

Intracellular pH Regulates Basolateral K^+ and Cl^- Conductances in Colonic Epithelial Cells by Modulating Ca^{2+} Activation

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ABSTRACT The role of intracellular pH as a modulator of basolateral K^+ and Cl^- conductances in epithelial cells was studied using digitonin-permeabilized colonic cell layers so that cytosolic pH could be clamped at specific values, while basolateral K^+ and Cl^- conductances were activated by stepwise increases in intracellular free Ca^{2+} . Increasing the intracellular pH from 6.6 to 8.0 enhanced the sensitivity of both ionic conductances to intracellular Ca^{2+} , but changing extracellular pH had no effect. Maximal K^+ and Cl^- currents activated by Ca^{2+} were not affected by changes in intracellular pH, suggesting that protons do not alter the conduction properties of the channels. Hill analysis of the Ca^{2+} activation process revealed that raising the cytosolic pH from 6.6 to 8.0 reduced the $K_{1/2}$ for Ca^{2+} activation. In the absence of Ca^{2+} , changes in intracellular pH did not have a significant effect on the basolateral K^+ and Cl^- conductances. These results are consistent with the notion that changes in cytosolic pH can modulate basolateral conductances by modifying the action of calcium, perhaps by acting at or near the activation site to provide a mechanism of variable "gain control."

INTRODUCTION

Cytosolic pH may play an important role as an intracellular messenger and modulator of ion channels in epithelial cells (Boron, 1986; Oberleithner et al., 1987, 1988; Harvey et al., 1988), but the mechanisms underlying the effects of pH on ion channels are not well understood. Any attempt to determine the effect of cytosolic pH on ion channels in intact epithelial cells is potentially complicated by parallel changes in intracellular Ca^{2+} , another important mediator (Grinstein and Goetz, 1985; Hesketh et al., 1985; Busa, 1986; Grinstein and Cohen, 1987). Ideally, it would be possible to study the effects of cytosolic pH and free Ca^{2+} activity under conditions in which both of these variables could be clamped at specified values.

Previously we showed that it was possible to study the conductive properties of the basolateral membranes of colonic epithelial cells using cell layers that had been

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apically permeabilized by exposure to the detergent, digitonin (Chang and Dawson, 1988). The extreme permeabilization of the apical membrane permitted us to use organic buffer systems to effectively clamp cytosolic pH and pCa. A cytosolic pH of 6.6 was chosen for these initial studies to optimize the Ca^{2+} buffering properties of EGTA, and we identified basolateral ionic conductances selective for K^+ and Cl^- which could be activated by cytosolic free Ca^{2+} in the range of 10–1,000 nM.

In this paper we present the results of experiments designed to evaluate the possible role of pH as a modulator of basolateral K^+ and Cl^- conductances. The limitations due to the pH sensitivity of EGTA were overcome by using BAPTA (Tsien, 1980) as a buffer so that it was possible to study Ca^{2+} activation at cytosolic pHs ranging from 6.6 to 8.0. The results are compatible with the notion that intracellular pH modulates these Ca^{2+} -activated conductances due to an action at or near the Ca^{2+} activation site, which, in effect, allows intracellular pH to control the “gain” of the Ca^{2+} activation process.

MATERIALS AND METHODS

Tissue Preparation and Measurement of Ion Currents

Colons were excised from freshwater turtles (*Pseudemys scripta*) and stripped of outer longitudinal and circular musculature as previously described (Dawson, 1977). Isolated tissue sections were mounted in lucite Ussing chambers (area 5.2 cm²) and bathed on both sides by a vigorously aerated, modified physiological Ringer's solution at room temperature (22–25°C). The transmural potential difference (PD) was clamped to 0 mV by an automatic voltage clamp adjusted to compensate for the fluid resistance. When asymmetric bathing solutions were used, the fluid resistance was determined for each and the average value was used as a correction. Measurement of the transmural PD was achieved with calomel half-cells, which were connected by 0.5 M KCl-agar salt bridges to both chamber halves. Current was passed across the epithelium with Ag/AgCl electrodes, which were connected to both chamber halves by 1.0 M KCl-agar salt bridges. The short-circuit current (I_{sc}) was continuously recorded on a stripchart recorder. Tissue conductance was measured every 100–200 s by measuring the change in current brought about by a brief (1 s) 10-mV change in clamping potential. The modified physiological Ringer's solution (adapted from Altman and Ditmer, 1961) contained (in millimolar): 81 NaCl, 4.2 KHCO_3 , 21.8 NaHCO_3 , 1.1 Na_2HPO_4 , 2.2 Mg acetate, 10 HEPES acid, 5 NaOH, 1.5 Ca^{2+} gluconate, 10 Na gluconate, and 10 glucose at pH 7.4.

After a steady-state I_{sc} was attained during a preincubation period (30–60 min), the mucosal and serosal baths were washed at least five times and replaced by their respective test solutions. To study basolateral K^+ currents, tissues were bathed in chloride-free solutions. A K^+ gradient was imposed by bathing the mucosal (or luminal) side with a Ca^{2+} -free, K aspartate Ringer's solution of the following composition (in millimolar): 120 K^+ , 90 aspartate, 20 organic Good's buffer, 5 BAPTA, and 10 glucose. The organic Good's buffers used were: PIPES (pH 6.6 and 7.0), HEPES (pH 7.4 and 7.7), and TAPS (pH 8.0). The serosal bath contained a Na aspartate Ringer's solution that was identical except for the replacement of K by Na and the addition of 6 mM Ca^{2+} gluconate (1 mM free Ca^{2+}) and 2 μM atropine (to block any effects of endogenous acetylcholine; Venglarik and Dawson, 1986). To study currents due to Cl^- , the cell layer was bathed by potassium-free solutions. A Cl^- gradient was imposed by placing a NaCl Ringer's solution in the serosal bath and a Ca^{2+} -free, Na aspartate Ringer's solution in the mucosal bath. The NaCl Ringer's solution contained (in millimolar): 120 Na^+ , 20 organic Good's buffer, 5 BAPTA, 6 Ca^{2+} gluconate, 90 Cl^- , 10 glucose, and 2 μM atropine. The Na aspartate solution

was identical except for the deletion of the calcium and the replacement of Cl^- by aspartate. The pH of the test solutions was buffered to 6.6, 7.0, 7.4, 7.7, or 8.0. Concentrations of Na^+ and K^+ were verified by flame photometry.

The Ca^{2+} -BAPTA Buffer System

In previous studies we used the Ca^{2+} chelator, EGTA, at pH 6.6 in the mucosal bathing solutions to clamp internal free Ca^{2+} at desired levels. This relatively acidic pH was chosen for two reasons. First, at this pH the slope of the titration curve (Chang et al., 1988) is such that it is relatively easy to obtain free Ca^{2+} concentrations in the 10–1,000-nM range and yet maintain adequate Ca^{2+} buffering capacity. Second, at this pH the conductance of the basolateral membrane in the absence of Ca^{2+} was relatively low. For the purposes of this study, EGTA was unsuitable as a buffering agent because in the desired pH range its Ca^{2+} buffering capacity is highly pH dependent due to amine groups that bind protons with pK_a 's of 8.96 and 9.58. To overcome this obstacle we used the relatively pH-insensitive Ca^{2+} chelator BAPTA (Tsien, 1980). In BAPTA's chemical structure benzene rings replace the methylene links between oxygen and nitrogen of EGTA. This structure confers upon the nitrogens of BAPTA pK_a 's of

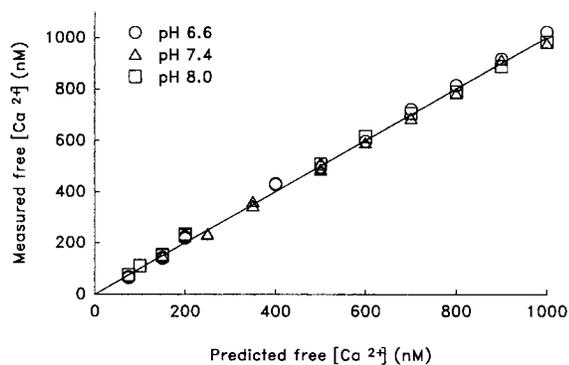


FIGURE 1. Fura-2 calibration of free $[Ca^{2+}]$ in mucosal bathing solutions containing 5 mM BAPTA. Free $[Ca^{2+}]$ in these solutions was designed using a computer program (see Materials and Methods) and the published stability constants for BAPTA (Tsien, 1980). Free Ca^{2+} levels predicted by this method were evaluated by dual-wavelength spectrofluorimetry with fura-2 free acid salts as

described by Grynkiewicz et al. (1985). Each data point represents the mean of three separate determinations of each sample.

5.47 and 6.36 and also provides BAPTA with faster rates of Ca^{2+} buffering than EGTA (Tsien, 1980).

The composition of the mucosal bathing solution was designed to yield any desired free Ca^{2+} concentration at a given pH with the aid of a computer program developed previously (Chang et al., 1988). This BASIC computer program was modified for use with BAPTA by incorporating equilibrium reactions between BAPTA, Ca^{2+} , Mg^{2+} , and H^+ and their respective stability constants as reported by Tsien (1980).

Fura-2 Measurement of Free Calcium

To verify the free Ca^{2+} concentrations used in mucosal bathing solutions designed with our computer program we tested these solutions with fura-2 spectrofluorimetry (Grynkiewicz et al., 1985). Test solutions were made by the addition of specified amounts of Ca^{2+} to a 10-ml volume of mucosal bathing solution. A 3-ml sample volume of this solution containing 1.333 μM fura-2 (cell impermeant pentapotassium or pentasodium salt; Molecular Probes, Inc., Eugene, OR) was placed in polyacrylate cuvettes (Sarstedt) and the dual-excitation fluorescent emission was collected at 0.5-s periods for 0.5–2 min at 22–25° (SPEX Fluorog II). The excitation

monochromators were set at 340 and 380 nm with 0.5-mm slit widths, while the emission monochromator was set at 505 nm with a 1.25-mm slit width. The F_{\min} was then obtained by the addition of 150 μ l 0.1 M EGTA (basic, pH 12.5) to the cuvette. Then 150 μ l of 1 M calcium acetate was added to the cuvette to obtain the F_{\max} . Free $[\text{Ca}^{2+}]$ was calculated from the ratio of the fluorescence at the two excitation wavelengths by the method of Tsien and co-workers (Grynkiewicz et al., 1985).

Comparisons between free Ca^{2+} concentrations predicted by the use of our computer program corresponded closely to those measured with fura-2 as shown in Fig. 1. There is an element of circularity in this procedure, however, because a Ca^{2+} -EGTA buffer system was used by Grynkiewicz et al. (1985) to characterize the Ca^{2+} binding properties of this indicator dye. In essence, therefore, our determination of free Ca^{2+} levels in the BAPTA buffered solutions using fura-2 is simply a comparison of our solutions with the free Ca^{2+} standard established by Tsien and co-workers (Grynkiewicz et al., 1985).

Digitonin Permeabilization

Permeabilization of the apical membrane was achieved by the addition of 10–20 μ l of a concentrated (20 mM) solution of digitonin (Fluka Chemical Corp., Ronkonkoma, NY) to the mucosal bathing solution. Because digitonin is poorly soluble in water, the stock solution was warmed before addition. As judged from the increase in tissue conductance, the onset of permeabilization occurred from 1 to 5 min after the addition of the detergent and was complete after 15 min. Despite the continued presence of digitonin in the mucosal bath, the tissue conductance was stable for up to 2–3 h. The permeabilizing effect of digitonin was irreversible.

RESULTS

Internal pH Modifies Calcium Activation of Basolateral K^+ and Cl^- Conductances

Representative traces in Fig. 2 show step-wise Ca^{2+} activation of the basolateral K^+ and Cl^- conductances at different pH values. As reported previously, basolateral conductance was low in the absence of Ca^{2+} ($[\text{Ca}^{2+}] < 10$ nM), but the addition of specified amounts of calcium acetate to the mucosal bath sufficient to raise the free Ca^{2+} concentration to values ranging from 50 to 1,000 nM activated currents due to K^+ and Cl^- . It is readily apparent from these traces that raising the pH from 6.6 to 8.0 increased the sensitivity of both the K^+ and Cl^- conductances to cell Ca^{2+} .

It was important to determine if the maximal Ca^{2+} -activated currents were altered by changes in intracellular pH. Ca^{2+} -activated K^+ currents were operationally defined as the quinidine-sensitive change in the maximal I_{sc} induced by the addition of Ca^{2+} (Chang and Dawson, 1988). In all but a few cases the Ca^{2+} -activated and quinidine-inhibited portions of the I_{sc} were identical. The magnitude of Cl^- currents was defined as the maximal current activated by Ca^{2+} in the absence of other permeant ions. Using paired tissues we compared the maximal I_{sc} activated by cytosolic Ca^{2+} at intracellular pH values ranging from 6.6 to 8.0. This comparison, presented in Fig. 3, indicated that the maximal, Ca^{2+} -activated K^+ and Cl^- currents were relatively unaffected by changes in intracellular pHs around 7.4. This finding is consistent with the notion that the reduction in the sensitivity to Ca^{2+} of the K^+ and Cl^- conductances brought about by a more acidic cytosolic pH was not a consequence of a change in the maximal currents such as would be expected, for example, if protons blocked the channels.

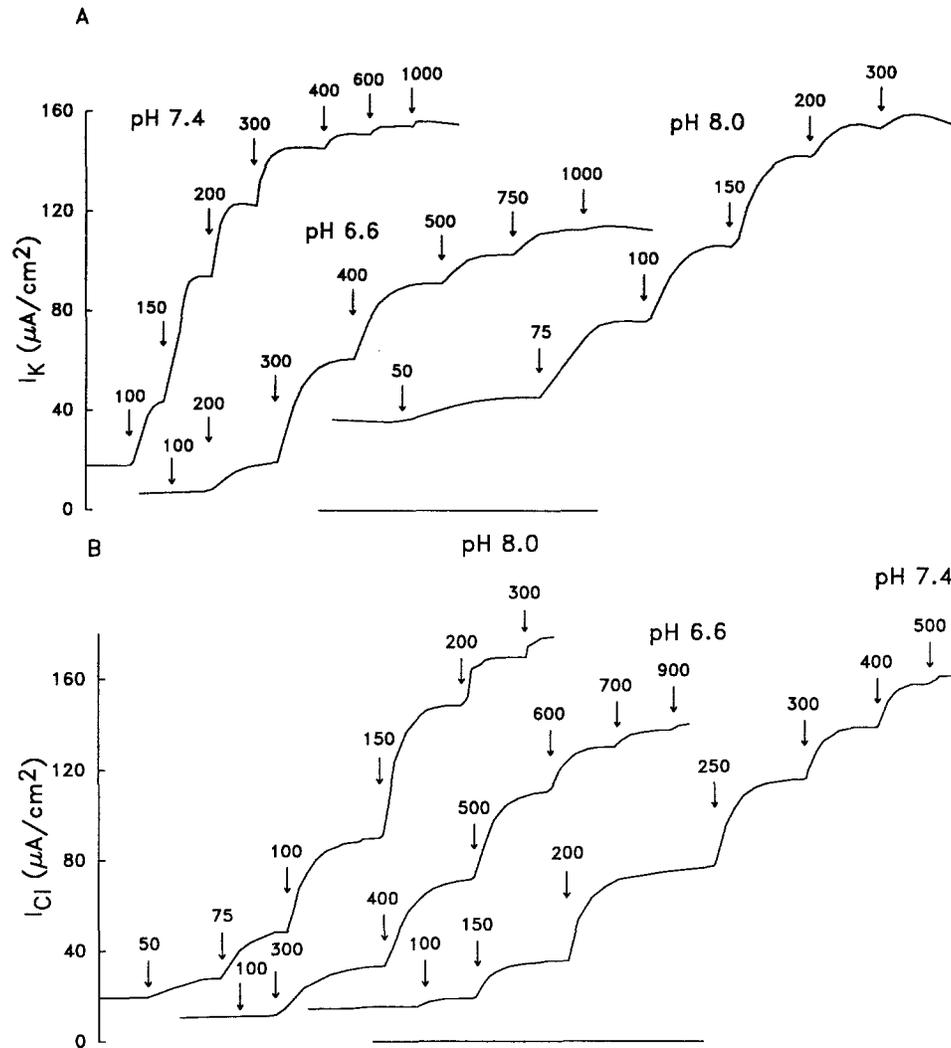


FIGURE 2. Dependence of basolateral K^+ and Cl^- conductances on pH and Ca^{2+} . Representative traces of stepwise activation of I_K (A) and I_{Cl} (B) by increasing free Ca^{2+} concentrations in the mucosal bathing solutions at various pH values. The pHs of the mucosal and serosal bathing solutions were identical in each trace. The pH dependence of the activation of the K^+ and Cl^- conductances was studied at pH values of 6.6, 7.0 (not shown), 7.4, 7.7 (not shown), and 8.0. The values above the arrows indicate the concentrations of free Ca^{2+} (nanomolar) achieved in the mucosal bathing solution by the addition of specific amounts of Ca^{2+} . The concentrations of total Ca^{2+} required to obtain these free Ca^{2+} levels at each particular pH level with 5 mM BAPTA were calculated as described in Materials and Methods. The accuracy of these solutions was tested by fura-2 measurements as shown in Fig. 1. Time bar represents 10 min.

The Site of Action of pH Is Intracellular

The initial experiments were conducted by manipulating the pH of the mucosal and serosal baths simultaneously to avoid possible time-dependent changes as a result of pH gradients across the basolateral membrane. To determine whether the intracellular or the extracellular face of the basolateral membrane was the site of action of the pH effect, we conducted additional experiments using asymmetric pH buffer solutions to bathe the mucosa (intracellular compartment) and serosa (extracellular). Ca^{2+} activation of the basolateral K^+ conductance in the presence of a pH gradient is shown in Fig. 4. With a mucosal pH of 6.6 and a serosal pH of 8.0, the K^+ conductance displayed a Ca^{2+} activation profile characteristic of symmetric pH 6.6 conditions. Conversely, Fig. 4 *B* shows that the sensitivity of the K^+ conductance to Ca^{2+} activation under pH 8.0 (mucosal) vs. pH 6.6 (serosal) conditions was typical of those obtained under symmetric pH 8.0 conditions. Fig. 4 exhibits data from a similar experiment for the Ca^{2+} -activated Cl^- conductance. Again, we observed that the Ca^{2+} activation of the basolateral Cl^- conductance was modified by changes in the mucosal (cytosolic), not the serosal, pH.

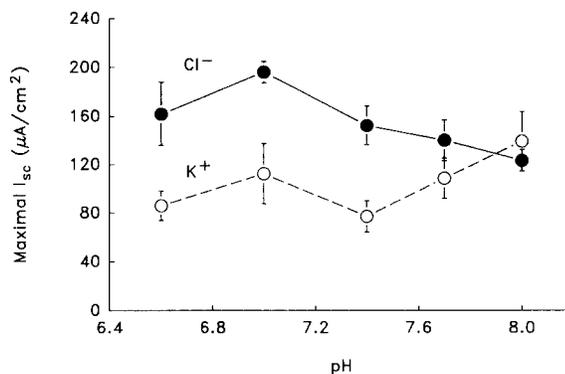


FIGURE 3. Comparison of maximally activated K^+ (open circles) and Cl^- (filled circles) conductances. Maximally activated basolateral K^+ and Cl^- currents are plotted versus cytosolic pH. Each point represents the mean and SEM of 4–10 experiments.

Evidence for pH Modulation of Calcium Activation Sites

Fig. 5 summarizes the effect of cellular pH in the 6.6–8.0 range on the activation of the basolateral K^+ and Cl^- conductances by free Ca^{2+} . As the cytosolic compartment was made more acidic, the sensitivity of both the K^+ and the Cl^- conductances to Ca^{2+} was reduced, such that greater concentrations of free Ca^{2+} were required to achieve half-maximal activation. Furthermore, at any pH the K^+ conductance was more sensitive to intracellular Ca^{2+} than the Cl^- conductance. Fig. 6 illustrates the effect of intracellular pH on the extent of Ca^{2+} activation at a fixed free Ca^{2+} concentration of 200 nM.

Because of the marked sigmoidal dependence of both the K^+ and Cl^- conductances on free $[\text{Ca}^{2+}]$, we used Hill analysis to examine the relationship between pH and Ca^{2+} in the activation of the two conductances. A Hill plot of one of the activation curves (K^+ conductance at pH 7.4) is presented in Fig. 7 *A* and shows that the Ca^{2+} activation data were well described by the Hill equation. Values for the apparent $K_{1/2}$

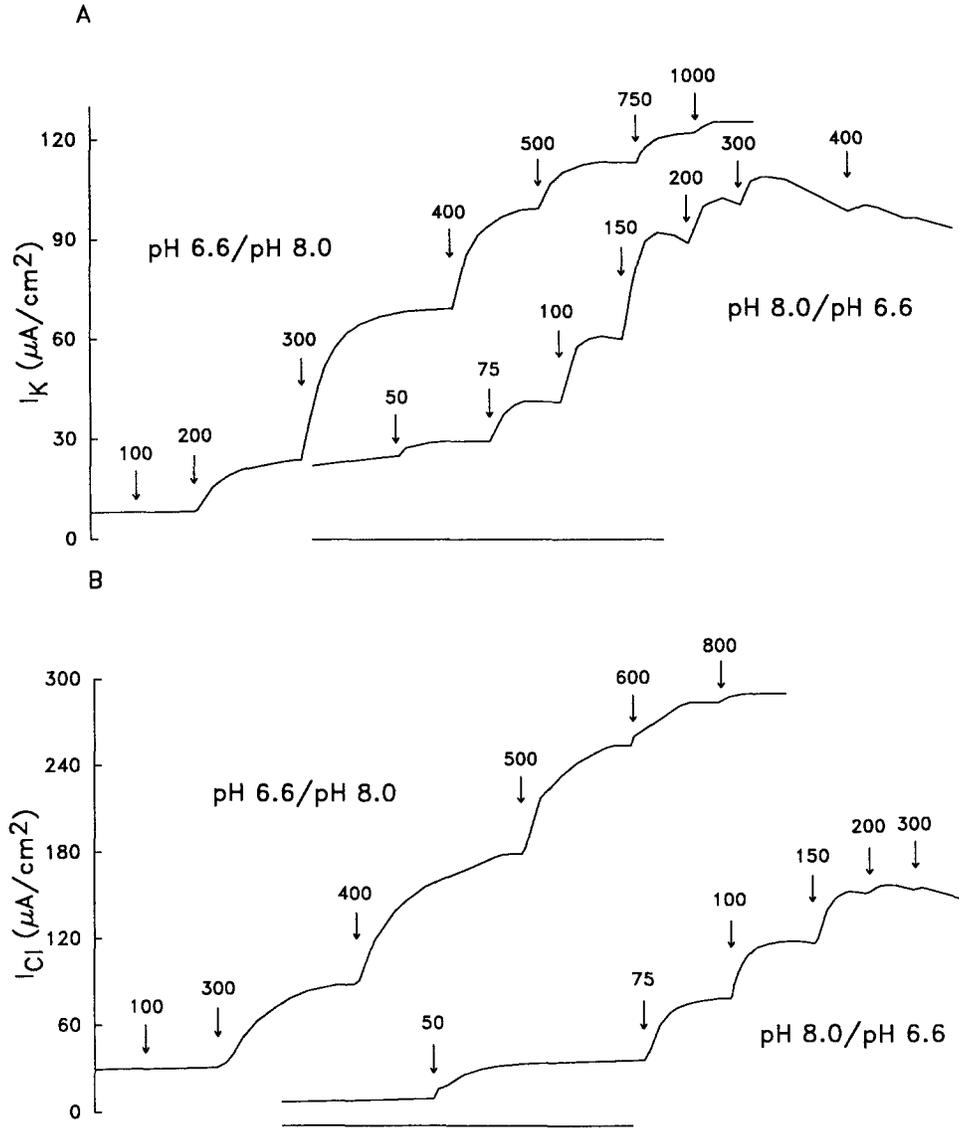


FIGURE 4. Representative experiments demonstrating the sidedness of action of pH on Ca^{2+} -activated basolateral K^+ and Cl^- conductances. (A) Stepwise activation of basolateral K^+ currents by intracellular Ca^{2+} under conditions where the pHs of the mucosal and serosal bathing solutions differed. The pH value appearing before the slant indicates the pH of the mucosal bath, while the value after the slant represents that of the serosal bath. The values above the arrows indicate the free $[Ca^{2+}]$ (nanomolar) in the mucosal bathing solution. (B) Parallel experiments for the Cl^- conductance. Time bar represents 10 min.

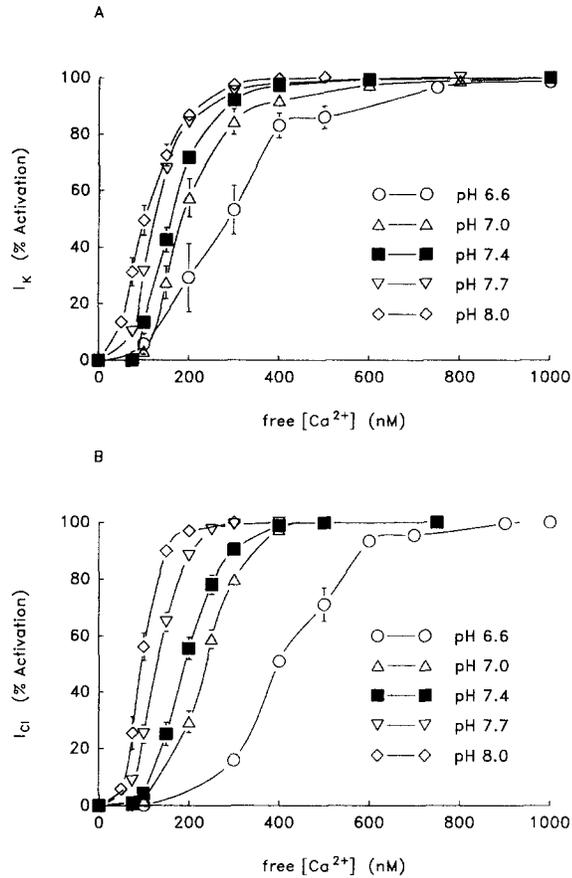


FIGURE 5. Dependence of Ca²⁺ activation on intracellular pH. Level of activation by Ca²⁺ of basolateral K⁺ (A) and Cl⁻ (B) conductances at various levels of internal pH. Results from stepwise activation experiments (shown in Fig. 2) were used to construct a dose-response curve for each internal pH condition. K⁺ and Cl⁻ currents were normalized to their individual maximally activated currents. Free [Ca²⁺] was determined as described in Materials and Methods and in Figs. 1 and 2. Each point represents the mean and SEM of 4–10 experiments.

for the activation by Ca²⁺ of the K⁺ and Cl⁻ conductances obtained from such plots are presented as a function of pH in Fig. 7 B. As the intracellular pH was made more acidic, $K_{1/2}$ values for the activation of K⁺ conductance increased from ~100 nM to 250 nM and those for activation of the Cl conductance increased from 65 to 400 nM.

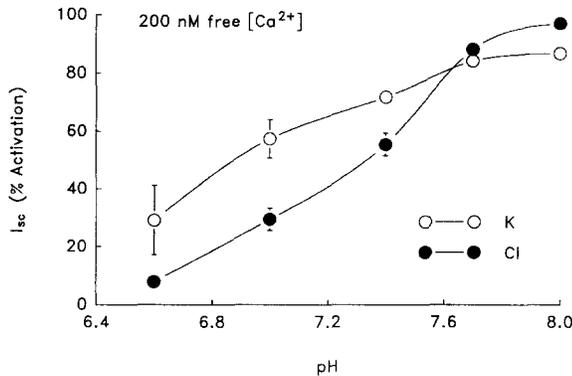


FIGURE 6. Effect of intracellular pH on the activation of K⁺ and Cl⁻ conductances at a fixed [Ca²⁺]. Data derived from Fig. 5 were used to illustrate how changes in intracellular pH may serve to modulate K⁺ and Cl⁻ conductances independent of changes in free [Ca²⁺].

The slope of the Hill plot provides an estimate of the apparent "molecularity" (Golowasch et al., 1986) of Ca^{2+} of the activation reaction. As the cytosolic pH was increased from 6.6 to 8.0, slopes of Hill plots did not vary appreciably, averaging ~ 3.5 for the K^+ conductances and 5.2 for the Cl^- conductance.

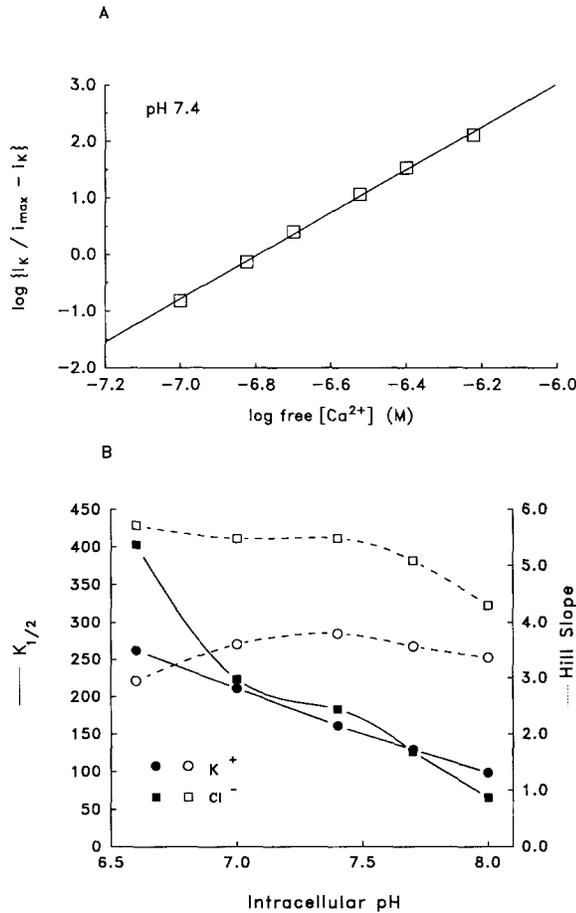


FIGURE 7. Hill analysis of the effect of pH_i on Ca^{2+} activation. (A) Representative Hill plot of Ca^{2+} activation of basolateral K^+ conductances at pH 7.4. Data in Fig. 5 are graphically presented as a Hill plot to demonstrate that activation of K^+ conductances are well-described by Hill kinetics. (B) Hill slopes (open symbols, dashed lines) and apparent K_D (filled symbols, solid lines) for Ca^{2+} activation as a function of pH. Results of Hill analysis applied to the activation curves are presented for the K^+ (circles) and the Cl^- (squares) conductances.

DISCUSSION

Protons Modify Basolateral Ca^{2+} -dependent Gating

Changes in intracellular pH within a "physiological" range (6.6–8.0) dramatically altered the Ca^{2+} activation properties of two distinct basolateral conductances, one selective for K and another that conducts Cl as well as other anions (Chang and Dawson, 1988). A useful framework for considering possible mechanisms for the effect of pH is found in the now well-known relation between macroscopic conductance, g , and single channel properties, i.e., $g = \gamma NP_0$, where γ is the single channel conductance, N is the number of channels, and P_0 is the probability of finding a

channel in the conducting state. In principle, changes in cytosolic pH could affect g by means of some effect on one or more of these parameters.

Cellular acidification might be expected to result in blockade of ion channels by protons. There is evidence for proton blockade of the conduction pathway of some Ca^{2+} (Hess et al., 1986; Krafe and Kass, 1988) and K^+ (Moody and Hagiwara, 1982; Davies, 1990) channels, but simple block of open channels does not appear to contribute to the pH effects reported here. First, maximal Ca^{2+} -activated K^+ and Cl^- currents were independent of pH. The on-rate for open-channel block by H^+ would be enhanced, if anything, at values of P_0 approaching unity so that one might expect to see block expressed as an attenuation of the maximal current as the pH is decreased. In addition, the effects of pH were qualitatively similar for both cation- and anion-selective conductances, a result not expected for a process involving protonation of a specific site in the conduction path.

Changes in cytosolic pH might also affect γ via an alteration in surface potential and a consequent change in the concentration of permeant ions at the mouths of the channels (McLaughlin, 1977). This sort of effect would be expected to alter cation and anion conductances in a reciprocal fashion, however, whereas we found that the effects of changes in cytosolic pH on g_{K} and g_{Cl} were qualitatively similar. The pH independence of the maximal currents also argues against an effect of pH on the total number of channels, although it is not possible to exclude opposing effects of pH on γ and N which gratuitously canceled.

The marked effect of cytosolic pH on the $K_{1/2}$ for calcium activation and the lack of effect on I_{max} suggests that the calcium activation process itself is pH sensitive. Although protons per se do not appear to gate the channels, the local proton concentration appears to determine the efficacy of calcium as an activator. Increasing cytosolic pH from 6.6 to 8.0 produced a decrease in the $K_{1/2}$ for calcium activation of g_{K} of about threefold and of about sixfold for g_{Cl} .

Studies of single Ca^{2+} -activated K^+ channels from other epithelial cells are also consistent with an effect of pH on channel gating rather than on conduction. In their study of the properties of a Ca^{2+} -activated maxi- K^+ channel from amphibian choroid plexus, Christiansen and Zeuthen (1987) showed that decreasing pH at the cytoplasmic face of a detached patch resulted in a substantial decrease in the fraction of time spent in the conducting state. In principle the increase in nonconducting (closed) intervals produced by the tenfold increase in proton activity could represent long-lived proton block, but the fractional conducting (open) time, which was substantially reduced at $V_m = 0$, nevertheless approached unity at depolarized potentials (+40 mV), which would be expected to increase the efficacy of channel blockade. The effect of pH appeared to be completely attributable to the pH dependence of Ca^{2+} -dependent gating. It is also noteworthy that in instances where apparent channel blockade by protons has been observed in Ca^{2+} channels (Hess et al., 1986; Prod'homme et al., 1987; Kaibara and Kameyama, 1988) and K^+ channels (Davies, 1990), the block appears to be a rapid, amplitude-reducing block which is observed as a decrease in the amplitude of single channel currents.

Cornejo et al. (1989) concluded that cytosolic pH influenced Ca^{2+} -dependent gating of a maxi- K^+ channel from cultured renal tubular cells. They found that lowering the pH at the cytoplasmic face of detached patches shifted the Ca^{2+}

activation curve to the right but did not prevent maximal activation by Ca²⁺ or voltage. In addition, they reported that lowering the pH reduced the Hill slope from 2.7 to 2.0 and concluded that pH affected the Ca²⁺ binding site.

Physiological Significance of Modulation of Ca²⁺ Activation by Cytosolic pH

It has been known for some time that [Ca²⁺] is a regulator of epithelial ion channels, and there is now abundant evidence to suggest that cytosolic pH is also an important modulator of ion channels in epithelial cells. Na⁺ conductances (Palmer and Frindt, 1987; Harvey et al., 1988) and K⁺ conductances (Christensen and Zeuthen, 1987; Harvey et al., 1988; Cornejo et al., 1989) are both reduced by decreasing cytosolic pH. Furthermore, aldosterone-induced upregulation of active ion transport in cells of the amphibian skin (Harvey and Ehrenfeld, 1988) and kidney (Oberleithner et al., 1987; Wang et al., 1989) is associated with alkalization of the cytoplasm. Cellular pH is a particularly attractive candidate modulator because, as pointed out by Harvey et al. (1988), changes in cytosolic pH could produce coordinate regulation of apical and basolateral membranes by causing parallel changes in apical g_{Na} and basolateral g_K .

In this study we obtained evidence for a specific mechanism which could account for the effect of cytosolic pH on ion conductance: modulation of Ca²⁺-dependent gating. The results suggest that pH can exert a form of "gain control" on the Ca²⁺ activation process. It is of interest to note that in contrast to some Ca²⁺-activated channels (particularly the large conductance maxi-K⁺ channels) which require micromolar concentrations of Ca²⁺ for activation at $V_m = 0$, the conductances identified in digitonin-permeabilized colonic cells could be active at pH 7.4, assuming, for example, resting Ca²⁺ levels in the range of 50–100 nM as reported by Wong et al. (1989, 1990). Cytosolic pH could be an effective modulator of the "resting" basolateral K⁺ conductance (Germann et al., 1986*a, b*), which is required to sustain Na absorption (Dawson and Richards, 1990; Dawson, 1991). Alterations in intracellular pH could also modulate the response of membrane channels to transient changes in cytosolic calcium, such as those induced by muscarinic agonists (Venglarik and Dawson, 1986; Venglarik et al., 1986; Wong et al., 1989, 1990). The basolateral membranes of turtle colonic cells and T84 cells contain a population of channels that are transiently activated by muscarinic agonists, presumably as a result of a transient rise in cytosolic calcium. The basolateral membranes of turtle colon also exhibit a substantial Cl conductance which is under inhibitory cholinergic control (Venglarik et al., 1986), but a role for intracellular Ca²⁺ in this process has not been established. The dual regulation of g_K and g_{Cl} by pH and pCa is consonant with the notion that that final value of the steady-state conductance of the plasma membrane is exquisitely sensitive to the internal state of the cell (Dawson and Richards, 1990). Interestingly, Garty et al. (1987) reported that in membrane vesicles from toad urinary bladder, inhibition of Na uptake by cytosolic calcium, which was evident at pH 7.8, was abolished at pH 7, an effect opposite to that reported here.

Expression of Basolateral Conductances in Permeabilized Cells

Initial studies of the Ca²⁺ activation of basolateral conductances in digitonin-permeabilized cells were conducted at pH 6.6 as described earlier. In this condition

the Ca^{2+} -activated conductances dominated the basolateral membrane and we speculated that this was due in part to the reduced cytosolic pH. The present studies, however, indicated that the situation is similar even at cytosolic pH values of 7 and above; i.e., regardless of cytosolic pH, the Ca^{2+} -activated g_{K} accounts for virtually all of the measurable K^{+} conductance in digitonin-permeabilized cells. Failure to observe other conductances under these conditions raises the possibility that their expression depends on as yet unidentified cytosolic factors. Single-channel recordings have shown, for example, that the 20-pS channel which appears to be responsible for the swelling-activated conductance (Richards and Dawson, 1986) rapidly inactivates in detached patches. This type of observation suggests that expression of the full complement of basolateral conductances may require careful and systematic reconstitution of the appropriate intracellular milieu, a task for which the digitonin-permeabilized cell layer may be an ideal tool.

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REFERENCES

- Altman, P. L., and D. S. Ditmer. 1961. *Biological Handbooks. Blood and Other Body Fluids.* Federation of American Societies for Experimental Biology, Washington, DC. 43.
- Boron, W. F. 1986. Intracellular pH regulation in epithelial cells. *Annual Review of Physiology.* 48:377–388.
- Busa, W. B. 1986. Mechanisms and consequences of pH-mediated cell regulation. *Annual Review of Physiology.* 48:389–402.
- Chang, D., and D. C. Dawson. 1988. Digitonin-permeabilized colonic cell layers: demonstration of calcium-activated basolateral K^{+} and Cl^{-} conductances. *Journal of General Physiology.* 92:281–306.
- Chang, D., P. S. Hsieh, and D. C. Dawson. 1988. CALCIUM: a program in BASIC for calculating the composition of solutions with specified free concentrations of calcium, magnesium and other divalent cations. *Computers in Biology and Medicine.* 18:351–366.
- Christensen, O., and T. Zeuthen. 1987. Maxi K^{+} channels in leaky epithelia are regulated by intracellular Ca^{2+} , pH and membrane potential. *Pflügers Archiv.* 408:249–259.
- Cornejo, M., S. E. Guggino, and W. B. Guggino. 1989. Ca^{2+} -activated K^{+} channels from cultured renal medullary thick ascending limb cells: Effects of pH. *Journal of Membrane Biology.* 110:49–55.
- Davies, N. W. 1990. Modulation of ATP-sensitive K^{+} channels in skeletal muscle by intracellular protons. *Nature.* 343:375–377.
- Dawson, D. C. 1977. Na and Cl transport across the isolated turtle colon: parallel pathways for transmural ion movement. *Journal of Membrane Biology.* 37:213–233.
- Dawson, D. C. 1991. Ion channels and colonic salt transport. *Annual Review of Physiology.* 53:321–329.
- Dawson, D. C., and N. W. Richards. 1990. Basolateral K conductance: role in regulation of NaCl absorption and secretion. *American Journal of Physiology.* 259:C181–C195.
- Garty, H., C. Asher, and O. Yeger. 1987. Direct inhibition of epithelial Na^{+} channels by a pH-dependent interaction with calcium, and by other divalent ions. *Journal of Membrane Biology.* 95:151–162.

- Germann, W. J., M. E. Lowy, S. A. Ernst, and D. C. Dawson. 1986a. Differentiation of two distinct K conductances in the basolateral membrane of the turtle colon. *Journal of General Physiology*. 88:237–251.
- Germann, W. J., S. A. Ernst, and D. C. Dawson. 1986b. Resting and osmotically induced basolateral K conductances in turtle colon. *Journal of General Physiology*. 88:253–274.
- Golowasch, J., A. Kirkwood, and C. Miller. 1986. Allosteric effects of Mg²⁺ on the gating of Ca²⁺-activated K⁺ channels from mammalian skeletal muscle. *Journal of Experimental Biology*. 124:5–13.
- Grinstein, S., and S. Cohen. 1987. Cytoplasmic [Ca²⁺] and intracellular pH in lymphocytes. *Journal of General Physiology*. 89:185–213.
- Grinstein, S., and J. D. Goetz. 1985. Control of free cytoplasmic calcium by intracellular pH in rat lymphocytes. *Biochimica et Biophysica Acta*. 819:267–270.
- Gryniewicz, G., M. Poenie, and R. Y. Tsien. 1985. A new generation of Ca²⁺ indicators with greatly improved fluorescence properties. *Journal of Biological Chemistry*. 260:3440–3450.
- Harvey, B. J., and J. Ehrenfeld. 1988. Role of Na⁺/H⁺ exchange in the control of intracellular pH and cell membrane conductances in frog skin epithelium. *Journal of General Physiology*. 92:793–810.
- Harvey, B. J., S. R. Thomas, and J. Ehrenfeld. 1988. Intracellular pH controls cell membrane Na⁺ and K⁺ conductances and transport in frog skin epithelium. *Journal of General Physiology*. 92:767–791.
- Hesketh, T. R., J. P. Moore, J. D. H. Morris, M. V. Taylor, J. Rogers, G. A. Smith, and J. C. Metcalfe. 1985. A common sequence of calcium and pH signals in the mitogenic stimulation of eukaryotic cells. *Nature*. 313:481–484.
- Hess, P., J. B. Lansman, and R. W. Tsien. 1986. Calcium channel selectivity for divalent and monovalent cations. Voltage and concentration dependence of single channel current in ventricular heart cells. *Journal of General Physiology*. 88:293–319.
- Kaibara, M., and M. Kameyama. 1988. Inhibition of the calcium channel by intracellular protons in single ventricular myocytes of the guinea-pig. *Journal of General Physiology*. 403:621–640.
- Krafte, D. S., and R. S. Kass. 1988. Hydrogen ion modulation of Ca channel current in cardiac ventricular cells. Evidence for multiple mechanisms. *Journal of General Physiology*. 91:641–657.
- McLaughlin, S. 1977. Electrostatic potentials at membrane-solution interfaces. *Current Topics in Membranes and Transport*. 9:71–144.
- Moody, W. J., and S. Hagiwara. 1982. Block of inward rectification by intracellular H⁺ in immature oocytes of the starfish *Mediaster aequalis*. *Journal of General Physiology*. 79:115–130.
- Oberhauser, A., O. Alvarez, and R. Latorre. 1988. Activation by divalent cations of a Ca²⁺-activated K⁺ channel from skeletal muscle membrane. *Journal of General Physiology*. 92:67–86.
- Oberleithner, H., M. Weigt, H.-J. Westphale, and W. Wang. 1987. Aldosterone activates Na⁺/H⁺ exchange and raises cytoplasmic pH in target cells of the amphibian kidney. *Proceedings of the National Academy of Sciences, USA*. 84:1464–1468.
- Palmer, L. G., and G. Frindt. 1987. Effects of cell Ca and pH on Na channels from rat cortical collecting tubule. *American Journal of Physiology*. 253:F333–F339.
- Prod'hom, B., D. Pietrobon, and P. Hess. 1987. Direct measurement of proton transfer rates to a group controlling the dihydropyridine-sensitive Ca²⁺ channel. *Nature*. 329:243–246.
- Richards, N. W., and D. C. Dawson. 1986. Single potassium channels blocked by lidocaine and quinidine in isolated turtle colon epithelial cells. *American Journal of Physiology*. 251:C85–C89.
- Tsien, R. Y. 1980. New calcium indicators and buffers with high selectivity against magnesium and protons: design, synthesis, and properties of prototype structures. *Biochemistry*. 19:2396–2404.
- Venglarik, C. J., and D. C. Dawson. 1986. Cholinergic regulation of Na absorption by turtle colon: Role of basolateral K conductance. *American Journal of Physiology*. 251:C563–C570.

- Venglarik, C. J., J. L. Keller, and D. C. Dawson. 1986. Muscarinic inhibition of basolateral conductances to K and Cl in turtle colon: possible roles for Ca^{++} and cAMP as intracellular mediators. *Federation Proceedings*. 45:2083.
- Wang, W., R. M. Henderson, J. Geibel, S. White, and G. Giebisch. 1989. Mechanism of aldosterone-induced increase of K^+ conductance in early distal renal tubule cells of the frog. *Journal of Membrane Biology*. 111:277–289.
- Wong, S. M. E., R. P. Lindeman, S. Parangi, and H. S. Chase, Jr. 1989. Role of calcium in mediating action of carbachol in T84 cells. *American Journal of Physiology*. 257:C976–C985.
- Wong, S. M. E., A. Tesfaye, M. D. DeBell, and H. S. Chase, Jr. 1990. Carbachol increases basolateral K^+ conductance in T84 cells. *Journal of General Physiology*. 96:1271–1285.