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^{2+} channel current by extracellular pH (pH_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_{Ca}. The efficacy of pH_o in modulating I_{Ca} was reduced when the concentration of the charge carrier was elevated ($[Ca^{2+}]_o$ or $[Ba^{2+}]_o$ varied between 2 and 110 mM). Analysis of whole cell and single Ca^{2+} channel currents suggested that more acidic pH_o values shift the voltage-dependent gating (~15 mV per pH-unit) and reduce the single Ca^{2+} channel conductance g_{Ca} due to screening of negative surface charges. pH_o effects on g_{Ca} depended on the pipette $[Ba^{2+}]$ ($[Ba^{2+}]_p$), pK*, the pH providing 50% of saturating conductance, increased with $[Ba^{2+}]_p$ according to $pK^* = 2.7 - 2 \cdot \log\{[Ba^{2+}]_p\}$ suggesting that protons and Ba^{2+} ions compete for a binding site that modulates g_{Ca}. The above mechanisms are discussed in respect to their importance for Ca^{2+} influx and vasotonus.

INTRODUCTION

Extracellular pH (pH_o) regulates blood flow through the contractile state of vascular smooth muscle cells (vsm). Vasodilation by $pH_o < 7.4$ (acidosis) and vasoconstriction for $pH_o > 7.4$ (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^{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_o 6 reduces the Ca^{2+} inward current (I_{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_o can modulate L-type I_{Ca}: a pH_o effect on voltage dependent channel gating, and an effect on single

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Ca^{2+} channel conductance. In the following paper (Klöckner and Isenberg, 1994), we present evidence that changes in pH_o also induce changes in intracellular pH (pH_i) and in the availability of the Ca^{2+} channel to open.

The vsm isolated from bovine pial arteries are excitable, and their contractions strongly dependent on $[Ca^{2+}]_o$ (Hirst and Edwards, 1989; our unpublished observations). Vsm from porcine coronary arteries are nonexcitable and contraction is less dependent on $[Ca^{2+}]_o$ (Ito, Kitamura, and Kuriyama, 1979). Despite those general differences, we find that pH_o-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 $[Ca^{2+}]_o$), due to both small surface area (membrane capacitance between 10 and 20 pF) and small density of Ca^{2+} 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 $[Ca^{2+}]_o$ to 3.6, 10, or 20 mM, we discovered an antagonism between H^+ and Ca^{2+} (or Ba^{2+}) 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 KH_2PO_4, 5 $MgCl_2$, 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 $MgSO_4$, 5 Na_2ATP, 0.2 EGTA, 5 Na-pyruvate, 5 succinate, 5 creatine, 20 glucose, 20 taurine, 1 g/l fatty acid free albumin, 30 K_2HPO_4/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 $CaCl_2$, 1.2 $MgCl_2$, 10 glucose, 5 HEPES/NaOH (pH 7.4). Changes in the concentration of $CaCl_2$ or $BaCl_2$ are indicated in the text. For solutions with pH_o more acidic or alkaline than pH_o 7.4, MES, HEPES, and TRIS buffers were used according to their pK_a 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 μM 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 μM nitrendipine or 1 μM D600, respectively). (*d*) Addition of 50 μM 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 pH_o changes could be studied. Therefore, data from different cells were normalized and pooled and the evaluation was focused on the pH_o 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 μM 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

pH_o Modulation of Peak I_{Ca}

Reduction of pH_o 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 pH_o 6.4 reduced average peak I_{Ca} to 26 ± 6% (mean from eight coronary and seven pial vsm). pH_o 6.4 almost suppressed late I_{Ca} (6 ± 4% of control). Extracellular alkalosis increased peak and late I_{Ca}, both in coronary (Fig.

1 *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 run down 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.

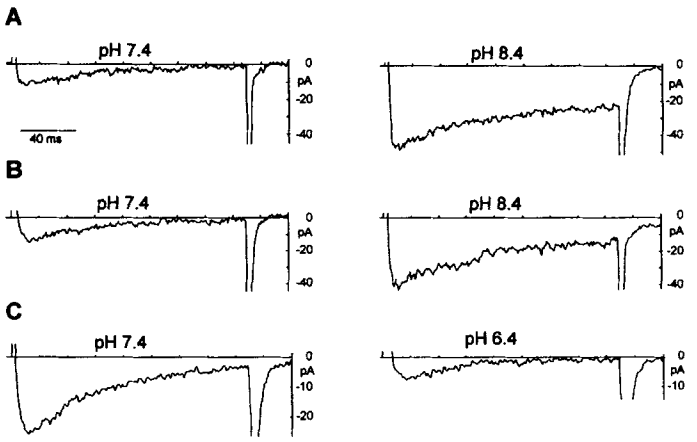

FIGURE 1. Effect of bath pH (pH_o) on peak I_{Ca}. (*A*) Coronary vsm, 3.6 mM $[Ca^{2+}]_o$. At pH_o 7.4 peak I_{Ca} was −15 pA and late I_{Ca} ~ zero. 30 s after the change to pH_o 8.4 (*right*) peak I_{Ca} was −49 pA and late I_{Ca} was −23 pA. (*B*) Pial vsm, 3.6 mM $[Ca^{2+}]_o$. At pH_o 7.4 peak I_{Ca} was −16 and late I_{Ca} was −2 pA. 30 s after the change to pH_o 8.4 (*right*) peak I_{Ca} was −44 pA and late I_{Ca} was −17 pA. (*C*) Pial vsm 1.5 mM $[Ca^{2+}]_o$. Reduction of peak I_{Ca} from −36 to −7 pA by changing pH_o from 7.4 to 6.4. 170-ms depolarizations from −60 to −5 mV were applied at 0.25 Hz.

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

$$I_{Ca}(pH)/I_{Ca}(pH\ 7.4) = 1.44/\{1 + 10^{[pK'-pH]\cdot n}\}. \quad (1)$$

Eq. 1 shows that peak I_{Ca} is sensitive to pH_o over a wide range (low Hill coefficient of

n = 0.63). 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.

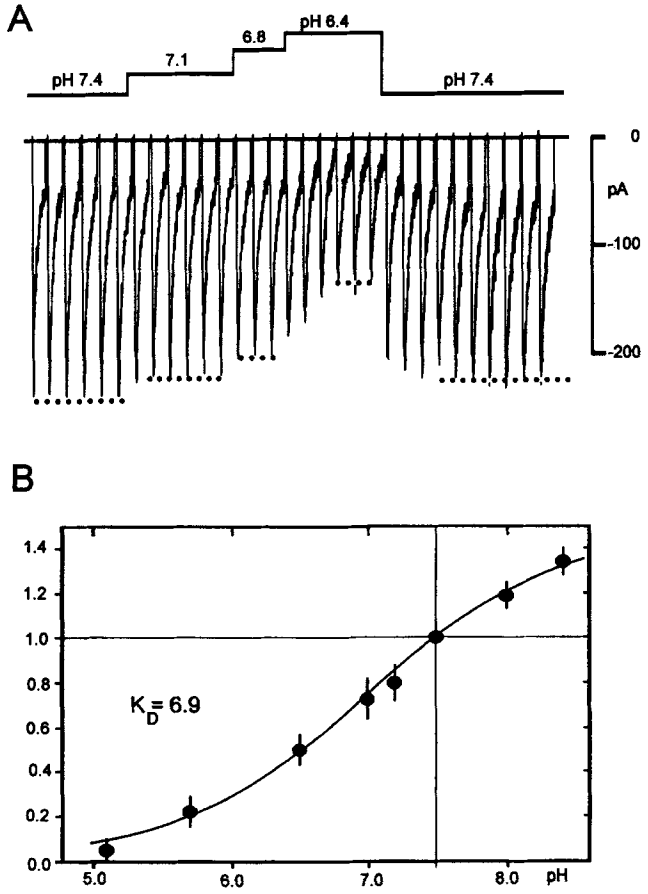

FIGURE 2. Modulation of I_{Ca} by pH_o is graded and reversible. 20 mM $[Ca^{2+}]_o$. 170-ms steps from −65 to +5 mV at 0.1 Hz. (*A*) Pial vsm, time course of the changes shown by a computer play-back of I_{Ca} (the intervals between the pulses are removed). (*B*) Normalized peak I_{Ca} as function of pH_o (mean ± SEM, n = 10 coronary and 8 pial vsm). Data I_{Ca}/I_{Ca}(pH 7.4) were fit by $1/[1 + 10^{(pK-pH)\cdot n}]$ 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_{Ca}, i.e., a larger pH_o change was necessary for a similar reduction of I_{Ca}. This idea is supported by the estimates for 50% reduction of I_{Ca} caused by more acidic pH_o; this pH_o was 7.0 in the absence of $[Ca^{2+}]_o$ (Na-currents through Ca^{2+} channels), 6.8 at 3.6 mM $[Ca^{2+}]_o$, and 6.4 at 20 mM $[Ca^{2+}]_o$. The results suggest that pH-modulation of I_{Ca} is much stronger at physiologial 1.5 mM $[Ca^{2+}]_o$ than indicated by measurements in media with elevated Ba^{2+} or Ca^{2+} content.

pH_o Shifts the Voltage-dependent Gating Parameter

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

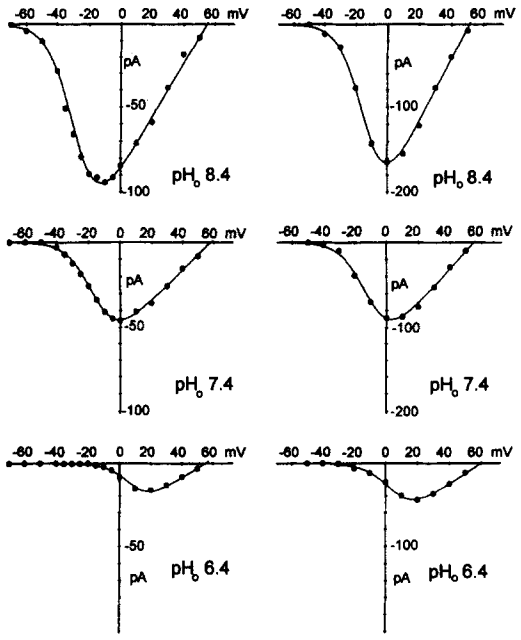

FIGURE 3. pH_o modulates the voltage-dependence of peak I_{Ca}. 170-ms pulses from −65 mV to the indicated potentials at a rate of 0.25 Hz. (*Left*) Pial vsm at 3.6 mM $[Ca^{2+}]_o$; (*right*) coronary vsm at 20 mM $[Ca^{2+}]_o$. The fitted curves were calculated by peak $I_{Ca} = A(V, pH_o) \cdot G_{max}(pH_o) \cdot (V - E_{rev})$. The maximal conductance G_{max} was approximated by the slope of the ascending branch; it was 210, 110, and 50 pS for the pial cell (*left*) and 400, 250, and 120 pS for the coronary vsm (*right*). The reversal potential (E_{rev}) was approximated by the intersection of the ascending branch with the voltage axis, it was essentially pH_o-insensitive. The bell-shaped part of the curve was fitted with an activation parameter $A(V, pH_o) = 1/\{1 + \exp([V - V_h]/k)\}$. For the parameters V_h and k see legend Fig. 4.

curves were fitted according to

$$\text{peak } I_{Ca} = A_p(V, pH) \cdot G_{max} \cdot (V_p - E_{rev}), \quad (2)$$

where A_p is an activation parameter, G_{max} the slope conductance of the ascending branch and E_{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

$$\text{peak } G(V)/G_{max} = A_p(V, pH) = 1/\{1 + \exp[(V - V_h)/k]\}. \quad (3)$$

The analysis of a total of 10 peak I_{Ca}-voltage curves suggests the following pH_o-effects: (*a*) The curves are shifted by pH_o 8.4 to more negative and by pH_o 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_{Ca} was 12 ± 6 mV more negative than

at pH_o 7.4; whereas at pH_o 6.4 V_h was 14 ± 7 mV more positive than at pH_o 7.4. (*b*) E_{rev} was almost pH_o insensitive, e.g., it was 60 ± 4 mV at pH_o 7.4 (20 mM $[Ca^{2+}]_o$), 58 ± 7 mV at pH_o 6.4 and 64 ± 4 mV at pH_o 8.4. These small changes were not significant (compare Krafte and Kass, 1988). (*c*) G_{max} was increased by pH_o 8.4 and reduced by pH_o 6.4. The efficacy of pH_o effect on G_{max} decreased when the extracellular Ca^{2+} or Ba^{2+} concentration was elevated.

Division of peak I_{Ca} by the pH_o-independent driving force ($V_p - E_{rev}$) and by G_{max} yields the normalized activation-variable $A_p(V,pH)$ of Fig. 4, *left* (*dashed line*, 3.6 mM $[Ca^{2+}]_o$). On average, at pH_o 7.4 $A_p(V)$ started from a threshold at −40 mV and reached 50% at V_h = −11 ± 4 mV (n = 21). pH_o 6.4 shifted V_h by +13 ± 6 mV (n = 4) and pH_o 8.4 by −18 ± 5 mV (n = 7). The slope factor (k = −8 mV) was not significantly influenced by pH_o. 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 pH_o 7.4. pH_o 6.4 shifted this potential by 9 ± 5 mV (n = 4) and pH_o 8.4 by −10 ± 6 mV (n = 6).

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

TABLE I

Effect of pH_o on the Voltage Dependent Gating Parameters of I_{Ca}

pH_o	$V_{h,act}$	$V_{h,act,norm}$	E_{rev}	$V_{h,inact}$	$G_{max,norm}$
	mV	*mV*	*mV*	*mV*	%
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. $V_{h,act}$ indicates the potential of half maximal activation, $V_{h,act,norm}$ the potential of half maximal activation of the normalized current, E_{rev} the reversal potential, $V_{h,inact}$ the potential of half maximal inactivation and $G_{max,norm}$ the normalized maximal conductance.

pH_o modulation of peak I_{Ca}. Fig. 3 shows that the slope of the ascending branch of the curves, i.e., the maximal conductance G_{max}, is pH_o-dependent. With $G_{max}(V, pH_o)$ we extend Eq. 2 to

$$\text{peak } I_{Ca}(V, pH_o) = A_p(V, pH_o) \cdot G_{max}(pH_o) \cdot (V - E_{rev}). \quad (4)$$

The pH_o-effect on G_{max} is shown more clearly in Fig. 4 where the data of Fig. 3 (*left*) are plotted as conductance (*left*) or activation parameter (*right*). If G_{max} was pH_o-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 pH_o 8.4, 105 pS at pH_o 7.4 and only 43 pS at pH_o 6.4 (3.6 mM $[Ca^{2+}]_o$). Data from four pial vsm and three coronary vsm in 3.6 mM $[Ca^{2+}]_o$ indicate a reduction of G_{max} to 52 ± 15% for the change from pH_o 7.4 to 6.4 and an increase of G_{max} to 146 ± 24% for the change to pH_o 8.4.

pH_o Modulation of Single Channel Conductance

The whole-cell conductance G_{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}

$$G_{max} = N_F \cdot P_o \cdot g_{Ca}. \quad (5)$$

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. 5 *A*). The pH_p-effect on single channel current was evaluated by amplitude histograms, the

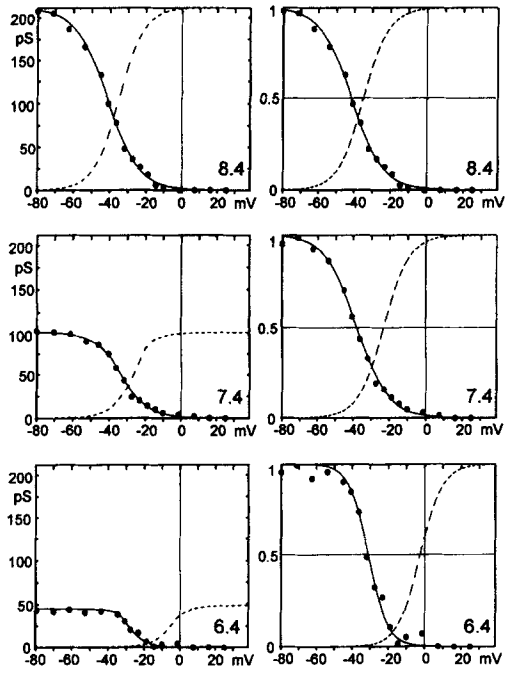

FIGURE 4. Influence of pH_o on the gating parameters. (*Left*) Steady state inactivation (*circles*). Peak conductance at +10 mV was modified by 170-ms prepulses to the potential indicated on abscissa. Intersection on left ordinate shows G_{max}. (*Right*) Normalized inactivation curves (G/G_{max}) fitted with the Boltzmann-curve $h_\infty(V, pH_o) = 1/\{1 + \exp([V - V_h]/k)\}$. 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, pH) (*right*). Data from coronary vsm in 20 mM $[Ca^{2+}]_o$ (Fig. 3, *right*) were fitted with a similar Boltzmann-equation as h_∞. 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.

results of which were plotted as *i-v* curves (Fig. 5 *B*). The slope of the *i-v* curves indicated a single-channel conductance of g_{Ca} = 22 ± 2 pS (n = 5) for pH_p 7.4. Acidic pH_p reduced g_{Ca} to 19 ± 3 pS (n = 4) at pH_p 6.4 and to 11 ± 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 ± 4 pS (n = 5).

The effect of pH_p on g_{Ca} was studied at a variety of $[Ba^{2+}]$ (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 $[Ba^{2+}]_p$ up to saturation (g_{sat} = 24 pS) according to

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

The half maximal conductance of 12 pS was found at an apparent K_D-value of 4.7 mM $[Ba^{2+}]_p$ (Fig. 6 *A;* 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. 6 *D* shows the modulation of K_D by pH by a series of curves evaluated for identical $[Ba^{2+}]_p$. If $pK^* = -\log(K^*_H)$ was used to indicate the

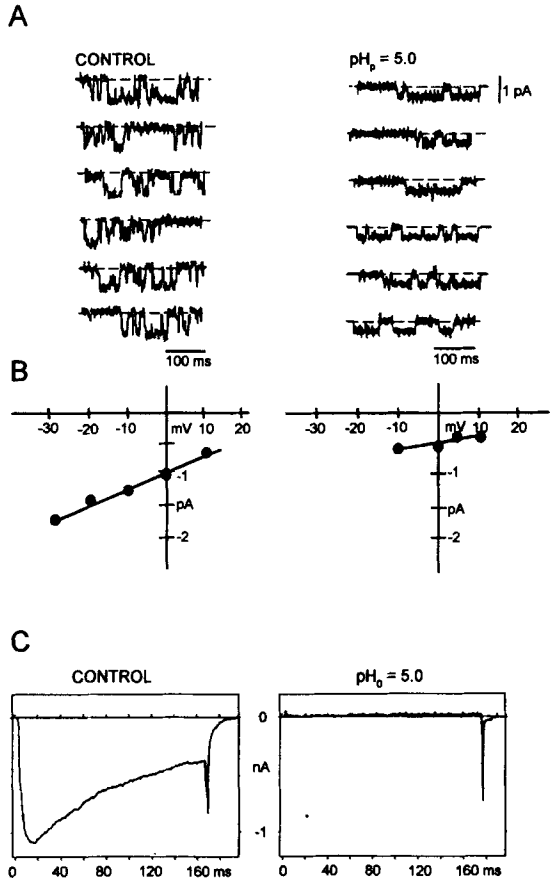

FIGURE 5. Single Ca^{2+} channel currents under the influence of pipette pH_o reduced from pH_p 7.4 to 5.0. Patch attached to coronary vsm. 50 mM $[Ba^{2+}]_o$ as charge carrier, 0.5 μM Bay K 8644, 22°C. In (*A* and *B*), the cell was bathed in a high K^+ solution (pH_o 7.4) zeroing the membrane potential, 400 ms pulses from −65 to 0 mV were applied at 0.1 Hz. (*A*) Representative current tracings obtained before (*control*) and after the change from pH_p 7.4 to 5.0. (*B*) *i-v* curves for single channel current, by linear regression a slope conductance of 21 pS is evaluated for pH_p 7.4 and of 10 pS for pH_p 5.0. (*C*) Reduction of bath pH_o blocks whole cell I_{Ca}, recorded at 22°C and 50 mM $[Ba^{2+}]_o$ (0.5 μM Bay K 8644).

pH-value that reduced g to 50% of g_{sat}, the data could be fitted with

$$g = g_{sat}/\{1 + ([H^+]_p/K^*_H)^{0.5}\} \quad \text{or} \tag{7}$$

$$g = g_{sat}/\{1 + 10^{(pK^* - pHp)\cdot 0.5}\}. \tag{8}$$

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^*_H; 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

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

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

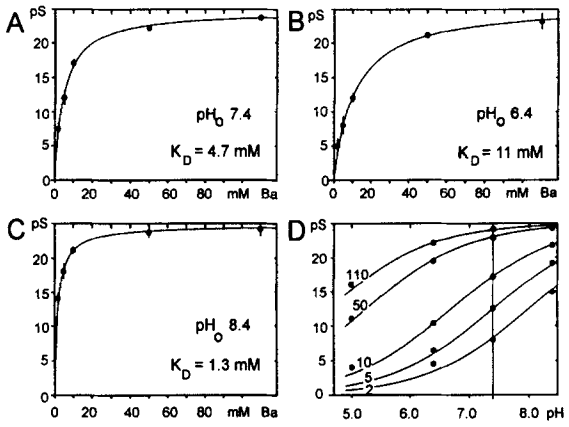

FIGURE 6. Influence of pH_o on single channel conductance (ordinate) is dependent on the Ba^{2+} concentration in the patch-electrode. $[Ba^{2+}]_p$ was varied between 2 and 110 mM. Mean ± SEM from seven cell-attached and six outside-out patches. (*A*, *B*, and *C*) Concentration dependence was fitted with $g = g_{sat}/\{1 + K_D/[Ba^{2+}]_p\}$. The saturating conductance g_{sat} of 24 ± 1.4 pS is pH independent. $[Ba^{2+}]_p$ for 50% of g_{sat} (K_D) increases with more acidic pH_p. (*D*) Conductance g as function of pH_p with $[Ba^{2+}]_p$ as parameter was fitted with $g = g_{sat}/\{1 + 10^{(pK^*-pH)0.5}\}$. pK* is the pH that reduces g to 50% of g_{sat}. pK* increases if $[Ba^{2+}]_p$ is reduced, pK* is 4.6 at 110 mM $[Ba^{2+}]_p$, 5.4 at 50 mM, 6.7 at 10 mM, 7.4 at 5 mM and 8.0 at 2 mM, respectively.

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^{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^{2+} channels can explain the preferential pH_o-effect, for example, by a suppression or facilitation of late re-openings of the L-type Ca^{2+} channels (Klöckner and Isenberg, 1994).

This study suggests that pH_o modulates I_{Ca} through (*a*) shifts of the voltage-dependent channel gating, and (*b*) changes in the single channel conductance. In addition, pH_o can modify Ca^{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; Krafte and Kass, 1988). The extent of the pH_o-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_o induced shifts were significant. The contribution of this shift to the pH_o-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_o 8.4 shifts the activation parameter to a more negative potential. Due to this shift, a substantial fraction (5%) of Ca^{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^{2+} current at −50 mV (compare Fig. 4, *left*). The effects on activation, inactivation and G_{max} predict that the continuous Ca^{2+} influx through L-type channels at −50 mV is seven times higher at pH_o 8.4 than 7.4. This substantial Ca^{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^{2+} influx at alkaline than at neutral pH_o 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_o 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_o effects can partially compensate each other and explain why peak I_{Ca} (+5 mV) followed pH_o with a Hill-coefficient of < 1. The shift of activation and the reduction of G_{max} can explain why acidic pH_o suppresses the generation of action potentials and the concomitant Ca^{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_o modulates G_{max}. pH_o 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_F 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. 6 *D* indicated that pK*, the pH_o reducing g_{Ca} to 50%, became more acidic with higher $[Ba^{2+}]_o$ in proportion to $-2 \cdot \log \{[Ba^{2+}]_p\}$. 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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