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Intracellular pH as a Regulator of Na^+ Transport

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Abstract. Na reabsorption by tight epithelia, such as frog skin and toad urinary bladder, is highly sensitive to the acid-base status of the cytoplasm. This can be observed in intact epithelia by acidifying the intracellular compartment with acute hypercapnia. Both apical membrane Na channels, which are responsible for the uptake of Na into the cell, and basolateral membrane K channels, which are required for the recycling of K that is actively transported into the cell through the Na/K pump, are shut down by low intracellular pH. This suggests the possibility that cell pH may serve as an important regulator of transport. One possible role is as a second messenger for rapid effects of the adrenal mineralocorticoid aldosterone.

Key words: Epithelial Na transport — Na channels — K channels — Aldosterone

Introduction

While we grieve the loss of Hans Ussing, this is also a time to celebrate the 50^{th} anniversary of the remarkable paper of Ussing and Zerahn (Ussing and Zerahn, 1951). That communication introduced the "Ussing chamber", the "short-circuit current" technique, and the use of isotopes to monitor epithelial ion transport. It also laid some of the groundwork for studies of the regulation of ion transport. It describes stimulation of sodium fluxes and short-circuit current (SCC) by adrenaline and antidiuretic hormone (applied as a neurohypophoseal extract). It also documents inhibition of transport by application of CO_2. In this contribution I will review what is now known about the mechanisms and physiological significance of this latter effect.

Early in my career as an independent investigator I spent a considerable effort in studying the modulation of Na transport and apical Na permeability by the intracellular ion composition in the intact isolated toad urinary bladder. One of the most consistent effects could be achieved simply by an acute increase in the ambient P_{CO_2}. This presumably lead to an intracellular acidification, as CO_2 will enter the cell much faster than the negatively charged HCO_3^- ion. Once inside the cell, the CO_2 will be hydrolyzed to H^+ and HCO_3^-, leading to a fall in intracellular pH. The maneuver always resulted in a decrease in short-circuit current and a fall in Na permeability, measured from current–voltage relationships across the K-depolarized bladder of about 50%. As I was preparing a manuscript describing these results, I learned, to my chagrin, that I had been scooped by Ussing more than 3 decades earlier! In fact, the effects described by Ussing and Zerahn were, if anything, larger and more impressive, as they found that just 5% CO_2 reversibly reduced the short-circuit current across the frog skin to zero. Fortunately for me, The Journal of Membrane Biology was willing to apply a kind of statute of limitations on this result, and allowed me to publish my findings (Palmer 1985).

CO_2 Decreases Intracellular pH

Using indirect but typically elegant (and correct) reasoning, Ussing and his colleagues (Funder, Ussing & Wieth, 1967) later suggested that the effect of CO_2 could involve acidification of the cytoplasm (Fig. 1). They based this deduction on the finding that the transport rate was affected by changes in P_{CO_2} either on the outside or on the inside of the skin, but that it was more sensitive to changes on the outside. This indicated a site of action between the two media but closer to the outside. This idea was later verified directly by Harvey Thomas and Ehrenfeld (1988), who measured intracellular pH in the principal cells of the intact, open-circuited frog skin using microelectrodes. Mucosal application of 5% CO_2 plus 24 mM HCO_3^- to keep extracellular pH constant provoked a prompt acidification of the cytoplasm by about 0.2 pH units. Simultaneously, apical membrane Na permeability

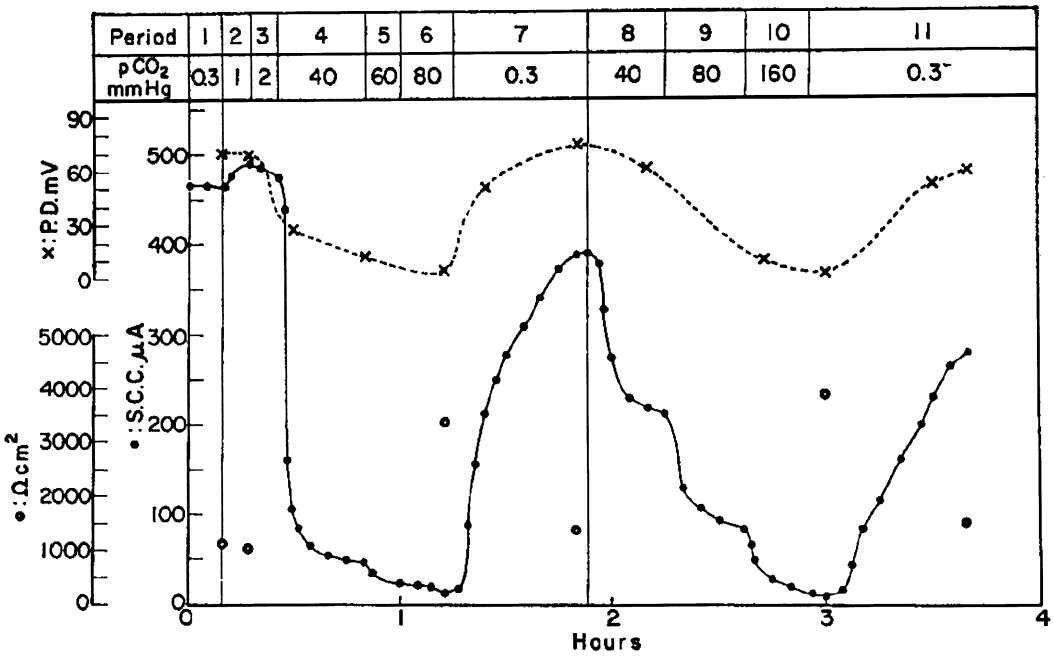

Fig. 1. Effect of extracellular CO_2 on electrical properties of the frog skin. In periods 2–6, P_{CO_2} was increased from 0.3 to 80 mm Hg on the mucosal side only. After a small stimulation at 1 mm Hg, the short-circuit current (●) decreased sharply to very low levels. There was a concomitant decrease in the transepithelial voltage (x) and an increase in resistance (○). In periods 8–10, P_{CO_2} was increased on the serosal side of the skin. Here, short-circuit current also decreased, but was less sensitive to P_{CO_2}. From Funder et al. (1967) with permission.

(P_{Na}) fell by about 70% while the basolateral membrane potential depolarized by about 10 mV. Other maneuvers that lowered cell pH, including removal of serosal Na and application of amiloride to the serosal solution to inhibit Na/H exchange, had similar effects. When CO_2/HCO_3 was added to the serosal side, neither the acidification nor the decrease in P_{Na} were observed, possibly because the HCO_3^- was highly permeable across the basolateral membrane. Taken together, these results suggest that the fall in SCC observed in frog skin and the decrease in the apical Na permeability of the toad urinary bladder can be accounted for by a reduced cytoplasmic pH.

Intracellular pH Regulates Apical Na Channels

The experiments of Harvey and Ehrenfeld, in which cell pH and the apical membrane current–voltage relationship were measured simultaneously, also revealed the quantitative relationship between these parameters. Cell pH was either decreased with CO_2/HCO_3 or increased with application of NH_4^+. Assuming a maximal conductance was reached at the highest pH achieved (pH 7.75), the titration curve had an apparent pK_a of 7.25 and an exponent (Hill coefficient) of 2. Thus the channels are highly sensitive to cell pH in the physiological range.

Similar results were obtained with the patch-clamp technique using inside-out patches from the rat cortical collecting tubule (CCT) (Palmer & Frindt 1987). Changing the pH bathing the cytoplasmic side of the patch between 7.4 and 6.4 produced parallel changes in Na channel open probabilities (P_o). Increased P_o values reflected increased mean open times and decreased mean closed times (Fig. 2). Although a complete titration curve was not obtained, the results are in good agreement with those obtained with the intact frog skin. Furthermore, they suggested that the effect of pH_i on the channel is direct, since most soluble regulatory factors would have been washed away from the membrane surface.

The pH dependence of Na channel activity was also observed with cloned ENaC channels heterologously expressed in *Xenopus* oocytes (Chalfant et al., 1999). Here, three types of experiments provided similar results. Macroscopic Na currents recorded from intact oocytes were reduced when external pH was lowered using a permeant buffer (acetate) but not an impermeant buffer (HEPES). The difference presumably reflects a parallel change in cytoplasmic pH with the permeant but not the impermeant buffers. Direct effects of pH were also observed in inside-out patches and after reconstitution of the channels into planar lipid bilayers. The ENaC channels are normally composed of three different but homologous subunits (α, β, and γ). Chalfant et al. (1999) observed pH-dependant activity with αβγ, αβ, and αγ multimers as well as with the homomeric α channel. This indicates that the α subunit responds to pH_i changes. Since no activity can be detected in the absence of this subunit, additional effects of pH_i on the β and γ subunits cannot be ruled out.

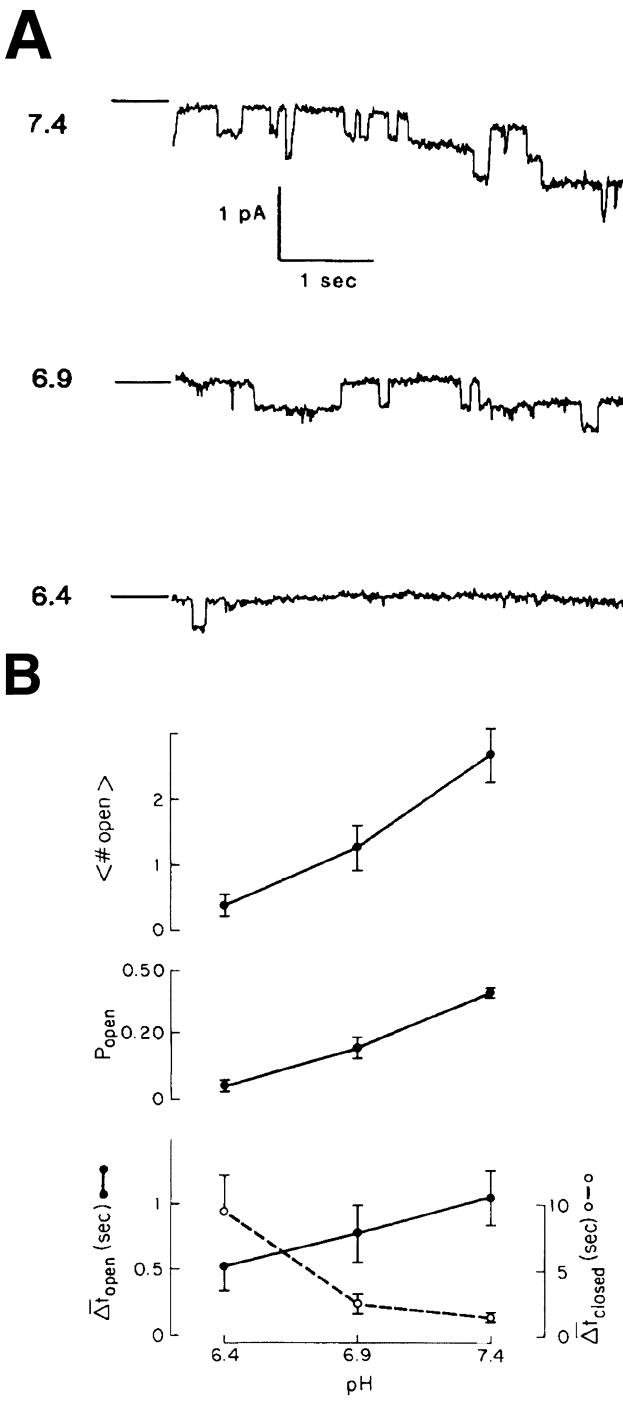

Fig. 2. Effect of intracellular pH on epithelial Na channels. Inside-out patches from the apical membrane of principal cells of the rat CCT were superfused with solutions buffered to pH 7.4, 6.9 and 6.4. Typical traces of Na channel activity are shown in *A*. Lines to the right of the traces indicate current levels when all channels are closed. Channel openings gave downward current deflections, In *B*, values for the mean number of open channels, $<\#\text{open}>$, the estimated open probability (P_o) and the mean open and closed times are plotted for 5 patches. From Palmer & Frindt (1987) with permission.

Intracellular pH Regulates Basolateral K Channels

An inhibition of basolateral membrane K conductance, accompanied by a depolarization of the basolateral membrane potential, probably also contributes to the reduction in short-circuit current with cell acidification. As mentioned above, Harvey et al. (1988) showed a depolarization of the basolateral membrane of the frog skin with acidification. The pH-dependence of the basolateral K conductance was similar to that of the apical Na conductance, with an apparent pK_a of 7.1 and a Hill coefficient of 2. Depolarization of the basolateral membrane will also depolarize the apical membrane through electrical coupling due to the finite conductance of the tight junctions. This will decrease the electrical driving force for Na entry into the cell, which will act in concert with the reduced Na permeability of the apical membrane to inhibit transepithelial Na transport.

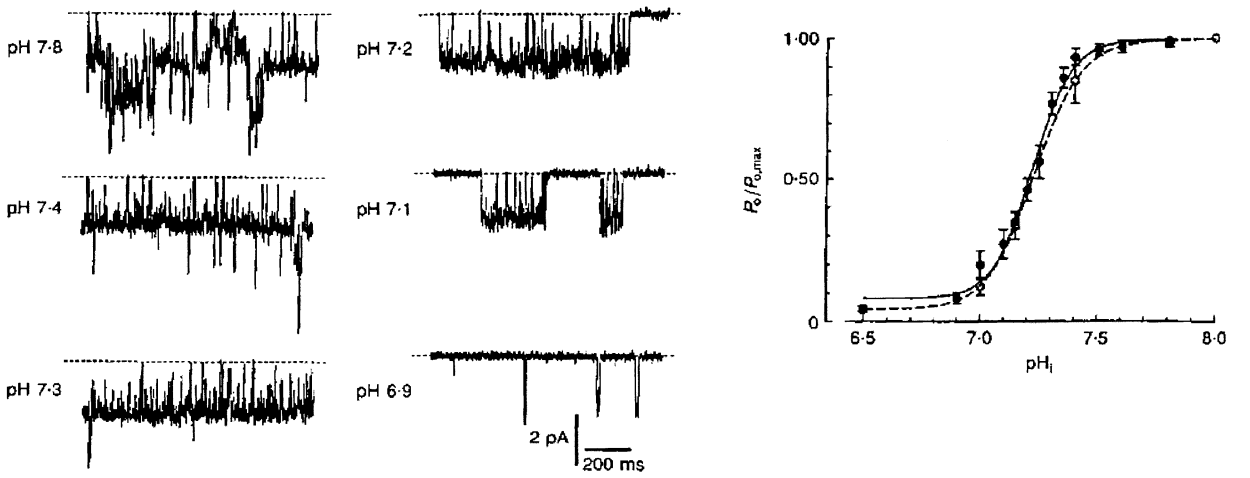

Fig. 3. Effect of intracellular pH on basolateral K channels in frog skin. Inside-out patches were obtained from the basolateral membrane of the isolated epithelium of the skin and superfused with solutions buffered to pH 6.9–7.8. Dotted lines indicate the current levels at which all channels are closed. Channel openings gave downward deflections. A plot of the normalized open probabilities (P_o) *vs.* pH shows a steep pH dependence in the same range as seen for the basolateral conductance of the intact skin. From Urbach et al. (1996) with permission.

Cytoplasmic pH appears to affect K channels directly as shown in patch-clamp experiments in isolated frog skin epithelia (Urbach et al., 1996). In inside-out patches, channel open probability increases steeply between pH 7.0 and 7.5, similarly to the macroscopic K conductance (Fig. 3). The steepness of the relationship is even greater in the single-channel studies (Hill coefficient 6 *vs.* 2). These channels are also inhibited by cytoplasmic ATP (Urbach, Van Kerkhove & Harvey 1994). A similar type of channel contributes to the basolateral K conductance of the mammalian proximal tubule (Beck et al., 1993). Its molecular identity is not known. The ATP sensitivity suggests a relationship to the K_{ATP} channels of the mammalian pancreatic β cell and heart, which are heteromultimers of Kir6.2 and the sulfonylurea receptor SUR1 (Inagaki et al., 1995). Those channels, however, are stimulated, rather than inhibited, by intracellular acidification in the physiological range (Xu et al., 2001).

The molecular mechanism underlying the pH dependence of K channel activity has been examined in some detail with the cloned renal K channel ROMK. This channel seems to be restricted to the apical membrane of the thick ascending limb and distal nephron segments and thus is presumably a gene product different from the basolateral channel, but it may serve as a model system for studying these effects. In the case of ROMK, a lysine group located at the interface between the cytoplasmic N terminus and the first transmembrane domain is absolutely required for regulation by internal pH and may be the "sensor" group that is titrated as the channel is inactivated (Fakler et al., 1996; Choe et al., 1997). While lysine in solution has a pK_a of >10, either the hydrophobic environment or the presence of other fixed charges may serve to lower the apparent pK_a into the physiological range (Choe et al., 1997; Schulte et al., 1999). How the titration of the lysine triggers the closure of the channel is so far unknown.

Intracellular pH as a Regulatory Factor

Although it is not surprising to find that ion channels, or any other proteins for that matter, are sensitive to cell pH, the strong dependence of the Na and K channels throughout the physiological pH range raises the possibility that intracellular pH may be an important regulator of these channels and, by inference, of Na transport.

One circumstance in which such a regulation may be desirable is during metabolic stress. If cells use ATP at rates that exceed those at which it can be produced through oxidative metabolism, intracellular acidosis may occur. Under these conditions shutting down active transport, which can constitute a strong drain on the metabolic resources of the cell, would make sense. There is strong evidence that epithelial Na transport rates are sensitive to cell metabolism, and that the apical Na permeability is decreased when metabolism is impaired. Hong and Essig (1976) showed that the metabolic inhibitor 2,3 DPG decreased the conductance of the active transport pathway in toad urinary bladder. Later studies showed that the apical Na permeability was directly affected under these conditions (Palmer, Edelman & Lindemann, 1980; Garty, Edelman & Lindemann, 1983). Patch-clamp studies revealed that the mean number of conducting Na channels in the apical

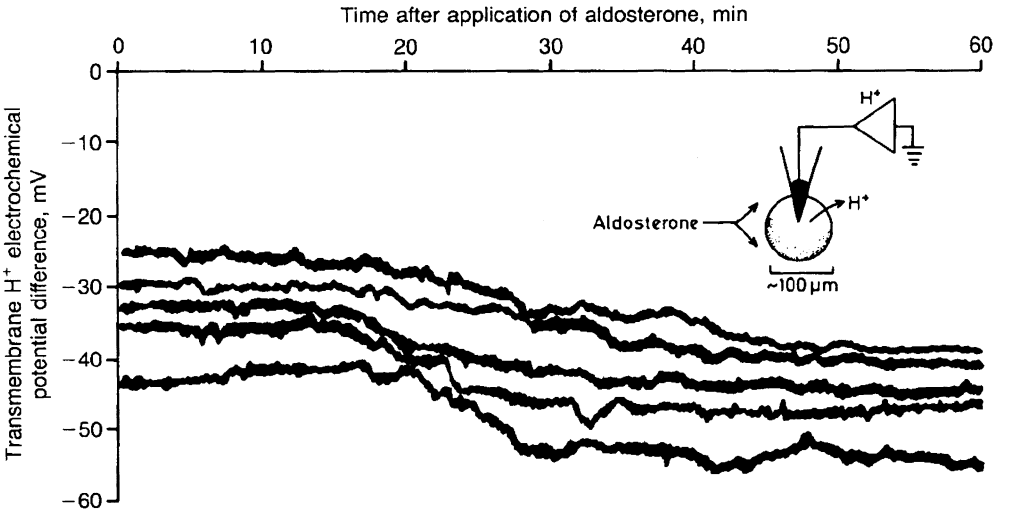

Fig. 4. Effect of aldosterone on cell pH in frog distal tubule cells. Giant cells were produced by fusion of cells from the frog diluting segment. Intracellular pH was measured with an ion-selective microelectrode. Application of aldosterone (300 nM) produced an alkalinization of the cell (decrease in transmembrane H^+ electrochemical potential difference) after a delay of 20 minutes. From Oberleithner et al. (1987) with permission.

membrane of the rat CCT was decreased when the metabolic cost of Na transport became excessive (Frindt et al., 1995). However, none of these studies provided a direct correlation between metabolic stress and cell pH, and the mechanisms linking metabolism to the activity of the Na channels are unknown. The situation with basolateral K channels is even more complicated. When metabolism is compromised, reduced intracellular ATP will tend to open the channels (Beck et al., 1993; Urbach et al., 1996). This will counteract the effects of acidosis.

Aldosterone Can Alter Cell pH

It is also conceivable that hormones could regulate epithelial Na transport through changes in cell pH. This concept is controversial. Growth factors were shown to be able to alkalinize the cytoplasm by stimulating Na–H exchange, and this effect was postulated to be important in mediating their biological effects (Busa 1986). However, studies in the laboratory of Boron (Ganz, Perfetto & Boron, 1990) indicated that alkalinization and cell proliferation could be dissociated in the presence of HCO_3^- and that a high cell pH may be permissive for cell growth but not necessarily a signal.

The adrenal mineralocorticoid aldosterone is a leading candidate for regulation of epithelial transport through cell pH. Oberleithner et al. (1987) showed that the hormone activates Na/H exchange in fused cells from the distal nephron of the frog kidney. This leads to alkalinization of the cytoplasm by 0.28 pH units (Fig. 4). Similar effects have been observed in a variety of epithelia, including the intact frog diluting segment (Cooper and Hunter 1996), cultured MDCK cells (Vilella et al., 1992; Gekle et al., 1996), and mammalian colon (Winter et al., 1999). The phenomenon is not limited to epithelia as it has also been documented in lymphocytes (Wehling, Käsmayr & Theisen 1991). Urbach et al. (1996) presented evidence that basolateral K channels in the frog skin itself are stimulated by aldosterone through alkalinization of the cell.

Although the classical pathway for aldosterone action involves increased synthesis of specific mRNA's and proteins, these effects on cell pH may arise from nongenomic mechanisms. They typically occur with a delay of only 10–20 minutes after addition of the hormone, compared to ~1 hour for most genomic effects. In many cases they were observed in the presence of inhibitors of new mRNA or protein synthesis, or of spironolactone, the classic mineralocorticoid receptor antagonist (but *see* Cooper and Hunter, 1996). They require only very low (1–10 nM) concentrations of the hormone, suggesting the participation of some kind of high-affinity receptor. The identity of the receptor has not been established.

Regulation of Na Transport through pH?

All the pieces of a regulatory pathway are in place: Aldosterone, whose major physiological function is to regulate epithelial Na reabsorption, can increase cytoplasmic pH. Alkalinization of the cell in turn is known to activate apical Na conductance as well as basolateral K conductance. Both of these actions should serve to augment the transepithelial Na^+ transport rate.

One important observation is missing, however. As mentioned above, the steroid-dependent alkalinization of the cell takes place quickly, with delays of

10 minutes or less. Increases in Na transport, both in vitro and in vivo, occur only after a characteristic ~1 hour lag period, which is attributed to the time required for synthesizing new proteins. Although the alkalinization response should be sufficient to increase transport, apparently it does not, at least in the commonly studied epithelia such as frog skin, toad urinary bladder, colon and kidney.

How can these findings be reconciled? One possibility is that the change in pH does not reach the apical membrane. Activation of Na/H exchange may provoke large increases in pH close to the basolateral membrane where the exchanger is located. Average changes in the cytoplasm, as measured by electrodes or by fluorescent dyes, may be less and at the apical membrane the changes could be very small. This argument is easiest to invoke in the frog skin, where the epithelium consists of a syncitium of several cell layers and the basolateral membranes of the innermost layer may be far from the outer membrane where the Na channels reside. Compartmentalization of cell pH may be possible even in a single cell layer, especially if there are different transporters that regulate pH at the apical and basolateral surfaces. It would be interesting to test this point using probes that specifically report pH in the vicinities of the two membranes.

If this is the case, then pH-mediated regulation of the Na channels would most likely occur through hormone receptors on the apical membrane. Evidence for several such apical receptors has been reported recently. Distinct apical and basolateral purinergic receptors have been documented in airway epithelia and can signal independently (Paradiso, Ribeiro & Boucher, 2001). In the renal distal nephron apical vasopressin receptors, with signaling pathways different from those of the basolateral membrane, appear to regulate K secretion (Amorim & Malnic, 2000).

Compartmentalization cannot explain why pH-dependent stimulation of basolateral K channels does not increase Na transport. Here the argument may be more quantitative. Increasing K permeability will affect the basolateral (and indirectly the apical) membrane potential only if the potential is significantly different from E_K, the K equilibrium potential. This may be the case for a rapidly transporting epithelial cell in which the voltages across the two membranes influence each other through the finite resistance of the paracellular pathway. A high apical membrane Na permeability will tend to depolarize the basolateral membrane while a high basolateral K permeability will tend to hyperpolarize the apical membrane. If the Na permeability is low to begin with, however, the basolateral membrane potential may already be close to E_K and increasing the activity of the K channels may have minimal effects.

According to this view, increases in the basolateral K conductance (through alkalinization) may be necessary to maintain high rates of Na transport, but will not itself be sufficient to initiate an increase in transport rate. This idea is also testable. It predicts that acute application of aldosterone should hyperpolarize the basolateral membrane in epithelia with high rates of Na transport but not in those with low rates of transport.

Conclusions

The high pH sensitivity of two of the transporters essential for transepithelial Na transport—apical Na channels and basolateral K channels—suggests the possibility that the overall transport rate could be regulated through mechanisms which alter cell pH. This concept has not been directly demonstrated, despite the observation that at least one hormone that is known to increase transport—aldosterone—can influence pH in a manner consistent with the natriferic response. Future research topics include identification of apical membrane hormone receptors that can affect subapical pH, and the documentation of apical to basolateral pH gradients within the epithelial cell.

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