

Aldosterone activates Na^+/H^+ exchange and raises cytoplasmic pH in target cells of the amphibian kidney

(signal transducer/ K^+ permeability/distal nephron/amiloride/spironolactone)

H. OBERLEITHNER, M. WEIGT, H.-J. WESTPHALE, AND W. WANG

University of Würzburg, Department of Physiology, Röntgenring 9, 8700 Würzburg, Federal Republic of Germany

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ABSTRACT The hypothesis was tested if the mineralocorticoid hormone aldosterone stimulates Na^+/H^+ exchange in “giant cells” fused from individual target cells of the distal nephron of the frog kidney. By means of microelectrodes, steady-state intracellular pH (pH_i) and pH_i recovery from an acid load were recorded continuously while the fused cells were exposed to aldosterone. Twenty minutes after addition of the hormone, pH_i started to rise and reached a new steady state after about 60 min ($\Delta\text{pH}_i = 0.28 \pm 0.01$). After hormone treatment, pH_i recovered significantly faster in response to an intracellular acid load. The diuretic drug amiloride blocked pH_i recovery. Experiments in intact tubules showed that aldosterone induces H^+ and K^+ secretion. Thus, intracellular alkalosis, mediated by Na^+/H^+ exchange, could serve as a signal that activates pH-sensitive K^+ channels of the luminal cell membrane.

Aldosterone enhances Na^+ reabsorption as well as K^+ and H^+ secretion in the distal nephron of the kidney. There it binds to cytoplasmic receptor proteins, which migrate into the nucleus as a hormone-receptor complex and then interact with specific chromatin acceptor sites. According to the current view (1–3), this could lead to “de novo” synthesis of transport proteins (e.g., Na^+/K^+ -ATPase) and/or to the induction of regulatory proteins that control the activity of ionic channels (e.g., luminal Na^+ channels).

In the amphibian kidney, K^+ and H^+ secretion can be induced in the distal nephron when animals are exposed chronically to a high K^+ environment (4, 5). Under these metabolic conditions, plasma aldosterone is increased (6). Recent experiments suggest that the secretion of H^+ and K^+ is functionally linked (7). The two transport systems, located in parallel in the luminal cell membrane and responsible for H^+ and K^+ secretion, are a Na^+/H^+ exchanger and a K^+ -conductive pathway (i.e., K^+ channels). Both are activated by K^+ adaptation (i.e., high aldosterone) (5, 8) and inactivated by the diuretic drug amiloride (9). In search of the intracellular signal for the linkage of these two transport systems, we tested the following hypothesis: the Na^+/H^+ exchanger is the primary target for aldosterone. Activation of this transport protein alkalizes the cell cytosol. This in turn increases the conductive properties of the luminal pH-sensitive K^+ channels (10).

METHODS

Cell Fusion and Perfusion Techniques. The fusion technique of isolated renal epithelial cells of the frog kidney to “giant cells” has been described in detail (11). Briefly, cells of the early distal portion of the nephron—the diluting segment—were obtained by mechanical dissection of collagenase-

treated tubules (Fig. 1 A and B). Cells were suspended for 3 min into a fusogenic medium containing 30% PEG. The cells shrink, attach to each other, and fuse to form large cells when the PEG is gently substituted by culture medium. The cells were allowed to rest for at least 4 hr in Leibovitz 15 medium, adjusted to 200 milliosmolar and to pH 7.8. Intracellular impalements were carried out while the cell under study was rapidly circumfused by use of a multichannel pipette system, its mouth about 500 μm away from the cell surface (Fig. 1 C and D). The amphibian control solution was composed of 97 mM NaCl, 3 mM KCl, 1.5 mM CaCl_2 , 1 mM MgCl_2 , 5.5 mM glucose, and 10 mM Hepes, titrated to pH 7.8 with 0.1 M NaOH. A reversible intracellular acidosis was induced by applying the ammonia-prepulse method (12). The cell under study was circumfused for 3 min with a solution containing the $\text{NH}_4^+/\text{NH}_3$ system (20 mM NH_4Cl substituted for NaCl). Then, on removal of the $\text{NH}_4^+/\text{NH}_3$ system, the cell cytosol acidifies transiently. The initial pH_i recovery (after cytoplasmic acidification) was measured in the absence and presence of 1 mM amiloride before and after treatment with aldosterone (0.3 μM). Steady-state pH_i and pH_i -recovery were measured in the individual fused cell before (control) and after addition of the steroid hormone.

Intracellular pH. The measurements were performed with single- and double-barreled pH-sensitive liquid ion-exchange microelectrodes. Manufacture, calibration, and properties of the H^+ -selective electrodes have been described (5, 13). We attempted to measure the cell membrane electrical potential (V_m) and the H^+ electrochemical potential (V_m^{H}) simultaneously in the same cell at the various experimental conditions. We accepted only those measurements in which V_m^{H} could be recorded continuously over the entire length of the aldosterone application (at least 60 min). V_m was measured either continuously or intermittently during the course of the experiments. pH_i was calculated from the equation: $\text{pH}_i = \text{pH}_0 - (V_m^{\text{H}} - V_m)/S$. The symbol S is the electrode slope, measured in calibration solutions with intracellular ionic background; $\text{pH}_0 = 7.8$.

Intraluminal pH and K^+ Activity in Intact Tubules. In another series of experiments, the limiting intraluminal pH (pH_{lu}) and intraluminal K^+ activity ($\text{K}_{\text{lu}}^{\text{a}}$) were measured in early distal nephron segments of the isolated perfused frog kidney. The kidney perfusion technique and the application of the ion-sensitive microelectrodes have been published in detail (4, 5). Tubules of the kidneys of NaCl-adapted (aldosterone-deprived) animals were impaled by conventional and H^+ - or K^+ -sensitive microelectrodes. From the transepithelial electrical potential (V_{te}) and the H^+ or K^+ electrochemical potentials (V_{te}^{H} and V_{te}^{K}) at static-head conditions (lumen flow rate = 0), pH_{lu} ($\text{pH}_{\text{lu}} = \text{pH}_0 - (V_{\text{te}}^{\text{H}} - V_{\text{te}})/S$) and $\text{K}_{\text{lu}}^{\text{a}}$ ($\log \text{K}_{\text{lu}}^{\text{a}} = \log \text{K}_0^{\text{a}} - (V_{\text{te}}^{\text{K}} - V_{\text{te}})/S$; $\text{K}_0^{\text{a}} = 2.3 \text{ mM}$) could be evaluated before and after peritubular perfusion of aldosterone (3–0.3 μM).

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Abbreviation: pH_i , intracellular pH.

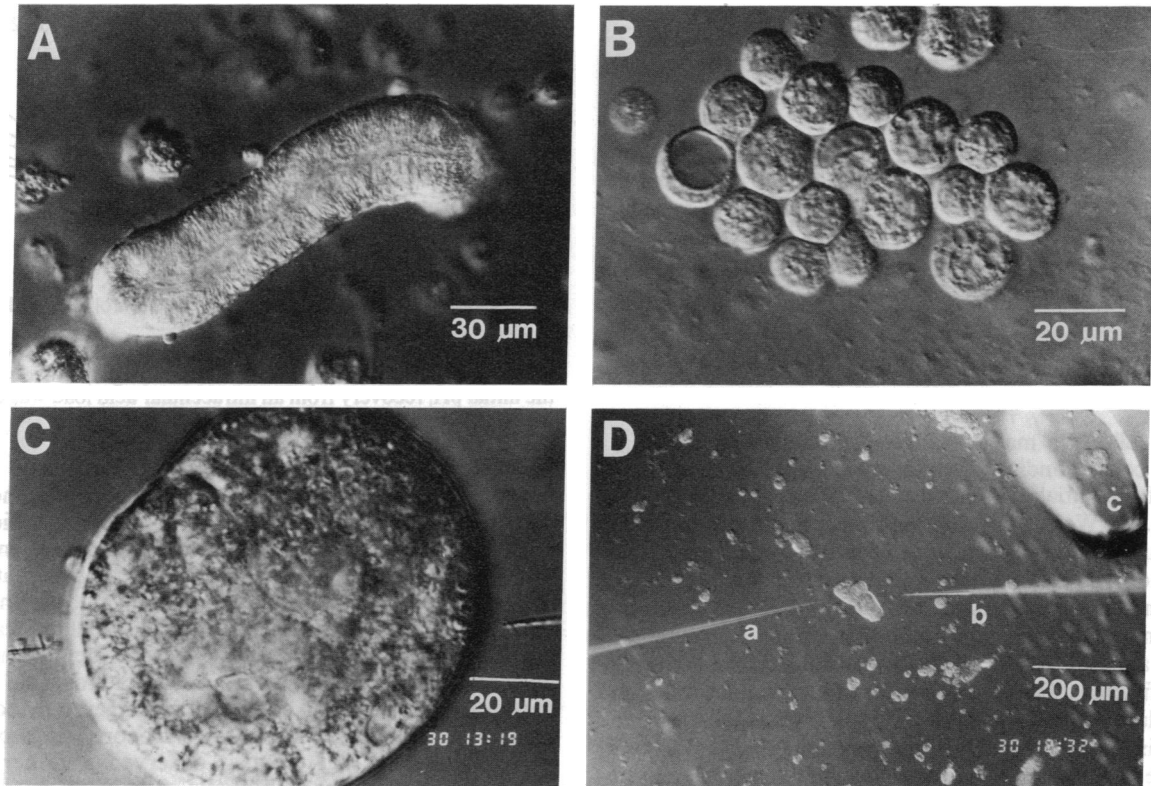


FIG. 1. Cell fusion and circumfusion technique. (A) Early distal tubule isolated from the frog kidney. (B) Single tubule cells after enzymatic dissociation. (C) "Giant cell", 6 hr after fusion. (D) Fused cell before impalement with conventional (a) and pH-sensitive (b) microelectrodes while circumfused with amphibian Ringer's solution by means of a glass pipette (c).

RESULTS

Steady-State pH_i and pH_i Recovery in Fused Cells. Impalement of a fused cell with a pH-sensitive microelectrode resulted in a negative shift of the electrode reading. This corresponds to the transmembrane V_m^H , maintained by an H^+ extrusion mechanism (i.e., Na^+/H^+ exchange). Fig. 2 shows continuous measurements of V_m^H while $0.3 \mu M$ aldosterone was superfused. Twenty minutes after application of the hormone, V_m^H started to rise and reached a new steady state after about 60 min. The corresponding mean values are summarized in Table 1. When one takes into account the V_m values measured at identical conditions in the same cells, pH_i

increased by 0.28 ± 0.03 in response to the steroid hormone. Intracellular pH approached extracellular pH under those conditions (Fig. 3 *Left*). To test whether this hormone-induced cytoplasmic alkalization is mediated by Na^+/H^+ exchange, we evaluated the ability of the cells to recover from an intracellular acid load. Fig. 4 displays four original tracings of V_m^H obtained in one individual fused cell. There was a transient alkalization of the cell cytosol when the NH_4^+/NH_3 system was offered to the cell. On removal of NH_4^+/NH_3 , the cytoplasm was acidified, followed by pH_i recovery. This initial recovery phase could be suppressed almost completely by 1 mM amiloride. After exposure to $0.3 \mu M$ aldosterone for 60 min, the experiment was repeated in

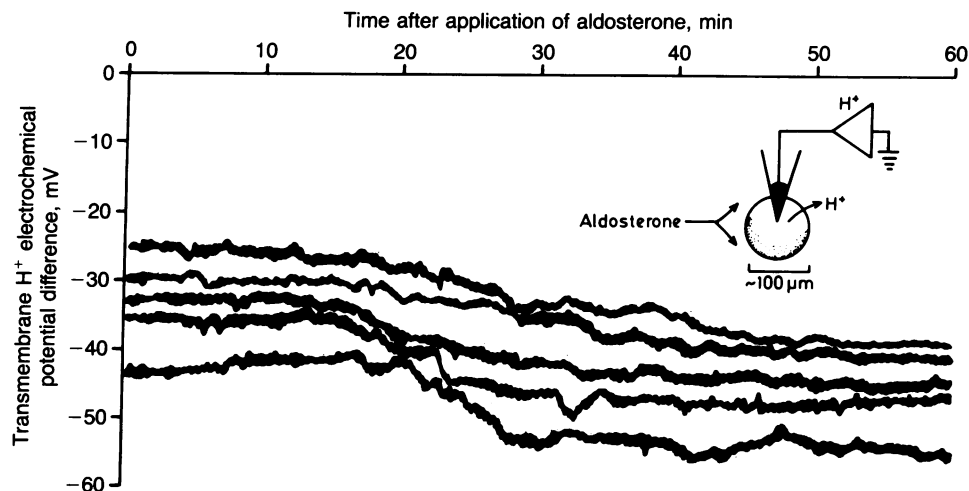


FIG. 2. The transmembrane H^+ electrochemical potentials of fused cells were continuously recorded by pH-sensitive microelectrodes. Note the consistent change of V_m^H 20 min after application of $0.3 \mu M$ aldosterone. This indicates cytoplasmic alkalization.

Table 1. Membrane potential, pH_i , and pH_i recovery in "giant" cells in the absence and presence of aldosterone

Aldosterone addition	Parameters			
	V_m , mV	V_m^H , mV	pH_i	ΔpH per min
Before	-51.8 ± 3.1	-33.4 ± 2.5	7.43 ± 0.01	0.14 ± 0.03
After	-48.0 ± 2.5	-43.8 ± 2.8	7.71 ± 0.01	0.31 ± 0.07
Difference	$3.8 \pm 1.4^*$	$10.4 \pm 1.1^\dagger$	$0.28 \pm 0.03^\dagger$	$0.17 \pm 0.02^\dagger$

V_m is the intracellular membrane potential; V_m^H , transmembrane H^+ electrochemical potential; ΔpH_i per min, pH_i recovery. Data are paired observations in five fused cells (\pm SEM) before and 60 min after application of $0.3 \mu M$ aldosterone.

* $P < 0.05$.

† $P < 0.001$.

the same cell. Two major changes are observed. First, V_m^H increased corresponding to an increase of pH_i , and second, the rate of the initial pH_i recovery had about doubled (Table 1 and Fig. 3). We also performed time-control experiments over 60 min without adding aldosterone. Then pH_i remained stable, and pH_i recovery was found to be unchanged.

Intraluminal pH and K^+ Activity in Intact Tubules. K^+ adaptation induces luminal acidification (5) and K^+ secretion (4) in the amphibian diluting segment. Aldosterone could be the specific signal because hormone concentrations are high in the plasma of K^+ -adapted animals (6). To test this hypothesis in the intact kidney, we applied $0.3 \mu M$ aldosterone to the peritubular side of the distal tubules in kidneys of NaCl-adapted aldosterone-deprived frogs. Within 20–60 min, pH_{lu} decreased from 7.73 ± 0.02 to 7.17 ± 0.08 . This decrease of pH_{lu} can be inhibited when 1 mM amiloride is added to the luminal compartment. The question arises whether aldosterone acts via intracellular receptors. Therefore, we perfused the kidney with spironolactone—a specific competitive antagonist of aldosterone—together with aldosterone in a con-

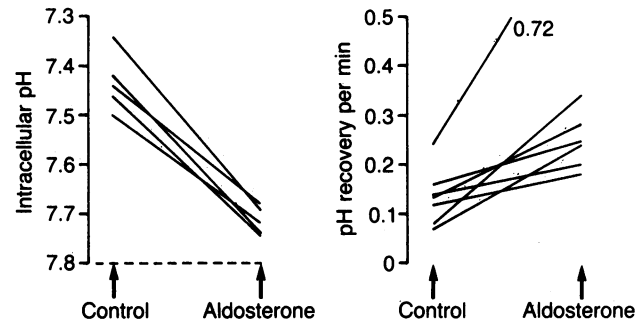


FIG. 3. (Left) pH_i was evaluated in five fused cells before and 60 min after exposure to $0.3 \mu M$ aldosterone. (Right) In the same cells, the initial pH_i recovery from an intracellular acid load was evaluated before and after administration of the steroid.

centration ratio of 250:1. The presence of spironolactone indeed could prevent the aldosterone-induced acidification of the tubule fluid (Fig. 5). Functional linkage between H^+ and K^+ secretory fluxes was indicated by the observation that luminal K^+ activity almost doubled when aldosterone ($3 \mu M$) was added to the kidney perfusate. This could be caused by the stimulation of the Na^+, K^+ -ATPase in the basolateral cell membrane and/or by an increase of the luminal K^+ conductance. K^+ net secretion could not be triggered by equivalent concentrations of the synthetic glucocorticoid dexamethasone, indicating that we had observed a rather specific mineralocorticoid effect (Fig. 6).

DISCUSSION

There is excellent documentation on the sites and mode of action of aldosterone in urinary epithelia. Target cells have been detected particularly along the mammalian distal nephron (2, 14) and the amphibian urinary bladder (15, 16). In the

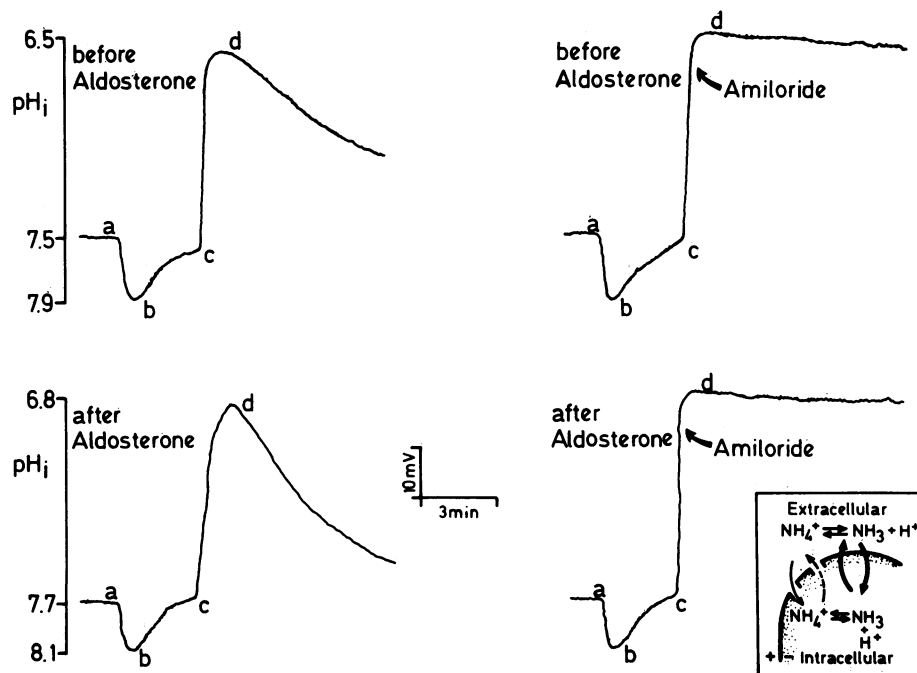


FIG. 4. Four original tracings obtained with pH -sensitive microelectrodes from an individual fused cell before and 60 min after application of $0.3 \mu M$ aldosterone. The ammonia-prepare method (see *Inset*) was used to acidify the cell cytosol. Upon exposure to NH_4^+/NH_3 , the cell alkalizes transiently (curve segment a to b). Three minutes later, after pH_i has returned to normal (curve segment b to c), NH_4^+/NH_3 was removed from the circumfusate. This causes a rapid acidification (curve segment c to d), followed by a pH_i recovery. The cell membrane potential depolarizes for 14 ± 2 mV after addition of NH_4^+/NH_3 but returns back toward control values after removal of NH_4^+/NH_3 from the extracellular fluid. Note that steady-state pH_i is elevated and that the initial pH_i recovery is faster after exposure to the steroid hormone; pH_i recovery is suppressed almost completely by 1 mM amiloride added to the circumfusate.

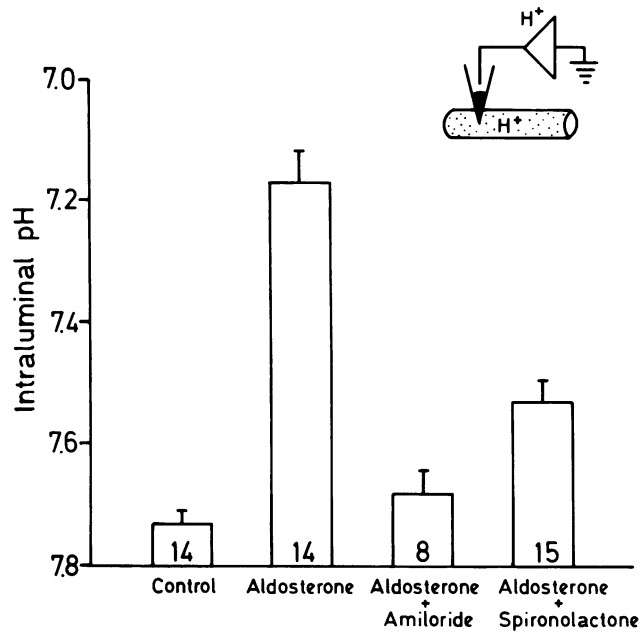


FIG. 5. Intraluminal pH was measured in distal tubules of NaCl-adapted frogs before (control) and 60 min after application of $0.3 \mu\text{M}$ aldosterone, aldosterone/ 1 mM amiloride (added to the lumen fluid), and aldosterone/ $75 \mu\text{M}$ spironolactone (added to the peritubular fluid). Aldosterone induces acidification of the tubule fluid, which can be inhibited by blocking luminal Na^+/H^+ exchange with amiloride or by competitive inhibition of the intracellular receptor sites with spironolactone.

kidney, acute administration of aldosterone increases the K^+ permeability (17) and stimulates K^+ secretion (18) in the distal portions of the nephron. The well-established long-term effects of aldosterone are the segment-specific increase of the Na^+/K^+ -ATPase activity in the kidney (19) and the induction of new and reversible recruitment of preformed Na^+ channels from intracellular reservoirs (16).

Although previous experiments have indicated strongly that the induction of K^+ and H^+ secretion is mediated by aldosterone (4, 5), we were surprised by the rapid onset of response. Cytoplasmic alkalinization occurred after a lag period of only 20 min. H^+ secretion, measured in the intact tubule, approached its maximal value 60 min after application of the hormone. This early response well fits recent observations made in amphibian epithelial cells in which Na^+ transport increased 2- to 3-fold after a lag period of about 60 min (20). The presence of specific mineralocorticoid binding sites either in cytoplasm or nucleus is evident because, on the one hand, the aldosterone-antagonist spironolactone (21) can successfully prevent H^+ secretion, and, on the other hand, the synthetic glucocorticoid dexamethasone cannot induce K^+ secretion as does aldosterone. In contrast to observations of renal proximal tubules (22), this is indirect evidence that the Na^+/H^+ exchange is rather insensitive to glucocorticoids. Rossier *et al.* (1) have demonstrated that the hormone's response in toad urinary bladder is pleiotropic. After the hormone-receptor complex has migrated to the nucleus and bound to chromatin acceptor sites, the synthesis is induced of regulatory proteins that modify transepithelial ion transport at different sites. With this in mind, it is tempting to speculate that activation of Na^+/H^+ exchange is in fact among the earliest responses in the action of aldosterone. This effect will not be detected by short-circuit current measurements because of the electroneutrality of Na^+/H^+ exchange. However, it possibly could be detected by transepithelial resistance measurements, if one assumes that the

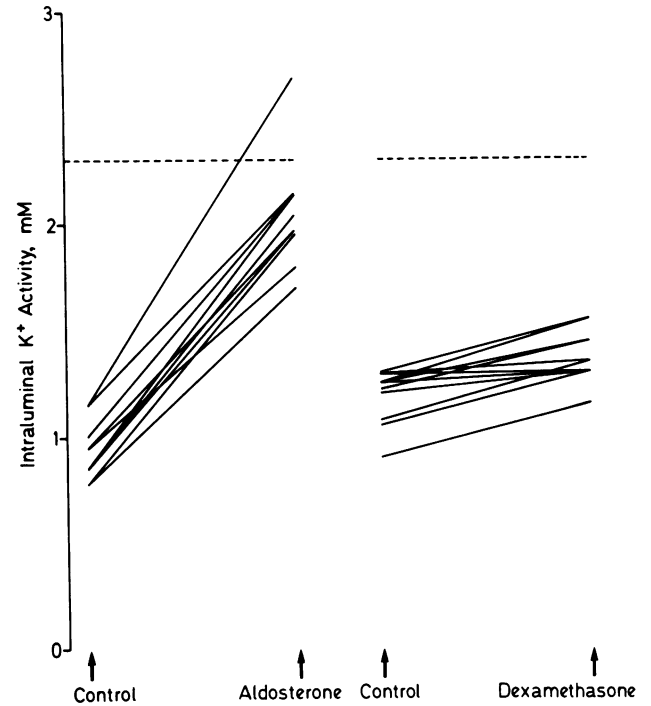


FIG. 6. The intraluminal K^+ activity was measured in distal tubules of NaCl-adapted frogs with K^+ -sensitive microelectrodes before (control) and 12 hr after addition of $3 \mu\text{M}$ aldosterone or dexamethasone. The dotted line represents the extracellular K^+ activity. The earliest measurements of intraluminal K^+ activity were performed 3 hr after application of the hormone. Those values were not significantly different from the 12-hr values indicating that the full response occurs within the first 3 hr. The synthetic glucocorticoid dexamethasone does not exert comparable effects on K^+ secretion.

cytoplasmic alkalinization increases the K^+ conductance of the cell membranes.

There is a striking parallelism between the steroid hormone-induced response reported and the response of various cells induced by growth hormone (23, 24) or insulin (25, 26). In cultured fibroblasts, growth factors selectively activate the Na^+/H^+ exchanger by increasing its affinity for intracellular H^+ . This results in sustained cytoplasmic alkalinization (23, 24). The question was raised whether pH_i could serve as a potential "messenger" in the control of cell proliferation. Later on, it was discovered that the Na^+/H^+ exchanger is activated by protein kinase C, a phospholipid- and Ca^{2+} -dependent enzyme, in response to phorbol ester and diacylglycerol; both substances raise cytoplasmic pH (27). Al-

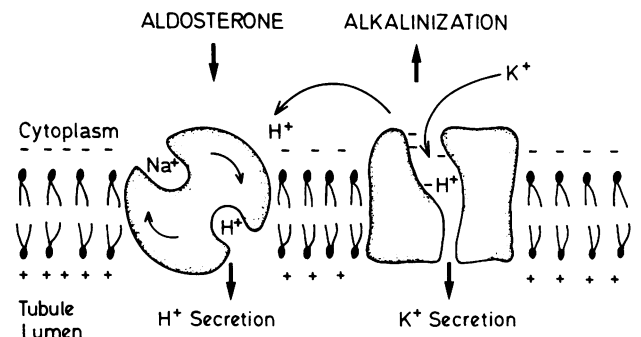


FIG. 7. Hypothesis on the action of aldosterone in target cells of the amphibian kidney. Aldosterone activates Na^+/H^+ exchange in the lumen cell membrane. H^+ ions are extruded, and the cell cytoplasm alkalinizes. The neighboring K^+ channel proteins are deprotonated. This improves the conductance properties and facilitates K^+ secretion.

though the second messenger role of diacylglycerol and inositol triphosphate—breakdown products from membrane phosphoinositides—in the action of various hormones is well documented (28), we have no direct indications so far that similar processes can be involved in the action of steroid hormones.

Our results raise the question of whether cytoplasmic alkalization mediated by Na^+/H^+ exchange could be a common denominator in the action of hormones (peptide as well as steroid hormones). The aldosterone-induced increase of pH_i in target cells of the amphibian kidney could serve as an intracellular signal regulating the open probability of K^+ channels (Fig. 7). This could explain the parallel induction of H^+ and K^+ net secretory fluxes in the distal nephron.

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