Calcium Channel Current of Vascular Smooth Muscle Cells: Extracellular Protons Modulate Gating and Single Channel Conductance

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ABSTRACT Modulation of L-type Ca\textsuperscript{2+} channel current by extracellular pH (pH\textsubscript{o}) was studied in vascular smooth muscle cells from bovine pial and porcine coronary arteries. Relative to pH 7.4, alkaline pH reversibly increased and acidic pH reduced \(I_{\text{Ca}}\). The efficacy of pH\textsubscript{o} in modulating \(I_{\text{Ca}}\) was reduced when the concentration of the charge carrier was elevated ([Ca\textsuperscript{2+}]\textsubscript{o} or [Ba\textsuperscript{2+}]\textsubscript{o} varied between 2 and 110 mM). Analysis of whole cell and single Ca\textsuperscript{2+} channel currents suggested that more acidic pH\textsubscript{o} values shift the voltage-dependent gating (~15 mV per pH-unit) and reduce the single Ca\textsuperscript{2+} channel conductance \(g_{\text{Ca}}\) due to screening of negative surface charges. pH\textsubscript{o} effects on \(g_{\text{Ca}}\) depended on the pipette [Ba\textsuperscript{2+}] ([Ba\textsuperscript{2+}]\textsubscript{p}), pK\textsuperscript{*}, the pH providing 50% of saturating conductance, increased with [Ba\textsuperscript{2+}]\textsubscript{p} according to 
\[pK^* = 2.7-2\log([\text{Ba}^{2+}]_p)\] suggesting that protons and Ba\textsuperscript{2+} ions compete for a binding site that modulates \(g_{\text{Ca}}\). The above mechanisms are discussed in respect to their importance for Ca\textsuperscript{2+} influx and vasotonus.

INTRODUCTION
Extracellular pH (pH\textsubscript{o}) regulates blood flow through the contractile state of vascular smooth muscle cells (vsm). Vasodilation by pH\textsubscript{o} < 7.4 (acidosis) and vasoconstriction for pH\textsubscript{o} > 7.4 (alkalosis) has been reported for a variety of vessels (Rooke and Sparkes, 1981; Dacey and Duling, 1982; Wahl, 1985). Vasoconstriction and -dilation have been attributed to the influx of extracellular Ca\textsuperscript{2+}, thought to be stimulated by alkalosis and reduced by acidosis (Betz and Csornai, 1978; Omote, Takizawa, Nagao, Nosoka, and Nakajima, 1981; Harder and Madden, 1986). This 'pH-hypothesis' was supported by voltage clamp experiments that showed that pH\textsubscript{o} 6 reduces the Ca\textsuperscript{2+} inward current (\(I_{\text{Ca}}\)) not only in cardiac (Vogel and Sperelakis, 1977; Kohlhardt, Haap, and Figulla, 1976; Irisawa and Sato, 1986; Tytgat, Nilius, and Carmeliet, 1990) but also in vascular myocytes (KLÖCKNER and ISENBERG, 1988; West, Leppla, and Simard, 1992). In this study, we analyze two mechanisms by which pH\textsubscript{o} can modulate \(I_{\text{Ca}}\): a pH\textsubscript{o} effect on voltage dependent channel gating, and an effect on single
Ca²⁺ channel conductance. In the following paper (Klöckner and Isenberg, 1994), we present evidence that changes in pH₀ also induce changes in intracellular pH (pHi) and in the availability of the Ca²⁺ channel to open.

The vsm isolated from bovine pial arteries are excitable, and their contractions strongly dependent on [Ca²⁺]₀ (Hirst and Edwards, 1989; our unpublished observations). Vsm from porcine coronary arteries are nonexcitable and contraction is less dependent on [Ca²⁺]₀ (Ito, Kitamura, and Kuriyama, 1979). Despite those general differences, we find that pH₀-modulation of I_Ca is very similar in both types of cells. In both types of vsm peak I_Ca is low (−20 pA to −30 pA in 2 mM [Ca²⁺]₀), due to both small surface area (membrane capacitance between 10 and 20 pF) and small density of Ca²⁺ channels (1.2 to 1.6 pA/pF). The small amplitude of I_Ca limited the analysis of the reduction of I_Ca by acidosis. When, therefore, I_Ca was enlarged by elevation of [Ca²⁺]₀ to 3.6, 10, or 20 mM, we discovered an antagonism between H⁺ and Ca²⁺ (or Ba²⁺) in regard to I_Ca and its single channel conductance.

Part of this work has been presented in abstract form (Klöckner and Isenberg, 1988).

MATERIALS AND METHODS

Cells and Solutions

Pial vsm: fresh bovine brains were obtained from the local slaughterhouse and transported in cold (4°C) bath solution to the laboratory. Arteries with a diameter between 0.2 and 2 mm were isolated, sliced and chopped into 2 × 2 mm pieces. Coronary vsm: the proximal part of the right and left coronary artery was taken out of porcine hearts at the slaughterhouse. In the laboratory, the vessels were sliced and the intima was removed. The muscularis was separated from the adventitia, then the tissue was chopped into chunks of ~ 2 × 2 mm. The cell isolation was performed at 36°C according to Klöckner and Isenberg (1985). The tissue chunks were stirred for six 5-min periods in 25 ml of a nominally “Ca-free solution” which contained (in millimolar) 90 NaCl, 1.2 KH₂PO₄, 5 MgCl₂, 20 glucose, 50 taurine, 5 HEPES/NaOH (pH 7.1). Then, the chunks were incubated in calcium-free solution complemented with collagenase (2 mg per ml, C0130, Sigma Chemical Co., St. Louis, MO) and protease (0.5 mg per ml, P4630, Sigma Chemical Co.). The enzyme treatment delivered only broken material within the first incubation period (30 min) but numerous elongated cells after 60 and 90 min. The cells were harvested in the supernatant and suspended for storage at 4°C in a “KB-medium” containing (in millimolar) 85 KCl, 5 MgSO₄, 5 Na₃ATP, 0.2 EGTA, 5 Na-pyruvate, 5 succinate, 5 creatine, 20 glucose, 20 taurine, 1 g/l fatty acid free albumin, 30 K₂HPO₄/KOH (pH 7.4).

All whole-cell experiments were performed at 36°C. In a 200 µl chamber, the cells were continuously superfused with a prewarmed bathing solution containing (in millimolar) 150 NaCl, 5.4 KCl, 3.6 CaCl₂, 1.2 MgCl₂, 10 glucose, 5 HEPES/NaOH (pH 7.4). Changes in the concentration of CaCl₂ or BaCl₂ are indicated in the text. For solutions with pH₀ more acidic or alkaline than pH 7.4, MES, HEPES, and TRIS buffers were used according to their pKₐ values. Electrodes with fire-polished tips of ~2-µm internal diameter were used. They had an electrical resistance of ~3 MΩ when they were filled with an electrode solution composed of (in millimolar) 130 CsCl, 5 Na-pyruvate, 5 Cs-oxalacetate, 5 Cs-succinate, 10 EGTA, 10 HEPES/CsOH (pH 7.4). Liquid junction potentials of ~8 mV (compare Fenwick, Marty, and Neher, 1982) were independent of the pH and subtracted.

An EPC7 patch-clamp amplifier (List electronics, Darmstadt, Germany) was connected to a PDP 11-73 minicomputer (Digital Equipment Corp., Marlboro, MA) that generated the pulse
protocol, digitized the recorded membrane currents (1,024 points of 12 bit resolution) and stored them. Data was not corrected for leakage and capacitive currents. Leakage currents were estimated from hyperpolarizing steps to -100 mV, typically they were <5 pA, when leakage current was >20 pA, experiments were discarded.

**Definition of I_{ca}**

At 36°C, the cells had resting potentials between -55 and -68 mV (pial vsm) or between -45 and -55 mV (coronary vsm), accordingly holding potentials of -65 mV were used. Voltage-clamp depolarizations to 0 mV induced both I_{ca} and potassium currents; the latter were blocked by release of Cs+ ions from the patch electrode within 30–60 s. The remaining net negative current wave was identified with I_{ca}. The most negative current surge defined peak I_{ca}, the negative current 170 ms after start of depolarization defined the late I_{ca}. I_{ca} was reversibly blocked by substitution of 3.6 mM [Ca\(^{2+}\)]_o by 3.6 mM [Mg\(^{2+}\)]_o; the difference current sensitive to Ca\(^{2+}\) removal differed from I_{ca} by no more than 5 pA. The voltage-dependence of steady state inactivation was evaluated from the influence of a 170-ms prepulse (varied between -90 and +30 mV) on peak I_{ca} during the test pulse (+10 mV).

We suggest that in these experiments I_{ca} is mostly of the L-type. This idea is supported by: (a) Substitution of 3.6 mM [Ca\(^{2+}\)]_o by 3.6 mM [Ba\(^{2+}\)]_o increased peak I_{ca} to 185 ± 12% and retarded the decay (five coronary and four pial vsm). (b) Exposure to 1 \mu M BAY K 8644 doubled peak I_{ca}. (c) Organic Ca\(^{2+}\) channel blockers suppressed I_{ca} by more than 85% (four coronary and four pial vsm treated with 0.1 \mu M nitrendipine or 1 \mu M D600, respectively). (d) Addition of 50 \mu M nickel, known to block T-type I_{ca} (Bean, 1985), did not reduce I_{ca} (n = 12). (e) Changing the holding potential from -60 to -95 mV did not significantly increase I_{ca} (n = 18). (f) Single channel currents (110 mM Ba\(^{2+}\) as charge carrier) could be attributed to a 24 pS channel (n = 54) which is representative for the L-type Ca\(^{2+}\) channel (see for references Pelzer, Pelzer, and McDonald, 1990). No currents through an 8 pS T-type Ca\(^{2+}\) channel were recorded.

The run-down of whole-cell I_{ca} limited the period of stable I_{ca} recordings to <15 min. Within such a period the effects of only 3–5 pHo changes could be studied. Therefore, data from different cells were normalized and pooled and the evaluation was focused on the pHo induced relative changes. At 3.6 mM [Ca\(^{2+}\)]_o average peak I_{ca} was -35 ± 15 pA in coronary vsm (n = 20) and -20 ± 10 pA in pial vsm (n = 18).

Single channel recordings were performed in the cell-attached configuration. The membrane potential was zeroed by superfusing a Ca-free high-potassium medium composed of (in mM) 85 K-glutamate, 40 KCl, 10 EGTA, 10 HEPES/KOH (pH 7.4). The patch pipettes were filled with either 110 mM BaCl\(_2\) solution or with a solution containing x mM BaCl\(_2\) plus (110 – x) mM NaCl (pH 7.4 by 10 mM HEPES/KOH). The pipette solution contained 1 \mu M BAY K 8644. In some experiments, the pH of the pipette solution (pH\(_p\)) was varied between 5 and 9. The single channel currents were filtered at 1 kHz, digitized at 5 kHz and stored for off-line analysis. Leakage and capacitive currents were corrected by subtracting blank records. Data are presented as mean values ± SEM.

**RESULTS**

**pHo Modulation of Peak I_{ca}**

Reduction of pHo from 7.4 to 6.4 decreased peak I_{ca} and late I_{ca} (Fig. 1 C, Fig. 2 A). At 3.6 mM CaCl\(_2\), the change to pHo 6.4 reduced average peak I_{ca} to 26 ± 6% (mean from eight coronary and seven pial vsm). pHo 6.4 almost suppressed late I_{ca} (6 ± 4% of control). Extracellular alkalosis increased peak and late I_{ca}, both in coronary (Fig.
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A) and pial vsm (Fig. 1 B). At 3.6 mM [Ca\(^{2+}\)\(_o\)], peak \(I_{Ca}\) was increased to 190 ± 65% in coronary vsm (n = 10) and to 180 ± 60% in pial vsm (n = 12). Late \(I_{Ca}\) was increased by a factor of 2.35 ± 1.05. When control peak \(I_{Ca}\) at pH\(_o\) 7.4 was smaller than -10 pA, the increase by alkalosis could be more than fourfold.

Fig. 2 A shows for 20 mM [Ca\(^{2+}\)\(_o\)] that reduction of \(I_{Ca}\) by acidic pH\(_o\) is graded and reversible. The reduction of \(I_{Ca}\) stabilized within ~ 20 s which is the time for complete solution change. Upon return to pH\(_o\) 7.4 peak \(I_{Ca}\) recovered with a similar time course. If recovery was incomplete (90% in Fig. 2 A) this was attributed to the rundown of \(I_{Ca}\) as it also occurs without the pH\(_o\) interventions. Similar conclusions can be drawn from results with more alkaline solutions: the increase in \(I_{Ca}\) completed within 20–30 s, sustained as long as the pH\(_o\) was more alkaline, and was reversed upon return to control pH\(_o\) of 7.4.

The dependence of peak \(I_{Ca}\) on pH\(_o\) is shown in Fig. 2 B, which summarizes results from 10 coronary and 8 pial vsm in bath solutions with 20 mM [Ca\(^{2+}\)\(_o\)]. For averaging, peak \(I_{Ca}(pH\(_o\))\) were normalized to their respective control peak \(I_{Ca}(pH\(_o\) 7.4). Fig. 2 B shows a 50% reduction of peak \(I_{Ca}(pH\(_o\) 7.4)\) at pH\(_o\) 6.4 and a total block of \(I_{Ca}\) at pH\(_o\) 5.0 or lower. Alkaline pH\(_o\) 8.4 increased \(I_{Ca}\) to 128%, on average. pH\(_o\) more alkaline than 8.4 damaged the cell, judged by a loss of optical density and a leakiness of the seal. The averaged data could be formally described according to

\[
I_{Ca}(pH)/I_{Ca}(pH\(_o\) 7.4) = 1.44/[1 + 10^{pK'-pH\(_o\)]}. \tag{1}
\]

Eq. 1 shows that peak \(I_{Ca}\) is sensitive to pH\(_o\) over a wide range (low Hill coefficient of
For alkaline solutions, Eq. 1 suggests that $I_{Ca}$ can maximally increase by a factor of 1.44 when it is studied at 20 mM $[Ca^{2+}]_o$ and with clamp-steps to $+10$ mV. Eq. 1 further suggests an apparent pK' value of 6.9 for half maximal $I_{Ca}$. In Fig. 2 B, the 50% reduction of $I_{Ca}(pH \, 7.4)$ is not reached at pK' but at 6.4 because $I_{Ca}(pH \, 7.4)$ is lower than maximal $I_{Ca}$ at alkaline pH.o.

\[ A \]
\[ pH \, 7.4 \quad 7.1 \quad 6.8 \quad pH \, 6.4 \quad pH \, 7.4 \]

\[ B \]
\[ pH \]

**Figure 2.** Modulation of $I_{Ca}$ by pH.o is graded and reversible. 20 mM $[Ca^{2+}]_o$. 170-ms steps from $-65$ to $+5$ mV at 0.1 Hz. (A) Pial vsm, time course of the changes shown by a computer play-back of $I_{Ca}$ (the intervals between the pulses are removed). (B) Normalized peak $I_{Ca}$ as function of pH.o (mean ± SEM, n = 10 coronary and 8 pial vsm). Data $I_{Ca}/I_{Ca}(pH \, 7.4)$ were fit by $1/[1 + 10^{(pK-pH\,o)/n}]$ with an apparent pK value of 6.9 and a Hill-coefficient $n = 0.63$. Note: the efficacy of pH.o is higher than that at 20 mM when 3.6 or 1.5 mM $[Ca^{2+}]_o$ are used.

The efficacy of pH.o on peak $I_{Ca}$ decreased with the concentration of extracellular Ca$^{2+}$ or Ba$^{2+}$ ions. For example, pH.o 8.4 increased $I_{Ca}$ by a factor of 3.7 at 1.5 mM $[Ca^{2+}]_o$, 2.85 at 3.6 mM and 1.38 at 20 mM $[Ca^{2+}]_o$. Acidic pH.o 6.4 reduced peak $I_{Ca}$ by 80 ± 5% ($n = 4$) at 1.5 mM $[Ca^{2+}]_o$, by 70 ± 4% ($n = 12$) at 3.6 mM, and by 50 ± 6% ($n = 16$) at 20 mM. Thus, elevated Ca$^{2+}$ or Ba$^{2+}$ concentrations attenuated the
efficacy of protons on peak $I_{\text{Ca}}$, i.e., a larger pHo change was necessary for a similar reduction of $I_{\text{Ca}}$. This idea is supported by the estimates for 50% reduction of $I_{\text{Ca}}$ caused by more acidic pHo; this pHo was 7.0 in the absence of $[\text{Ca}^{2+}]_o$ (Na-currents through Ca$^{2+}$ channels), 6.8 at 3.6 mM $[\text{Ca}^{2+}]_o$, and 6.4 at 20 mM $[\text{Ca}^{2+}]_o$. The results suggest that pH-modulation of $I_{\text{Ca}}$ is much stronger at physiological 1.5 mM $[\text{Ca}^{2+}]_o$ than indicated by measurements in media with elevated Ba$^{2+}$ or Ca$^{2+}$ content.

**pHo Shifts the Voltage-dependent Gating Parameter**

Fig. 3 shows a series of peak $I_{\text{Ca}}$-voltage curves measured in pHo 6.4, 7.4, and 8.4 in a pial vsm (3.6 mM $[\text{Ca}^{2+}]_o$, left) and a coronary vsm (20 mM $[\text{Ca}^{2+}]_o$, right). The curves were fitted according to

$$\text{peak } I_{\text{Ca}} = A_p(V, \text{pH}) \cdot G_{\text{max}}(V_p - E_{\text{rev}}),$$

where $A_p$ is an activation parameter, $G_{\text{max}}$ the slope conductance of the ascending branch and $E_{\text{rev}}$ the intersection of this branch with the voltage axis. The voltage-dependence of the activation parameter $A_p$ was fitted with a Boltzmann-formula

$$\text{peak } G(V)/G_{\text{max}} = A_p(V, \text{pH}) = 1/[1 + \exp \{(V - V_h)/k\}].$$

The analysis of a total of 10 peak $I_{\text{Ca}}$-voltage curves suggests the following pHo-effects: (a) The curves are shifted by pHo 8.4 to more negative and by pHo 6.4 to more positive potentials (Table I; see also Fig. 4, right, dashed lines). That is, at pH 8.4 the potential $V_h$ activating half maximal peak $I_{\text{Ca}}$ was 12 ± 6 mV more negative than
at pHo 7.4; whereas at pHo 6.4 Vh was 14 ± 7 mV more positive than at pHo 7.4. (b) 
Erev was almost pHo insensitive, e.g., it was 60 ± 4 mV at pHo 7.4 (20 mM [Ca²⁺]o), 58 ± 7 mV at pHo 6.4 and 64 ± 4 mV at pHo 8.4. These small changes were not significant (compare Krafte and Kass, 1988). (c) Gmax was increased by pHo 8.4 and reduced by pHo 6.4. The efficacy of pHo effect on Gmax decreased when the extracellular Ca²⁺ or Ba²⁺ concentration was elevated.

Division of peak ICa by the pHo-independent driving force (Vp - Erev) and by Gmax yields the normalized activation-variable A_p(V, pH) of Fig. 4, left (dashed line, 3.6 mM [Ca²⁺]o). On average, at pHo 7.4 A_p(V) started from a threshold at -40 mV and reached 50% at Vh = -11 ± 4 mV (n = 21). pHo 6.4 shifted Vh by +13 ± 6 mV (n = 4) and pHo 8.4 by -18 ± 5 mV (n = 7). The slope factor (k = -8 mV) was not significantly influenced by pHo. Fig. 4 (dots and solid lines) shows also the voltage-dependence of steady state inactivation measured and fitted in analogy to Eq. 3. 50% inactivation was found at -32 ± 6 mV for pHo 7.4. pHo 6.4 shifted this potential by 9 ± 5 mV (n = 4) and pHo 8.4 by -10 ± 6 mV (n = 6).

The shifts in the voltage-dependent gating alone do not adequately describe the

| pHo | Vh,act | Vh,act,norm | Erev | Vh,inact | Gmax,norm |
|-----|--------|-------------|------|----------|-----------|
| 6.4 | +1 ± 7 | +2 ± 6      | 58 ± 7 | -23 ± 5 | 52 ± 15   |
| 7.4 | -13 ± 5 | -11 ± 4    | 60 ± 4 | -32 ± 6 | 100       |
| 8.4 | -25 ± 6 | -29 ± 5    | 64 ± 4 | -42 ± 10 | 146 ± 24  |

The values shown are the means ± SD of 6 to 10 cells from pial and coronary arteries. Vh,act indicates the potential of half maximal activation, Vh,act,norm the potential of half maximal activation of the normalized current, Erev the reversal potential, Vh,inact the potential of half maximal inactivation and Gmax,norm the normalized maximal conductance.

pHo modulation of peak ICa. Fig. 3 shows that the slope of the ascending branch of the curves, i.e., the maximal conductance Gmax is pHo-dependent. With Gmax(V, pHo) we extend Eq. 2 to

\[
\text{peak } I_{\text{Ca}}(V, \text{pH}_o) = A_p(V, \text{pH}_o)G_{\text{max}}(\text{pH}_o)(V - E_{\text{rev}}).
\] (4)

The pHo-effect on Gmax is shown more clearly in Fig. 4 where the data of Fig. 3 (left) are plotted as conductance (left) or activation parameter (right). If Gmax was pHo-insensitive, the dashed conductance curves (left) should reach saturation at a constant value as the normalized activation curves do (right). However, the curves reached 215 pS at pHo 8.4, 105 pS at pHo 7.4 and only 43 pS at pHo 6.4 (5.6 mM [Ca²⁺]o). Data from four pial vsm and three coronary vsm in 3.6 mM [Ca²⁺]o indicate a reduction of Gmax to 52 ± 15% for the change from pHo 7.4 to 6.4 and an increase of Gmax to 146 ± 24% for the change to pHo 8.4.
**pH modulation of single channel conductance**

The whole-cell conductance $G_{\text{max}}$ is the product of the number of functional Ca$^{2+}$ channels ($N_F$), the open probability ($P_o$) and the single channel conductance $g_{CA}$

$$G_{\text{max}} = N_F P_o g_{CA}. \quad (5)$$

The possible effects of pH$_o$ on $g_{CA}$ were initially studied with 50 mM BaCl$_2$ as the charge carrier in the patch pipette. At clamp-steps to 0 mV, reduction of pH$_p$ from 7.4 to 5.0 diminished the open channel current from $-1.0$ to $-0.55$ pA (Fig. 5A). The pH$_p$-effect on single channel current was evaluated by amplitude histograms, the results of which were plotted as i-v curves (Fig. 5B). The slope of the i-v curves indicated a single-channel conductance of $g_{CA} = 22 \pm 2$ pS ($n = 5$) for pH$_p$ 7.4. Acidic pH$_p$ reduced $g_{CA}$ to $19 \pm 3$ pS ($n = 4$) at pH$_p$ 6.4 and to $11 \pm 2$ pS ($n = 4$) at pH$_p$ 5.0. The more alkaline pH$_p$ 8.4 had little effect, i.e., $g_{CA}$ was $23 \pm 4$ pS ($n = 5$).

The effect of pH$_p$ on $g_{CA}$ was studied at a variety of [Ba$^{2+}$]$_i$ (2, 5, 10, 20, 50, and 110 mM, seven measurements cell attached, six outside-out). At control pH$_p$ 7.4, $g_{CA}$ increased with [Ba$^{2+}$]$_i$ up to saturation ($g_{\text{sat}} = 24$ pS) according to

$$g = g_{\text{sat}} /[1 + K_D/[\text{Ba}^{2+}]_i]. \quad (6)$$

**Figure 4.** Influence of pH$_o$ on the gating parameters. (Left) Steady state inactivation (circles). Peak conductance at +10 mV was modified by 170-ms prepulses to the potential indicated on abscissa. Intersection on left ordinate shows $G_{\text{max}}$. (Right) Normalized inactivation curves ($G/G_{\text{max}}$) fitted with the Boltzmann-curve $h_o(V, \text{pH}_o) = 1/(1 + \exp ([V - V_h]/k))$. $k$ was 10, 10, and 7 mV for pH 8.4, 7.4, and 6.4, respectively. The potential of 50% inactivation ($V_h$) was $-43$ mV (pH 8.4), $-36$ mV (pH 7.4) and $-30$ mV (pH 6.4). Dashed curves show peak conductance (left) and activation parameter $A(V, \text{pH})$ (right). Data from coronary vsm in 20 mM [Ca$^{2+}$]$_o$ (Fig. 3, right) were fitted with a similar Boltzmann-equation as $h_o$. $k$ was $-8$, $-8$, and $-7$ mV for pH 8.4, 7.4, and 6.4, respectively. The potential of half maximal conductance was $-15$ mV at pH$_o$ 8.4, $-10$ mV at pH$_o$ 7.4 and $+4$ mV at pH$_o$ 6.4, respectively.
The half maximal conductance of 12 pS was found at an apparent $K_D$-value of 4.7 mM $[Ba^{2+}]_p$ (Fig. 6A; compare $K_D = 9.6$ mM $Ba^{2+}$ by Ganitkevich, Shuba, and Smirnov, 1988; $K_D = 6$ mM $Ba^{2+}$ by Kuo and Hess, 1993).

Changes in $pH_o$ did not change the $g_{sat}$ of 24 pS, however, they modulated the apparent $K_D$. At alkaline $pH_p$ 8.4 the $K_D$ was 1.3 mM $[Ba^{2+}]_p$ whereas at acidic $pH_p$ 6.4 the $K_D$ was 11 mM $[Ba^{2+}]_p$. Fig. 6D shows the modulation of $K_D$ by $pH$ by a series of curves evaluated for identical $[Ba^{2+}]_p$. If $pK^* = -\log(K_D)$ was used to indicate the $pH$-value that reduced $g$ to 50% of $g_{sat}$, the data could be fitted with:

$$g = g_{sat}/\left[1 + ([H^+]_p/K_D)^{0.5}\right]$$

or

$$g = g_{sat}/\left[1 + 10^{(pK^*-pH_p)^{0.5}}\right].$$

For all curve fits, a $g_{sat}$ of 24 pS and a Hill coefficient of 0.5 could be used. Elevation of $[Ba^{2+}]_p$ increased $K_D$; for example, at 110 mM $[Ba^{2+}]_p$ $pK^*$ was 4.6, at 50 mM $pK^*$ was 5.4, at 10 mM it was 6.7, at 5 mM 7.4 and at 2 mM $[Ba^{2+}]_p$ it was 8. Linear
regression in double logarithmic coordinates yielded the dependence of $pK^*$ on the pipette $Ba^{2+}$ concentrations as

$$pK^* = 2.7 - 2 \log [Ba^{2+}]_p.$$  \hspace{1cm} (9)

**Bath pH Has Effects Beyond Pipette pH**

Although pipette solutions of pH 5.0 reduced the amplitude of the single-channel currents they did not block them (Fig. 5 A). This result contrasts to the complete block of whole cell $I_{Ba}$ that was found when the whole cell was exposed to a bath pH of 5.0 (Fig. 5 C, similar block for steps between −20 and +60 mV). The conditions for whole cell and single-channel recordings were similar: 50 mM $[Ba^{2+}]_o$ as charge carrier, 0.5 μM Bay K 8644 and 22°C. Therefore, the discrepancy suggests that the exposure of a whole cell to a bath pH of 5.0 has effects beyond those that can be attributed to the interaction of protons with the $Ca^{2+}$ channel and its surroundings in the isolated patch. These additional pH effects are attributed to a change in pH$_i$ and are analyzed in the following paper (Klöckner and Isenberg, 1994).

**DISCUSSION**

In this paper, we have studied $I_{Ca}$ in vsm from porcine coronary and bovine pial arteries. The current was interpreted as L-type $I_{Ca}$ because there was no evidence for T-type channels (see Materials and Methods). Our results indicate that peak $I_{Ca}$ was less sensitive to pH$_o$ changes than late $I_{Ca}$, confirming the results of West et al. (1992). However, we do not interpret our results as the “rapidly inactivating component” flowing through a distinct population of “B-channels” that are less pH$_o$ sensitive than the “slowly inactivating component” (Simard, 1991; West et al., 1992).
Ca\textsuperscript{2+} currents through single L-type channels decay along a similar double exponential time course as the whole cell \(I_{Ca}\) does (Klöckner and Isenberg, 1991). The complex kinetic properties of L-type Ca\textsuperscript{2+} channels can explain the preferential pH\textsubscript{e} effect, for example, by a suppression or facilitation of late re-openings of the L-type Ca\textsuperscript{2+} channels (Klöckner and Isenberg, 1994).

This study suggests that pH\textsubscript{e} modulates \(I_{Ca}\) through (a) shifts of the voltage-dependent channel gating, and (b) changes in the single channel conductance. In addition, pH\textsubscript{e} can modify Ca\textsuperscript{2+} channel availability through a change in intracellular pH (following paper, Klöckner and Isenberg, 1994). Proton-induced shifts of the voltage-dependent gating of peak \(I_{Ca}\) and steady state inactivation have been described from a variety of preparations (Iijima, Ciani, and Hagiwara, 1986; Kraftie and Kass, 1988). The extent of the pH\textsubscript{e}-shifts were small in cardiac ventricular myocytes (Irisawa and Sato, 1986) and almost zero in vsm from guinea-pig basilar artery (West et al., 1992). Presumably, the density of the surface charges differs between the preparations. In this study on coronary and pial vsm, the pH\textsubscript{e} induced shifts were significant. The contribution of this shift to the pH\textsubscript{e}-modulation of \(I_{Ca}\) may be illustrated with examples, given firstly for a nonexcitable vsm with a membrane potential of \(-50\) mV. Alkalosis: the more alkaline pH\textsubscript{e} 8.4 shifts the activation parameter to a more negative potential. Due to this shift, a substantial fraction (5%) of Ca\textsuperscript{2+} channels should open at \(-50\) mV (compare Fig. 4, right, dashed line). The simultaneous shift of the steady state inactivation attenuates this current but does not prevent it. The 60% enlargement of \(G_{max}\) further augments the steady Ca\textsuperscript{2+} current at \(-50\) mV (compare Fig. 4, left). The effects on activation, inactivation and \(G_{max}\) predict that the continuous Ca\textsuperscript{2+} influx through L-type channels at \(-50\) mV is seven times higher at pH\textsubscript{e} 8.4 than 7.4. This substantial Ca\textsuperscript{2+} influx could explain why alkalosis can cause sustained contractions of vsm in the absence of electrical or humoral stimuli (McCulloch, Edvinson, and Watt, 1982; Smeda, Lombard, Madden, and Harder, 1987). In the presence of these stimuli, a moderate depolarization would induce more Ca\textsuperscript{2+} influx at alkaline than at neutral pH\textsubscript{e} because of the shift of the activation parameter. Further, the shift of the activation parameter, together with the increase in \(G_{max}\), would facilitate generation of action potentials in the excitable vsm from pial arteries. Acidosis: Acidic pH\textsubscript{e} 6.4 reduces the extent of steady state inactivation at \(-50\) mV, an effect that should facilitate \(I_{Ca}\). On the other side, the simultaneous shift of the activation parameter and the reduction of \(G_{max}\) reduce \(I_{Ca}\). The superimposition of these pH\textsubscript{e} effects can partially compensate each other and explain why peak \(I_{Ca}\) (+5 mV) followed pH\textsubscript{e} with a Hill-coefficient of \(<1\). The shift of activation and the reduction of \(G_{max}\) can explain why acidic pH\textsubscript{e} suppresses the generation of action potentials and the concomitant Ca\textsuperscript{2+} influx in excitable vsm (Smeda et al., 1987).

The ascending part of the peak \(I_{Ca}\) curves (Fig. 3) and the un-normalized steady state inactivation curves (Fig. 4, left) demonstrated that pH\textsubscript{e} modulates \(G_{max}\). pH\textsubscript{e} effects on whole-cell \(G_{max}\) have been described in detail by Iijima et al. (1986). According to Eq. 5, the change in whole-cell \(I_{Ca}\) could result from effects on single channel conductance (\(g_{Ca}\)), open probability (\(P_o\)) and channel availability (\(P_f\)). Separation of the effects on \(g_{Ca}\) and \(P_o\) has been performed on single cardiac T-type
Ca\(^{2+}\) channels (Tytgat et al., 1990). An analysis of pH effects on vascular L-type \(P_o\) and \(P_v\) is provided in the following paper (Klöckner and Isenberg, 1994).

In this paper we have shown that the efficacy of pH\(_o\) in modulating \(g_{Ca}\) depends on the concentration of extracellular Ca\(^{2+}\) or Ba\(^{2+}\) ions. Fig. 6D indicated that \(pK^*\), the pH\(_o\) reducing \(g_{Ca}\) to 50\%, became more acidic with higher [Ba\(^{2+}\)]\(_p\) in proportion to \(-2\log([Ba^{2+}]_p)\). For example, \(g_{Ca} = 12\) pS (50% of saturating \(g_{Ca} = 24\) pS) was found at 5 mM [Ba\(^{2+}\)]\(_p\) with pH\(_o\) 7.4 whereas at 50 mM [Ba\(^{2+}\)]\(_p\) \(pK^*\) was 5.4 (100-fold increase in [H\(^+\)]). These results favor the idea that \(g_{Ca}\) is modulated by a binding site for which two protons and one Ba\(^{2+}\) ion compete.

According to present knowledge, H\(^+\)-Ba\(^{2+}\) competition could affect the single channel conductance through a change of the intra-channel proton block (Kuo and Hess, 1993) or through a change in the local concentration of the charge carrier (Dani, 1986). The hypothesis of a modulated proton block (Kuo and Hess, 1993) postulates that protons deter the entry of Ba\(^{2+}\) ions into the pore, Ca\(^{2+}\), Ba\(^{2+}\) and H\(^+\) ions competing for the same intra-channel high-affinity binding site. The single channel recordings of this study do not favor a blocking mechanism, i.e., the life-time of the open state was essentially pH\(_o\)-independent.

The second hypothesis postulates that negative charges of the channel protein increase the local Ba\(^{2+}\) concentration at the channel mouth beyond the bulk concentration. If we assume that changes in bulk pH\(_o\) protrude into the channel mouth, acidic pH\(_o\) would facilitate protonation of these sites. The consequence would be a lower local Ba\(^{2+}\) concentration and a reduced conductance due to the limited diffusional access of Ba\(^{2+}\) to the pore. The hypothesis is supported by our result that the current reversal potential was essentially insensitive to pH\(_o\) as if the change in the electric field across the membrane would be associated with a proportional change in the concentration of the permeant Ca\(^{2+}\) or Ba\(^{2+}\) ions at the channel mouth. In addition, one can estimate (Ohmori and Yoshii, 1977) from the 14-mV shift in surface potential by the change from pH\(_o\) 7.4 to 6.4 (3.6 mM [Ca\(^{2+}\)]\(_o\)) that the local calcium concentration was lowered by 24\%. This reduction is close to the 26\% reduction of the single channel conductance, observed during the same pH\(_o\) change. However, one should bear in mind that the single channel currents used Ba\(^{2+}\) and the whole cell currents Ca\(^{2+}\) ions as the charge carrier. The dependence of \(pK^*\) on [Ba\(^{2+}\)]\(_o\) or [Ca\(^{2+}\)]\(_o\) suggests that pH\(_o\)-effects can be extrapolated from elevated [Ba\(^{2+}\)]\(_o\) or [Ca\(^{2+}\)]\(_o\) to the physiological 2 mM [Ca\(^{2+}\)]\(_o\) only with care.

In vivo, pH\(_o\) will modulate conductance, gating and channel availability of L-type channels simultaneously. In addition, the nonclamped vsm may hyperpolarize during acidosis (e.g., from −60 to −65 mV for a pH\(_o\) change from 7.4 to 6.6) and depolarize during alkalosis (−52 mV at pH\(_o\) 8.2; Siegel, Kämpe, and Ebeling, 1981). The pH\(_o\)-effect on membrane potential adds to the effect on surface potential. That is, Ca\(^{2+}\) influx through L-type Ca\(^{2+}\) channels, that open between −45 and −65 mV in the absence of action potentials, is more sensitive to pH\(_o\) in the unclamped vsm than indicated by the voltage-clamp results of Figs. 3 and 4.

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