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# Fusion of Cultured Dog Kidney (MDCK) Cells: II. Relationship between Cell pH and $K^+$ Conductance in Response to Aldosterone

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**Summary.** We have chosen the MDCK cell line to investigate aldosterone action on $H^+$ transport and its role in regulating cell membrane $K^+$conductance ($G_m^K$). Cells grown in a monolayer respond to aldosterone indicated by the dose-dependent formation of domes and by the alkalinization of the dome fluid. The pH sensitivity of the plasma membrane $K^+$ channels was tested in "giant cells" fused from individual MDCK cells. Cytoplasmic pH ($pH_i$) and $G_m^K$ were measured simultaneously while the cell interior was acidified gradually by an extracellular acid load. We found a steep sigmoidal relationship between $pH_i$ and $G_m^K$ (Hill coefficient 4.4 ± 0.4), indicating multiple $H^+$ binding sites at a single $K^+$ channel. Application of aldosterone increased $pH_i$ within 120 min from 7.22 ± 0.04 to 7.45 ± 0.02 and from 7.15 ± 0.03 to 7.28 ± 0.02 in the absence and presence of the $CO_2/HCO_3^-$ buffer system, respectively. We conclude that the hormone-induced cytoplasmic alkalinization in the presence of $CO_2/HCO_3^-$ is limited by the increased activity of a $pH_i$-regulating $HCO_3^-$ extrusion system. Since $G_m^K$ is stimulated half-maximally at the $pH_i$ of 7.18 ± 0.04, internal $H^+$ ions could serve as an effective intracellular signal for the regulation of transepithelial $K^+$ flux.

**Key Words** cytoplasmic pH · aldosterone · $K^+$ channels · cell fusion · MDCK cells

## Introduction

Aldosterone maintains salt balance and acid-base homeostasis by regulating ion transport in the distal nephron of the kidney. After a lag period of about 30 min aldosterone activates the $Na^+/H^+$ exchanger located in the luminal cell membrane of the diluting segment of frog kidney [34]. Transepithelial $H^+$ secretion is induced paralleled by cytoplasmic alkalinization and by increased secretory $K^+$ net flux [25]. Recently we have obtained evidence for a pH-sensitive $K^+$ conductance in the pH range modified by the mineralocorticoid [24]. Therefore, we conclude that aldosterone action in amphibian renal tubules involves stimulation of $Na^+/H^+$ exchange and subsequent cytoplasmic alkalinization, which in turn facilitates cell-to-lumen $K^+$ secretory flux through the pH-sensitive $K^+$ channels. In order to test this hypothesis in a mammalian preparation we have chosen the MDCK cells; an established cell line derived from dog kidney [10, 19, 22]. These cells possess cytoplasmic receptors for mineralocorticoids [2, 21] and respond with increased transepithelial salt transport when exposed to the hormone [14, 31]. Furthermore, the MDCK cells are characterized by transport pathways similar to $Na^+/H^+$ exchange, $Cl^-$ and $K^+$ conductance [15, 16, 26, 29, 30] when compared with amphibian diluting segments. We have studied aldosterone-induced formation of domes in the intact MDCK cell monolayer and pH in the dome fluid as criteria for transepithelial $H^+$ transport. In order to make simultaneous intracellular pH ($pH_i$) and $K^+$ conductance measurements technically feasible, we have fused single MDCK cells, isolated from subconfluent monolayers, into "giant cells." The results demonstrate aldosterone-induced transepithelial $H^+$ transport paralleled by cytoplasmic alkalinization and describe the pH sensitivity of the cell membrane $K^+$ conductance.

## Materials and Methods

### Experiments on Domes: Dome Formation and pH Measurements in the Dome Fluid

In order to find out whether MDCK cells are suitable to investigate aldosterone-induced $H^+$ transport, we studied dome formation and pH in the dome fluid in response to the hormone. To study dome formation MDCK cells were seeded at high density ($10^6$ cells/ml) on 24-well plexiglass dishes and incubated for 48 hr in MEM medium plus 10% fetal calf serum (37°C, 5% $CO_2$). Over this time period confluent monolayers were established. Fetal calf serum was then removed to deprive the cells from growth factors and other hormonal transport stimuli. 24 hr later aldosterone at various concentrations was added to the MEM medium and the monolayers were again incubated. 24 hr after hormone addition the number of domes per $cm^2$ was evaluated using an

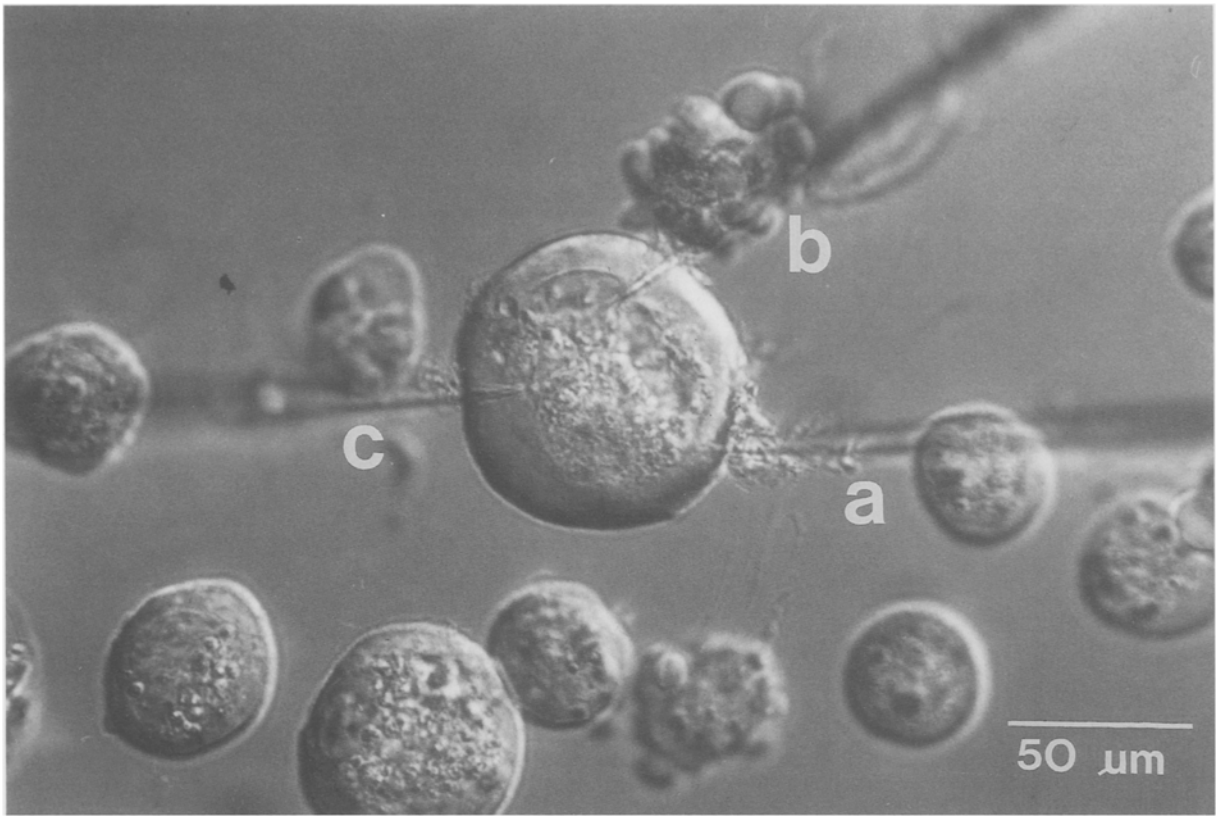


**Fig. 1.** Simultaneous $pH_i$ and plasma membrane $K^+$ conductance measurements in a fused MDCK cell. (*a*) current-injecting microelectrode; (*b*) voltage-sensing microelectrode; (*c*) pH-sensitive microelectrode

inverted microscope at low magnification. Only domes with diameters of 100 $\mu$m or beyond were taken.

To study pH in the dome fluid MDCK cells were grown to confluency on thin glass-cover slips in MEM medium plus 10% fetal calf serum (37°C, 5% $CO_2$). As soon as multiple dome formation was observed, fetal calf serum was removed and cells were maintained at serum-free conditions 24 hr prior to the experiments. Dome-pH measurements were performed with pH-sensitive microelectrodes [1] in the presence of HEPES-buffered $CO_2/HCO_3^-$-free Ringer's solution. Dome pH was calculated from the equation: $pH_d = pH_o - (V_d^H - V_d)/S$. The $pH_o$ is the extracellular pH, $V_d^H$ and $V_d$ are the transepithelial $H^+$ electrochemical and electrical potential differences, respectively. The symbol $S$ is the electrode slope, measured in HEPES-buffered Ringer's solution with a pH range between 6 and 8. $V_d$ was measured with conventional microelectrodes (filled with 1 mol/liter KCl) ranging between 0.5 and 2 mV, oriented dome negative, in reference to a grounded Ag/AgCl wire facing the apical bathing solution.

## Cell Fusion and Superfusion Solutions

The preceding paper describes culture and fusion techniques in detail [13]. In short MDCK cells were grown in plastic culture dishes until subconfluency was approached. MEM medium plus 10% fetal calf serum was used. Then cells were harvested by a two-step procedure applying first EDTA and the trypsin containing phosphate-buffered saline. Cells were fused to "giant cells" according to the fusion technique recently developed [13]. Intracellular measurements were carried out at least 4 hr after the fusion procedure. Then, cells were round-shaped, between 50 and 100 $\mu$m in diameter and the cytoplasmic compartment appeared homogeneous. Suitable cells were transferred on thin, microscopic glass-cover slips and superfused with solutions basically composed of (mmol/liter): 130 NaCl, 5.4 KCl, 1.2 $CaCl_2$, 0.8 $MgCl_2$, 10 HEPES, 5.5 glucose; pH 7.4. In one series of experiments NaCl was substituted by equivalent amounts of $K^+$ gluconate$^-$ and 7 mmol/liter $CaCl_2$ was added to compensate for $Ca^{2+}$ gluconate$^-$ binding. pH of the gluconate$^-$-containing solutions ranged between 6 and 8 set by appropriate addition of KOH (1 mol/liter) to the solutions. In another set of experiments 24 mmol/liter $HCO_3^-$ was added, and the solution was gassed with 5% $CO_2$ (pH 7.35). This solution contained no HEPES buffer, and NaCl was reduced to maintain an osmolality of about 285 mosm/kg $H_2O$.

## Simultaneous $K^+$ Conductance and $pH_i$ Measurements in Fused MDCK cells

Fused cells were penetrated with three microelectrodes (Fig. 1). One conventional microelectrode (filled with 1 mol/liter KCl) was used to inject negative current pulses ($1–5 \times 10^9$ A, 200 msec duration), another one was used to monitor the cell membrane potential ($V_m$) and a third one, a pH-sensitive liquid ion-exchange

**Table 1.** Aldosterone-induced dome formation in MDCK-cell monolayers

| | Control | Aldosterone (mol/liter) | | | |
|---|---|---|---|---|---|
| | | $10^{-8}$ | $10^{-7}$ | $10^{-6}$ | $10^{-7}$ + spiron. |
| Domes/cm$^2$ | 111 ± 67 | 281 ± 41[a] | 436 ± 43[a] | 578 ± 101[a] | 60 ± 15[b] |

MDCK-cell monolayers grown on glass were kept for 24 hr in MEM medium without serum in the absence (control) and presence of various concentrations of aldosterone. The competitive inhibitor spironolactone (spiron.) was applied at a concentration 250 times the hormone concentration, as suggested previously [8]. ± SEM, $n = 4$ (number of monolayers studied).

[a] Significantly different from control.

[b] Significantly different from the corresponding value ($10^{-7}$ mol/liter aldosterone).

microelectrode, was applied to measure $pH_i$ when the cell cytoplasm was gradually acidified. High impedance amplifiers were used to measure $V_m$ and $pH_i$. The constant current pulses were delivered by a current injecting device (Frankenberger electrometer, Germering, FRG.)

Intracellular pH was measured with single-barreled pH-sensitive liquid ion-exchange microelectrodes [1]. $pH_i$ was calculated from the equation

$$pH_i = pH_o - (V_m^H - V_m)/S.$$

The $pH_o$ is the extracellular pH, $V_m^H$ and $V_m$ are the cell membrane $H^+$ electrochemical and electrical potential differences, respectively; the symbol $S$ is the electrode slope, measured in calibration solutions mimicking the cytosolic composition. The cell input resistance (i.e., the reverse of the conductance) was calculated according to Ohm's law

$$R_m = \Delta V_m/I$$

where $\Delta V_m$ is the cell membrane potential deflection in response to the injected current pulse ($I$). Then, $R_m$ was related to the cell surface ($F$)

$$R_m(\Omega cm^2) = R_m(\Omega) \cdot F(cm^2).$$

Since the fused cells were approximately spherical, $F$ was calculated as the surface of a sphere ($F = 4\ \pi r^2$) where $r$ (measured optically) is the radius of the sphere.

### Statistics

Data are presented as mean values ± SEM. Significance of difference was tested by either paired or unpaired student $t$ test if applicable. Significantly different is $P < 0.05$.

## Results

### Aldosterone-Induced Dome Formation and Transepithelial $H^+$ Transport

Domes represent local regions of fluid accumulation between the cell monolayer and the culture dish [20]. It was recently shown that aldosterone can indeed induce dome formation in MDCK cells [14]. We performed a dose-response curve and applied the aldosterone-antagonist spironolactone at a concentration 250 times the concentrations of aldosterone. This should lead to the complete occupation of the mineralocorticoid-specific cytoplasmic receptors [8]. Table 1 shows the results. In the absence of serum, dome formation can be enhanced by aldosterone in a dose-dependent manner. Spironolactone can inhibit aldosterone-induced dome formation, indicating that hormone binding to cytoplasmic receptors is stereospecific. For our experiments we have chosen the supra-maximal dose of $10^{-6}$ mol/liter aldosterone to trigger the cytoplasmic signal chain as fast as possible. In another series of experiments we studied static-head pH in the dome fluid while the monolayer was constantly superfused with HEPES-buffered $CO_2/HCO_3^-$-free Ringer's solution (pH 7.4). MDCK monolayers (with domes) were preincubated 24 hr prior to experiments in medium lacking serum (hormone-depleted cells). Thus, neither aldosterone nor growth factors were present over this time period. Under these conditions dome pH is close to the pH of the superfusate (Table 2). However, within 120 min of aldosterone treatment ($10^{-6}$ mol/liter) the dome fluid alkalinizes by 0.42 ± 0.04. This indicates that aldosterone stimulates transepithelial $H^+$ transport. From these experiments we derived that MDCK cells could be a suitable model for studying mineralocorticoid-induced $H^+$ transport and its possible relation to the cell membrane $K^+$ conductance.

### Relationship between $pH_i$ and $G_m^K$

Since we wanted to study the whole cell $K^+$ conductance, $Cl^-$ ions were largely removed from the perfusate and substituted by impermeant anions ($gluconate^-$). Furthermore, $Na^+$ ions were substituted by $K^+$ ions to increase cell membrane $K^+$ con-

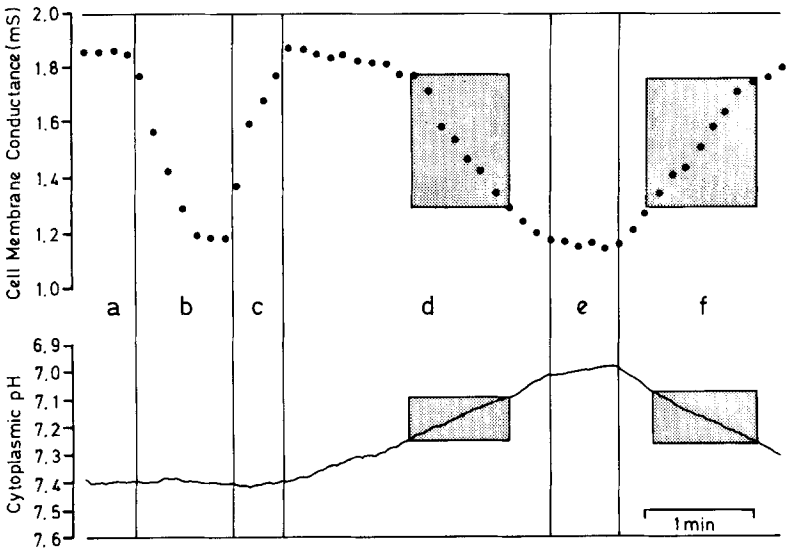


**Fig. 2.** Simultaneous $pH_i$ and cell membrane conductance ($G_m$) measurements in a fused MDCK cell, bathed in $K^+$ gluconate$^-$ Ringer solution. (*a*) The giant cell is superfused with a solution of pH 8. (*b*) 10 mmol/liter $Ba^{2+}$ is added. (*c*) $Ba^{2+}$ is removed. (*d*) The bath solution is acidified to pH 6. The shaded areas indicate corresponding $pH_i$ and $G_m$ changes. (*e*) $Ba^{2+}$ is added at acidic $pH_i$; there is no further reduction of $G_m$. (*f*) $Ba^{2+}$ and $H^+$ ions are removed (superfusion with $Ba^{2+}$-free solution, pH 8.0); $pH_i$ and $G_m$ recover to the initial values

**Table 2.** Aldosterone induces transepithelial $H^+$ transport measured in domes of MDCK-cell monolayers

| Dome | Aldosterone depleted | ΔpH | Aldosterone supplemented |
|---|---|---|---|
| 1 | 7.30 | 0.60 | 7.90 |
| 2 | 7.20 | 0.65 | 7.85 |
| 3 | 7.25 | 0.45 | 7.70 |
| 4 | 7.40 | 0.35 | 7.75 |
| 5 | 7.55 | 0.25 | 7.80 |
| 6 | 7.30 | 0.40 | 7.70 |
| 7 | 7.35 | 0.45 | 7.80 |
| 8 | 7.45 | 0.30 | 7.75 |
| 9 | 7.35 | 0.35 | 7.70 |
| 9 | 7.35 ± 0.04 | 0.42 ± 0.04[a] | 7.76 ± 0.02 |
| *n* | mean ± SEM | mean ± SEM | mean ± SEM |

Steady-state pH in the dome (i.e., fluid enclosed between the basolateral cell membrane and the glass surface) was measured at static-head conditions while the apical surface of the monolayer was superfused with HEPES-buffered $CO_2/HCO_3^-$-free Ringer's solution (pH 7.4). Monolayers were studied 24 hr after removal of fetal calf serum from the culture medium (depleted) and then 120 min after the application of $10^{-6}$ mol/liter aldosterone (supplemented). [a]$\Delta = P < 0.0001$.

ductance and thus to magnify the pH-induced conductance changes. Figure 2 represents a typical experiment in which $G_m$ and $pH_i$ were simultaneously recorded. In order to cover a wide pH range, the fused cell was initially exposed to an extracellular pH of 8. Under these conditions $pH_i$ is about 7.4 and total cell membrane conductance is 1.8 mS (Fig. 2*a*). Since the major cation in these experiments is $K^+$, total $G_m$ closely reflects the cell membrane $K^+$ conductance. Addition of 10 mmol/liter $Ba^{2+}$ to the superfusate decreases $G_m$ while cytoplasmic pH remains constant (Fig. 2*b*). Removal of $Ba^{2+}$ reverses the conductance change (Fig. 2*c*). Acidifying extracellular pH to 6.0 (Fig. 2*d*) leads to a gradual acidification of the cell cytoplasm. Note that in the narrow intracellular pH range of about 7.25 and 7.15 there is a dramatic shift in $G_m$ (shaded areas). Addition of $Ba^{2+}$ (Fig. 2*e*) at acidic $pH_i$ does not further change $G_m$, indicating that the target-structure (the $K^+$ channel) is obviously identical for $H^+$ and $Ba^{2+}$ ions. Simultaneous removal of $Ba^{2+}$ and $H^+$ ions leads to a shift of cytoplasmic pH and $G_m$ back to the initial values (Fig. 2*f*). From such experiments the relationship between $pH_i$ and $G_m^K$ can be determined. Figure 3 demonstrates that $G_m^K$ is highly sensitive to $H^+$ ions in a rather narrow range of $pH_i$. The sigmoidal shape of the curve indicates that there is a positive cooperative interaction between the $K^+$ channel proteins and the $H^+$ ions. 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. This can be derived from the slope (i.e., Hill coefficient) of the nonlinear regression between $pH_i$ and the corresponding $G_m^K$ values. A Hill coefficient of approximately 4.4 is calculated. The $IC_{50}$ value indicates that $G_m^K$ is half-maximally inhibited at $pH_i$ of 7.18 ± 0.04. The potential physiological relevance of this experimental value will be disclosed when compared with the $pH_i$ measurements of hormone-deprived and hormone-supplemented cells.

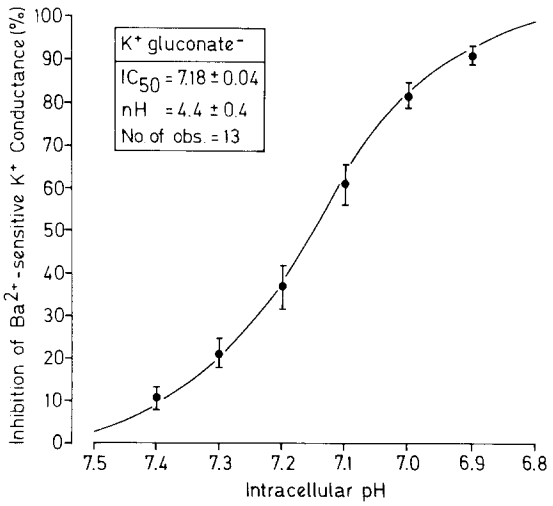


**Fig. 3.** Relationship between $K^+$ conductance and $pH_i$ in fused MDCK cells

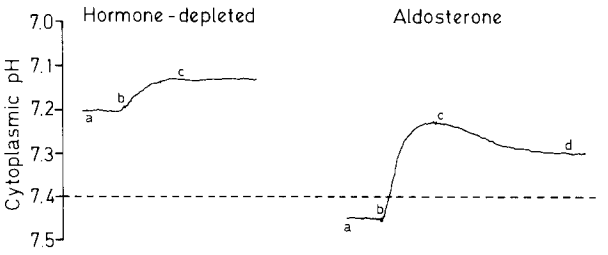


**Fig. 4.** Two tracings of cytoplasmic pH obtained in a fused MDCK cell before (left) and 120 min after (right) addition of $10^{-6}$ mol/liter aldosterone to the bath solution. (*a*) steady-state $pH_i$ in HEPES-buffered Ringer's solution; (*b*) addition of the $CO_2/HCO_3^-$ buffer system (at constant pH of 7.4); (*c*) peak acidic $pH_i$ (left and right) and steady-state $pH_i$ (left); (*d*) right: steady-state $pH_i$ bathed in the $CO_2/HCO_3^-$ buffered solution

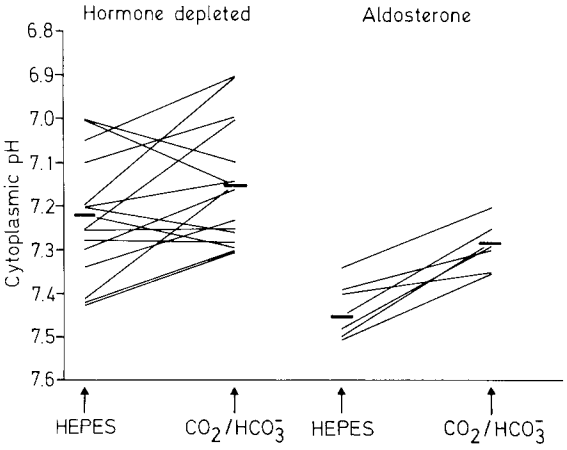


**Fig. 5.** Cytoplasmic pH measurements at steady-state conditions in fused MDCK cells before and 120 min after (right) addition of $10^{-6}$ mol/liter aldosterone to the bath perfusate. Cells were exposed either to HEPES-buffered or $CO_2/HCO_3^-$-buffered perfusates (pH in both solutions, 7.4)

### $pH_i$ in Hormone-Depleted and Hormone-Supplemented Cells

One of the key questions is whether aldosterone can lead to sustained cytoplasmic alkalinization in MDCK cells as found in amphibian tubule cells. In order to increase the sensitivity of the MDCK cells to aldosterone, cells were hormone deprived 48 hr prior to fusion. Over this time period cells were grown in MEM-culture medium but in the absence of fetal calf serum and aldosterone. Figure 4 shows two original tracings of $pH_i$ in hormone-depleted and aldosterone-exposed cells (i.e., 120 min after the exposure to $10^{-6}$ mol/liter aldosterone at 24°C). Some remarkable observations can be made: segment *a* of the tracing indicates that in the absence of $CO_2/HCO_3^-$, $pH_i$ is alkaline after hormone treatment compared to $pH_i$ of the hormone-depleted cell. Exposure to $CO_2/HCO_3^-$ (segment *b*) reduces $pH_i$ in both conditions; however, cytoplasmic acidification (segment *c*) is significantly more profound in the aldosterone-treated cell. The results of this series of experiments are summarized in Fig. 5. Note that $pH_i$ after hormone treatment is increased, but aldosterone-induced cytoplasmic alkalinization is blunted in the presence of $CO_2/HCO_3^-$. This gives evidence for a potent $HCO_3^-$ extrusion mechanism operative in the hormone-stimulated cell. Such a mechanism might also be present in the plasma membrane of hormone-depleted cells but not turned on due to the comparatively acidic intracellular pH.

## Discussion

We thought that MDCK cells could be a suitable model for investigating transport processes underlying aldosterone action for the following reasons: The cells possess cytoplasmic receptors for mineralocorticoids [2, 21], respond to aldosterone with an increased formation of domes (ref. 14 and Table 1), a parameter most likely related to transepithelial ion transport and maintain hormone-stimulated transepithelial pH gradients (Table 2), indicating that $H^+$ transport is affected by the steroid. Therefore, we fused MDCK cells to giant cells and tested for the hormone-dependent interaction between $pH_i$ and the cell membrane $K^+$ conductance.

A significant pH sensitivity of the cell membrane $K^+$ conductance is found in a variety of epithelial and nonepithelial cells. Studies in frog skeletal muscle [5], in squid giant axon [33], in rat and mouse pancreatic $\beta$-cells [7, 28], in rat lens [3], in retinal pigment epithelium [11], and in *Amphiuma* diluting segment [11] indicate that $K^+$ channels are blocked by internal $H^+$ ions independent of whether the external bath is made more acidic or basic. This suggests the existence of titratable groups at the inner (cytoplasmic) face of the $K^+$ channel proteins. In squid axon membrane [33] and frog skeletal muscle [5] the steepness of the $pH_i$-induced block of the $K^+$ conductance suggests that more than one $H^+$ ion is required to bind to the blocking site. A steep sigmoidal relationship between cytoplasmic pH and overall $K^+$ conductance was recently found also in frog diluting segment [24]. The Hill coefficient of the $pH_i$-$G_m^K$ relationship obtained in fused MDCK cells with the value of $4.4 \pm 0.4$ indicates multiple $H^+$ binding sites at a single $K^+$ channel protein or at separate proteins within a microdomain. Interestingly, half-maximal inhibition of $K^+$ conductance in fused MDCK cells is achieved at considerably lower $pH_i$ values compared to the amphibian preparation [24]. This is not unexpected since physiological pH values in amphibians are usually well above those of mammals. Thus, if cytoplasmic $H^+$ ions are likely to play a crucial role in intracellular signal transduction and transcellular transport, $pH_i$ must be at values that can affect the function of transport proteins significantly. Although at first sight different, the comparison of $pH_i$ measurements in the presence and absence of $CO_2/HCO_3^-$ reveals that the $pH_i$-induced $K^+$ conductance changes due to aldosterone treatment may be quantitatively similar. In the absence of $CO_2/HCO_3^-$, aldosterone-induced alkalinization is significantly larger than the one in the presence of $CO_2/HCO_3^-$. However, the $pH_i$ of fused cells in $CO_2/HCO_3^-$ solutions is lower compared to the corresponding $pH_i$ of cells in $CO_2/HCO_3^-$-free solutions. Thus, steady-state $pH_i$ in hormone-depleted cells (in the presence of $CO_2/HCO_3^-$) is close to the $IC_{50}$ value of the $K^+$ conductance and thus most sensitive to $pH_i$ changes. Although aldosterone-induced cytoplasmic alkalinization is more marked in the absence of $CO_2/HCO_3^-$, $K^+$ conductance under these circumstances becomes increasingly insensitive at high $pH_i$ values.

How can we explain the observation that the aldosterone-induced $pH_i$ increase is significantly larger in absence than in presence of $CO_2/HCO_3^-$? It is likely that $pH_i$ regulation is defective in the absence of $HCO_3^-$. Recent studies in MDCK cells indicate the presence of $Na^+/H^+$ and $Cl^-/HCO_3^-$ exchange and underline their crucial roles in $pH_i$ regulation [17, 30]. Whereas $Na^+/H^+$ exchange is activated by cytoplasmic acidification, $Cl^-/HCO_3^-$ exchange becomes active when the cell interior is threatened by alkalosis. Aldosterone activates the $H^+$ extrusion mechanism obviously by modifying its "set point" determined by $pH_i$. In the absence of $HCO_3^-$ the cell lacks an effective $HCO_3^-$ extrusion mechanism and thus fails to defend itself against increasing cytoplasmic alkalinization. With the addition of the $CO_2/HCO_3^-$ buffer system the cell regains its full $pH_i$-regulatory function and "resets" $pH_i$ at a less alkaline value due to the activation of $Cl^-/HCO_3^-$ exchange. Yet unpublished observations from our laboratory indicate that DIDS, a stilbene derivative known to block $Cl^-/HCO_3^-$ exchange inhibits the $HCO_3^-$ extrusion mechanism more marked in the aldosterone-treated MDCK cells as compared to the nonexposed cells. This is consistent with the view that $Cl^-/HCO_3^-$ exchange is more active at hormone-stimulated conditions. Such conclusions are suggested also by studies in sodium-depleted rats with secondary hyperaldosteronism [9]. Therefore, sodium depletion stimulates electroneutral chloride-dependent sodium absorption, most likely as a result of aldosterone-induced stimulation of $Na^+/H^+$ and $Cl^-/HCO_3^-$ exchange.

In amphibian oocytes the steroid hormone progesterone induces meiotic maturation characterized by cytoplasmic alkalinization and complex changes of the ion permeabilities of the plasma membrane [4, 18, 27]. Thus, the immature oocyte reinitiates meiotic division and reorganizes its plasma membrane and cytoplasm to become a mature oocyte, which will finally fertilize and develop normally. Since the rate of protein synthesis in the maturing oocyte increases it was speculated that $pH_i$ could play a role in oocyte maturation by modifying protein synthesis [18]. Assuming that steroid action is similar in various target cells—binding to cytosolic receptors, gene activation, induction of protein synthesis—it is tempting to infer from the experiments in oocytes that aldosterone may act in renal distal tubules to stimulate transport in a similar way. Recent patch-clamp experiments in amphibian distal tubules indicate that aldosterone leads to the insertion of $K^+$ channel proteins, a process triggered by cytoplasmic alkalinization and successfully prevented by cell acidification [32]. Our study does not allow us to distinguish whether cytoplasmic alkalinization has increased the single $K^+$ channel conductance or has led to the rapid insertion of preformed $K^+$ channel proteins in the plasma membrane.

Our studies link intracellular pH regulation to transepithelial transport. From the literature we

know that mineralocorticoids induce protein synthesis in MDCK cells [35] after binding to cytosolic receptors [2]. At least two membrane proteins with molecular weights of 35 and 14 kD are induced by the hormone [6]. Studies in the intact monolayer indicate that transepithelial transport is increased after hormone treatment [4]. Yet unpublished observations from our laboratory indicate that both $pH_i$ regulating transport systems (i.e., $Na^+/H^+$ and $Cl^-/HCO_3^-$ exchangers) are located in the apical cell membrane. Aldosterone-induced activation of $Na^+/H^+$ exchange increases $pH_i$, which in turn stimulates $Cl^-/HCO_3^-$ exchange [17]. $Cl^-$ ions recycle across the apical cell membrane [15]. The basolateral $K^+$ conductance [16] is increased by cytoplasmic alkalinization. The specific permeability properties of the apical and basolateral cell membranes determine a transepithelial potential difference (apical side negative; [23]), which finally drives paracellular $K^+$ secretion. However, this transport model is still hypothetical and has yet to be tested.

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