Effects of External pH on Ionic Currents in Smooth Muscle Cells from the Basilar Artery of the Guinea Pig

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\( \text{pH}_i \) is an important determinant of vascular tone in cerebral blood vessels. We investigated the effects of changes in \( \text{pH}_i \) on isolated smooth muscle cells from the basilar artery of the guinea pig. Single cells contracted rapidly in response to an elevation in \( \text{pH}_i \) (constant \( \text{CO}_2 \)), and contraction was blocked by nifedipine, suggesting a role for dihydropyridine-sensitive \( \text{Ca}^{2+} \) channels. In whole-cell patch-clamp experiments, changes in \( \text{pH}_i \) (\( \text{pH} \), 5.7–8.1, \( \text{pH} \), 7.2 with 10 mM HEPES) strongly affected the amplitude of the peak \( \text{Ca}^{2+} \) channel current (10 mM \( \text{Ba}^{2+} \), +15 mV, holding potential of −55 mV), with an apparent \( \text{pK} \) of 6.9. The current–voltage curves were minimally shifted, indicating no important effect of surface charge. To separate the slowly inactivating \( L \)-type \( \text{Ca}^{2+} \) channel current from the more rapidly inactivating \( B \)-type current, the decaying portions of inward currents from cells studied with repetitive 1-second pulses (+15 mV, holding potential of −55 mV) were fit to a two-component model. Titration curves for the \( L \)-type and \( B \)-type currents indicated maximum increases by factors of 3.65 and 1.28 at alkaline \( \text{pH}_i \), and gave apparent \( \text{pK} \) values of 7.71 and 6.47 (Hill coefficient unity). The time constant of inactivation for the \( B \)-type current at +15 mV was little affected by \( \text{pH}_i \), whereas that for the \( L \)-type current increased somewhat with increasing \( \text{pH}_i \). Additional experiments showed no significant effect of \( \text{pH}_i \) on holding current or on voltage-activated outward currents (\( \text{pCa} \), 7 with 11 mM EGTA). Our results provide additional evidence for participation of \( \text{Ca}^{2+} \) channels in regulating basal tone in cerebral smooth muscle and indicate that \( \text{pH}_i \) regulates current through slowly inactivating, dihydropyridine-sensitive \( L \)-type \( \text{Ca}^{2+} \) channels. (Circulation Research 1992;71:201–209)

**Key Words** • \( \text{Ca}^{2+} \) channels • \( K^+ \) channels • \( \text{pH} \) • smooth muscle cells • basilar artery

The importance of acid–base balance in determining cerebral vascular tone has long been recognized. In 1849, Donders1 observed in cranial window preparations that hyperventilation resulted in dilation of pial vessels, and in 1881, Mosso2 reported that hyperventilation caused a decrease in cerebrospinal fluid pulsations in patients with cranial defects. In the modern practice of neurosurgery, the relation between acid–base balance and cerebral blood flow remains axiomatic and is frequently exploited for therapeutic purposes. In patients with increased intracranial pressure, mechanical hyperventilation is used to induce respiratory alkalosis, resulting in cerebral vasoconstriction, a decrease in cerebral blood volume, and a decrease in intracranial pressure.

An important question that gained early attention concerned the relative role of \( \text{pH}_i \) versus \( \text{pH}_o \) in mediating changes in tone. It is well known that the membrane potential of smooth muscle cells is significantly affected by changes in \( \text{CO}_2 \).3–5 From studies in Harder’s laboratory, evidence has accumulated that \( \text{pH}_i \) is important in determining membrane potential6 but that, under certain conditions, membrane potential can be dissociated from contractile effects caused by changes in \( \text{CO}_2 \). Conversely, in experiments using constant levels of \( \text{CO}_2 \), elevation of \( \text{pH}_i \) is associated with a decrease in vessel diameter7 as well as an increase in force production.8 Together, these observations have been taken to indicate that changes in \( \text{pH}_i \), although important, are insufficient to account for the observed effects of \( \text{CO}_2 \) or \( \text{pH} \) on the cerebral circulation9 and that, as postulated by Lassen,7 \( \text{pH}_o \) appears to be a key determinant of cerebrovascular tone.

Extracellular \( \text{Ca}^{2+} \) is an important source of activator \( \text{Ca}^{2+} \) and is required for maintaining basal tone in cerebral vessels.8–10 In vascular smooth muscle, the influx of \( \text{Ca}^{2+} \) required for basal tone is thought to occur by way of voltage-dependent \( \text{Ca}^{2+} \) channels,11 which in other tissues are known to be sensitive to \( \text{pH} \).12 That \( \text{Ca}^{2+} \) channels may be important for changes in tone in response to changes in \( \text{pH} \) is suggested by the findings that \( \text{CO}_2 \) reactivity obtained from measurements of cerebral blood flow may be blunted by administration of \( \text{Ca}^{2+} \) channel blockers13,14 and that vasoconstriction caused by alkalosis in isolated cerebral arterioles is abolished by the absence of \([\text{Ca}^{2+}]_o\).10 Evidence also has

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Supported by grants from the Moody Foundation (89-61) and the National Heart, Lung, and Blood Institute (HL-42646). G.A.W. was supported by National Institutes of Health training grant NS-07144.

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Received June 17, 1991; accepted March 10, 1992.
been presented in unpublished or preliminary form that the amplitude of the Ca\(^{2+}\) action potential or of the Ca\(^{2+}\) current in small cerebral arteries varies inversely with [H\(^+\)].\(^{15-17}\)

In the present study, we investigated the role of Ca\(^{2+}\) channels in cerebrovascular responses to changes in pH,

using isolated smooth muscle cells from the basilar artery of the guinea pig. We observed that contractions of single cells due to elevated pH\(_{c}\) (constant CO\(_2\)) were blocked by nifedipine, consistent with an important role of transmembrane flux in supplying activator Ca\(^{2+}\) for augmentation of tone.\(^{10}\) In patch-clamp experiments, we found that Ca\(^{2+}\) channel currents were strongly modulated by changes in pH\(_{c}\). As in a previous report,\(^{18}\) we separated the Ca\(^{2+}\) channel current into two components, L-type current and B-type current. T-type Ca\(^{2+}\) channels, identified in some vascular smooth muscle,\(^{19-22}\) are not present in basilar artery cells of the guinea pig, but a second component, termed B-type current, is distinguishable from L-type current by differences in voltage dependence of activation, rates of inactivation and deactivation, and sensitivity to dihydropyridines.\(^{18}\) In the present study, we show that an additional feature distinguishing B-type from L-type current is their different sensitivities to [H\(^+\)].

**Materials and Methods**

**Cell Isolation**

Single smooth muscle cells were isolated from basilar arteries of guinea pigs (*Cavia porcellus*, Hartley strain, 200–400 g) as reported.\(^{18}\) The basic isolation solution (PS1) contained (mM) NaCl 116.3, KCl 5.4, Na\(_2\)HPO\(_4\) 10.4, MgSO\(_4\) 0.83, glucose 5.5, and NaHCO\(_3\) 26.2, equilibrated with 95% O\(_2\)–5% CO\(_2\), pH 7.4. In brief, the animals were killed with an overdose of pentobarbital, and the basilar artery was dissected in PS1 plus 0.2 mM CaCl\(_2\) and 24 \(\mu\)g/ml papaverine–HCl (PAP). Enzymatic digestion was carried out at 37°C in PS1 plus 0.2 mM CaCl\(_2\) and 2.4 \(\mu\)g/ml PAP (PS2) with (mg/ml) collagenase 2, elastase 0.4, DNaese 0.2, soybean trypsin inhibitor (STI) 1, and fatty-acid–free bovine serum albumin (BSA) 1 for 75–95 minutes, followed by PS1 plus 2.4 \(\mu\)g/ml PAP without added Ca\(^{2+}\) (PS3) plus (mg/ml) protease 0.4, STI 0.4, and BSA 0.4 for 5–10 minutes. After enzymatic treatment, the artery was transferred to PS3 and trituated to release single myocytes. Cells were stored at 4°C in either PS2 or, for most voltage-clamp experiments, in KB solution\(^{23}\) containing (mM) KCl 85, K\(_2\)HPO\(_4\) 30, MgSO\(_4\) 5, sodium pyruvate 5, taurine 20, \(\beta\)-hydroxybutyrate 5, creatine 5, and Na\(_2\)ATP 2, along with 1 mg/ml BSA, pH 7.4.

**Cell-Contraction Experiments**

At ~7 minutes, an aliquot of cells was transferred to PS2 in a closed chamber\(^{24}\) (200 \(\mu\)l) on the stage of an inverted microscope (Nikon Diaphot). The floor of the chamber was a glass slide previously coated with poly-L-lysine, allowed to dry, and then rinsed with BSA (2 mg/ml) to prevent attachment of the cells. Cells were allowed to settle for 2 minutes before beginning superfusion. Cells were chosen for study only if they appeared elongated and phase bright. To measure spontaneous, unstimulated contraction of the cells, at ~5 minutes the solution was changed to PS1 plus 1 mM CaCl\(_2\) (PS4), pH 7.2; then, to check contractibility, at 10 minutes the solution was changed to a high [K\(^{+}\)] solution similar to PS4 except that K\(^{+}\) was increased to 100 mM by equimolar replacement of Na\(^{+}\). For the experiments shown in Figures 1 and 2, at ~5 minutes the solution was changed to PS4, pH 7.2, without (control cells) or with (experimental cells) 1 \(\mu\)M nifedipine. At 0 minutes the solution was changed to PS4, pH 7.9, without (control cells) or with (experimental cells) 1 \(\mu\)M nifedipine.

Control and experimental conditions were alternated for successive aliquots of cells. PS4 contained only the CO\(_2\)/HCO\(_3\)\(^-\) buffer given above and was adjusted to the desired pH with NaOH or HCl immediately before use with each aliquot of cells. At 10 minutes the solution was changed to the 100 mM K\(^{+}\) solution without nifedipine, to confirm that cells whose contraction had been blocked by nifedipine could indeed contract. Experiments were performed at room temperature, and images were recorded on videotape. Curvilinear cell length, obtained using an electronic map wheel (Runmate Instruments, Rijlaarsdam, Holland), was measured from video recordings at times just preceding −5, 0, 3, 10, and 15 minutes. For each cell, values at different times were expressed as percent decrease in length with respect to the value at ~5 minutes. The time course of the change in pH inside the chamber was measured using a separate apparatus that differed only in having the floor constructed using a flat-surface pH electrode (Markson), keeping volume and flow rate the same. With the same solutions as used to study the cells, a change in pH from 7.2 to 7.9 required 2 minutes to be 95% complete.

**Voltage-Clamp Experiments**

Cells were voltage-clamped using the whole-cell variant of the patch-clamp technique.\(^{25}\) Pipettes (1.5–4 M\(\Omega\)) were made from borosilicate glass (Kimax, Fisher Scientific, Pittsburgh, Pa.) and were fire-polished. An aliquot of cells was added to a 35-mm culture dish with paraffin inserts that reduced the volume to ~200 \(\mu\)l. The culture dishes previously had been coated with poly-L-lysine, allowed to dry, and washed briefly with BSA (2 mg/ml) to prevent attachment of cells to the dish. Cells were viewed with an inverted microscope (Nikon Diaphot). The liquid junction potential was nullled before seal formation. Seals were formed in 10BaNa, which contained (mM) NaCl 125, KCl 5, MgCl\(_2\) 1, BaCl\(_2\) 10, HEPES 10, and glucose 12.5, pH 7.2 with NaOH. After seal formation, typically 20–50 \(\Omega\) and rupture of the patch, the cell was lifted to near the surface of the bath to reduce capacitance. The superfuse was then changed for recording either inward or outward currents at various values of pH (see below). Bath solutions were superfused through multiple glass capillary tubes positioned near the cell with a micromanipulator, and flow was controlled by electronic switches. The pH of the superfusate was monitored with a pH microelectrode (Lazar Research Laboratories, Los Angeles, Calif.) to determine the time course of pH changes. Recording solutions containing tetrathyamlammonium chloride (NETA) introduced a ~5-mV liquid junction potential that was later subtracted.

Currents were amplified (EPC-7 amplifier, List, Darmstadt, FRG), filtered (eight-pole Bessel filter, Frequency Devices Inc., Haverhill, Mass.), and sampled
on-line by a microcomputer (model PDP 11/73, Digital Equipment Corp., Marlboro, Mass.). The sampling rate (2.5 and 0.5 kHz) varied with the duration of test pulses (200 and 1,000 msec). All voltage pulse protocols were menu-driven and generated by the computer. Except when measuring the holding current, leak and capacitative currents were automatically subtracted by the computer before storage using a procedure similar to that described by Bezanilla and Armstrong.26 Except during test and leak-subtraction pulses, the transient compensation and series resistance were monitored constantly throughout the recording period using repetitive 10-msec +3-mV pulses. Series resistance compensation was not used. Cell capacitance was 15–22 pF, as previously reported.18 Current recordings shown in the figures or fit to equations were filtered with a Gaussian digital filter.27 All experiments were performed at room temperature (22–25°C).

**Solutions**

Membrane seals were obtained in 10BaNa. For recording inward currents, the bath solution contained (mM) NaCl 125, 4-amino pyridine 5, MgCl2 1, BaCl2 10, and glucose 2.5, in addition to 10 mM MES (pH 5.7 and 6.1), HEPES (pH 6.5 and 7.2), or Tris (pH 7.7 and 8.1), adjusted with NaOH or HCl for the values of pH indicated. For recording outward currents, the bath solution contained (mM) NaCl 130, KCl 5, MgCl2 1, CaCl2 1.8, and glucose 12.5, in addition to 10 mM MES (pH 6.1), HEPES (pH 7.2), or Tris (pH 8.1), adjusted with NaOH or HCl for the values of pH indicated. The pH of recording solutions was measured daily and adjusted as necessary. For inward currents, the pipette solution contained (mM) CsCl 135, MgCl2 4, HEPES 10, Na2ATP 2, [ethylene bis(oxyanilino)tetraacetic acid (EGTA)] 11, and CaCl2 1 (pCa 7.8), adjusted to pH 7.2 with CsOH. For outward currents, the pipette solution contained (mM) KCl 145, NaCl 5, MgCl2 1, HEPES 10, Na2ATP 2, EGTA 2.48, CaCl2 1 (pCa 7.0), and glucose 10, adjusted to pH 7.2 with KOH. Stock solutions of nifedipine were prepared daily as 1-mM solutions in 50% polyethylene glycol (molecular weight, 400). At final dilution (0.05%), polyethylene glycol did not affect contraction of the cells. All chemicals were from Sigma Chemical Co., St. Louis, Mo., or Fisher Scientific.

**Data Analysis**

Data were given as mean±SD unless otherwise noted. Data were fit to equations using the nonlinear least-squares method of Marquardt (SAS, Cary, N.C.). As previously shown,18 the decaying portions of current recordings during 1-second pulses to +15 mV could be fit to a double exponential equation:

\[ I(t) = I_1 \cdot \exp(-t/\tau_1) + I_2 \cdot \exp(-t/\tau_2) \]

(1)

where \( I(t) \) is the total current during the test pulse, \( I_1 \) and \( I_2 \) are the amplitudes of the slowly inactivating (L) and more rapidly inactivating (B) components at time zero, \( \tau_1 \) and \( \tau_2 \) are the time constants of inactivation, and \( t \) is time. At low pHs, however, when currents were small, fits to Equation 1 were unreliable, especially for \( I_2 \), as judged by consistency of fits within a family of successive recordings from one cell. Reliability was greatly improved by modeling the slowly inactivating component as a linear function, i.e., by fitting current recordings to the following equation:

\[ I(t) = I_1 \cdot (1 - t/\tau_2) + I_2 \cdot \exp(-t/\tau_6) \]

(2)

Approximating the slowly inactivating component as a linear function results in underestimating its value and overestimating the value for \( I_0 \). This discrepancy was small, however, as may be seen by comparing the superimposed fits for the slowly inactivating component obtained using the two methods on data from the same cell (Figures 6A and 7A). For the sake of uniformity, data at all values of pH, were fit to Equation 2 for the analyses shown in Figures 6, 7C, and 8.

**Results**

**Contraction Experiments**

To study the contractile effects of elevated pH, we measured the shortening responses of single isolated myocytes. Under control conditions (pH 7.2), isolated cells shortened slightly (<5%) when exposed to 1 mM [Ca2+]0 for 15 minutes, but when the superfusate was made alkalotic, isolated cells contracted rapidly. An example of an isolated cell undergoing contraction in response to a change in pH, from 7.2 to 7.9 is shown in Figure 1, and the results for a group of cells (n=9) are shown in Figure 2. As indicated in “Materials and Methods,” the change in pH in the chamber was relatively slow, requiring >2 minutes to be complete, and so changes in length were assessed at 3 minutes. In response to a change in pH, from 7.2 to 7.9, isolated myocytes shortened 16±3% (mean±SEM) within 3 minutes, with no further change in length during an additional 7 minutes of observation (Figure 2, open bars).

The contractile response to alkalosis was blocked completely by the dihydropyridine nifedipine. In the presence of 1 μM nifedipine, a change to pH 7.9 resulted in cell shortening of <4% over 10 minutes (n=7; Figure 2, right-hatched bars), a value that was not different from unstimulated cells at pH 7.2. After washout of nifedipine, all cells contracted in response to 100 mM K+ [pH 7.2 (Figure 2, cross-hatched bars), indicating that cells whose response to alkalosis was blocked by nifedipine were indeed functional and could contract. In these experiments, pH was not directly manipulated beyond assuring that all solutions contained 5% CO2; thus, changes in pH, accompanying the changes in pHs, cannot be fully excluded. Nevertheless, our results strongly favor the hypothesis that alkalosis-induced contraction requires dihydropyridine-sensitive influx of Ca++. 

**Effect of pH on Inward Current**

Macroscopic inward currents were recorded in response to depolarizing step potentials. With 10 mM Ba2+ at pH 7.2, measurable inward currents appeared at approximately ~30 mV and were maximum at +15 mV, similar to values reported earlier.18 Decreasing pH caused a decrease in the inward current, as shown by original current recordings during 200-msec pulses to +15 mV from a holding potential (Eh) of ~55 mV at pH 7.2 (Figure 3A, recording a) and pH 6.5 (Figure 3A, recording b). Plots of the peak inward current (Imax) versus voltage at pH 7.2 and 6.5 are shown in Figure 3B. The extrapolated reversal potential (Erev) changed <5 mV, indicating no important effect on permeability, and values for the midpoint poten-
nifedipine significantly after \[K^+\], in increase mean nifedipine for change a was one with cells from contractile effect of were obtained by fitting run-up was taken into length cell to to 7.2. As -55 clamp the effect of ing pH, was attenuated in cells shown in cells that can be contractible with elevated \[K^+\], after removal of nifedipine.

![Figure 1](https://example.com/figure1.png)

**Figure 1.** A single smooth muscle cell from the basilar artery of the guinea pig contracting in response to a change in pH, from 7.3 to 7.9. Bright field micrographs were obtained at times just preceding -5, 0, 3, 10, and 15 minutes in panels A–E, respectively. Panel A: After 2 minutes in PS2, which contained 0.2 mM CaCl₂ and 2.4 μg/ml papaverine-HCl. Panel B: After 5 minutes in PS4, which contained 1 mM CaCl₂ without papaverine-HCl, pH, 7.2, just before the change to pH, 7.9. Panel C: Three minutes after change to pH, 7.9. Panel D: Ten minutes after change to pH, 7.9. Panel E: Five minutes after change to a 100 mM K⁺ solution. Curvilinear cell lengths were 89, 89, 63, 63, and 53 μm in panels A–E, respectively, giving 0%, 29%, 29%, and 40% changes in cell length at times 0, 3, 10, and 15 minutes, respectively.

![Figure 2](https://example.com/figure2.png)

**Figure 2.** Bar graph showing that nifedipine blocks the contractile effect of increased pH, in isolated smooth muscle cells from the basilar artery of the guinea pig. Two groups of cells were studied, one without nifedipine (open bars, nine cells) and one with 1 μM nifedipine (right-hatched bars, seven cells); cell length was measured at pH, 7.2 at -5 and 0 minutes, at 3 and 10 minutes after a change to pH, 7.9, and then 5 minutes after a change to a 100 mM K⁺ solution, pH, 7.2, which contained no nifedipine for either group (cross-hatched bars). Data are given as mean±SEM of the percent change with respect to length at -5 minutes; a positive number indicates shortening of the cell. Nifedipine significantly attenuated shortening in response to an increase in pH, in cells shown to be contractible with elevated \[K^+\], after removal of nifedipine.

![Figure 3](https://example.com/figure3.png)

**Figure 3.** Effect of a decrease in pH, on Ca²⁺ channel current in a smooth muscle cell from the basilar artery of the guinea pig. Panel A: Original current recordings during 200-msec pulses to +15 mV in control solution at pH, 7.2 (recording a) and after a change to pH, 6.5 (recording b). Linear leak and capacitative currents were subtracted; recordings were filtered at 0.2 kHz (–3 dB). Panel B: Peak current–voltage curves at pH, 7.2 (■) and pH, 6.5 (○). Note the block of current but absence of shift in voltage dependence at pH, 6.5. Panel C: Peak inward current during 200-msec test pulses to +15 mV plotted as a function of time after starting whole-cell voltage clamp. The pH, was changed from 7.2 to 6.5 and back to 7.2 at the times indicated by the vertical lines. Note that the initial run-up was followed by run-down of current. The time course of run-down (solid line) was computed from values at pH, 7.2 after run-up was complete, by fitting values to a simple exponential function with a run-down time constant of 15 minutes. Values at a and b correspond to recordings in panel A and to the maxima of the current–voltage curves in panel B. The holding potential was -55 mV, and all data were from the same cell.
the effect of changes in pH_{10}. For the example in Figure 3C, the time constant of run-down was 15 minutes (solid line in Figure 3C), the predicted value of I_{max} before return to pH_{10} 7.2 (at 24 minutes) was -109.5 pA, and the measured value of I_{max} at pH_{10} 6.5 was -56.5 pA, giving a relative current (I_{r}) of 0.33.

With an increase in pH_{10}, there was a corresponding increase in I_{max}, as shown by original current recordings during 1-second pulses to +15 mV from E_{n} of -55 mV, at pH_{10} 7.2 (Figure 4A, recording a) and pH 8.1 (Figure 4A, recording b). The current–voltage curves at pH_{10} 7.2 and 8.1 gave values of E_{u2} (10 and -9 mV), E_{max}, and E_{rec}, that were not appreciably different (Figure 4B). Successive values of I_{max} during the change to pH_{10} 8.1 are also shown for this cell (Figure 4C).

Similar results were obtained in other cells; i.e., the relative value of I_{max} measured at +15 mV (E_{n} of -55 mV) depended strongly on pH_{10}, but no substantial shift in E_{rec} or in voltage dependence was observed. Decreasing pH_{10} from 7.2 to 6.1 (n=4) changed E_{u2} from -7.2±1.9 to -3.1±3.7 mV, whereas increasing pH_{10} from 7.2 to 8.1 (n=6) changed E_{u2} from -5.0±2.6 to -5.2±2.0 mV. After taking run-down into account, lowering pH_{10} to 5.7 (n=5), 6.1 (n=10), and 6.5 (n=5) gave mean values of I_{r} for the peak current of 0.16, 0.36, and 0.42, whereas raising pH_{10} to 7.7 (n=8) and 8.1 (n=13) gave mean values of 1.21 and 1.54 (Figure 5). Data on the concentration–response relation between pH_{10} and I_{r} were fit to the equation

\[ I_{r} = I_{\text{max}} / (1 + 10^{pK_{a} - pH}) \]  

(3)

where pK is the negative logarithm_{10} of the apparent dissociation or ionization constant and I_{max} is the maximum increase observable. Values of I_{max} of 1.55 and pK 6.9 were found (solid line, Figure 5).

The time course of the response of the inward current to a change in pH was fairly rapid but was noticeably slower than the change in pH of the bath solution. Moreover, the response to a decrease in pH was consistently more rapid than that to an increase in pH. Although the kinetics of the response were not studied systematically, a decrease in inward current after lowering pH_{10} was apparent usually in the pulse immediately after the solution change and reached a steady-state level at \approx 3.5 minutes (consecutive pulses were given every 30 seconds). By contrast, the change in pH in the recording chamber measured with a pH microelectrode was first detected at 5–16 seconds and reached a steady-state value near 1 minute, with a time for 95% completion of 46 seconds (30–65 seconds).

**Effect of pH_{10} on the Two Components of Inward Current**

In gastric myocytes, the effect of pH_{10} on the peak inward current was previously found to be less pronounced than the effect on the sustained current.28 A similar observation was made here, using data collected
during 1-second pulses to +15 mV from $E_v$ of −55 mV. After correcting for run-down, lowering pH, to 5.7 ($n=3$) and 6.1 ($n=7$) gave mean values of I$_b$ for end-of-pulse current of 0.006±0.009 and 0.04±0.04, whereas raising pH, to 7.7 ($n=8$) and 8.1 ($n=6$) gave values of 1.5±0.32 and 2.79±0.97. Comparing these data with values given above for I$_{ba}$ (see also Figure 5) indicates that pH, had a much stronger effect on maintained current than on peak current.

One explanation for this differential effect of pH, on peak versus maintained current would be the presence of two distinct components of current. As previously shown, the inward Ba$^{2+}$ current in smooth muscle cells from the basilar artery of the guinea pig can be separated into two components, termed I$_b$ and I$_{ba}$, that differ in voltage dependence of activation and time constants of inactivation and deactivation.\(^{18}\) One indication that pH, differentially affected I$_b$ and I$_{ba}$ was that current tracings during inactivation tended to remain “parallel” on going to either low or high pH, indicating a greater effect of pH, on I$_b$. This is illustrated in Figures 7A and 7B, where tracings from two cells are scaled to the same peak value for the measurements at pH, 7.2.

To further examine this hypothesis, data from cells studied with repetitive 1-second pulses to +15 mV from $E_v$ of −55 mV were fit to Equation 2. Original current recordings at pH, 7.2 and 6.1 are shown in Figure 6A, with the fit for the slowly inactivating component superimposed on each tracing. Values for I$_b$ and I$_{ba}$ for the entire family of recordings are plotted in Figure 6B, and values for $\tau_i$ and $\tau_n$ are shown in Figure 6C. As previously shown,\(^{18}\) run-down of I$_b$ was faster; the run-down time constant was 35 and 97 minutes for I$_b$ and I$_{ba}$, respectively. I$_b$ for each component was computed as indicated above after run-down into account. The change to pH, 6.1 gave values of I$_b$ of 0.24 and 0.53 for I$_b$ and I$_{ba}$, respectively. A small reduction in $\tau_i$ was noted, but $\tau_n$ was relatively insensitive to the change in pH, (Figure 6C).

Data from a series of cells at different values of pH, obtained during 1-second pulses to +15 mV from $E_v$ of −55 mV, were analyzed in a similar way except that, for each cell, three to five current recordings were averaged before, at the end of, and after the change from pH, 7.2, and only the averaged recordings were fit to Equation 2. Averaged recordings from two cells are shown in Figures 7A and 7B. (For Figures 7A and 7B only, I$_b$ superimposed on current tracing was actually from a fit to Equation 1, as discussed in “Materials and Methods.”) Mean values of I$_b$ for cells at pH, 5.7 ($n=3$), 6.1 ($n=6$), 7.7 ($n=4$), and 8.1 ($n=5$) are shown in Figure 7C, for I$_b$ (I) and I$_{ba}$ (C). The data for each component were fit to Equation 3 with $I_{max}$ of 3.65 and pK 7.71 for I$_b$ and I$_{ba}$ of 1.28 and pK 6.47 for I$_{ba}$ (solid lines in Figure 7C). A Hill plot of these data is shown in the inset of Figure 7C, where the mean values for each component were computed as $S_1/I_1/(I_{max1} - I_1)$, and log(S) was plotted against pH,. The lines through the data (inset) represent the equation

$$\log(S) = h \cdot pH - pK$$  

(4)

where $h$, the Hill coefficient, is unity. The good correspondence between the data and the model provides further justification for the distinction of two components of inward Ba$^{2+}$ current in these cells and indicates that the L-type current is considerably more sensitive to pH, than the B-type current.

Figure 8 is a semilog plot of the mean time constants of inactivation for the two components of current at various values of pH, obtained from the analysis of Figure 7C. Values at pH, 7.2 agreed well with previous findings.\(^{18}\) As shown, $\tau_i$ at +15 mV was little affected by pH,, whereas $\tau_n$ at +15 mV tended to increase with increasing pH,.\(^{18}\)

**Figure 6.** Effect of decrease in pH, on L- and B-type Ca$^{2+}$ channel currents in a smooth muscle cell from the basilar artery of the guinea pig. Panel A: Original current recordings at pH, 7.2 (recording a) and pH, 6.1 (recording b) obtained at the times indicated by the vertical marks along the abscissa of panel C. One-second pulses were to +15 mV, and the holding potential was −55 mV. Linear leak and capacitative currents were subtracted. The current recordings were filtered at 50 Hz (−3 dB), and the decaying portions were fitted to Equation 2, with the slowly inactivating L-type current (I$_L$)=−170 and −24 pA, the time constant of inactivation for the L-type current ($\tau_L$)=2.252 and 1.543 msec, the more rapidly inactivating B-type current (I$_B$)=−181 and −82 pA, the time constant of inactivation for the B-type current ($\tau_B$)=140 and 188 msec, for recordings a and b, respectively. Fits for the slowly inactivating component are superimposed on each recording. Panels B and C: The decaying portion of successive current recordings, recorded at 30-second intervals during 1-second pulses to +15 mV from a holding potential of −55 mV, were fitted to Equation 2. Values of I$_L$ (I) and I$_B$ (C) are plotted in panel B, and values of $\tau_i$ (I) and $\tau_n$ (C) are plotted in panel C as a function of time after starting whole-cell voltage clamp. pH, was changed from 7.2 to 6.1 and back to 7.2 at the times indicated by the vertical lines. The time course of run-down of I$_b$ and I$_{ba}$ (solid lines in panel B) was computed from values at pH, 7.2 after run-up was complete, by fitting values to a simple exponential function with run-down time constants of 35 and 97 minutes for I$_b$ and I$_{ba}$, respectively. In this cell, dividing the fitted values for I$_b$ and I$_{ba}$ at pH, 6.1 by the expected values based on the calculation of run-down gave relative currents at pH, 6.1 of −35.2/−148.8=0.24 and −86.7/−164.1=0.53 for I$_b$ and I$_{ba}$, respectively.
Effect of pH<sub>i</sub> on Holding and Outward Current

Effects on holding and on outward currents were examined in five cells during changes in pH<sub>i</sub> between 6.1, 7.2, and 8.1, with pH<sub>i</sub> 7.2 and pCa<sub>i</sub> 7.0. The holding current, measured at −60 and −40 mV, was not significantly affected by changes in pH<sub>i</sub> (data not shown). Similarly, there was no significant effect of pH<sub>i</sub> on voltage-activated outward currents recorded from E<sub>0</sub> of −60 mV during 200-msec pulses to potentials between −50 and +30 mV (data not shown).

Discussion

This report presents the first systematic description of the effects of pH<sub>i</sub> on Ca<sup>2+</sup> channel currents in vascular smooth muscle cells. Our data are consistent with previous reports of unpublished or preliminary findings that the amplitude of the Ca<sup>2+</sup> action potential or Ca<sup>2+</sup> current in cerebral arterioles is inversely related to [H<sup>+</sup>]<sub>i</sub>. We found that a decrease in pH<sub>i</sub> caused a strong decrease in total inward current, with near complete block at pH<sub>i</sub> 5.7, whereas an increase in pH<sub>i</sub> caused an increase in total inward current.

The inward Ba<sup>2+</sup> current in basilar artery cells is separable into two components with distinct physiological properties. One component has characteristics identical to L-type channels in heart and other cells, including slow inactivation, fast deactivation, and sensitivity to dialysis (i.e., fast run-down) and dihydropyridines. The second component, termed B-type current, is different from T-type current described in other preparations, including peripheral vascular smooth muscle. Compared with T-type current, I<sub>B</sub> activates at more positive potentials and inactivates more slowly, with a time constant of 160 msec at +15 mV. In the present study, we found that I<sub>B</sub> and I<sub>B'</sub> were differentially sensitive to pH<sub>i</sub>, providing further support for the distinction of two components of inward Ba<sup>2+</sup> current in these cells. I<sub>B'</sub> was blocked completely between pH<sub>i</sub> 6.1 and 5.7, whereas I<sub>B</sub> was reduced 40–60%. Also, the apparent pK for each component differed by more than one log unit (7.71 versus 6.47 for I<sub>B</sub> and I<sub>B'</sub>, respectively). Notably, for the L-type current, the apparent pK near physiological pH and the Hill coefficient of unity were similar to values found in ventricular myocytes in single-
channel studies using Na\(^+\) as the charge carrier.\(^{35}\) Therefore, it appears that an important effect of a change in pH\(_r\) in cerebral smooth muscle involves modulation of the slowly inactivating L-type current. Only at more extreme, unphysiological values of pH\(_r\) would there be a significant effect on I\(_{b}\).

In cardiac tissue, the effects of pH on Ca\(^{2+}\) currents are more complex than those reported here in cerebral smooth muscle. Apart from a block by external H\(^+\), changes in pH\(_r\) also affect gating, presumably because of an effect on surface charge.\(^{12,34}\) In the present study, we found no effect of pH\(_r\) on the voltage dependence of activation, and similar results were obtained in experiments at pH\(_r\) 7.2 in which 10 mM Ba\(^{2+}\) was replaced by equimolar Ca\(^{2+}\) (J.M. Simard, unpublished observations). These data suggest an absence of surface charge effects on gating of Ca\(^{2+}\) channels in these cells, although disruption of surface charges by enzymatic treatment during isolation cannot be excluded.

Tytgat et al\(^{34}\) have recently reported that, in guinea pig ventricular myocytes, the T-type channel is more sensitive to pH\(_r\) than the L-type channel. From measurements of both single-channel and macroscopic currents, they found that a decrease in pH\(_r\) caused a decrease in conductance and open probability, as well as a shift in activation and inactivation of the T-type channel. The apparent pK for the effect on conductance was 7.1–7.5; maximal T-type conductance was completely blocked at pH\(_r\) 5.8 and was increased by a factor of 10 for a change in pH\(_r\) from 6.5 to 9. By contrast, maximal L-type conductance was completely blocked at pH\(_r\) 4.0 and was increased by only 1.18 for a change in pH\(_r\) from 6.5 to 9. Thus, it appears not only that L-type channels differ between cardiac myocytes and cerebral smooth muscle but the data presented here further substantiate the earlier report\(^{18}\) that T-type channels, as described in cardiac and other cells, are not present in smooth muscle from guinea pig basilar artery and that B-type current is quite different from T-type current.

In cardiac myocytes, decreasing pH\(_r\) decreases Ca\(^{2+}\) current,\(^{35–38}\) and Ca\(^{2+}\) currents may be more sensitive to pH\(_r\) than to pH\(_{i}\).\(^{37}\) All of the data in the present study were obtained by changing pH\(_r\), with the pipette solution maintained at pH 7.2 with 10 mM HEPES. The effects that we observed on inward currents with changes in pH\(_r\) are unlikely to have been mediated by changes in pH\(_i\) because 10 mM HEPES provides adequate buffering.\(^{12,34}\) Clearly, our data do not address the important effects of pH\(_r\) on Ca\(^{2+}\) current or on contractility in the cells.\(^{5,17}\)

Several systems that contribute to cerebrovascular tone are sensitive to pH\(_r\) or pH\(_i\), but questions remain concerning the relative role of each in affecting changes in tone with changes in pH. Free [Ca\(^{2+}\)]\(_r\) is likely to rise at least transiently with intracellular acidification, as H\(^+\) ions displace Ca\(^{2+}\) that is bound.\(^{39,40}\) Based on measurements of membrane potential, it has been shown that a K\(^+\) conductance in cerebral smooth muscle is sensitive to pH\(_r\) (increasing with acidification) but that this conductance is not affected by changing pH\(_i\) when CO\(_2\) is constant.\(^{4}\) In the present study, we also found no effect of pH\(_r\) on the holding current at negative potentials with buffering of pCa, and pH\(_i\).

Bonnet et al\(^{41}\) recently reported on outward currents in isolated myocytes from feline cerebral vessels. No Ca\(^{2+}\)-sensitive outward current was found, but in most cells, an outward rectifier current was present that increased with extracellular acidification. It is not clear, however, whether this outward rectifier is activated at potentials at which changes in resting potential were previously observed.\(^{4,5}\) In the present study, we could not confirm the presence of an outward current sensitive to changes in pH\(_r\), but other differences, possibly due to species or methods, are apparent. Unlike the cells studied by Bonnet et al,\(^{41}\) the cells that we studied from guinea pig basilar artery also have large-conductance Ca\(^{2+}\)-activated K\(^+\) (K\(_{Ca}\)) channels that contribute to the outward current (Y. Song and J.M. Simard, unpublished observations), as do bovine pial\(^{42}\) and rat basilar\(^{43}\) artery cells.

Our results on the modulation of Ca\(^{2+}\) channel currents by pH\(_r\) do not imply that changes in resting potential\(^{4,5}\) resulting from changes in pH or CO\(_2\) can be attributed to changes in Ca\(^{2+}\) influx. Rather, it is the membrane potential, which is determined at rest by K\(^+\) conductance, that regulates activation of voltage-dependent Ca\(^{2+}\) channels.\(^{11,15}\) Transmembrane Ca\(^{2+}\) flux may contribute indirectly via activation of K\(_{Ca}\) channels, which do participate in regulating the membrane potential in cerebral smooth muscle.\(^{42}\) Unless pH\(_r\) is constant, however, the role of K\(_{Ca}\) channels is likely to be complex, because these channels may be blocked by internal H\(^+\),\(^{44}\) resulting in an effect on membrane potential that opposes that expected from an increase in [Ca\(^{2+}\)].

The effect of pH\(_r\) on Ca\(^{2+}\) channel currents in cerebral vascular smooth muscle, as previously noted\(^{15–17}\) and as elaborated on here, suggests an additional mechanism by which changes in pH\(_r\) may affect basal tone in the cerebral vasculature, i.e., by modulating tonic steady-state influx of Ca\(^{2+}\). In cerebral vascular smooth muscle, activator Ca\(^{2+}\) originates predominantly from outside the cell rather than from intracellular stores.\(^{8,9}\) Extracellular Ca\(^{2+}\) is required for maintaining basal tone in cerebral arterioles, with relaxation observed on removal of Ca\(^{2+}\),\(^{10}\) and isolated basilar artery cells studied without load exhibit a gradual contraction that is prevented by nifedipine or by removal of Ca\(^{2+}\) (J.M. Simard and D.C. Leppa, unpublished observations). Such data have been taken as indicating that tonic influx of Ca\(^{2+}\) is crucial for maintenance of basal tone. Moreover, CO\(_2\) reactivity of cerebral blood flow is reported to be blunted by administration of Ca\(^{2+}\) channel blockers\(^{13,14}\); alkalosis-induced vasoconstriction in isolated cerebral arterioles is blocked by either nifedipine (G.A. West and A.C. Ngai, unpublished observations) or zero-calcium solution\(^{10}\); and in our single cell contraction experiments, increasing pH\(_r\) with constant CO\(_2\) caused contraction of the cells that was blocked by nifedipine. Together, these data provide evidence that influx of Ca\(^{2+}\) via a dihydropyridine-sensitive pathway is required for the increase in tone due to alkalosis.

An attractive hypothesis that accounts for effects of Ca\(^{2+}\) removal and of Ca\(^{2+}\) channel blockers on cerebrovascular tone is that tone is due to steady-state influx of Ca\(^{2+}\) via voltage-dependent L-type Ca\(^{2+}\) channels.\(^{11}\) We propose that external H\(^+\) ions modulate this steady-state Ca\(^{2+}\) current and note that internal H\(^+\) ions could have a similar effect.\(^{17}\) As with dihydropyridines, block of steady-state current by an increase in H\(^+\) would favor a decrease in tone; removal of H\(^+\) ion block by raising pH would favor an increase in tone. Implicit in this hypothesis is that a
fraction of voltage-dependent dihydropyridine-sensitive Ca\textsuperscript{2+} channels is open near resting membrane potentials and that opening of these channels allows sufficient influx of Ca\textsuperscript{2+}. Macroscopic Ca\textsuperscript{2+} channel currents at resting membrane potentials, which are difficult to study because of their small size, were not examined in this report. As indicated in a recent review,\textsuperscript{11} however, even the low probability of opening of L-type Ca\textsuperscript{2+} channels that obtains near resting membrane potentials (p=0.001–0.003) can allow surprisingly large inflows of Ca\textsuperscript{2+}, which, depending on mechanisms of extrusion, may be sufficient to account for maintenance of tone. Quantitative measurements of Ca\textsuperscript{2+} channel currents in basilar artery cells at resting membrane potentials will be required to substantiate this hypothesis. Our observations on the sensitivity of Ca\textsuperscript{2+} channels to pH\textsubscript{o}, however, suggest that steady-state influx of activator Ca\textsuperscript{2+} via these channels would be affected by changes in [H\textsuperscript{+}].

Acknowledgment

We thank Ms. Christy Saydjari for assistance in computer programming.

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Circ Res. 1992;71:201-209
doi: 10.1161/01.RES.71.1.201

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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