Intracellular pH Controls Cell Membrane Na⁺ and K⁺ Conductances and Transport in Frog Skin Epithelium

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ABSTRACT We determined the effects of intracellular respiratory and metabolic acid or alkali loads, at constant or variable external pH, on the apical membrane Na⁺-specific conductance (g₁) and basolateral membrane conductance (g₂), principally due to K⁺, in the short-circuited isolated frog skin epithelium. Conductances were determined from the current-voltage relations of the amiloride-inhibitable cellular current pathway, and intracellular pH (pHᵢ) was measured using double barreled H⁺-sensitive microelectrodes. The experimental set up permitted simultaneous recording of conductances and pHᵢ from the same epithelial cell. We found that due to the asymmetric permeability properties of apical and basolateral cell membranes to HCO₃⁻ and NH₄⁺, the direction of the variations in pHᵢ was dependent on the side of addition of the acid or alkali load. Specifically, changing from control Ringer, gassed in air without HCO₃⁻ (pHₒ = 7.4), to one containing 25 mmol/liter HCO₃⁻ that was gassed in 5% CO₂ (pHₒ = 7.4) on the apical side caused a rapid intracellular acidification whereas when this maneuver was performed from the basolateral side of the epithelium a slight intracellular alkalinization was produced. The addition of 15 mmol/liter NH₄Cl to control Ringer on the apical side caused an immediate intracellular alkalinization that lasted up to 30 min; subsequent removal of NH₄Cl resulted in a reversible fall in pHᵢ, whereas basolateral addition of NH₄Cl produced a prolonged intracellular acidosis. Using these manoeuvres to change pHᵢ we found that the transepithelial Na⁺ transport rate (Iₑ), and g₁, and g₂ were increased by an intracellular alkalinization and decreased by an acid shift in pHᵢ. These variations in Iₑ, g₁, and g₂ with changing pHᵢ occurred simultaneously, instantaneously, and in parallel even upon small perturbations of pHᵢ (range, 7.1–7.4). Taken together these results indicate that pHᵢ may act as an intrinsic regulator of epithelial ion transport.

INTRODUCTION

In epithelia it has long been realized that variations in ion movements at opposing apical and basolateral cell membranes may influence each other. Such intrinsic regulation has been termed transcellular “cross-talk” or coupling and is known to

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occur in a wide variety of epithelia (reviewed by Schultz 1981; and Diamond 1982). Definite evidence for cross-talk was first obtained from work on frog skin (MacRobbie and Ussing, 1961; Helman et al., 1979), toad urinary bladder (Finn, 1974; Reuss and Finn, 1975; Davis and Finn, 1982), and Necturus urinary bladder (Thomas et al., 1983). In the frog skin it was found that inhibition of the basolateral Na⁺/K⁺ ATPase was accompanied by a decrease in apical Na⁺ permeability and conductance. A converse type of cross-talk was described in the toad bladder where block of the apical Na⁺ conductance was accompanied by a decrease in basolateral K⁺ conductance, whereas in Necturus urinary bladder the basolateral membrane conductance was found to increase with the conductance of the apical membrane when Na⁺ transport was stimulated. Another type of "parallel coupling" has been described within the basolateral membrane between "pump" (Na⁺/K⁺-ATPase) and "leak" (K⁺ electrodiffusion). In renal proximal tubules ouabain inhibition of the Na⁺/K⁺ ATPase pump was accompanied by a parallel decrease in apparent K⁺ conductance (Messner et al., 1985).

Up until now, however, the intracellular signal that controls cell membrane conductances has not been identified. In this study we examined the possibility that intracellular pH may act as such a signal.

Intracellular pH is closely regulated in animal cells and much information exists on the mechanisms that control pH, within narrow limits (Roos and Boron, 1981; Thomas, 1984). In spite of the importance of this tight regulation of pH, considering its profound effects on cell properties, such as excitability and ion transport (for review see Moody, 1984), there is little information on the role of normal physiological or experimentally induced changes in pH in controlling cell membrane conductances, especially in epithelia. Experimental maneuvers designed to create an intracellular acid load have been shown to decrease the short-circuit current in frog skin (Funder et al., 1967; Mandel, 1978) and to decrease apical Na⁺ permeability in the K⁺-depolarized toad urinary bladder (Palmer, 1985). Furthermore, an intracellular acidification was reported to reduce the K⁺ transference in the Necturus proximal tubule (Kubota et al., 1983) and in cultured bovine retinal pigment cells (Keller et al., 1986). Since no quantitative study has yet been reported on these responses, our aim was to characterize the effects of respiratory and metabolic acid-base disturbances on pH, in the short-circuited isolated frog skin epithelium and, having done so, to study the effects of such experimentally induced changes in pH on apical Na⁺ and basolateral K⁺ conductances.

In the companion paper (Harvey and Ehrenfeld, 1988) we report on the evidence for Na⁺/H⁺ exchange at the basolateral membrane and its role in pH regulation, and, as a consequence, the effect of its activity on Na⁺ and K⁺ conductances.

GLOSSARY
Current is expressed in units of μA.cm⁻² surface area, voltage in mV, and conductance in mS.cm⁻².

Iₑ: short-circuit current.
Iᵥ: transepithelial clamp current.
Iᵥ, Iₕ: amiloride-sensitive current across the apical and basolateral cell membranes, respectively.
Intracellular pH and Ionic Conductances

$V_t$: transepithelial potential difference.
$V_a$: apical cell membrane potential.
$V_b$: basolateral cell membrane potential.
$V_r$: reversal potential, equivalent to Nernst potential for passive Na⁺ distribution across the apical membrane. Determined from $I_a - V_a$ relations at $V_a$ when $I_a = 0$.
$g_s$: slope conductance of the amiloride-sensitive Na⁺ transport pathway at the apical membrane. Determined from the partial derivative of the Goldman-Hodgkin-Katz (GHK) flux equation.
$P_{Na}^*$: apical membrane Na⁺ permeability determined from GHK fit to $I_a - V_r$ relations.
$g_b$: slope conductance of the basolateral membrane determined from linear regression analysis of $I_b - V_b$ relations.
$G_{Na}$: chord conductance of the amiloride-sensitive Na transport pathway at the apical membrane.
$G_t$: total transepithelial conductance.
$G_c$: transcellular conductance; $(G_t$ in absence of amiloride) - $(G_t$ in presence of 50 μM amiloride in apical bath).
$G_a, G_b$: apical and basolateral membrane conductance, respectively, obtained from circuit analysis.
$R_a, R_b$: reciprocal of $G_a$ and $G_b$ (resistance)
$F(R_a)$: fractional resistance of apical membrane, equivalent to $G_a/G_t$.
$[\text{Na}^+]_i$: intracellular sodium concentration calculated from $V_a$ at constant external apical sodium concentration.
$pH_i$: intracellular pH measured with double-barreled ion-sensitive microelectrodes.
$[\text{K}^+]_b$: potassium concentration in the basolateral Ringer solution.

**METHODS**

The experiments were carried out on in vitro *Rana esculenta* ventral skin ("whole skin") and on the epithelium ("isolated epithelium") isolated by treating the corial side with 1.0 mg/ml collagenase (Worthington Biochemical Corp., St. Louis, MO) at 30°C under a 10-cm hydrostatic pressure. The whole skin or isolated epithelium was mounted in a modified Ussing chamber that permitted cell penetration from above, via either the apical or basolateral sides, by microelectrodes. The transepithelial potential ($V_t$) was clamped to zero by short-circuiting the tissue using an automatic voltage clamp (model VC600; Physiologic Instruments, Houston, TX). The Na⁺ transport rate was measured as being equivalent to the short-circuit current ($I_s$), which was sensitive to amiloride (50 μM) applied to the apical side.

**Solutions and Drugs**

The tissue was normally perfused on both sides with a Ringer solution designated "control" in the text, which had the following composition (in millimolar): 83 NaCl, 2.5 KCl, 2 CaCl₂, 11 Na₂SO₄, 2 MgSO₄, 1.2 KH₂PO₄, 2.5 Na₂HPO₄, 11 glucose. This solution was gassed in air and buffered to pH 7.4 with BES (10 mM), pH adjustment was made with 1 N NaOH (when 1 mM BaCl₂ was added to the basolateral side, this Ringer solution had sulfate salts replaced by the corresponding chloride salt). When intracellular acid-base disturbances were produced at constant external pH, the control Ringer on the apical side was changed for a Ringer of similar composition except that it was gassed in 5% CO₂ and contained 24 mM NaHCO₃ in place of the 11 mM Na₂SO₄. Alternatively, an intracellular alkalinization or acidification was produced at constant external pH by adding 15 mM NH₄Cl to the control Ringer on the apical or basolateral side.
Amiloride was purchased from Merck, Sharp and Dohme (West Point, PA) and 4,4'-diiso-
thiocyanostilbene-2,2'-disulfonic acid (DIDS) from Sigma Chemical Co. (St. Louis, MO).

**Microelectrode Recording Arrangements**

Apical and basolateral cell membrane potential differences ($V_a$ and $V_b$, respectively) were recorded with borosilicate glass (Hilgenberg, FRG) microelectrodes filled with 1 M KCl (60–80 MΩ tip resistance when immersed in Ringer solution) and connected via Ag/AgCl wire to a dual microprobe amplifier (model 750; World Precision Instruments (WPI), New Haven, CT). Current and potential differences were measured with reference to the apical Ringer solution connected to virtual ground in the VC 600 clamp. Microelectrodes were advanced into the cells using Huxley Goodfellow micromanipulators (HG-3000; Goodfellow Metals, Cambridge, UK).

$pH_i$ was measured with double-barreled H$^+$ ion-sensitive microelectrodes using the proton ionophore tridodecylamine as the H$^+$ sensor (Proton cocktail 82500; Fluka, Switzerland). Manufacture and calibration of pH microelectrodes was similar to that described previously (Harvey and Ehrenfeld, 1986) and is described in greater detail in the companion paper. The electrodes responded with non-Nernstian slopes of 50–54 mV per pH unit when tested in calibration solutions over the pH range 5–8. The reference barrel was filled with 1 M KCl and was used to measure $V_a$ or $V_b$.

The ion-sensitive barrel was backfilled with 1 M NaCl and Na citrate at a pH of 6.5. This latter barrel, when present within a cell and referenced to the external Ringer solution, measures the transmembrane electrochemical potential for H$^+$ when corrected for the non-Nernstian calibration response. The outputs from the reference and ion-sensitive barrels were fed via Ag/AgCl wires to a high input impedance differential electrometer (FD 223; NPI) and then sent to a driven shield (FC 25; WPI) around the microelectrode. We used double-barreled ion-sensitive microelectrodes in preference to two separate single barrels. The accurate recording of $pH_i$ requires subtraction of the membrane potential output of both reference and ion-sensitive barrels, and this is best obtained when both barrels record from the same cell.

Membrane potentials were displayed on an oscilloscope (model 5115; Tektronix, Inc., Beaverton, OR), and $I_o$ cell membrane potentials and $pH_i$ were monitored on a potentiometric pen recorder (type 2065; Linseis, FRG), and stored on floppy disk by an Apple IIe computer, or on hard disk by an IBM AT 3 computer using a Unisoft program.

**Current-Voltage Analysis**

The recording of current-voltage ($I$-$V$) curves was performed under short-circuit current conditions with a computer program adapted from the one used by Thomas et al. (1983) and an Apple IIe computer for data storage and analysis. The current-pulse train consisted of transepithelial bipolar current pulses ($I_t$) of 50-ms duration, 100-ms interval between pulses, and of sufficient strength to clamp the $V_i$ over the range 0 to ±200 mV in steps of 5, 10, or 20 mV under computer command.

The $I$-$V$ relations of the apical membrane amiloride-sensitive Na$^+$ conductive pathway ($I_a$-$V_a$) were obtained from the difference in the $I$-$V$ curves recorded under control spontaneous Na$^+$ transport conditions and those recorded when $I_a$ had fallen to a steady level between 30–90 s after the addition of 50 µM amiloride to the apical Ringer solution. We assume that the amiloride-inhibited $I_a$ is equal to the transcellular Na$^+$ current pathway. For short applications of amiloride this assumption appears valid for a wide variety of tight-junctioned epithelia (Thompson et al., 1982; Nagel et al., 1983; Thomas et al., 1983). In agreement with recent reports in whole frog skin (De Long and Givan, 1984; Schoen and Erlij, 1985), the $I_a$-$V_a$ relationship recorded in whole skin or isolated epithelium could be accurately described.
by the GHK equation for a single permeant ion (Na\(^+\)) over the range of \(V_a\) between -200 and +100 mV.

The GHK flux equation (Eq. 1) was fitted to the \(I_a-V_a\) relations by obtaining the best-fit values for apical Na\(^+\) permeability (\(P_{Na_a}\)) and cell sodium concentration ([Na]\(_i\))

\[
I_a = \left( \frac{P_{Na_a} \cdot V_a \cdot F^3}{RT} \right) \left( \frac{[Na]_o - [Na]_i \exp(x)}{1 - \exp(x)} \right),
\]

where \(x = V_a \cdot F/RT\). The slope conductance of the amiloride-sensitive apical Na\(^+\) transport pathway (\(g_a\)) was calculated from the following differential forms of the GHK equation: for \(g_a\) at any \(V_a\) except \(V_a = 0\)

\[
\frac{dI}{dV} = \left( \frac{F^3 \cdot P_{Na_a}}{RT} \right) \left[ \frac{\exp(x)(1 - x) - 1}{[Na]_i \exp(x) - [Na]_o} \right]
\]

\[
+ \left\{ \frac{x}{[\exp(x) - 1]} \cdot \frac{[Na]_i \exp(x)}{[Na]_o} \right\}
\]

For \(g_a\) at \(V_a = 0\):

\[
\frac{dI}{dV} = \left( \frac{F^3 \cdot P_{Na_a}}{RT} \right) \left( \frac{[Na]_o + [Na]_i}{2} \right)
\]

For \(g_a\) at the reversal potential:

\[
\frac{dI}{dV} = \left( \frac{F^3 \cdot P_{Na_a}}{RT} \right) \left( \frac{[Na]_o \cdot [Na]_i \cdot \ln [Na]_o}{[Na]_o - [Na]_i \cdot [Na]_i} \right)
\]

An alternative measure, used by some workers, of apical membrane Na\(^+\) conductance is the chord conductance (\(G_{Na}\)), which relates the measured transcellular Na\(^+\) current to the transmembrane Na\(^+\) electrochemical driving force (\(\Delta \mu Na\)). \(G_{Na}\) was determined from Eq. 5.

\[
G_{Na} = \frac{I_a}{\Delta \mu Na} = \frac{I_a}{(V_a - V_b)} = \frac{F^3 \cdot P_{Na_a} \cdot x}{RT} \left( \frac{[Na]_o - [Na]_i \exp(x)}{[1 - \exp(x)] - [\ln [Na]_o/Na]_i} \right)
\]

In general, we chose to determine the slope conductance at \(V_a = V_b\) since the chord and slope conductances are closely related only in the range of membrane voltages near the reversal potential (Thompson, 1986).

The \(I-V\) relations of the basolateral cell membranes (\(I_b-V_b\)) were recorded simultaneously with those of the apical membrane \(I_a-V_a\) relations and were obtained from the difference between those determined before and after the addition of amiloride (50 \(\mu\)M) to the apical bath. These “difference” \(I_a-V_a\) relations were linear and stabilized for sample times >20 ms after the onset of the voltage clamp, which is in agreement with the observations of Schoen and Erlij (1985). The slope conductance of the basolateral membranes (\(g_b\)) was calculated from linear regression analysis of the difference \(I_a-V_a\) relations over the range of basolateral membrane voltage of 0 and 150 mV.

**Apparent Relative K\(^+\) Conductance**

To a large extent, \(g_b\) reflects K\(^+\) conductance since this membrane is K\(^+\) permselective. We attempted to estimate the relative K\(^+\) conductance of the basolateral cell membranes by determining the K\(^+\)-dependent partial potential ratio \(T_K\):

\[
T_K = \frac{\Delta V_b}{F} \ln \frac{[K]_i}{[K]_c},
\]
where $\Delta V_b$ is the response of the basolateral membrane potential (under short-circuit conditions) to the sudden increase in the K⁺ concentration of the basolateral Ringer from $[K]_t = 3.7$ to $[K]_t = 37$ mM made by adding K gluconate or KCl.

The apparent relative rubidium conductance was calculated in the same manner as the $T_X$ by substituting all K⁺ with Rb⁺ in the control Ringer and then adding 33.3 mM RbCl to the test solution.

It is more correct to consider K⁺ transference ($t_X$) rather than K⁺-dependent partial potential ratio ($T_X$) as indicative of the apparent relative K⁺ conductance, since $t_X$ is calculated from changes in basolateral membrane electromotive force rather than membrane potential. From equivalent circuit analysis in open-circuit conditions $t_X = 0.97 \pm 0.03 \ (n = 8)$, and in short-circuit conditions $t_X = 0.94 \pm 0.04 \ (n = 8)$. Thus, the basolateral membrane is K⁺ permselective. The normalized values of $T_X/T_c^\text{max}$ and $t_X/t_c^\text{max}$ showed the same relative change as a function of pH; for this reason we used the more easily determined $T_X$ as an expression of variations occurring in K⁺ conductance.

**Circuit Analysis**

In some experiments when we were not measuring $I-V$ relations we obtained a measurement of apical and basolateral membrane conductances ($G_a$ and $G_b$, respectively) by equivalent circuit analysis (Schultz et al., 1977). This method requires measurements of the fractional resistance of the apical membrane ($F_{R_a}$) and of the transcellular conductance ($G_t$).

The $F_{R_a}$ was determined from the voltage divider $\Delta V_a/\Delta V_t$ measured from the displacement of $V_t$ when $V_t$ was voltage clamped to $\pm 10$ mV under short-circuit current conditions. The difference between the transepithelial conductance ($G_t$) measured before and after block of $I_a$ with amiloride was assumed to give the transcellular conductance (see Nagel et al., 1983 for criticism). These measurements of $F_{R_a}$ were performed for 500 ms every 5 s in the absence or presence of apical amiloride (50 µM), and were displayed and stored by the computer. From this analysis we obtain:

\[
F_{R_a} = \frac{\Delta V_a}{\Delta V_t} = \frac{G_c}{G_a} \quad (7a)
\]

\[
G_a = \frac{G_c}{F_{R_a}} \quad (7b)
\]

\[
G_b = \frac{G_c}{(1 - F_{R_a})} \quad (7c)
\]

Cell impalements were accepted if $F_{R_a}$ approached unity after the addition of amiloride (50 µM) to the apical Ringer solution.

**22Na Unidirectional Flux**

The transepithelial unidirectional sodium fluxes were measured in whole frog skin with the isotope $^{22}$Na (0.2 µCi/ml) added to the mucosal solution. After a 20-min equilibration period, the appearance of the isotope was followed in the serosal solution as a function of time with an automatic gamma-counter (MR 252; Kontron, France). The fluxes were calculated in nanoequivalents per hour $^1$ per centimeters$^2$ and compared with simultaneous measurements of short-circuit current. Data are expressed as mean values $\pm SE$ of the mean and $n$ is the number of experiments.

**RESULTS**

**Intracellular Acid-Base Disturbances**

In these experiments, we used, where indicated, the short-circuit whole frog skin or isolated epithelium when acid-base disturbances were produced from the apical
side, and we always used the isolated epithelium when these perturbations were made from the basolateral side. Control conditions mean that the tissue was superfused on both sides with bicarbonate-free Ringer equilibrated with air and buffered to pH 7.4 with BES (N,N-bis[2-hydroxyethyl]-2 aminoethane sulfonic acid).

**CO₂:HCO₃⁻ Load**

When the control Ringer bathing the apical side of the isolated epithelium was switched to one buffered in 5% CO₂ and 24 mmol/liter HCO₃⁻ at constant external conditions, the pH fell rapidly to 6.85 and Vᵐ depolarized with a small increase in FR. The pH recovered to normal values over a period of 10 min after return to the control Ringer. On the contrary, superfusion of CO₂:HCO₃⁻ Ringer on the basolateral side had little effect on pHₗ producing only a slight alkalinization, which increased further immediately after return to control Ringer. The opposite effects of apical and basolateral CO₂:HCO₃⁻-buffered Ringer on pHₗ may be due to HCO₃⁻ ions being permeable only at the basolateral membranes. In support of this conclusion it can be seen that superfusion of the basolateral side with Ringer gassed in 5% CO₂ without HCO₃⁻ (pHₗ 6.4) produced a reversible fall in pHₗ comparable to that produced by apical CO₂:HCO₃⁻-buffered Ringer. After removal of the electrode from the cell (at arrow "out"), the electrode was calibrated by switching to a Ringer buffered to pH 6.8 with MES.

pH (7.4), an immediate and prolonged intracellular acidification was produced (Fig. 1). This marked fall in pHₗ was readily reversible upon return to control Ringer. By contrast, when the basolateral side of the epithelium was superfused with the same CO₂:HCO₃⁻-buffered Ringer, the pH increased slightly (Fig. 1). The relative lack of effect of basolateral CO₂ on pHₗ in this case is probably due to simultaneous CO₂
and HCO₃⁻ entry across the basolateral membrane and a subsequent increase in intracellular buffering power. In support of this conclusion we found that superfusion of the basolateral side with a Ringer equilibrated in 5% CO₂ without HCO₃⁻ (pH 6.4) caused a rapid and reversible cell acidification (Fig. 1). Since apical CO₂:HCO₃⁻-buffered Ringer produced similar effects on pHᵢ, the HCO₃⁻ permeability of the apical cell membranes must be low.

Concomitant with the changes in pHᵢ, the intracellular potential also varied. Since the experiments were carried out under short-circuit current conditions this change in potential reflects variations in both apical and basolateral membrane potentials. Surprisingly, however, the ratio of apical to transcellular resistances (FRₐ) changed only slightly. Thus, if changes in transcellular resistance occurred during perturbations of pHᵢ, the resistances of both the apical and basolateral membranes must have been affected simultaneously and in the same direction.

NH₃/NH₄⁺ Load

To cause intracellular acid-base disturbances, we followed the now classical method described for other tissues of using an NH₄Cl prepulse to alkalinize the cell followed
by its washout to create a rebound intracellular acidification. Exposure of the apical side to 15 mM NH₄Cl in whole frog skin was associated with a rapid and transient intracellular alkalinization that lasted between 15 and 30 min (Fig. 2). For incubation periods longer than 15 min, we observed a decrease in pHᵢ. Upon removal of NH₄Cl the pHᵢ rapidly acidified and then recovered slowly to control values (Fig. 2). The degree of this acidification depended on the duration of exposure to NH₄Cl. An incubation time of at least 30 min was found to produce the maximum acidification after washout.

Exposure of the basolateral side to NH₄Cl (15 mM) in isolated epithelia produced a similar but faster pattern of pHᵢ changes to that observed during NH₄Cl addition on the apical side. During the first 2 min of exposure to NH₄Cl a rapid and transient intracellular alkalinization was produced (Fig. 3). For longer incubation times, the pHᵢ slowly decreased to more acidic values. After washout of the NH₄Cl, the pHᵢ fell even further before returning to control levels. In both cases of NH₄Cl addition to the apical or basolateral Ringer, the disturbances in pHᵢ were accompanied by changes in membrane potential without a great deal of variation in FRₐ (Figs. 2 and 3).

The degree of intracellular acidification produced by apical CO₂ or basolateral NH₄⁺ was dependent on the intracellular buffering power (see Appendix). The latter was increased approximately twofold when a CO₂:HCO₃⁻-buffered Ringers solution was present on the basolateral side. In this condition, gassing with CO₂ on the apical side or the addition of NH₄Cl to the basolateral side produced only very slight changes in pHᵢ. Thus, to produce sizeable variations in intracellular pH, the Ringer
curves, producing an increase in slope conductance and current at any given membrane potential. An acid load produced the opposite effects. Detailed characteristics of the GHK fit to the apical membrane I-V curves and linear regression analysis of the basolateral membrane I-V relations of such acid- and alkali-loaded cells are given in Table I. (C) Relations between $g_a$ and $V_a$ that were normalized by dividing $g_a$ by its maximum theoretical value calculated from Eq. 8 under control conditions (O) and acid (A) or alkali (●) loads. Since $g_a/g_{max}$ vs. $V_a$ relations under all three conditions are superimposable over the normal range of membrane voltages (−100 to −20 mV), the effect of pH$_i$ on $g_a$ is voltage independent. In other words, the degree of inhibition of $g_a$ by low pH$_i$ or stimulation by high pH$_i$ is practically constant over the range of negative membrane potentials. This does not appear to be true for positive membrane potentials that are not normally encountered in the transporting epithelium. The maximum theoretical deviation from the voltage independence of the pH$_i$ effect shown at large and positive $V_a$ values was calculated from Eq. 9. At a large and negative $V_a$ value:

$$\text{max } dI/dV = (F^2/RT) \cdot \frac{P_{\text{Na}}}{[\text{Na}]}$$

At a large and positive $V_a$ value:

$$\text{min } dI/dV = (F^2/RT) \cdot \frac{P_{\text{Na}}}{[\text{Na}]}$$
bathing the basolateral side must contain a nonpermeable buffer. It should be noted that application of CO₂ from the apical side was found to be the most effective way of producing a rapid intracellular acidification that remained at a steady level during the presence of CO₂. On the other hand, the production of an alkaline shift in pHᵢ was best obtained during the first 20 min of exposure to NH₄Cl from the apical side. The response of pHᵢ to apically applied CO₂ or NH₄Cl was similar in experiments on whole skin and on the isolated epithelium.

An intracellular acid load could also be effectively produced after the washout of NH₄Cl after preloading from the apical or basolateral sides. Moreover, a slowly changing acidification was best observed during long-term exposure (>5 min) of the isolated epithelium to NH₄Cl from the basolateral side.

These methods of creating intracellular acid-base disturbances at constant external pH allowed us to examine the effects of pHᵢ variations on the I-V relations and ionic conductances of the apical and basolateral cell membranes.

### Table I

| Condition          | Lᵢₑ | pₐₑ | gₐ | gᵦₐ | [Naᵢ] | pHᵢ |
|--------------------|-----|-----|----|-----|-------|-----|
| Control            | 20 ± 2 | 0.569 ± 0.067 | 0.156 ± 0.019 | 0.166 ± 0.017 | 0.29 ± 0.04 | 11 ± 2 | 7.23 ± 0.09 |
| (n = 8)            |     |     |    |     |       |     |       |
| Alkali load        | 25 ± 2 | 0.853 ± 0.075 | 0.432 ± 0.036 | 0.507 ± 0.041 | 0.45 ± 0.04 | 23 ± 3 | 7.65 ± 0.11 |
| (n = 8)            |     |     |    |     |       |     |       |
| Acid load          | 3 ± 0.5 | 0.265 ± 0.011 | 0.039 ± 0.005 | 0.036 ± 0.004 | 0.08 ± 0.01 | 5 ± 2 | 6.85 ± 0.25 |

Apical Na⁺ permeability (pₐₑ), slope (gₐ), and chord (Gᵦₐ) conductance, basolateral membrane slope conductance (gᵦ), intracellular Na⁺ concentration [Naᵢ], were determined from amiloride-sensitive cell I-V relations. pHᵢ was measured using double-barreled H⁺-sensitive microelectrodes. Control conditions were in bicarbonate-free Ringer solutions bathing both sides of the frog skin. An intracellular alkali load was induced by adding 15 mM NH₄Cl to the control Ringer solution on the apical side. The I-V relations were recorded between 2 and 15 min after NH₄Cl addition when pHᵢ and short-circuit current (Iₛₑ) had reached stable maximum values. An intracellular acid load was produced by switching the control Ringer solution bathing the apical side to a solution buffered at the same pH with 5% CO₂ and 24 mM HCO₃⁻. Data analysis was performed between 2 and 5 min after CO₂ equilibration when pHᵢ and Lᵢₑ had reached stable minimum values.

**Effects of pHᵢ on Apical Membrane Na⁺ Conductance and Basolateral Membrane Conductance at Constant External pH**

The amiloride-sensitive I-V relationship of the apical cell membranes could be accurately fit by the GHK flux equation for Na⁺ in control and in conditions of acid-base disturbances (Fig. 4 A). Using the equation as described in the Methods, we calculated apical Na permeability (pₐₑ), slope conductance (gₐ), chord conductance (Gᵦₐ), and [Naᵢ] when the cells were acid or alkali loaded (Table I). The effects of acid and alkali load on the I-V relationships of the apical Na conductive pathway and of the basolateral cell membranes recorded in the same cell of an isolated epithelium are shown in Fig. 4, A and B. An alkali load (produced by NH₄Cl addition to the apical side) always increased pₐₑ, gₐ, Na transport rate (Iₛₑ), and basolateral conductance (Table I) whereas a respiratory acid load (produced by CO₂:HCO₃⁻ added to apical side) had the opposite effects on all of these parameters (Table I).
Voltage Independence of the pH, Effect

The membrane potential changed with variations in pH, and, since the experiments were conducted under short-circuit conditions, the direction and magnitude of this change depended on both apical and basolateral membrane conductances. For example, inhibition of apical Na⁺ entry would tend to hyperpolarize the apical membrane, whereas block of K⁺ channels at the basolateral membrane would depolarize Vb. Do these voltage changes influence the effects of pH on apical membrane Na⁺ conductance? With dissimilar concentrations of Na⁺ on either side of the membrane, a voltage dependence of gₐ is implicit in the GHK curves shown in Fig. 4A and can be calculated from the first derivative of the I-V plot as a function of voltage (Eqs. 2-4). When plots of gₐ vs. Vₐ were normalized by dividing gₐ by the maximum (theoretical) value of gₐ calculated for a large and negative Vₐ, the relations of gₐ/gₐmax vs. Vₐ were found to be practically superimposable for control, alkali, and acid load conditions over the Vₐ range of -100 to -20 mV (Fig. 4C). Thus, the voltage dependence of gₐ was not distorted by intracellular acidification or alkalinization over the normal intracellular voltage range. Moreover, the relative inhibition or stimulation of gₐ by acid or alkali loads, respectively, was constant over the Vₐ range normally encountered, indicating that the effects of intracellular H⁺ on gₐ were not affected by membrane potential changes.

Dependence of gₐ on pHₐ

Simultaneous measurements of pHₐ and gₐ using double-barreled H⁺-sensitive microelectrodes and I-V analysis revealed a close covariance between these parameters. An intracellular acid load produced by CO₂: HCO₃⁻-buffered Ringer on the apical side was accompanied by a decrease in gₐ and short-circuit current (Fig. 5). Upon return to control Ringer, the gₐ and Iₛ both increased in parallel with the recovery of pH to control values. The change in Iₛ is expected to follow gₐ if the apical Na⁺ entry step is rate-limiting for overall transepithelial Na⁺ transport. On the other hand, both gₐ and Iₛ were increased when CO₂: HCO₃⁻ Ringer was present either simultaneously on both sides or on the basolateral side alone. These changes were accompanied by a slight intracellular alkalinization (Fig. 5).

The effects of a long-term increase in pH on gₛ and Iₛ were investigated in the presence of NH₄Cl on the apical side. Immediately after the addition of 15 mmol/liter NH₄Cl, the gₛ and Iₛ increased in parallel with the rise in pH and remained at a steady level during the "plateau-phase" alkalinization (Fig. 6). After a 20-min incubation with NH₄Cl, the pH began to decline, as did the gₛ and Iₛ. After washout of NH₄Cl, the "rebound" acidification was accompanied by a decrease in gₛ and Iₛ, and subsequently these parameters showed a similar time-dependent recovery. The dependence of gₛ and Iₛ on pH variations in both alkaline and acidic directions could also be observed in the same cell by loading from the basolateral side with NH₃:NH₄⁺. The addition of 15 mmol/liter NH₄Cl to the basolateral side produced a similar but much faster pattern of changes in pH, gₛ, and Iₛ (Fig. 7) than when NH₄Cl was present on the apical side. Again, the variations in gₛ and Iₛ were covariant with the induced perturbations in pH. The effect of NH₄Cl on pHₐ and transport parameters was greatly diminished when the basolateral side was superfused in CO₂: HCO₃⁻-buffered Ringer (Fig. 7), a condition in which intracellular buffering...
power is more than double that found in control BES-buffered Ringer (cf. Appendix). This result emphasizes the role of permeable buffers in dampening the effects of acid or alkali loads on pH and, as a consequence, on Na⁺ transport.

The relationship obtained between $g_a$ and pH determined in these experiments is given in Fig. 8. The specific Na⁺ conductance of the apical cell membranes was found to be a steep sigmoidal function of pH, especially over the physiological range of pH values between 7.1 and 7.4 where $g_a$ varied 10-fold. The maximum value of $g_a$ was obtained at a pH of 8.0 and the minimum at pH 6.5.

The relation $g_a/g_{\text{max}}$ vs. pH could be described by a titration curve fit by the equation $g_a/g_{\text{max}} = K^n/[\text{H}^+]^n$, where $K = 10^{-pK}$ and $n$ is a binding constant (Moody...
and Hagiwara, 1982). The best fit was obtained with a pK of 7.25 and n = 2 (Fig. 8).

From $I_{ap}-V_{ap}$ relations, we also calculated apical $P_{Na}$, $G_{Na}$, and $[Na]_i$ (from the reversal potential). An intracellular acid load always reduced $P_{Na}$ and $G_{Na}$ (Table I), whereas its effects on $[Na]_i$ were biphasic. $[Na]_i$ tended to decrease during the first 5 min of acid load (Figs. 5 and 6, and Table I), and thereafter increased for longer periods of intracellular acidosis. Conversely, an alkaline load increased $P_{Na}$ and $G_{Na}$ (Table I) and again the changes in $[Na]_i$ were biphasic and time dependent. Initially, during the intracellular alkalosis the $[Na]_i$ increased and after 5–10 min decreased. These biphasic effects of changing pH on the calculated $[Na^+]_i$ may be due to a pH

![Diagram of pH and NaCl effects](image)
sensitivity of both apical Na⁺ entry and basolateral Na⁺ exit (via Na⁺/K⁺ ATPase). Another possibility is that pH-regulating mechanisms, such as Na⁺/H⁺ exchange, may modify the intracellular Na⁺ transport pool (Ehrenfeld et al., 1987; Harvey and Ehrenfeld, 1988).

**FIGURE 7.** Response of pHᵢ, membrane potential, Na⁺ conductance, and transport rate to the application of 15 mM NH₄Cl to the basolateral Ringer at a constant external pH 7.4 in the short-circuited isolated epithelium. Cell impalement was from the basolateral side with a double-barreled H⁺-sensitive microelectrode. The addition of NH₄Cl to a CO₂-free Ringer gassed in air (first arrow) resulted in a transient intracellular alkalinization lasting 2–3 min, followed by a steady decrease in pHᵢ. A rapid rebound acidification occurred upon the removal of NH₄Cl. The changes in Na⁺ conductance and transport rate were covariant with the variations in pHᵢ. Cell acidification produced a decrease, and alkalinization an increase, in these parameters. The effects of basolateral superfusion and washout of NH₄Cl on pHᵢ, gₐ, and Iₛₐ were considerably diminished when performed in a CO₂/HCO₃⁻-buffered Ringer on the basolateral side (record after HCO₃⁻/CO₂ arrow). The inset shows the experimental protocol.

*Dependence of gₘ on pHᵢ*

The I-V relations of the amiloride-sensitive current pathway across the basolateral cell membranes (Iₛₐ-Vₐ) were determined simultaneously with those of the apical cell membranes described above. The pattern of stimulation or inhibition of basolateral slope conductance after an intracellular alkali or acid load, respectively, was similar to that found for the apical cell membranes (Table I). The effect of a respiratory
intracellular acid load on \( g_b \) was instantaneous. Evidence for the covariance of \( g_b \) with \( pHi \) was obtained from simultaneous recordings of these parameters as a function of time (Fig. 9).

The response of \( g_b \) to changes in \( pHi \) was just as rapid as that found for \( g_a \), since the \( g_a \) and \( g_b \) described in Fig. 4, A and B were determined simultaneously and in the

\[
\frac{g_a}{g_{max}} = \frac{(10^{-pK})^n}{[10^{-pK}]^n + [H^+]^n}
\]

giving a \( pK \) of 7.25, with \( n = 2 \), the number of protons interacting with each titratable site.

**Figure 8.** Apical membrane \( Na^+ \) conductance plotted as a function of \( pHi \) (mean values in eight tissues). The \( g_a \) was determined from amiloride-sensitive \( I-V \) relations of the apical membrane as \( pHi \) was varied at constant external \( pHi \) by respiratory (\( CO_2 \)) or metabolic (\( NH_4Cl \)) acid-base disturbances. Values of \( g_a \) were normalized by expressing them as a fraction of the maximum conductance measured at a \( pHi \) of 7.75. The relation \( g_a/g_{max} \) vs. \( pHi \) was best fit by a titration curve described by the equation:

**Figure 9.** Computer print out of \( pHi \) (●) and basolateral membrane conductance (++) (calculated every 5 s from circuit analysis), plotted as a function of time in the short-circuited frog skin. The record shows the rapid and covariant nature of \( pHi \) and \( g_b \) variations after a respiratory acid load (at a constant external \( pHi \) of 7.4) produced by switching from control Ringer (\( HCO_3^- \)-free, gassed in air) to a Ringer buffered with \( CO_2: HCO_3^- \) on the apical side. From this record an estimate of intrinsic (non-\( CO_2: HCO_3^- \)) intracellular buffering power (\( \beta_i \)) may be obtained from the change in \( pHi \) of 0.21 units and the calculated [\( HCO_3^- \)] of 9 mM (\( pCO_2 = 38 \text{ torr} \)) to give a \( \beta_i \) of 45 slykes.
same cells. Therefore, both $g_b$ and $g_a$ vary in parallel with pH$_i$. The near constancy of $FR_a$ during acid-base disturbances supports this conclusion (Figs. 1–3).

The K$^+$ ion partial potential ratio ($T_K$) of the basolateral cell membranes showed a dependence on pH$_i$ similar to that found for $g_b$, and both parameters were extremely sensitive to pH$_i$, especially over the physiological range (Fig. 10). The best-fit curve relating $g_b/g_{max}$ to pH$_i$ gave a pK of 7.1 with two H$^+$ binding to each titration site. The $I_t/V_b$ relations used to calculate $g_b$ thus appear to provide a good description of the state of the K$^+$ conductive pathway and its pH$_i$ dependence because of the close correlation between $g_b$ and $T_K$.

**DISCUSSION**

**Response of pH$_i$ to Acid-Base Disturbances**

We found that the effects of CO$_2$ and NH$_4$Cl (at constant external pH) on pH$_i$ were dependent on the side of addition of the weak acid or base (Fig. 11). Apical application of CO$_2$/HCO$_3^-$ Ringer caused an immediate and prolonged intracellular acidification, which was reversible upon the removal of CO$_2$/HCO$_3^-$ . This response is similar to that found in the salamander proximal tubule (Boron and Boulpaep, 1983). The rapid acidification we observed could be explained by CO$_2$ entering the cells alone without HCO$_3^-$ (Fig. 11A). The increased pCO$_2$ within the cells would favor the formation of H$^+$ and HCO$_3^-$ and intracellular acidification would be maintained if HCO$_3^-$ can subsequently leave the cells. We have previously shown that HCO$_3^-$ efflux can occur across the basolateral cell membranes via a DIDS-sensitive Cl$^-$/HCO$_3^-$ exchanger (Duranti et al., 1986). The asymmetry of the epithelium with respect to HCO$_3^-$ permeability and the continuous loss of cell HCO$_3^-$ via Cl$^-$/HCO$_3^-$ exchange could provide an intracellular sink for CO$_2$, thus reinforcing the acid load.

For basolateral application of CO$_2$/HCO$_3^-$ in the isolated epithelium, we usually observed an intracellular alkalization after a rapid but small initial acidification. The HCO$_3^-$ permeability of the basolateral membrane must be very high in order to
FIGURE 11. Schema of the experimental maneuvers designed to cause intracellular acid-base disturbances at constant external pH in frog skin epithelium. (a) The addition of CO$_2$:HCO$_3^-$-buffered Ringer solution to the apical side causes a rapid and prolonged intracellular acidification. The apical and basolateral membranes possess asymmetric permselectivity to HCO$_3^-$, and the basolateral membranes would tend to create an intracellular "sink" for CO$_2$ and thus reinforce the acid load. (b) CO$_2$:HCO$_3^-$ addition from the basolateral side increases pHi slightly. Since both partners of the buffer pair enter the cell, the $K_b$ is increased. (c) NH$_4$Cl addition from the apical side produces an immediate and prolonged intracellular alkalinization. The apical membrane is normally impermeable to K$^+$ and therefore NH$_4^+$ may not enter the cells from this side. The rapid diffusion of NH$_3$ into the cell will capture intracellular H$^+$, thus raising pHi. Loss of NH$_4^+$ out across the basolateral membranes will favor the continued entry of NH$_3$ and alkalinization. (d) The addition of NH$_4$Cl from the basolateral side causes an initial transient intracellular alkalinization due to NH$_4^+$ entry. The basolateral cell membranes, however, are highly permeable to K$^+$ and NH$_4^+$ entry via K$^+$ channels is greatly favored by a large inward electrochemical driving force. Intracellular accumulation of NH$_3$ and its dissociation to NH$_4^+$ and H$^+$ will tend to acidify the cell and this process will be reinforced by NH$_3$ exit into the NH$_4$Cl-free apical solution.

counteract acidification arising from entry of CO$_2$ alone, and both partners of the buffer pair must have entered the cell almost simultaneously (Fig. 11B). This would lead to an increase in the intracellular buffering power (see Appendix), which, of itself, may not change pHi but could serve to limit cell acidification.

Apical application of NH$_4$Cl produced an intracellular alkalinization lasting up to 30 min, and rebound acidification occurred upon washout of the weak base. The pattern of these pH$_i$ transients in response to NH$_4$Cl was similar to that described...
for NH\textsubscript{4}Cl exposure in mouse soleus muscle (Aickin and Thomas, 1977), giant barnacle muscle fibres (Boron, 1977), frog muscle (Bolton and Vaughan-Jones, 1977), squid giant axon (Boron and DeWeer, 1976), crayfish slow muscle fibers (Moody, 1980), crayfish neurons (Moody, 1981), and renal proximal tubule (Boron and Boulpaep, 1982). The prompt intracellular alkalinization in response to apical NH\textsubscript{4}Cl exposure may be explained by the rapid entry of NH\textsubscript{3} and subsequent capture of cell H\textsuperscript{+} (Boron and DeWeer, 1976). The NH\textsubscript{3} \textsuperscript{+} thus formed may leave the cell across the basolateral cell membranes (via K\textsuperscript{+} channels) and thereby reinforce the intracellular alkalinization process (Fig. 11 C).

Exposure of the isolated epithelium to NH\textsubscript{4}Cl from the basolateral side produced a biphasic pattern in the pH\textsubscript{i} response similar to, but faster than that seen in apical application (Fig. 11 D). In this case, however, a rapid entry of NH\textsubscript{3} \textsuperscript{+} and accompanying intracellular acidification is to be expected since the basolateral membrane is highly permselective to K\textsuperscript{+}. Prolonged preexposure (>30 min) of the epithelium to NH\textsubscript{4}Cl on the basolateral side produced a profound acidification. In this case, the recovery of pH\textsubscript{i}, I\textsubscript{sc}, and cell membrane conductances were delayed for periods of up to 1 h. Since a very high [NH\textsubscript{3} \textsuperscript{+}] is expected at equilibrium, an increase in cell volume may have complicated the response to long term NH\textsubscript{4}Cl exposure. Finally, the magnitude of the pH\textsubscript{i} changes induced by CO\textsubscript{2}:HCO\textsubscript{3} or NH\textsubscript{3}:NH\textsubscript{3} \textsuperscript{+} also depends on the intracellular buffering power (see Appendix).

**pH\textsubscript{i} Effects on Cell Membrane Conductances**

The amiloride-sensitive I-V curves of the apical cell membranes were accurately described by the GHK equation for Na\textsuperscript{+} flux, in agreement with the findings reported for frog skin by DeLong and Civan (1984), Schoen and Erij (1985), and other tight junction epithelia such as rabbit colon (Thompson et al., 1982), and Necturus and toad urinary bladder (Palmer et al., 1980; Thomas et al., 1983).

As was the case for the pH\textsubscript{i} response, we found that the changes in the apical and basolateral membrane conductances depended on the side of the epithelium from which acid-base disturbances were induced. However, under short-circuit conditions, an intracellular acidification, no matter how produced, always caused an inhibition in Na\textsuperscript{+} transport and in Na\textsuperscript{+} and K\textsuperscript{+} conductances. Likewise, an intracellular alkalinization always brought about an increase in these transport parameters.

Since $I_{\text{sc}}$, $P_{\text{Na}}$, $g_{\text{sc}}$, $g_{\text{Na}}$, and pH\textsubscript{i} could be determined simultaneously, we were able to show that slight variations of pH\textsubscript{i} in the physiological range (7.1–7.4) produced instantaneous and parallel changes in all these parameters. The relationship describing Na\textsuperscript{+} and K\textsuperscript{+} conductances as a function of pH\textsubscript{i} could be fit by titration curves. Intracellular protons may titrate a charged group (at a pK of 7.2, histidine is a candidate) on the cytoplasmic side of the Na\textsuperscript{+} and K\textsuperscript{+} channels.

Previous studies have shown that Na\textsuperscript{+} transport is sensitive to maneuvers designed to change pH\textsubscript{i}. For example, CO\textsubscript{2} applied to the apical side at constant external pH was reported to inhibit Na\textsuperscript{+} transport in frog skin (Funder et al., 1967; Mandel, 1978) and Na\textsuperscript{+} conductance in K\textsuperscript{+} depolarized toad urinary bladder (Palmer, 1985). However, Garty et al. (1985) recently reported that pH\textsubscript{i} changes had no effect on amiloride-sensitive 22Na fluxes in toad urinary bladder vesicles. This latter report is at variance with our findings in frog skin since unidirectional
Na influxes were inhibited by intracellular acidification (Table II). The reason for this discrepancy is not clear. Perhaps the sensitivity of the Na⁺ channel is modified by the vesicle preparation procedure or perhaps hydrogen ions do not act directly on the channel but via a cytoplasmic mediator. The latter possibility seems unlikely because of the extreme rapidity of the pHᵢ effect in our study on whole epithelium and the recent report by Palmer and Frindt (1987) of the pH sensitivity of whole cell and cell-detached patch-clamped Na⁺ channels in rabbit renal cortical collecting tubule.

Both the apparent K⁺ conductance and basolateral membrane conductance were also found to be very sensitive to pHᵢ changes. This result agrees with previous reports on the effects of pHᵢ on K⁺ conductances in excitable tissues, oocytes and epithelia. K⁺ conductance was reported to be blocked by lowering pHᵢ in frog skeletal muscle (Blatz, 1980), starfish oocytes (Moody and Hagiwara, 1982), squid axon (Wanke et al., 1979), and in cultured bovine retinal pigment cells (Keller et al., 1986).

**TABLE II**

| Condition       | \( \frac{J_{\text{Na}}^{\text{app}}}{\text{F} \cdot \text{h}^{-1} \cdot \text{cm}^{-2}} \) | Difference from control |
|-----------------|-----------------------------------------------------|-------------------------|
| Control (30 min) | 1,431 ± 173                                         | --                      |
| NH₄Cl (20 min)   | 1,609 ± 185                                         | +177 ± 45               |
| NH₄Cl (50 min)   | 1,360 ± 142                                         | -72 (NS) ± 65           |
| Washout (30 min) | 839 ± 95                                            | -595 ± 114              |

\(^{22}\)Na unidirectional influxes (apical to serosal) measured in frog skins \( (n = 4) \) during a control period lasting 30 min (control Ringer, pH 7.4 both sides), and in the presence of 15 mM NH₄Cl, to the apical Ringer at 20 and 50 min after addition, and 30 min after washout (return to control Ringer). The change in Na⁺ fluxes are consistent with the variations in Na⁺ conductance found in the experiment described in Fig. 6, as a result of NH₄/ NH₂⁻-induced plateau phase alkalinization and subsequent acidification, followed by rebound acidification after washout.

*Significantly different from control at level \( P < 0.025 \).

Our results indicate that, because of the high sensitivity of Na⁺ and K⁺ conductances to pHᵢ, pHᵢ is an important intrinsic regulator of transepithelial ion transport.

**Trancellular Cross-Talk: pHᵢ as Mediator**

Can intracellular H⁺ act directly to effect changes in \( I_{\text{c}} \), and Na⁺ and K⁺ conductances or is there a secondary intracellular mediator involved? The possible role of cell Ca²⁺ as a regulator of epithelial Na⁺ transport has recently been reviewed (Chase, 1984; Taylor and Windhager, 1985; Windhager et al., 1986; Frindt et al., 1988). Experimental maneuvers designed to increase [Ca²⁺], are reported to inhibit apical Na⁺ entry (Grinstein and Erlij, 1978; Taylor and Windhager, 1979; Chase and Al-Awqati, 1983). It has been claimed that cell Ca²⁺ varies inversely with pHᵢ in nervous tissue (Helix neurons) (Meech and Thomas 1977, 1980), and such changes can influence membrane conductance (Meech, 1978). On the other hand, cell acidification has been reported to produce a fall in cell Ca²⁺ in *Helix* neurons (Alvarez-
Moreover, there are few data available on the relationship between pH\text{\text{\text{c}},} and [Ca\text{\text{\text{++\text{\text{}}\text{\text{}}}}} in epithelia, and the effect of changing pH\text{\text{\text{c}},} on [Ca\text{\text{\text{++\text{\text{}}\text{\text{}}}}} in excitable tissues are not consistent and cannot be predicted with certainty (for reviews see Moody, 1984; Busa, 1986). Palmer and Frindt (1987) recently reported that Ca\text{\text{\text{++\text{\text{}}\text{\text{}}}}} had no effect on the activity of patch-clamped cell-detached epithelial Na\text{\text{\text{+\text{\text{}}\text{\text{}}}}} channels. Moreover, we have found that the response of \( g_a \) and \( P_{Na} \) to an intracellular acid load in frog skin epithelium was similar in the presence of 20 mmol/liter Ca\text{\text{\text{++\text{\text{}}\text{\text{}}}}} or in a Ca\text{\text{\text{++\text{\text{}}\text{\text{}}}}}-\text{free EGTA Ringer on the basolateral side, with or without the intracellular Ca\text{\text{\text{++\text{\text{}}\text{\text{}}}}} chelator MAPTAM (\text{bis-(2-amino-5-methyl-phenoxyl-ethane-N,N,N','N'-tetraacetic acid tetraacetoxymethyl ester}) and the Ca\text{\text{\text{++\text{\text{}}\text{\text{}}}}} ionophore ionomycin (Harvey and Thomas, 1987). This agrees with recent studies in which doubt was cast on the role of Ca\text{\text{\text{++\text{\text{}}\text{\text{}}}}} in controlling Na\text{\text{\text{+\text{\text{}}\text{\text{}}}}} transport in frog skin (Hogan et al., 1985; Nagel, 1987).

An increase in [Na\text{\text{+\text{\text{}}\text{\text{}}}}], has been proposed to act either directly or indirectly at the apical membrane to inhibit Na permeability (for review see Schultz, 1981). Here we found that changes in [Na\text{\text{+\text{\text{}}\text{\text{}}}}] (calculated from the \( I_oV_o \) relations) during an acid load and the recovery phases were variable and tended to lag behind the changes in pH\text{\text{c},}. We interpret the variations in [Na\text{\text{+\text{\text{}}\text{\text{}}}}], to result from pH\text{\text{c}}-induced changes in Na\text{\text{+\text{\text{}}\text{\text{}}}} conductance and Na\text{\text{+\text{\text{}}\text{\text{}}}:/K\text{\text{+\text{\text{}}\text{\text{}}}}} ATPase activity. At the onset of an acid load the [Na\text{\text{+\text{\text{}}\text{\text{}}}}] decreased, possibly as a result of the decreased apical \( P_{Na} \). As the acid load was prolonged, [Na\text{\text{+\text{\text{}}\text{\text{}}}}] increased; this may have been due to inhibition of the basolateral Na\text{\text{+\text{\text{}}\text{\text{}}}:/K\text{\text{+\text{\text{}}\text{\text{}}}} ATPase. An increase in [Na\text{\text{+\text{\text{}}\text{\text{}}}}], at low pH\text{\text{c}}, could also occur from activated basolateral Na\text{\text{+\text{\text{}}\text{\text{}}}:/H\text{\text{+\text{\text{}}\text{\text{}}}}} exchange (Ehrenfeld et al., 1987; Harvey and Ehrenfeld, 1988). In these studies we showed that an acid load increases basolateral \text{\text{\text{23\text{\text{}}\text{\text{}}}Na}} uptake to 1,600 neq/h\text{\text{\text{\text{\text{-1\text{\text{\text{}}\text{\text{}}\text{\text{}}\text{\text{}}}\cdot cm^{-2}}}, which is in excellent agreement with the rate of Na\text{\text{+\text{\text{}}\text{\text{}}}} uptake calculated from changes in [Na\text{\text{+\text{\text{}}\text{\text{}}}}] (Figs. 4 and 5) at 5 mM/min corresponding to 1,800 neq/h\text{\text{\text{\text{-1\text{\text{\text{}}\text{\text{}}\text{\text{}}\text{\text{}}}\cdot cm^{-2}} (for a volume to surface ratio of 6 \mu l\cdot cm^{-2}} (Harvey and Ehrenfeld, 1988). The gain in [Na\text{\text{+\text{\text{}}\text{\text{}}}}], despite reduction of \( g_a \) at acidic pH\text{\text{c}}, is analogous to that found during ouabain inhibition of Na\text{\text{+\text{\text{}}\text{\text{}}}:/K\text{\text{+\text{\text{}}\text{\text{}}}} ATPase in the presence of apical amiloride (Harvey and Kerman, 1984). These results support the conclusion that Na\text{\text{+\text{\text{}}\text{\text{}}}:/K\text{\text{+\text{\text{}}\text{\text{}}}} ATPase is inhibited at acidic pH\text{\text{c}}, and that the gain in [Na\text{\text{+\text{\text{}}\text{\text{}}}}], occurs via stimulated basolateral Na\text{\text{+\text{\text{}}\text{\text{}}}:/H\text{\text{+\text{\text{}}\text{\text{}}}}} exchange. Increased [H\text{\text{\text{+\text{\text{}}\text{\text{}}}}], is reported to reduce Na\text{\text{+\text{\text{}}\text{\text{}}}:/K\text{\text{+\text{\text{}}\text{\text{}}}} ATPase activity (Eaton et al., 1984; Homareda and Matsui, 1985), which could lead to an increased [Na\text{\text{+\text{\text{}}\text{\text{}}}}], if apical Na\text{\text{+\text{\text{}}\text{\text{}}} entry continued unrestricted. The sensitivity of \( P_{Na} \) to pH\text{\text{c}}, would serve to limit such a gain in [Na\text{\text{+\text{\text{}}\text{\text{}}}}]. These effects are important in that previous studies have shown near constancy of [Na\text{\text{+\text{\text{}}\text{\text{}}}}], with increasing transport rate (Wills and Lewis, 1980; Thomas et al., 1983; Turnheim et al., 1983). For this to occur, the apical entry and basolateral extrusion of Na\text{\text{+\text{\text{}}\text{\text{}}}} must be kept in step. The signal for such transcellular coupling of ion movements may be pH\text{\text{c}.}

**APPENDIX**

**Intracellular Buffering Power**

*Weak acid method.* Assuming that the initial change in pH\text{\text{c}}, upon superfusing the apical side with 5% CO\text{\text{2}} at constant external pH (24 mM HCO\text{\text{3}}) is due solely to the entry of CO\text{\text{2}} and that HCO\text{\text{3}} (OH\text{\text{-}}) and H\text{\text{-}} do not cross the cell membranes during this time, then the
change in pH will depend on the intrinsic buffering power ($\beta$) of the cell and the initial pH$_i$. Thus the non-CO$_2$/HCO$_3^-$ (intrinsic) buffering power may be calculated from $\beta = [\text{HCO}_3^-]/pH_i$. For the CO$_2$ application the calculated buffering power was $35 \pm 4$ meq H$^+$/pH unit ($n = 6$). Since CO$_2$ entry most likely gives rise to activation of pH$_i$ regulatory mechanism(s) and basolateral HCO$_3^-$ efflux, the calculation of $\beta$ from the initial and final pH$_i$ values may be erroneous. We therefore calculated $\beta_i$ by taking the final pH$_i$ value from the sharp peak obtained at the intersection of the tangent to the acidifying and plateau phases in response to a brief pulse of CO$_2$/HCO$_3^-$-buffered Ringer.

Weak base method. In the absence of CO$_2$/HCO$_3^-$ buffer, the buffering power calculated from the alkaline pH$_i$ response to an NH$_4$/NH$_4^+$ load on the apical side also gives the intrinsic or non-NH$_4$/NH$_4^+$ buffering power. The magnitude of the NH$_4$-induced alkalinization is determined by the intrinsic buffering power $\beta = [\text{NH}_4^+]/pH_i$. The change in [NH$_4^+$], was determined by taking the initial [NH$_4^+$]$_i$ to be zero and the final [NH$_4^+$], to be $([\text{NH}_4^+]-10 \exp (pK-pH_i))$ with $pK = 9.15$. The value of $\beta$, calculated by this method was $38 \pm 4$ meq H$^+$/pH unit ($n = 6$) and was not different from that determined by the weak acid method.

When a CO$_2$/HCO$_3^-$ buffer is present and the cell membrane is permeable to both CO$_2$ and HCO$_3^-$, the total intracellular buffering power ($\beta_t$) is given by $\beta_t = \beta_i + \beta_{CO_2}$ where $\beta_{CO_2}$ is the CO$_2$/HCO$_3^-$ buffering power. For any pCO$_2$, $\beta_{CO_2}$ is given by 2.3 ([HCO$_3^-$]$^2$). At a pH$_i$ of 7.26 $\pm$ 0.03 and a 5% CO$_2$/24 mM HCO$_3^-$ buffer on the basolateral side, the $\beta_{CO_2}$ was $38 \pm 5$ meq H$^+$/pH unit ($n = 6$). This gives a $\beta$, of $76 \pm 9$ meq H$^+$/pH unit ($n = 6$). Thus, in the presence of CO$_2$/HCO$_3^-$ Ringer solution on the basolateral side the $\beta$, is doubled. Under these conditions we have seen that an acid load produced by basolateral NH$_4$/NH$_4^+$ application produced small changes in pH$_i$ (Fig. 7). For the case of apical applied CO$_2$ (which normally produces a large intracellular acidification), the pH$_i$ response was practically abolished when the epithelium was previously bathed in CO$_2$/HCO$_3^-$-buffered Ringer solution on the basolateral side (Figs. 1 and 5). The increase in $\beta$, was produced only if CO$_2$/HCO$_3^-$ Ringer was added to the basolateral side. Consequently, reduced effects of acid load on $I_e$ and conductances occur when compared with the effects produced when a CO$_2$/HCO$_3^-$-free Ringer was present on the basolateral side. Under the latter conditions the addition of NH$_4$Cl to the apical side allows measurement of the total buffering power of the cell. Calculated in this way, $\beta_i = 80 \pm 7$ meq H$^+$/pH unit ($n = 4$), which agrees with the predicted value given above.

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