Hydrogen Ion Modulation of Ca Channel Current in Cardiac Ventricular Cells

Evidence for Multiple Mechanisms

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ABSTRACT We have investigated the effects of H ions on (L-type) Ca channel current in isolated ventricular cells. We find that the current amplitude is enhanced in solutions that are alkaline relative to pH 7.4 and reduced in solutions acidic to this pH. We measured pH_o-induced shifts in channel gating and analyzed our results in terms of surface potential theory. The shifts are well described by changes in surface potential caused by the binding of H ions to negative charges on the cell surface. The theory predicts a pK of 5.8 for this binding. Gating shifts alone cannot explain all of our observations on modulation of current amplitude. Our results suggest that an additional mechanism contributes to modification of the current amplitude.

INTRODUCTION

It is now well established that Ca channel currents are important to many aspects of cellular function in a wide variety of cells (Reuter, 1985). In the heart, Ca channel currents have been shown to play a role in excitation-contraction coupling (Fozzard, 1977; Fabiato and Fabiato, 1979) and electrical activity (Reuter, 1979; McDonald, 1982); consequently, perturbations that modulate their function have received much attention. One such perturbation is extracellular acidosis, which can occur during myocardial ischemia. Chesnais et al. (1975), Kohlhardt et al. (1976), and Yatani and Goto (1983) have all shown that acidosis depresses Ca channel current and, therefore, overall cardiac performance.

We have investigated the mechanisms by which H ions can modulate Ca currents in dialyzed cardiac myocytes and designed our experiments to test for H ion–induced changes in channel gating. We analyzed our results within the framework of surface potential theory and found shifts in gating consistent with the titration of negative charges on the external surface of the membrane within the vicinity of (L-type) Ca channels. Within the limitations of the theory, an estimation of the pK and density of these sites is given. In addition, we have found that another mechanism of current modulation must be acting in conjunction with the gating shift.

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These results have been presented in preliminary form to the Biophysical Society (Krafte and Kass, 1986).

METHODS

Single ventricular myocytes were isolated from either ventricle of adult female or male Sprague-Dawley rats (Charles River Breeding Laboratories, Charles River, MA) weighing ~200–225 g. The isolation procedure was a modification of methods described by Farmer et al. (1983) and Sheu et al. (1984). Myocytes were also obtained from adult guinea pigs using an isolation procedure similar to that described by Mitra and Morad (1985).

The recording methods were as described by Hamill et al. (1981) for the whole-cell configuration. Patch pipettes were made from Gold Seal Accu-fill 90 Micropipets (Clay Adams, Inc., Parsippany, NJ). The resistance of the pipettes was typically 1–3 MΩ when they were filled with 140 mM CsCl. Series resistance compensation was used in all experiments and was adjusted to give the fastest possible capacity transients without producing ringing. Data were sampled once every 0.3 ms and filtered at 1–2 kHz with an eight-pole Bessel filter (Frequency Devices, Inc., Haverhill, MA).

Solutions and Drugs

The standard pipette solution contained (millimolar): 100 CsCl, 40 CsOH, 2 $MgCl_2$, 1 $CaCl_2$, 11 EGTA, 10 HEPES (pH 7.4). We tripled our HEPES concentration in the pipettes to test for possible secondary effects of changes in extracellular pH (pH_o). Under these conditions, we found comparable effects on Ca channel currents for given changes in pH_o and concluded that intracellular pH (pH_i) was adequately buffered with 10 mM HEPES.

The bath solution contained (millimolar): 132 NaCl, 4.8 CsCl, 1.2 $MgCl_2$, 1 $CaCl_2$, 5 glucose, 5 HEPES (pK_a 7.5), and one of the following buffers where appropriate: CAPS (pK_a 10.1), TAPS (pK_a 8.55), MOPS (pK_a 7.2), MES (pK_a 6.15), or propionic acid (pK_a 4.9). These buffers were all purchased from Sigma Chemical Co., St. Louis, MO. For each pH change, the solutions contained 5 mM HEPES and 5 mM of the appropriate buffer. Thus, both buffers were present in control, test, and recovery runs, the only difference being titration of the solution to the desired pH. Liquid junction potentials were measured and found to be <2 mV with the solutions used. This value was independent of pH and was not corrected for in the subsequent analysis.

Na currents were eliminated by the choice of holding potentials and by the addition of 10–50 μM tetrodotoxin (TTX; Sigma Chemical Co.). Cs^+ substitution for K^+ on both sides of the membrane was used to block K channel currents (see Kass and Krafte, 1987).

Nisoldipine, a gift from Miles Laboratories, Inc., New Haven, CT, was dissolved in polyethylene glycol 400 (PEG) to make a concentrated stock and diluted in the bath to the final concentration. PEG at the concentration used (>1,000× dilution) has been shown to have no effects of its own on Ca channel currents (Kass, 1982).

Estimation of Leak Currents

Nisoldipine, a dihydropyridine compound known to block I_{Ca} (Kass, 1982), was used to determine leakage, or background, currents. The current that remains in the presence of nisoldipine (under the ionic conditions described above) is time independent and linear over a voltage range of 200 mV (see also Matsuda and Noma, 1984). This is the most direct measurement of background currents, but it is not useful if Ca channel and background currents are to be measured in several solutions in the same cell because the effects of nisoldipine are very difficult to reverse.

We therefore also used two other less direct methods that we found adequate to estimate background currents. The first was a linear extrapolation of currents measured during hyperpolarizing pulses. The second method estimated background currents after I_{Ca} was inactivated by positive conditioning pulses.

Voltage Protocols

T-type Ca channels were avoided by restricting holding potentials to voltages positive to −60 mV (Bean, 1985), and holding potentials were adjusted in experiments in which large shifts in surface potential were expected. Thus, in this article, current referred to as Ca channel current (I_{Ca}) corresponds to the L-type Ca channel current according to the terminology suggested by Nilius et al. (1985).

Inactivation was determined by measuring current in response to a constant test pulse, usually to the peak of the current-voltage relationship, preceded by 500-ms conditioning pulses to a series of voltages. A gap of 10 ms was present between the conditioning pulse and the test pulse. Peak current measured during the test pulse was plotted against the conditioning pulse potential and normalized to the current measured after the most negative conditioning pulse. We found that, in rat ventricular myocytes, 500-ms conditioning pulses were sufficient to reach steady state inactivation at room temperature when Ca was the charge carrier. We did not use pulses long enough to cause slow inactivation of the channels (Scheuer, 1983). High intracellular EGTA concentrations (11 mM) were used to minimize the contributions of Ca-dependent inactivation. Under these conditions, steady state inactivation has been shown to be a good assay of voltage-dependent gating (Kass and Krafte, 1987).

The procedure to calculate activation was as follows. Current was measured in response to a series of test pulses, background current was subtracted, and the reversal potential for Ca current was determined. Activation was determined by dividing peak current by driving force. This procedure is subject to errors due to possible nonlinearity of the single-channel conductance (Hess et al., 1986), but was used to provide an approximation to activation. Most of our experiments on channel gating focused on shifts in inactivation.

Computational Procedures

Surface potential calculations. The relationship between membrane surface charges, ions in the medium, and surface potential is described by the Grahame (1947) equation (McLaughlin, 1977):

$$\sigma_a^2 = kT\epsilon/2\pi\Sigma C_i \,(\exp\,[-\psi_o \cdot Z_i \cdot e/kT] - 1), \tag{1}$$

where σ_a is the apparent surface charge density in electronic charges per square angstrom, kT/e = 25.3 mV at T = 22°C, C_i is the concentration of the ith ion and Z_i is its valence, ϵ is the dielectric constant of aqueous solution, and ψ_o is the surface potential in millivolts.

If cations bind to (and thus neutralize) negative surface charges, the apparent charge density that contributes to the surface potential is reduced. The relationship between the apparent and true negative surface charge density is then (Begenisich, 1975; Hille et al., 1975):

$$\sigma_a = \sigma_{T1}/(1 + K_H\,[H^+]^*) + \sigma_{T2}/(1 + K_{Ca}[Ca^{++}]^*). \tag{2}$$

In this equation, K_H equals the apparent association constant of H ions for a negatively charged group that has surface charge density σ_{T1}. K_{Ca} equals the apparent association constant of Ca ions for a second group of charges with a surface charge density σ_{T2}. In our calculations, the value of K_{Ca} was fixed at 1 M^{-1} and σ_{T2} was set to −1 e/300 Å^2, values well within the range we have previously reported (Kass and Krafte, 1987). $[H^+]^*$ and $[Ca^{++}]^*$ are the surface

concentrations of H^+ and Ca^{++}, respectively. Eqs. 3*a* and 3*b* show how the bulk concentrations $[H^+]$ and $[Ca^{++}]$ are related to the surface concentrations of these ions:

$$[H^+]^* = [H^+] \exp(-\psi_o \cdot e/kT); \quad (3a)$$

$$[Ca^{++}]^* = [Ca^{++}] \exp(-2 \cdot \psi_o \cdot e/kT). \quad (3b)$$

For comparison, we also determined estimations of surface charge density if we assumed one negatively charged group to which both H^+ and Ca^{++} can bind. In this case, the relationship between apparent and true negative surface charge was:

$$\sigma_a = \sigma_T/(1 + K_H [H^+]^* + K_{Ca}[Ca^{++}]^*). \quad (4)$$

Curve-fitting procedures. In order to obtain estimates of the surface potential, surface charge density, and binding constants in Eqs. 1 and 2, we assumed that the measured shifts in channel gating were due to pH_o-induced changes in surface potential (ψ_o). To begin the fit of theory to experiment, we assumed values of σ_{Tl} and K_H, calculated σ_a with Eq. 2, and used a Newton-Raphson iteration procedure (Dorn and McCracken, 1972) to solve Eq. 1 for ψ_o. Then, with σ_{Tl} and K_H fixed, we computed changes in surface potential as pH_o was varied. These values for surface potential were compared to the measured shifts in gating for comparable changes in pH_o. A simplex algorithm (Caceci and Cacheris, 1984) was then used to vary K_H and σ_{Tl} systematically and minimize the difference between the computed changes in surface potential and the measured gating shifts. A similar procedure was followed when the apparent surface charge density was defined by Eq. 4.

Boltzmann functions were fitted to inactivation and activation data to obtain the half-maximal voltage (V_h) and the slope factor (k) using a Marquardt (1963) algorithm as described by Bevington (1969). The smooth curves in the current vs. voltage plots were obtained by spline interpolations and are included only as visual aids.

RESULTS

Ca Channel Current Amplitude Is Modulated by pH_o

The two current traces and current-voltage relationships in Figs. 1 and 2 show that changes in pH in either the alkaline or the acidic direction alter the Ca current amplitude. Fig. 1 shows the effects of extracellular alkalization and Fig. 2 shows the effects of extracellular acidification on membrane current. Peak current is reversibly enhanced in the alkaline solution and reduced in the acidic solution. Other pH_o-induced effects on the current-voltage relationship are discussed later. In 14 of 15 cells, alkalization increased the current magnitude, while in 40 out of 40 cells, acidification reduced the current magnitude.

Fig. 3 is a plot of the change in the Ca current amplitude vs. pH_o over a wide pH range. The change at the test pH is relative to the average of currents measured in control and recovery runs that were obtained at pH 7.4. It can be seen from the figure that raising pH above 7.4 increased the current amplitude, while lowering pH depressed the current amplitude. The relationship between the current magnitude and acidification was quite steep around pH 7.4. No attempt was made to fit a single-site binding curve to these data since the changes in amplitude are a function of more than one process (see below). The curve shown is intended only as a visual aid.

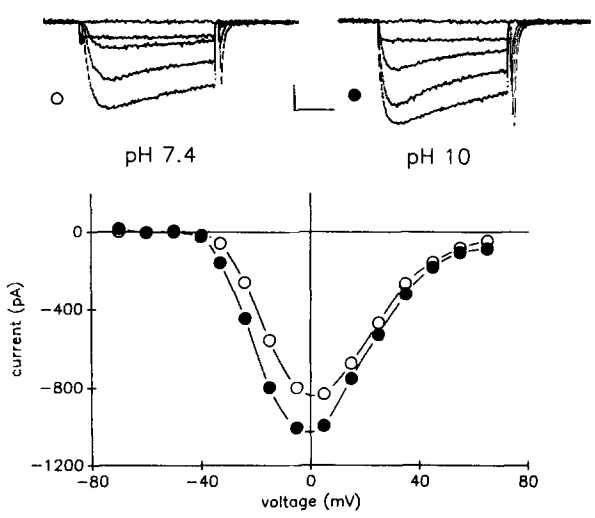


FIGURE 1. Enhancement of I_{Ca} in an alkaline solution. Currents were recorded after steady state was reached in control (pH 7.4) solution and in solution buffered to pH 10. The inset shows a family of leak-subtraced current traces in each solution in response to voltage pulses to −50, −24, −14, +4, and +45 mV. Currents are in response to 40-ms pulses. The curve shows peak current plotted against test voltage. The holding potential was −40 mV. 10 μM TTX. HEPES/CAPS. Calibration bars: 250 pA, 10 ms. Cell 8201.

V_{rev} Is Insensitive to pH_o

One possibility for the changes in current amplitude is that pH_o alters the selectivity of the channel, changes its reversal potential (V_{rev}), and alters the driving force for currents. We tested for pH_o-induced changes in V_{rev}, and, as shown in Fig. 4, we found that this did not underlie the changes in current amplitude that we observed. The figure shows the results of an experiment in which we measured a reversal potential of +60 (±5) mV in solutions of normal and acidic pH. The data also show a

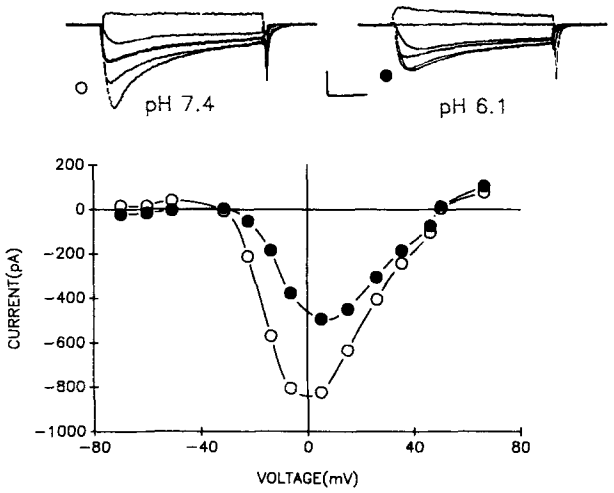


FIGURE 2. Reduction of I_{Ca} in an acidic solution. Currents were measured in control (pH 7.4) and acidic (pH 6.1) solutions after steady state was reached in each case. The inset shows leak-subtracted current traces in response to 40-ms voltage pulses to −17, 0, 10, 20, 30, and 70 mV. The curve shows peak current plotted against pulse voltage. The holding potential was −50 mV. 10 μM TTX. HEPES/MES. Calibration bars: 250 pA, 10 ms. Cell 8202.

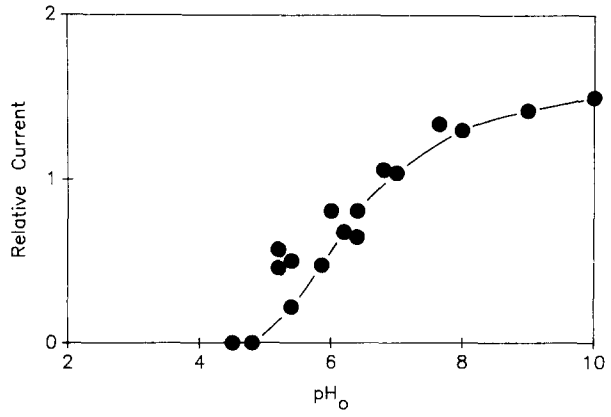


FIGURE 3. Relationship between pH_o and peak I_{Ca} amplitude. Leak-subtracted currents were used to determine the maximal I_{Ca} amplitudes near the peak of the current-voltage relationship in control and test pH solutions. The current amplitudes in control and recovery runs were averaged. The current in the test run was then normalized to this average and plotted against the test pH. Each point in the figure represents a value from a different cell. The smooth curve was obtained by spline interpolation to the data. It is included only as a visual aid. Complete block of current occurred for pH values of 4.8 or lower.

50% reduction in the peak current amplitude despite the constant reversal potential. Thus, in this experiment, a change in channel selectivity cannot account for the decrease in current magnitude.

We repeated similar tests of the effects of pH_o on V_{rev} in a total of 13 cells over a pH_o range of 5.2–10.0. The mean change in V_{rev} was -1.4 ± 41 mV (SEM), confirming the results of Fig. 5.

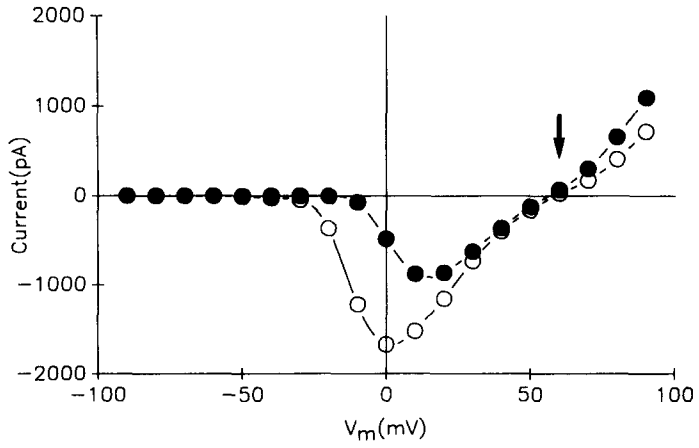


FIGURE 4. I_{Ca} reversal potential is insensitive to alterations in pH_o. Peak leak-subtracted currents were measured and plotted against pulse voltage in control (pH 7.4; open circles) and test (pH 6.0, HEPES/MES; filled circles) solutions. The holding potential was −50 mV in control and −40 mV at pH 6.0. The arrow indicates the reversal potential, which is not affected by the change in pH_o. 20 μM TTX. Cell 1185A9.

H Ions Shift Ca Channel Gating

We next tested for pH-induced shifts in gating. Fig. 5 shows a series of inactivation curves at different pH values that indicate that pH-induced gating shifts do in fact occur. In the experiments illustrated in this figure, shifts in the $V_{½}$ values of the inactivation curves of −5.0, +2.0, +14, and +23 mV were measured when pH_o was changed from 7.4 to 10.0, 7.1, 6.2, and 5.4, respectively. The magnitude of the shift in gating was a nonlinear function of pH and appeared to saturate for pH values of ~5.0. Table I provides a summary of these shifts in steady state inactivation.

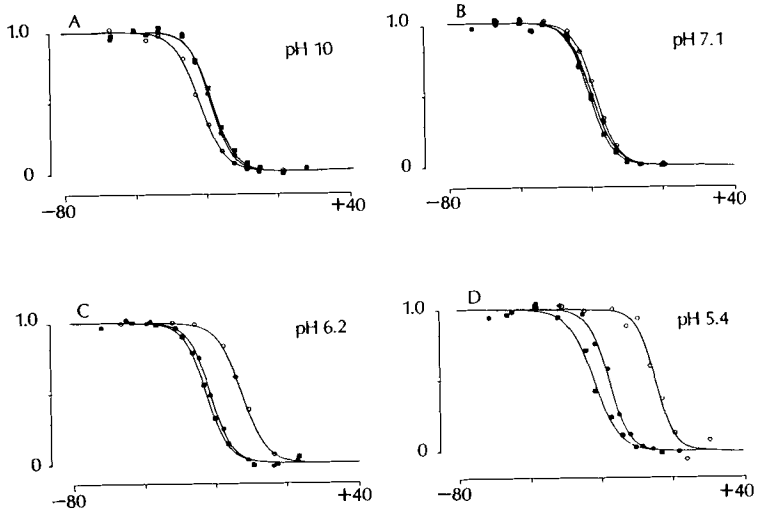


FIGURE 5. Ca channel inactivation is shifted by pH_o. Ca channel inactivation was determined as described in the Methods. Each panel shows normalized inactivation curves obtained in control (filled circles) and recovery (filled squares) solutions of pH 7.4 and data obtained in a solution of test pH (open circles). Curves are nonlinear least-squares fits of Boltzmann functions to the data as described in the Methods. (*A*) Test pH = 10.0 (HEPES/CAPS); 30 μM TTX; cell 0885EA. (*B*) Test pH = 7.1, (HEPES); 30 μM TTX; cell 0885E1. (*C*) Test pH = 6.2 (HEPES/MES); 50 μM TTX; cell 0785C2. (*D*) Test pH = 5.4 (HEPES/propionic acid); 30 μM TTX; cell 0885C3. $V_{½}$ and k values are given in Table I.

The shift in gating is seen in the activation curves as well. Fig. 6 is a plot of normalized conductance (determined as described in the Methods) vs. test potential in solutions of pH_o 7.4 (filled circles) and pH_o 6.2 (open circles). The activation curve was shifted in this experiment by +9.9 mV, which is slightly less than the shift of inactivation for a comparable pH change (+13.9 mV). Several activation curves were determined and their $V_{½}$ and slope factors (k) are given in Table II.

Predictions of Surface Potential Theory: Attraction and Binding to Negative Surface Charges

Fig. 7 summarizes our measurements of pH_o-induced shifts in the voltage dependence of channel gating and compares the experimental results to the predictions of surface potential theory. The figure plots shifts in inactivation that were measured

TABLE I

Summary of Shifts in Inactivation

File	Test pH	Control (pH 7.4)		Test		Recovery (pH 7.4)		$\Delta V_{½}$
		$V_{½}$	k	$V_{½}$	k	$V_{½}$	k	
0885EA	10.0	−19.0	4.2	−23.6	4.9	−18.3	4.5	−4.95
0985C1	9.0	−14.2	5.4	−20.2	6.3	−17.6	5.0	−4.3
0885E4	8.0	−23.6	4.0	−24.7	4.2	−22.3	4.2	−1.75
0985B1	7.8	−15.9	4.7	−20.4	5.1	−19.2	5.2	−2.85
0885E1	7.1	−19.7	4.8	−18.3	4.5	−20.8	4.6	+1.95
0785F8	6.8	−21.5	4.7	−19.0	4.6	−25.2	4.6	+4.35
0785B1	6.4	−22.6	4.9	−14.7	5.3	−32.0	7.4	+12.6
0785B7	6.4	−21.7	4.6	−15.9	4.3	−29.1	5.1	+9.5
0785C3	6.2	−22.0	4.4	−9.1	5.0	−23.9	4.5	+13.85
0785E4	6.0	−20.5	4.8	−6.3	5.1	−25.3	5.0	+16.6
0785F2	5.8	−20.4	4.2	−1.9	4.0	−24.2	5.0	+20.4
0885A1	5.6	−14.9	4.6	+3.8	3.5	—	—	+18.7
0885C3	5.4	−16.4	4.2	+3.1	3.9	−22.7	5.3	+22.65
0885DH	5.3	−19.6	4.0	+3.3	4.1	−19.3	4.4	+22.75
0885DE	5.3	−19.5	4.5	−4.1	4.4	−24.0	4.9	+17.65
0485C4	5.2	−23.8	4.8	−8.0	4.6	−27.7	4.4	+17.75
0485CD	5.2	−22.3	4.3	−1.8	5.2	−25.0	4.5	+21.85
Mean ± SEM		−19.85 ± 0.17				−23.5 ± 0.25		

relative to values determined in control and recovery solutions. Each point represents results obtained from a different cell. Data for shifts in activation are also included for comparison. Our experiments show that extracellular solutions with a pH greater than 7.4 shifted gating in the hyperpolarizing direction, while solutions with acidic pH values caused depolarizing shifts. If binding to negative charges occurs, variation

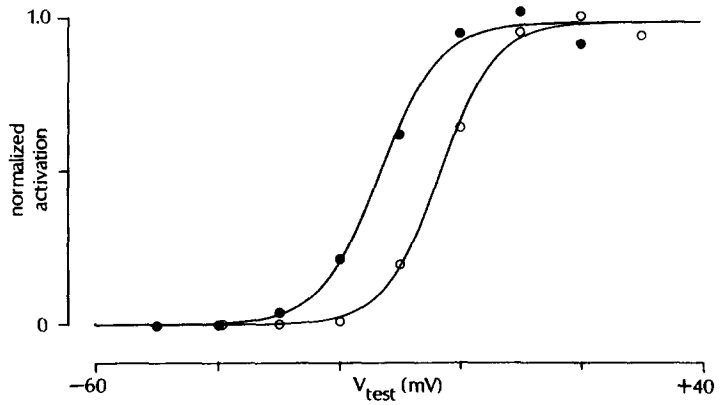


FIGURE 6. Acidification shifts the Ca channel conductance vs. voltage relationship in the depolarizing direction. Filled circles: values at pH 7.4; open circles: values at pH 6.2. Calculation of conductance was as described in the Methods. The smooth curves are fits of Boltzmann functions to the data. $V_{½}$ and k values are given in Table II. The holding potential was −60 mV. HEPES/MES. 50 μM TTX. Cell 0785C1.

TABLE II

Summary of Shifts in Activation

File	pH	Control		Test		$\Delta V_{½}$
		$V_{½}$	k	$V_{½}$	k	
0585B3	8.6	−5.8	5.7	−7.4	5.5	−1.6
0585A3	8.6	−7.1	5.5	−12.1	6.7	−5.0
0785F7	6.8	−13.0	5.3	−10.3	5.1	+2.7
0785C1	6.2	−13.1	5.0	−3.2	4.6	+9.9
1185A9	6.0	−11.4	4.9	+2.3	4.5	+13.7
0485CA	5.2	−7.3	7.0	+11.1	5.9	+18.4
Mean ± SEM		−9.6 ± 1.3				

in the extracellular H ion concentration should affect the cell surface potential and alter the voltage dependence of channel gating. Eqs. 1–3 (Methods) provide a theoretical framework in which to test for this possibility.

The smooth curve in the figure is the predicted change in surface potential that occurs for the changes in pH_o indicated along the abscissa relative to a control pH_o of 7.4. In this computation, two negative charge groups are assumed (see Eq. 2 in Methods). The theory predicts that H ions bind to negative surface charges. The pK for the titratable group is 5.8 and the density of this site is −1 e/250 Å^2. The titratable surface potential at pH 7.4 for the ionic composition given in the Methods is then calculated to be −67 mV.

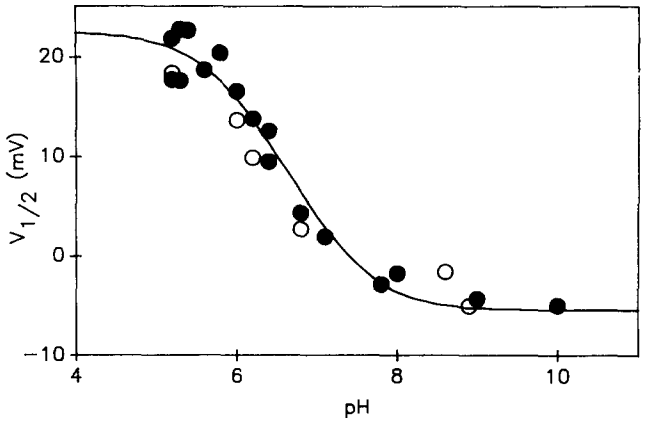


FIGURE 7. pH_o-induced gating shifts are well described by predictions of surface potential theory. Shifts in the $V_{½}$ value for inactivation (filled circles) and activation (open circles) are plotted vs. pH_o. The shift was determined by taking the difference between the average of the control and recovery runs and the value obtained at the test pH. The smooth curve is derived from surface potential theory as described by Eqs. 1–4 in the Methods. Two separate charge groups were assumed. The values for the association constant and charge density of the charges neutralized by Ca ions are given in the Methods. The following parameters were used for the charges titrated by H: σ_T = −1 e/250 Å^2 and pK = 5.8.

Evidence for H Ion Block of Ca Channels

The data in Fig. 7 indicate that lowering pH_o from 7.4 to 5.0 shifts voltage-dependent gating by ~24 mV. We were unable to measure gating shifts in more acidic solutions because further acidification completely eliminated the Ca channel current. A 24-mV positive shift in gating would be expected to cause the peak of the current-voltage relationship to occur near +24 mV as compared with 0 mV in pH_o 7.4. The reduced driving force at this voltage should cause the peak in this curve to be smaller, but not eliminated (see Fig. 9 *C*). Nevertheless, Ca channel currents are abolished under these conditions.

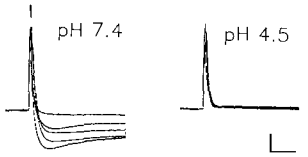
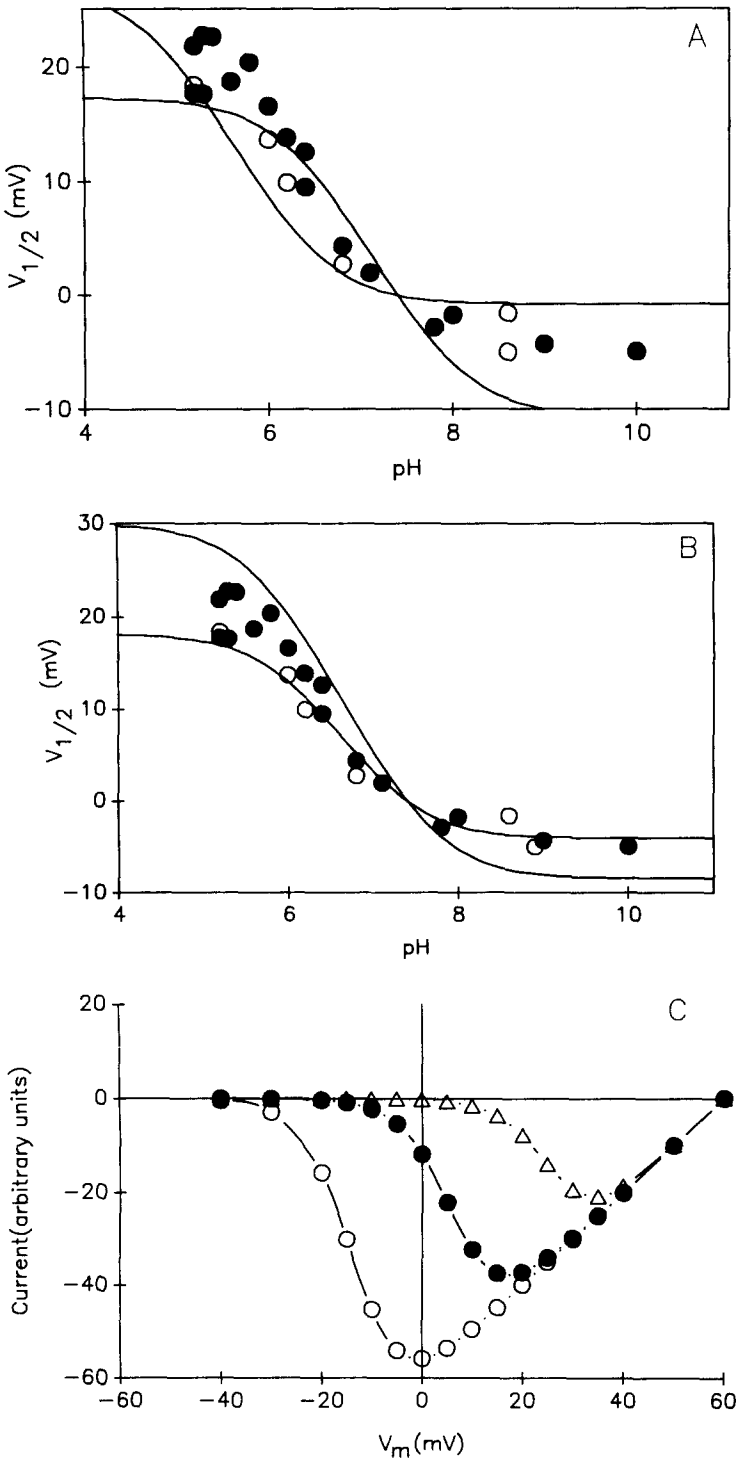


FIGURE 8. I_{Ca} is completely inhibited at very low pH: evidence for H ion block of the channel. Leak-subtracted traces of membrane current are shown in response to voltage pulses to −20, −10, 10, 20, and 30 mV in solutions buffered to pH 7.4 and pH 4.5. The holding potential was −40 mV. Calibration bars: 500 pA, 10 ms. HEPES/propionic acid. 20 μM TTX. Cell 862.

Fig. 8, which illustrates this point, shows that a pH change from 7.4 to 4.5 inhibits Ca current. In the figure, the currents shown were measured at voltages above and below the expected peak of the I_{Ca} current-voltage curve. I_{Ca} was inhibited at all voltages tested in the acidic solution (up to +40 mV). This observation was consistent in each of four other cells for which it was tested: I_{Ca} was completely inhibited at pH_o <4.8. These results suggest a mechanism in addition to pH_o-induced gating shifts.

In support of this view, we found that the I_{Ca} maximal conductance was reduced in acidic solutions over a less extreme pH_o range. In each of three cells in which it was measured, the maximal conductance was found to decrease in acidic solutions. At pH_o 5.5, there was an average reduction in maximal conductance of 28 ± 1%. Maximal conductance is measured at voltages sufficiently positive to ensure the opening of all available Ca channels. It thus avoids complications due to gating shifts and is a good assay for additional mechanisms of channel modification.

FIGURE 9. (*opposite*) Limits and predictions of surface potential theory. In the computations that follow, the parameters for charges neutralized by Ca ions were kept constant (see Methods). (*A*) Sensitivity of theoretical fit to variation in pK. Surface charge density was fixed at −1 e/250 Å^2 and pK was varied: 4.8 (upper curve) and 6.3 (lower curve). (*B*) Influence of surface charge density on the fit of the theory to experimental data. pK was fixed at 5.8 and the surface charge density of titratable charge was varied: −1 e/150 Å^2 (lower curve) and −1 e/350 Å^2 (upper curve). The circles in *A* and *B* are the experimental data described in Fig. 7. (*C*) Influence of predicted gating shifts on current-voltage curves. A Boltzmann function was used to approximate steady state activation (Methods). V_h was shifted +20 mV (filled circles) and +40 mV (triangles) relative to control values (open circles). The reversal potential was +60 mV in all simulations and an arbitrary and constant maximal conductance was used to compute the peak currents shown.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20
10
0
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$V_{1/2}$ (mV)
4
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8
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pH
B
30
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−10
$V_{1/2}$ (mV)
4
6
8
10
pH
C
20
0
−20
−40
−60
Current(arbitrary units)
−60
−40
−20
0
20
40
60
V_m(mV)

FIGURE 9.

DISCUSSION

Mechanisms of H Ion Modulation of Heart Ca Channels

We find that the modulation of Ca current in heart cells is due to both pH_o-induced shifts in voltage-dependent gating parameters and to at least one additional mechanism. The gating shifts we measured were consistent with changes in surface potential caused by the binding of H ions (pK 5.8) to fixed sites on the membrane surface.

Curve-fitting procedures (described in the Methods) were used to obtain the best fit of Eqs. 1–3 to our experimental data. However, because two parameters were varied, a range of values provides acceptable fits to the data. Fig. 9, *A* and *B*, is included to illustrate this range and to show the effects of maximal shifts in gating predicted by this theory. As can be seen in the figure, estimates of surface charge density for a titratable group ranging from -1 e/150 Å^2 to -1 e/350 Å^2 provide reasonable approximations to the measured gating shifts. Acceptable estimates of the pK for H ion binding ranged from 6.0 to 5.1. Additional possibilities are discussed below.

The effect of shifts in gating is quite clear, but alone it is not sufficient to explain all of our results. To illustrate this point, we computed predicted changes in the I_{Ca} peak current-voltage relationship caused by H ion–induced shifts in channel gating predicted by surface potential theory. According to the theoretical curve shown in Fig. 7, the maximal shift in gating caused by titration of fixed negative charges is ~24 mV for the curve that best fits our experimental data. Fig. 9 *C* shows that I_{Ca} is not abolished over the voltage range of 0 to +40 mV, even if gating is shifted by +40 mV, a value much larger than the theoretical maximum. Thus, it is not likely that, by themselves, gating shifts can account for the complete abolition of I_{Ca} measured in very acidic solutions.

At least two possibilities exist for an additional effect of H ions: changes in the surface concentration of Ca^{++} and H^+ block of the pore. A recent study by Iijima et al. (1986) investigated the effects of extracellular H ions on Ca currents in a mouse hybridoma cell line. They came to the conclusion that gating shifts and surface concentration changes were responsible for the increase or decrease of the Ca current magnitude as a function of pH_o. The main basis for this statement was the fact that they saw no block of outward current through the channel at positive test potentials. However, an alternative interpretation of these data is that H ion block is voltage dependent, as has been shown to be the case in squid axon for Na channels (Begenisich and Danko, 1983). As the membrane potential is made more positive, H ions are repelled out of the channel and block is relieved.

The data in Fig. 7 allow an estimation of the contribution of pH_o-induced changes in surface concentration to the reduction of I_{Ca} at low pH_o. The figure shows that titratable surface changes can alter the surface potential by a maximum of 24 mV. When all negative charges are neutralized by H ions (at pH_o 5.0 and lower), the reduction in surface potential causes a roughly fivefold decrease in the surface concentration of Ca ions (Eqs. 3*a* and 3*b*). Is this reduction enough to cause a complete inhibition of I_{Ca}? To answer this, consider the contribution of change in the surface concentration to the I_{Ca} magnitude at pH_o 5.5. At this pH, currents are clearly

large enough to measure, despite the fact that the surface concentration of Ca^{++} is reduced by a factor of 4.9 (Eqs. 3*a* and 3*b*), within 10% of the maximal reduction under the most acidic conditions. Another mechanism is likely to underlie the complete elimination of I_{Ca} at very low pH_o.

In addition, sizable increases in current magnitude were seen on alkalization to pH 10.0 (35% increase), while gating (and thus surface potential) was only slightly affected (+5 mV shift). It is, of course, possible that the surface potential seen by the permeation pathway is not the same as that affecting gating. We have no information about such a potential, though, and given the complete elimination of currents at very low pH, we feel that H ion block of the pore is just as likely an explanation. Consistent with this interpretation are the observations by Hess et al. (1986) that H ions appear to block monovalent ion flow through cardiac Ca channels.

Relationship to Previous Work in Heart

Depression of cardiac contractility by acidosis and enhancement by alkalosis was observed by Gaskell as early as 1880. A review of the earlier work has been presented by Vaughn Williams (1955), and since that time a substantial degree of effort has been directed at understanding the mechanisms involved.

The present study confirms the results of earlier work, which showed that decreasing pH_o depresses Ca channel current in heart (Chesnais et al., 1975; Kohlhardt et al., 1976; Vogel and Sperelakis, 1977; Yatani and Goto, 1983). One of our most interesting observations was that small changes in pH, within 0.6 units of 7.4, were shown to affect the magnitude of Ca channel current. Large extracellular acidification to about pH 5.5 reduced current by ~60–70%, a value somewhat larger than that previously reported.

Several recent studies have investigated the relationship between pH_i and Ca current magnitude (Kurachi, 1982; Sato et al., 1985; Irisawa and Sato, 1986). The two earlier reports indicated that when normal extracellular Na was present, Ca currents were less sensitive to changes in pH_i than when Na concentrations were reduced. This indicates the possible presence of an Na/H exchanger that tends to buffer pH_i (see Ellis and MacLeod, 1985; Piwnica-Worms et al., 1985). Irisawa and Sato (1986) found that changes in pH_i secondary to changing pH_o could be a problem when the cell buffering capacity was lowered by reducing the pipette buffer concentration. They also found that extracellular Na tends to maintain physiological pH_i. Under our conditions, secondary changes in pH_i following a change in pH_o do not appear to be a major problem since we saw similar effects when we tripled the buffer concentration in our pipettes. In addition, all experiments were done under conditions of normal extracellular Na concentrations.

Relationship to Previous Work in Noncardiac Preparations

H ion modulation of voltage-dependent ion channels has been studied in a number of preparations, most being invertebrates. Carbone et al. (1981) summarize some of these results in a table giving pK values for groups on the inside and/or outside of the membrane that influence permeation and gating. In general, increasing the H ion concentration reduces the amplitude of Na, K, and Ca currents (Hille et al., 1975; Campbell and Hille, 1976; Schauf and Davis, 1976; Ohmori and Yoshii, 1977; Brown

and Noble, 1978; Hagiwara et al., 1978; Begenisich and Danko, 1983). Cl currents have been shown to be reduced by extracellular acidification (Hutter and Warner, 1967) as well. The last study is noteworthy since, for negatively charged ions, the surface concentration change on extracellular acidification is opposite to what would be expected for a reduction in current amplitude. The pK of 5.8 obtained here is similar to that reported by Shrager (1974) for H^+ effects on the gating of K channels in crayfish axons.

The effects of alkalization have received much less attention than decreases in pH. The work presented in this study has shown that alkalization can influence gating and the amplitude of the Ca channel current. Furthermore, increases in current upon alkalization are not unique to cardiac Ca channels. Iijima et al. (1986) have reported the same observation for Ca channels in a mouse hybridoma cell line and Hille et al. (1975) found the same effect for Na channels. Presumably there is a site that is only partially titrated at pH 7.4. This implies a "tonic" reduction in current magnitude at physiological pH. It will be interesting to explore the effects of H ions at the single-channel level more thoroughly. If some block is present at pH 7.4, it may be evident as rapid block and unblock of the pore.

Limitations and Interpretations of the Theory

As discussed in the text, the analysis of the data presented in Fig. 7 assumed two independent charge groups accessible to H and Ca ions. Another approach to the analysis that provides a very good fit to the H ion–induced shift in gating is to assume only one charge group accessible to both Ca and H ions (Eq. 4). When this analysis is carried out, however, the predicted surface charge density for this charge group is -1 e/535 Å^2. Although this estimate of surface charge density is adequate to describe the H ion data, it is a factor of ~2 lower than the density predicted by previous measurements in which divalent ion concentrations were varied. A direct test of these two possibilities will be to probe interactions between divalent ions and H ions in the modulation of surface potential. These experiments will determine whether separate charge groups do in fact exist, as the analysis of our two independent sets of experimental data suggest.

The Gouy-Chapman theory as used in this article applies to a smeared layer of charges (uniform surface charge density) and is not appropriate for the analysis of discrete charged groups. We therefore have an oversimplified view of the influence of charges on lipids and on the Ca channel protein itself. Complex factors such as channel geometry will certainly influence surface potential (see Dani, 1986) and will have to be considered to probe more deeply into channel structure. In addition, the interaction of ions with charges may be more specific than the surface potential theory implies. Gilly and Armstrong (1982*a*, *b*) have described kinetic changes for Na and K channels of squid giant axon that are more consistent with a direct interaction of ions with the gating machinery of the channel. We certainly cannot rule out this type of interaction from our data. Our estimates of charge densities and binding constants should be used with these limitations in mind. A detailed understanding of the exact location and nature of the charges on Ca channels will have to await more detailed molecular information. However, these data are still very useful in comparing the effects of ions on the gating and permeation of channels in general,

even given the probable differences in the charge composition of the proteins and, possibly, the surrounding lipid environment.

Conclusions

We have investigated the effects of extracellular H ions on the cardiac Ca current. Gating shifts were seen that are consistent with titration of negatively charged sites on the membrane surface on or near the channel complex. These sites were estimated to have a pK of 5.8 and a density of -1 e/250 Å^2. In addition, H ions act on Ca channels by an additional mechanism that may be an actual block of the pore. The sensitivity of the Ca channel current to extracellular H ions around pH 7.4 indicates that these channels are likely to be involved in the effects of acidosis and alkalosis on cellular properties of the intact myocardium.

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Note added in proof: After submission of this manuscript, direct measurement of dihydropyridine-sensitive Ca channel block by protons has been reported in single-channel experiments by Prod'hom et al. (1987).

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