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Cytoplasmic pH determines K^+ conductance in fused renal epithelial cells

(aldosterone/K^+ channels/Na^+/H^+ exchange/cell fusion/distal nephron)

H. OBERLEITHNER*†, U. KERSTING*, AND M. HUNTER‡

*Department of Physiology, University of Würzburg, Röntgenring 9, 8700 Würzburg, Federal Republic of Germany; and ‡Department of Physiology, Leeds University, Leeds LS2 9NQ, United Kingdom

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ABSTRACT **The mineralocorticoid hormone aldosterone maintains acid–base balance and K^+ homeostasis by regulating H^+ and K^+ secretory mechanisms in kidney epithelial cells. We have shown recently in the amphibian distal nephron that aldosterone activates a Na^+/H^+ exchange system in the luminal cell membrane, thus leading to transepithelial H^+ secretion and cytoplasmic alkalinization. Since H^+ secretory fluxes were paralleled by K^+ secretion, it was postulated that the hormone-induced increase of intracellular pH activates the luminally located K^+ channels. In "giant" cells fused from individual cells of the distal nephron, we measured simultaneously cytoplasmic pH and cell membrane K^+ conductance during acidification of the cell cytoplasm. The experiments show that cell membrane K^+ conductance is half-maximal at an intracellular pH of 7.42 and that a positive cooperative interaction exists between K^+-channel proteins and H^+ (Hill coefficient = 6.5). Moreover, the cellular K^+ conductance is most sensitive to cytoplasmic pH in the range modified by aldosterone. This supports the hypothesis that intracellular H^+ activity, regulated by the Na^+/H^+ exchanger, serves as the signal to couple aldosterone-induced K^+ secretory flux to H^+ secretion in renal tubules.**

In the vertebrate kidney, aldosterone stimulates Na^+ retention and H^+ and K^+ secretion. In the collecting duct system of the mammalian kidney, aldosterone stimulates Na^+ entry across the luminal cell membrane (1), thereby depolarizing the cell membrane potential (2). This depolarization facilitates cell-to-lumen K^+ secretion (3) via hormone-activated (4) K^+-selective channels and H^+ extrusion via a rheogenic H^+ pump (5). From experiments in the early distal nephron of the amphibian kidney, we have evidence that transepithelial fluxes of Na^+, K^+, and H^+ ions are also functionally linked, but that the mechanisms involved are different from those in the mammalian collecting-duct system (6).

We propose a model in which hormone-induced stimulation of the Na^+/H^+ exchanger is the primary event leading to cytoplasmic alkalinization and subsequent activation of the pH-sensitive K^+ channels. If the mechanism whereby the hormone-induced interaction between a counter-transport mechanism (Na^+/H^+ exchanger) and an ionic channel (K^+ channel) is to be physiologically relevant, the K^+ channels must be sensitive to intracellular pH (pH_i) within the range encompassed during exposure to the hormone (7.45–7.73).

METHODS

To test our hypothesis, we simultaneously measured the intracellular pH and K^+-conductance of fused distal tubules from frog kidney during gradual acidification of the cytoplasm.

The fusion procedure has been described elsewhere (6). Briefly, kidneys of *Rana pipiens* were first isolated and perfused with amphibian Ringer's solution composed of 97 mM NaCl, 3 mM KCl, 5 mM Hepes, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 5 mM glucose. After the blood was rinsed off, 3 ml of the same solution containing 0.1% collagenase [Sigma or Seromed (Berlin); 180–300 units/mg] was injected. With fine scissors, the ventral surface of both kidneys containing primarily diluting segments was removed in small pieces and incubated for 50 min in the collagenase-containing solution. Then, the pieces were gently sucked up and down with a 200-μl Eppendorf pipette until a suspension of small tubule fragments was obtained. The tubules were centrifuged at 400 $\times$ g for 2 min, and the supernatant was removed. To the supernatant was slowly added 1 ml of the fusion medium [30% (wt/vol) polyethylene glycol (M_r 4000) dissolved in Leibovitz-15 medium diluted to 170 milliosmolar (pH 8.2)], and the cells again were centrifuged at 400 $\times$ g for 2 min. Finally, they were suspended in Leibovitz-15 medium (200 milliosmolar, pH 7.8) and transferred onto a thin microscope coverslip pretreated with poly(L-lysine) (0.1 g/liter; Serva, Heidelberg) and incubated for 24–48 hr at 6°C. Over this time period, membrane fusion proceeded and tubules were ready for impalements. By using high-resolution differential interference contrast microscopy (inverted microscope, IM 35, Zeiss; objective 63/1.4, oil), cell membranes remaining in the intracellular compartments because of incomplete cell-to-cell fusion were disclosed. Those tubules were not used for the experiments.

Such fused tubules (Fig. 1) can be impaled with three microelectrodes, and pH_i and K^+-conductance measurements can be performed simultaneously while pH_i is altered. We penetrated the fused cells with three microelectrodes. One conventional microelectrode (filled with 1 M KCl) was used to inject negative current pulses (1–5 nA, 200-ms duration); another one was used to monitor the cell-membrane potential, V_m; and a third one, a pH-sensitive liquid ion-exchange microelectrode, was applied to measure pH_i when the cell cytoplasm was gradually acidified. High impedance amplifiers were used to measure V_m and pH_i. Currents of 1×10^{-9} to 100×10^{-9} A were delivered by a current-injecting device (Frankenberger Electrometer, Germering, F.R.G.).

RESULTS AND DISCUSSION

The passive membrane conductance of early distal tubule cells is determined by Cl^- and K^+ channels (7). Since we wanted to study the whole-cell K^+ conductance, Cl^- ions were largely removed from the kidney perfusate and substituted by impermeant monovalent anions (gluconate). Fur-

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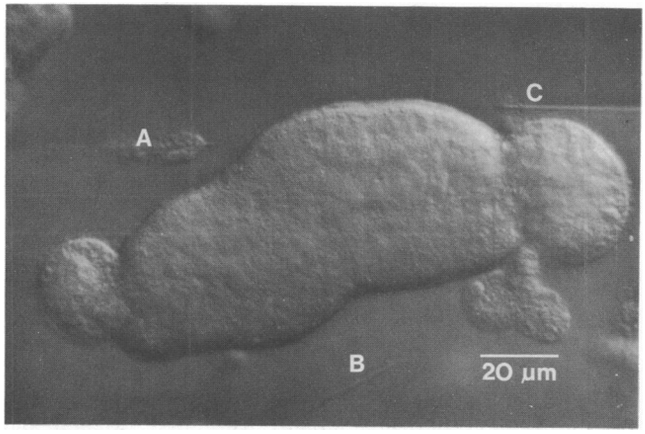
Abbreviations: pH_i, intracellular pH; G_K, Ba^{2+}-sensitive K^+ conductance.

†To whom reprint requests should be addressed.



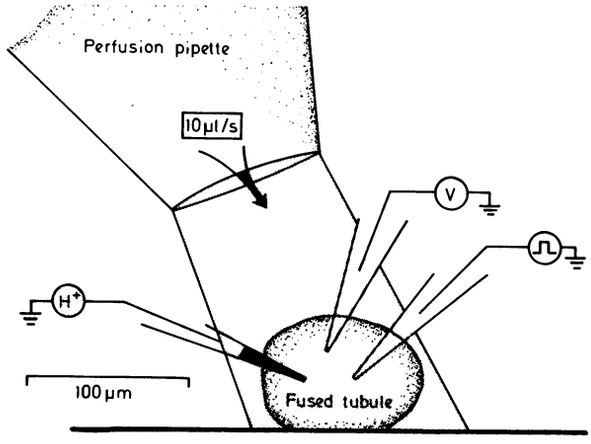


FIG. 1. (*Upper*) Fused renal tubule and three microelectrodes: A, electrode for voltage measurement; B, current injection electrode; and C, pH electrode after the impalements. (*Lower*) Fused tubules were superfused with high perfusion rates (intact tubules stick firmly to the glass surface), and extracellular solutions were exchanged within seconds. The superfusion solutions were similar to the kidney perfusate (see *Methods*) except that NaCl was substituted by either sodium gluconate or potassium gluconate. Since Ca^{2+} binds to monovalent gluconate, $CaCl_2$ (about 8 mM) was added under the control of a Ca^{2+}-sensitive macroelectrode to obtain the same Ca^{2+} activity as in the gluconate-free perfusion solution. KOH or NaOH (0.1 M) was used to titrate the solutions to the appropriate pH (between 6.5 and 8.0). $BaCl_2$ (3 mM) was added if applicable.

thermore, experiments were performed in sodium gluconate and potassium gluconate solutions with hyperpolarized and depolarized cell membrane potentials, respectively. These two series of experiments should elucidate whether the H^+-induced block of the K^+ conductance is voltage dependent.

Fig. 2 *Upper* shows an original recording of cell membrane potential, V_m; cell-membrane resistance, R_m; and pH_i when extracellular pH was changed from 7.8 to 6.8 (sodium gluconate). pH_i decreased gradually concomitant with a large cell-membrane depolarization. The magnitude of the current-induced V_m changes (i.e., voltage spikes), corresponding to the resistance of the cell membrane, R_m, increased sharply between pH_i 7.50 and 7.40 and then remained fairly constant. Addition of Ba^{2+} to the superfusion medium increased the current-induced voltage changes only slightly after the cytoplasmic pH was rendered acidic. Obviously, acidic pH_i has inactivated this portion of the cell membrane conductance (the reverse of R_m), which is Ba^{2+}-inhibitable and which most likely is the K^+ conductance.

In this paper we relate the pH_i-induced conductance changes to the Ba^{2+}-inhibitable conductance and term it the Ba^{2+}-sensitive K^+ conductance (G_K). Table 1 relates the pH_i values to the corresponding V_m and R_m measurements. When pH_i is acidic, the K^+ conductance decreases and V_m depolarizes. This is reflected by the increase of R_m, which is close to its maximum at acidic pH_i. Similar results were obtained when the pH_i–G_K relationship was tested in a fused cell with a depolarized cell-membrane potential. In the presence of potassium gluconate in the superfusate, V_m was -8.5 ± 2.0 mV and the pH_i was 7.69 ± 0.02 when the pH of the extracellular medium was 7.8. Acidification of pH_i depolarized V_m only slightly (Table 1) while a sharp K^+ conductance change was observed. Fig. 2 *Lower* shows that the K^+ conductance decreased dramatically within a rather narrow range of pH_i—namely, between 7.50 and 7.45. Further acidification of the cytosol and subsequent addition of Ba^{2+} to the superfusate did not additionally increase the membrane resistance. This again shows that a rather small change of pH_i in the physiological range can affect G_K dramatically. The data are summarized in Table 1. Cytoplasmic acidification by 0.1 pH units in the physiological range increased the cell membrane resistance by almost 30%. R_m was insensitive to Ba^{2+} at such acidic conditions, indicating that the R_m change was due to the decrease of the cell-membrane K^+ conductance.

Table 1. Intracellular pH, cell-membrane voltage, and cell-membrane resistance in 12 fused renal tubules

Condition				
Gluconate salt	pH	pH_i	V_m, mV	R_m, kΩ·cm²
Sodium	7.8	7.68 ± 0.02	−68.3 ± 2.7	1.64 ± 0.11
	6.8	7.24 ± 0.01	−36.0 ± 2.8	2.87 ± 0.23
+ $BaCl_2$	6.8	7.22 ± 0.01	−29.8 ± 2.9	3.21 ± 0.27
Potassium	7.8	7.69 ± 0.02	−8.5 ± 2.0	0.81 ± 0.06
	6.8	7.28 ± 0.01	−5.7 ± 1.6	1.43 ± 0.21
+ $BaCl_2$	6.8	7.25 ± 0.01	−7.2 ± 1.7	1.59 ± 0.22

Intracellular pH was measured with single-barreled pH-sensitive liquid ion-exchange microelectrodes. pH_i was calculated from the equation: $pH_i = pH_o - (V_m^H - V_m)/S$ in which pH_o is the extracellular pH, V_m^H is the cell membrane H^+ electrochemical potential difference, and S is the electrode slope measured in calibration solutions mimicking the cytosolic composition. The cell input resistance was calculated according to Ohm's law: $R_m = \Delta V_m/I$, where ΔV_m is the cell-membrane potential deflection in response to the injected current pulse (I). The R_m was related to the cell surface (F): R_m (Ω·cm²) = R_m (Ω)·F (cm²). Since fused tubules were either spherical or cylindrical, F was calculated as the surface of a sphere ($F = 4\pi r^2$) or of a cylinder ($F = 2r\pi h$), where r (measured optically) is the radius of the sphere or cylinder and h is the height of the cylinder. Results are given as means ± SEM.

Fig. 3 shows the relationship between pH_i and G_K. In both conditions, in the hyperpolarized (sodium gluconate) and in the depolarized (potassium gluconate) cell, G_K is highly sensitive to H^+ in a rather narrow range of pH. From the similar shape of the two curves, we deduce that the H^+-induced block of G_K is not voltage-dependent. The sigmoidal shape indicates that there is a positive cooperative interaction between the K^+-channel proteins and H^+. Binding of one H^+ ion to the channel causes a conformational alteration so that the affinity for the next H^+ ion to bind is increased. Furthermore, the curves suggest that multiple H^+ ions bind simultaneously to a single K^+ channel protein or to separate proteins within a microdomain. This can be derived from the slope (i.e., Hill coefficient) of the nonlinear regression between intracellular pH and the corresponding G_K values. A Hill coefficient of ≈6.5 was calculated for both conditions (i.e., hyperpolarized or depolarized cell membrane potential). Hunter and Giebisch (8) have postulated recently that the K^+ channel in the same nephron portion of the kidney has a multibarreled structure including a main gate that controls the overall open probability of the channel and at least four individual channel subunits. It is tempting to speculate that

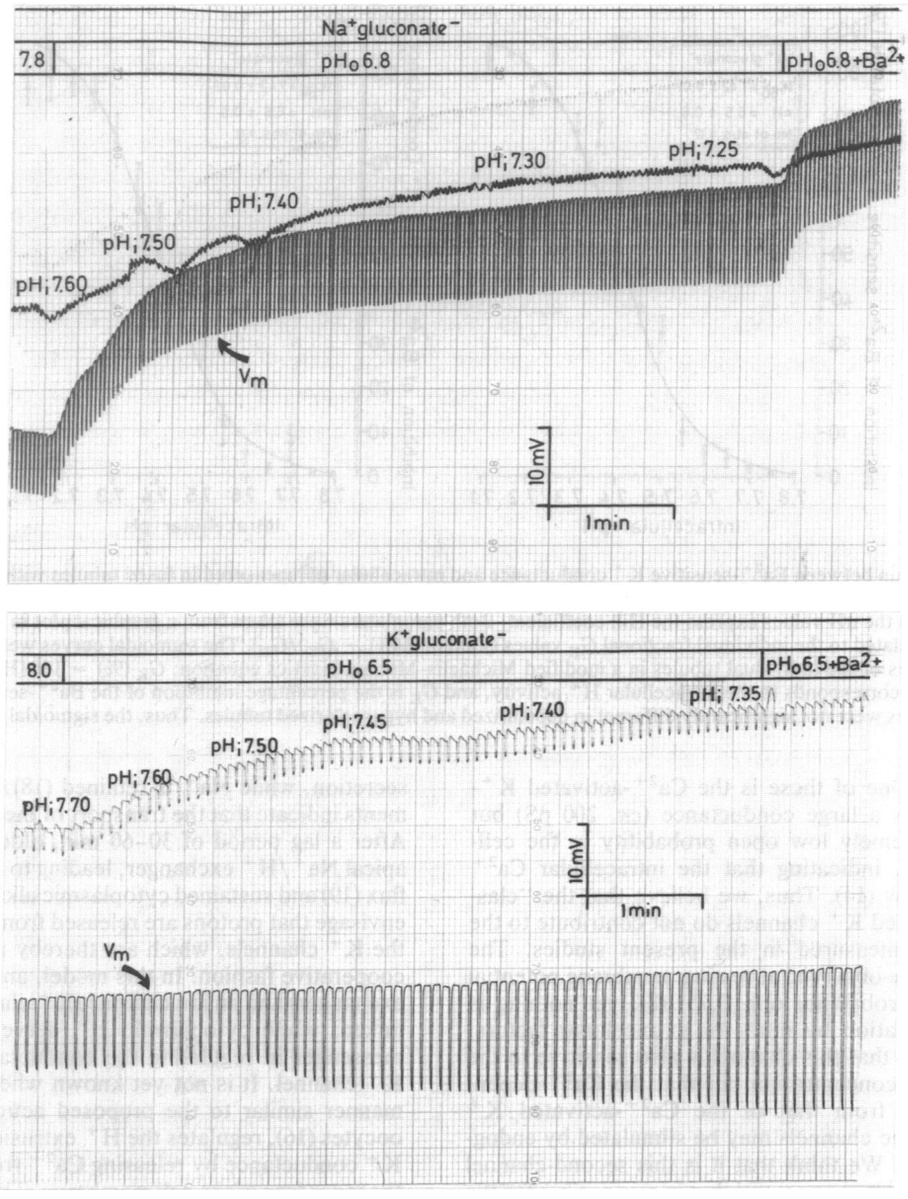


FIG. 2. Two original recordings of pH_i, V_m, and R_m (calculated from ΔV_m) obtained from a fused tubule (cylindrical shape; height, 150 μm). (*Upper*) Experiment in the hyperpolarized state (sodium gluconate). V_m depolarizes from -70 mV to -40 mV, while the cell cytoplasm is acidified by 0.35 pH units. ΔV_m increases dramatically between pH 7.50 and 7.40, indicating a major change of the cell-membrane conductance. Addition of 3 mM Ba^{2+} does not further increase the current-induced ΔV_m. (*Lower*) Experiment in the depolarized state (potassium gluconate). V_m remains fairly constant (at about -5 mV) when the cell cytoplasm is acidified. A large conductance change can be observed in the rather narrow pH_i range between 7.50 and 7.45. There is only a small increase of the current-induced ΔV_m changes when Ba^{2+} is added under acidic conditions.

cytosolic H^+ may interact with the individual subunit gates in a positive cooperative manner. This would represent a most suitable, fine-tuning mechanism over a wide K^+ conductance range, while maintaining the high selectivity of the K^+ channels.

Inhibition of cell-membrane K^+ conductance by acidification of the cytoplasmic membrane surface has been shown in perfused squid giant axon (9) and more recently in pancreatic B cells (10, 11). The fused cell preparation allows simultaneous measurements of intracellular pH and cell membrane G_K in the renal epithelium. Although we cannot distinguish between apical and basolateral K^+ permeabilities, we assume that quantitatively in this preparation the apical G_K dominates over basolateral G_K. This assumption is based on observations in both amphibian (7) and mammalian (12) preparations that the major basolateral membrane conductance is for chloride ions. Since we deleted chloride ions from the perfusates, apical G_K was the major cell membrane conductance in this study.

It is important to note that the experiments were performed with an intact cytoplasmic compartment and thus at physiological Ca^{2+} activities. It has been observed recently in patch–clamp experiments that whole-cell K^+ currents in renal diluting-segment cells are only moderately sensitive to cytosolic pH changes when cytosolic Ca^{2+} is absent (13). Addition of physiological concentrations of Ca^{2+} to the perfusion medium more than doubles the overall G_K (H.O., unpublished observation). This indicates that the inhibitory action of H^+ could be due to the competition between H^+ and Ca^{2+} at the multiple binding sites of Ca^{2+}-activated K^+ channels. It must be appreciated that there are two dominant K^+ channel species in the apical membrane of the amphibian

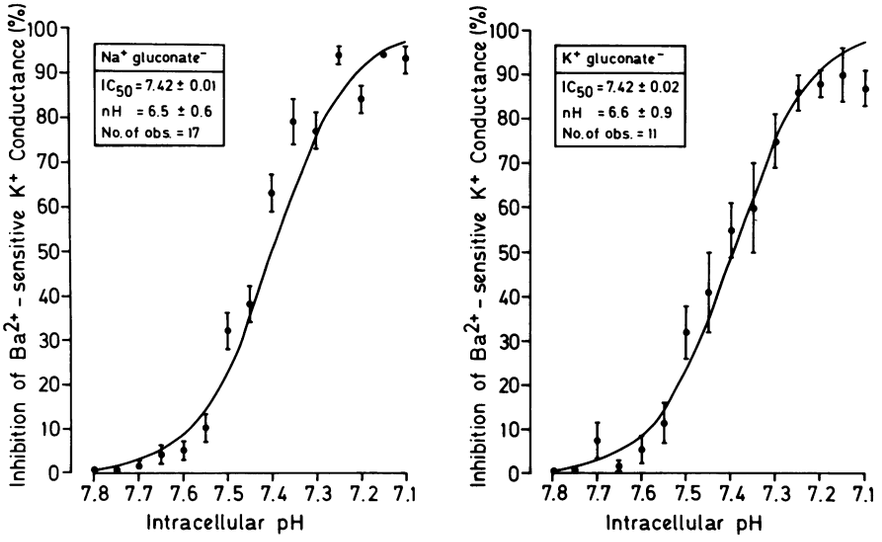


FIG. 3. Relationship between Ba^{2+}-sensitive K^+ conductance and intracellular pH measured in fused tubules with hyperpolarized (sodium gluconate) and depolarized (potassium gluconate) cell membrane potentials. The IC_{50} values indicate the half-maximal inhibition of the K^+ conductance, whereas the nH values express the Hill coefficient. Both parameters were taken from a graphical plot in which the individual pH_i values (x axis) were related to the individual fractional G_K values (y axis): $\log[(1 - G_K)/G_K]$. The sigmoidal curves were calculated by applying the IC_{50} and nH values of the individual tubules in a modified Michaelis–Menten kinetics equation: $G_K\ (\%) = 100 \cdot [H^+]^{nH}/(IC_{50}^{nH} + [H^+]^{nH})$. In this equation $[H^+]$ corresponds to the intracellular H^+ activity, and G_K is the percentage inhibition of the Ba^{2+}-sensitive K^+ conductance. The IC_{50} and nH values were not significantly different in depolarized and hyperpolarized tubules. Thus, the sigmoidal curve applies under both conditions.

diluting segment. One of these is the Ca^{2+}-activated K^+-channel, which has a large conductance (*ca.* 200 pS) but which has an extremely low open probability in the cell-attached condition, indicating that the intracellular Ca^{2+} concentration is low (14). Thus, we believe that the "classical" Ca^{2+}-activated K^+ channels do not contribute to the K^+ conductance measured in the present studies. The second channel type observed at resting membrane potential has a high open probability (*ca.* 0.75) (8), yet nothing is known of its regulation by intra- or extracellular factors. Thus, it is possible that this channel is also sensitive to the intracellular Ca^{2+} concentration but that the Ca^{2+} dependence is different from that of the Ca^{2+}-activated K^+ channels, so that the channels may be stimulated by endogenous Ca^{2+} levels. We think that it is this second-channel type [the multisubunit channel (8)] that is responsible for the majority of the cellular K^+ conductance.

Free H^+ and Ca^{2+} activities are well balanced by cytoplasmic buffers, and both may serve as intracellular messengers to control epithelial transport at the molecular level. Such a regulatory system in which H^+ competes with Ca^{2+} for specific binding sites has been proposed (15). Steroids (e.g., progesterone) induce meiotic division in amphibian oocytes by increasing cytoplasmic Ca^{2+} (16) activity while decreasing intracellular H^+ activity. Transient intracellular alkalinization is a characteristic feature of the postprogesterone period (17) and may be explained by the activation of quiescent Na^+/H^+ exchangers in the cell membrane.

We have reported cytoplasmic alkalinization by 0.28 pH units in renal epithelial cells induced by the steroid hormone aldosterone (6). The present study indicates that the G_K of the diluting segment cell is most sensitive to cytoplasmic pH well within the range of the physiological fluctuations. Aldosterone increases cytoplasmic pH from 7.45 to 7.75, which according to Fig. 3 decreases the percentage inhibition of the K^+ conductance by >2 orders of magnitude. Indeed, K^+ secretory fluxes are induced by the steroid (6). It is well accepted that aldosterone is the key hormone involved in the regulation of Na^+, H^+, and K^+ homeostasis. In the mammalian distal nephron, aldosterone induces K^+ and H^+ net secretion, while Na^+ is retained (18). Our previous experiments indicate that the transport of these three ions is linked. After a lag period of 30–60 min, aldosterone activates the apical Na^+/H^+ exchanger, leading to transepithelial H^+ net flux (19) and sustained cytoplasmic alkalinization (6). We can envisage that protons are released from their binding sites on the K^+ channels, which are thereby activated in a positive cooperative fashion. In this model, an ion carrier (Na^+/H^+ exchanger) and an ion channel (K^+ channel) are functionally linked, while cytoplasmic H^+ serves as the intracellular messenger in regulating the conductance properties of the K^+ channel. It is not yet known whether aldosterone, in a manner similar to the proposed action of progesterone in oocytes (16), regulates the H^+ extrusion mechanism and the K^+ conductance by releasing Ca^{2+} from binding sites along the inner face of the cell membrane. The Na^+/H^+ exchanger can indeed be activated by Ca^{2+} (20), and the conductance of a Ca^{2+}-activated and H^+-inactivated K^+ channel will most likely depend on the concentration ratio of cytoplasmic Ca^{2+} over H^+.

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