0 mV was converted to current density and is plotted against pH_o in Fig. 5.
Effects of pH_o on activation and inactivation of L-type Ca^{2+} channels were found (Tables 1 and 2). These results strongly suggest that changes in the pH_i either did not occur in 10 mM HEPES or did not affect the properties of the current we recorded. This implies that the observed changes in the voltage-dependent characteristics during external acidification or alkalinization were entirely caused by the effects of external protons on L-type Ca^{2+} channels.

Apparent voltage dependence of the effect of pH_o is due to the shift in activation gating. As noted above, the effect of pH_o on Ca^{2+} channel currents was more prominent at negative than at positive potentials. A comparison of the pH_o dependency of Ca^{2+} (1.5 mM Ca^{2+}) and Ba^{2+} (10 mM Ba^{2+}) current ratios at −10 and +10 mV is presented in Fig. 7 (left). Ca^{2+} and Ba^{2+} currents were clearly more pH_o dependent at −10 than at +10 mV. This is unlikely to be due to differences in the concentration of divalent cations at the membrane surface (see below), since the surface potentials at −10 and +10 mV should be the same for any given pH_o. One possible explanation could be that the current amplitude at any one potential would be influenced by the pH_o-mediated shifts in current activation that we have described above (effects on current availability with a holding potential of −60 mV were small enough to be ignored). To examine this possibility, we divided Ca^{2+} or Ba^{2+} current at −10 mV by the fraction of Ca^{2+}

Role of pH_i. Although the pipette solution contained 10 mM HEPES to buffer pH_i, it is possible that the changes in pH_i were causing corresponding alterations in pH_i that contributed to the observed effects of different pH_o on the Ca^{2+} channel current. To evaluate this possibility, the concentration of HEPES in the pipette solution was reduced to 80 mM accordingly. Effects of pH_o 6.2, 7.2, and 8.2 on activation and inactivation of Ba^{2+} current in 10 mM Ba^{2+} were then compared using the same experimental approach described above. Acidification of the external solution to 6.2 decreased the peak Ba^{2+} current amplitude by 46 ± 7% (n = 4), and alkalinization increased the peak current by 38 ± 3% (n = 6; data not shown). These values were not significantly different from those measured with 10 mM HEPES in the pipette solution (54 ± 2 and 42 ± 7%; n = 10 and 8, respectively). In addition, similar shifts in activation and inactivation dependencies were found (Tables 1 and 2). These results strongly suggest that changes in the pH_i either did not occur in

Fig. 6A. I_{Ba} (10 mM) measured using 30-s steps to −10 mV from the holding potential of −60 mV at pH_o 6.2, 7.2, and 8.2 and in the presence of 0.5 mM Cd^{2+}. Cell capacitance = 32 pF. B: window I_{Ba} (measured as Cd^{2+}-sensitive current at the end of 30-s voltage step and presented as current densities) recorded at test potentials of −10 and 0 mV at pH_o 6.2, 7.2, and 8.2. Each symbol represents a different HMA cell. Solid lines connect mean values calculated for each pH_o.
channels activated at this potential (calculated using Eq. 2). This was done at each pHo tested. The resulting currents thus adjusted for activation or inactivation were then expressed as a fraction of the adjusted current at pHo 7.2. This process was then repeated for both currents at +10 mV. The resulting corrected pHo dependencies for Ca2+ and Ba2+ currents are shown in Fig. 7 (right) and represent effects of pHo on the current that were independent of shifts in current activation. This correction resulted in the pHo dependencies of the current at −10 and +10 mV becoming almost superimposable (Fig. 7, right). Once corrected as described, the pHo dependency of the current amplitude at various potentials followed closely that shown in Fig. 4 for the peak current. Similar results were also obtained from the analysis of Ca2+ and Ba2+ recorded in 10 mM Ca2+ and 1.5 mM Ba2+, respectively (not shown). These results therefore suggest that the apparent potential dependence of the effect of pHo on Ca2+ and Ba2+ current amplitudes is mainly due to the shift in current activation.

**Role of the surface potential in the effects of pHo on Ca2+ channel currents.** It is generally accepted that the shift in I-V relationships for voltage-gated Ca2+ channels, caused by changing the extracellular concentration of divalent cations or protons, is due to the interaction of these cations with negative membrane surface charges that form a surface potential in the vicinity of the channel (6, 7, 20, 31). Increasing the concentration of protons, for example, results in a decline of the surface potential due to binding to and/or screening of negative surface charges. This leads to an increased transmembrane potential (as the potential between the bulk extra- and intracellular solutions remains the same) and, as a result, a shift of activation and inactivation dependencies toward more-positive voltages.

Another theoretically predicted effect of decreasing or increasing the surface potential is an alteration of the local concentration of divalent cations near the Ca2+ channel mouth. This would therefore affect the amplitude of the Ca2+ channel current carried by these cations. As previously described (20), there is an exponential relationship between the surface membrane divalent cation concentration (SC) and the surface potential (ϕo)

\[
S_C = C_C \cdot \exp \left(\frac{-zF\phi_o}{RT}\right) \quad (3)
\]

where z is the valency (equal to 2), CC is the bulk concentration of divalent cation, and F, R, and T have their usual thermodynamic definitions. As reported previously (20), Vm or Vh can be chosen as a measure of changes of ϕo. In case of shifts in Ca2+ channel activation, \(\phi_o = V_{m} + B\), where B is a constant (20). If the peak amplitude of Ca2+ and Ba2+ currents at different pHo shown in Fig. 4 is directly related to the SC, Eq. 3 predicts a linear relationship between the shift in the voltage-dependent characteristics of L-type Ca2+ channels and changes in the logarithm of the peak current amplitude with a slope of \(RT/2F\), equal to 25 mV. Figure 8 shows semilogarithmic plots of the relationship between the peak current amplitude and its relative shifts in activation and inactivation for each of the four solutions used. The line in each panel depicts the exponential relationship predicted by Eq. 3, and it is apparent that, in general, most of the data points lay very close to this line.

Do these changes in the current amplitude correlate with the changes in SC predicted to occur as a result of pHo modulation of ϕo? Because the absolute value of ϕo is unknown, it is impossible to calculate SC directly by Eq. 3 at each particular pHo (20). However, it is possible to calculate relative changes with respect to a particular pHo (e.g., 7.2). In this case, Eq. 3 becomes

\[
\frac{S_{C,\text{pH}_o}}{S_{C,7.2}} = 1 \cdot \exp \left(\frac{-zF(V_{m,\text{pH}_o} - V_{m,7.2})}{RT}\right) \quad (4)
\]

Because CC at each tested pHo and 7.2 is the same, its ratio will be equal to 1. Figure 9 illustrates the mean changes in SC, normalized relative to pHo 7.2, based on the shift in activation calculated in each cell and inactivation, for each of the four conditions tested. The
mean changes in the peak current amplitude at different pHo, again normalized relative to pHo 7.2, are also shown. It is apparent from Fig. 9 that the peak current amplitude during acidification and alkalinization conforms closely to that which would be predicted on the basis of pHo-mediated changes in φo and resulting alterations in S_C.

**DISCUSSION**

These experiments provide the first detailed measurements of the effect of changing pHo on the vascular smooth muscle L-type Ca2+ channel in the presence of a physiological concentration of Ca2+ (1.5 mM). The results suggest that in the presence of a normal or raised (10 mM) concentration of external Ca2+, as well as in the same concentrations of Ba2+, the degree of inhibition or enhancement of the Ca2+ channel current during acidification or alkalinization in HMA cells can be mainly explained in terms of the effect of protons on the surface membrane potential.

As described by the Gouy-Chapman equation (10, 11, 20), an increase in the proton concentration should screen negative membrane surface charges in the vicinity of the Ca2+ channel, resulting in a decrease in the surface potential and a consequent increase in the genuine transmembrane potential (since the potential between the bulk extra- and intracellular solutions is clamped). This would decrease the number of open Ca2+ channels in the physiological range of membrane potentials because of the shift of the activation to the right along the voltage axis (a “stabilizing” effect of protons on the cell membrane). Channel inactivation would also be shifted in a depolarizing direction. Opposite effects on gating would be expected for alkalinization. As summarized in Tables 1 and 2, alteration of pH shifted the activation and inactivation curves in HMA cells as expected in each of the four solutions examined. The magnitude of the shifts was comparable to those measured previously in a number of types of cells, including other vascular smooth muscles (14, 17, 28, 33).

This type of shift in gating parameters should not greatly affect the amplitude of the current at the peak of the I-V curve, since this should be little affected by shifts in activation gating and since steady-state inactivation at a holding potential of −60 mV is almost pHo insensitive (Fig. 5). However, in addition to modifying the voltage dependency of channel gating, acidification-mediated diminution of the surface potential should also reduce the concentration of divalent cations near the extracellular channel mouth, depressing the current amplitude (3, 9). This phenomenon has been visualized as a reduction in the amplitude of the unitary current on the single Ca2+ channel level, in, for example, guinea pig ventricular (29) and bovine and porcine vascular myocytes (14). Similarly, acidification of the trans-chamber, which corresponds to the external side, reduced the unitary conductance of the cardiac and skeletal sarcoplasmic reticulum Ca2+ release channels incorporated into planar lipid bilayers (24).

The extent of this reduction in the divalent cation concentration at the external surface of the membrane can be predicted using the shift in gating parameters, as described by Eq. 4, and assuming also that the channel mouth and the gating mechanism “see” similar surface potentials. If it is assumed that the current amplitude is linearly related to the surface concentration of divalent cation, it is possible to compare the observed effects of pHo on peak current amplitude with the effects that are expected on the basis of surface potential-mediated changes in the surface divalent cation concentration. As illustrated in Fig. 9, a generally good correlation between changes in peak current amplitude and the predicted changes in the surface concentration near the channel mouth was found for both concentrations of Ca2+ and Ba2+ tested. This result is analogous to the similarity between the observed change in the unitary channel conductance and the surface divalent cation concentration calculated from the shift in activation dependencies that was previously found in bovine pial and porcine coronary arterial cells (14).

An alternative explanation for the effect of pHo on Ca2+ channel currents is that a competition between protons and divalent cations for binding to negatively charged sites near or within the channel pore would
directly or indirectly block the movement of Ca\(^{2+}\) through the channel. However, most of the reports that have considered this type of interaction have concluded that significant alterations in Ca\(^{2+}\) permeation would not occur unless pH\(_o\) is decreased to <6 or unless highly artificial conditions are employed (e.g., Ca\(^{2+}\)-free conditions in which a monovalent cation is used as the permeant species) (14, 17, 21–23, 33). On the other hand, Klöckner et al. (16) more recently observed a progressive decrease in the conductance of the human cardiac L-type channel at pH\(_o\) > 6 when 10 mM Ba\(^{2+}\) was used as the charge carrier, which was abolished by replacement with alanine of one of the array of four glutamate residues thought to constitute the intrapore Ca\(^{2+}\) selectivity filter (32). This study (16) therefore suggested that a direct effect of protons on the permeation mechanism might occur with physiological levels of pH\(_i\) and extracellular Ca\(^{2+}\) concentration.

It is unlikely, however, that this type of interaction can explain the effects we have demonstrated in HMA cells, at least in the pH\(_o\) range of 6.2–9.2. The most striking finding of this study is that the relationship between pH\(_i\) and the amplitude of the Ca\(^{2+}\) channel current at the peak of the I-V curve was very similar in Ca\(^{2+}\) and Ba\(^{2+}\) and at 1.5 and 10 mM. Under conditions in which a competition between protons and divalent cations is influencing the permeation mechanism, the effect of pH\(_o\) on channel conductance should be sensitive to the identity and concentration of the divalent cation. The affinity of Ba\(^{2+}\) for the binding sites within the L-type channel that govern permeation is lower than that of Ca\(^{2+}\) (3, 9), and protons should be less able to competitively displace Ca\(^{2+}\) and Ba\(^{2+}\) from common binding sites when these divalent cations are present at higher concentrations. Thus, if protons compete for binding sites related to the permeation mechanism, it would be predicted that the effect of pH\(_o\) on the peak current amplitude should be larger in Ba\(^{2+}\) than in Ca\(^{2+}\) and should also be larger at the lower concentration of both ions. Instead, the effect of pH\(_o\) was similar, even when the extreme cases of 1.5 mM Ba\(^{2+}\) and 10 mM Ca\(^{2+}\) were compared. This finding is therefore consistent with the previous suggestions that a competition between protons and Ca\(^{2+}\) for the permeation pathway does no make an important contribution to the effect of pH\(_o\) on the Ca\(^{2+}\) current under normal conditions. By the same token, this invariance of the effects of pH\(_o\) on the current through L-type Ca\(^{2+}\) channels under all conditions tested further suggests that pH\(_i\)-dependent changes in the concentrations of divalent cation at the outer mouth of the channel are likely to be due to screening of the surface potential rather than a competition for binding sites.

In summary, our overall results suggest that the inhibition or enhancement of Ca\(^{2+}\) channel currents during extracellular acidification or alkalization in HMA myocytes occurs mainly via alteration of the membrane surface potential, resulting in a shift in potential-dependent channel gating and in changes in the concentration of divalent cation near the external Ca\(^{2+}\) channel mouth.

The proposed explanation of the effects of pH\(_o\) on the Ca\(^{2+}\) channel currents in HMA myocytes can be directly related to the regulation of vascular tone in general, since the mechanism of action of pH\(_o\) described above reflects a common feature of Ca\(^{2+}\) channels in a number of different tissues. It has been shown that Ca\(^{2+}\) channels are slightly activated at the resting membrane potential in vascular SMCs and that this activation is likely to play a role in the regulation of vascular tone by vasoconstrictors and vasodilators (19). We previously described in HMA cells a window current that could contribute to a steady-state Ca\(^{2+}\) influx over the physiological range of the membrane potentials (25). The results of the direct measurement of the effect of pH\(_o\) on the nonactivating window Ba\(^{2+}\) current through L-type Ca\(^{2+}\) channels showed that this occurs in HMA myocytes (Fig. 6). An analogous effect on the window Ca\(^{2+}\) current would be also predicted, since a similar pH\(_o\) dependence of the peak amplitude of Ca\(^{2+}\) current and significant shifts in its activation dependencies were observed. In this case, even a quite small effect of pH\(_o\) on the voltage dependence of current activation that we have measured will lead to marked alterations in Ca\(^{2+}\) influx.

Despite the well-known fact that acidosis causes vasodilatation and alkalosis causes vasoconstriction in isolated arteries and also in vivo (1), controversy exists as to whether these effects are due to changes in pH\(_o\) or in pH\(_i\). Under our experimental conditions, the effects of pH\(_o\) were independent of the changes of pH\(_i\), as our results with 10 and 100 mM HEPES in the pipette solution demonstrated. In intact vessels, however, effects on pH\(_i\) associated with changes in pH\(_o\) (4) could also have an important impact on the overall effects of acidification and alkalization. It has been recently shown that intracellular acidification also suppresses the Ca\(^{2+}\) current in vascular SMCs, largely by reducing channel availability (15). It is interesting, however, that Ino et al. (12) showed a marked suppression of the voltage-gated Ca\(^{2+}\) current in rabbit portal vein cells during intracellular acidification with propionate and, at the same time, reported that high K\(^{+}\) depolarization-mediated increases in intracellular Ca\(^{2+}\) concentration and tension development in the intact tissue are paradoxically enhanced under these conditions (12). Tian and co-workers (27) showed that reduction of pH\(_i\) under conditions where pH\(_o\) was kept constant caused vasodilatation. Furthermore, although application of the sodium salts of the weak acids lactate and butyrate caused vasodilatation of rat mesenteric resistance arteries, this response persisted even if the resulting intracellular acidification was prevented by simultaneous addition of ammonium chloride to the solution (2, 18). These observations point to a predominant role of pH\(_o\) rather than pH\(_i\) in mediating the vasodilating effects of acidosis. In agreement with this possibility, the present results and, in particular, the steep dependency on pH\(_o\) of the Ca\(^{2+}\) channel window current (Fig. 6) indicate that physiologically relevant levels of acidification are likely to result in significant inhibition of Ca\(^{2+}\) influx and a resulting vasodilata-
tion. The relative contribution of pH\textsubscript{i} vs. pH\textsubscript{o} to the control of vascular tone during acidification and alkalinization remains, therefore, a subject worthy of additional investigation.

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