Nongenomic regulation by aldosterone of the epithelial NHE3 Na\(^+\)/H\(^+\) exchanger

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Good, David W., Thampi George, and Bruns A. Watts III. Nongenomic regulation by aldosterone of the epithelial NHE3 Na\(^+\)/H\(^+\) exchanger. Am J Physiol Cell Physiol 290: C757–C763, 2006.—The relevance of nongenomic pathways to regulation of epithelial function by aldosterone is poorly understood. Recently, we demonstrated that aldosterone inhibits transepithelial HCO\(_3^-\) absorption in the renal medullary thick ascending limb (MTAL) through a nongenomic pathway. Here, we examined the transport mechanism(s) responsible for this regulation, focusing on Na\(^+\)/H\(^+\) exchangers (NHE). In the MTAL, apical NHE3 mediates H\(^+\) secretion necessary for HCO\(_3^-\) absorption; basolateral NHE1 influences HCO\(_3^-\) absorption by regulating apical NHE3 activity. In microperfused rat MTALs, the addition of 1 nM aldosterone rapidly decreased HCO\(_3^-\) absorption by 30%. This inhibition was unaffected by three maneuvers that inhibit basolateral Na\(^+\)/H\(^+\) exchange and was preserved in MTALs from NHE1 knockout mice, ruling out the involvement of NHE1. In contrast, exposure to aldosterone for 15 min caused a 30% decrease in apical Na\(^+\)/H\(^+\) exchange activity over the intracellular pH range from 6.5 to 7.7, due to a decrease in \(V_{\text{max}}\). Inhibition of HCO\(_3^-\) absorption by aldosterone was not affected by 0.1 mM lumen Zn\(^{2+}\) or 1 mM lumen DIDS, arguing against the involvement of an apical H\(^+\) conductance or apical K\(^+\)-HCO\(_3^-\) cotransport. These results demonstrate that aldosterone inhibits HCO\(_3^-\) absorption in the MTAL through inhibition of apical NHE3, and identify NHE3 as a target for nongenomic regulation by aldosterone. Aldosterone may influence a broad range of epithelial transport functions important for extracellular fluid volume and acid-base homeostasis through direct regulation of this exchanger.

Aldosterone plays an important role in the maintenance of Na\(^+\), K\(^+\), and acid-base balance through its effects on renal electrolyte excretion. This regulation involves the stimulation of Na\(^+\) absorption, K\(^+\) secretion, and H\(^+\) secretion by aldosterone in segments of the collecting duct through the induction of gene transcription and the synthesis of new proteins (2, 23, 29). In addition to these classic genomic actions, aldosterone has been demonstrated to induce rapid cellular effects that are not dependent on transcription or translation and not mediated through the classic intracellular mineralocorticoid receptor (7, 20). Nongenomic effects of aldosterone have been demonstrated in a variety of epithelial and nonepithelial cells, and include effects on signal transduction pathways and ion transporters, such as the epithelial Na\(^+\) channel, Na\(^+\)/H\(^+\) exchange, and the vacuolar H\(^+\)-ATPase (7, 9, 20, 24, 40, 41, 43). However, whether nongenomic pathways are relevant to the regulation of transepithelial transport by aldosterone in renal tubules has not been determined. Identification of the sites and mechanisms of nongenomic regulation by aldosterone in the kidney is important because it provides a pathway for aldosterone-specific regulation of electrolyte transport or other cell functions in nephron segments that do not express the classic mineralocorticoid receptor.

Na\(^+\)/H\(^+\) exchangers (NHE) are integral membrane proteins that mediate the electroneutral exchange of Na\(^+\) for H\(^+\). At least eight mammalian isoforms of Na\(^+\)/H\(^+\) exchange have been identified (NHE1/SLC9A1-NHE8/SLC9A8), which differ in their membrane and tissue distribution, regulatory properties, and physiological functions (26, 32). NHE1 is expressed ubiquitously in the plasma membrane of nonpolarized cells and the basolateral membrane of epithelial cells, where it mediates basic functions such as regulation of cell volume and cell pH and influences cell processes such as growth and adhesion (26, 27, 32). Other isoforms (e.g., NHE2–5) exhibit a more restricted tissue distribution. In particular, NHE3 is expressed selectively in the apical membrane of epithelial cells in the kidney and gastrointestinal tract, where it mediates transepithelial absorption of NaCl and/or NaHCO\(_3^-\) (2–4, 12, 26, 30, 32, 34, 42). Regulation of NHE3 activity in the kidney is important for the control of acid-base balance, Na\(^+\) balance, extracellular fluid volume, and blood pressure. Recently, Na\(^+\)/H\(^+\) exchange has been identified as a target for nongenomic regulation by aldosterone. Aldosterone rapidly increases Na\(^+\)/H\(^+\) exchange activity in a variety of nonpolarized and polarized cells, including endothelial and vascular smooth muscle cells, mononuclear leukocytes, distal colon, and renal epithelial cell lines (7, 20), effects that are attributed to stimulation of the ubiquitously expressed NHE1 (8, 20, 24, 40). However, the physiological significance of Na\(^+\)/H\(^+\) exchange activation for aldosterone-induced regulation of cellular or epithelial function remains to be defined. In addition, it has not been determined whether aldosterone can regulate NHE isoforms other than NHE1 through nongenomic pathways.

The medullary thick ascending limb (MTAL) of the mammalian kidney participates in acid-base regulation by reabsorbing most of the filtered HCO\(_3^-\) not reabsorbed by the proximal tubule (2, 12). Absorption of HCO\(_3^-\) by the MTAL depends on H\(^+\) secretion mediated by the apical membrane NHE3 Na\(^+\)/H\(^+\) exchange (3, 4, 12, 18, 38). The MTAL also expresses basolateral NHE1, and we have identified a novel role for this exchange in HCO\(_3^-\) absorption. Inhibition of NHE1 with amiloride or nerve growth factor, or by NHE1 knockout, results secondarily in inhibition of apical NHE3, thereby decreasing HCO\(_3^-\) absorption (16, 19, 35, 36). Recently, (17) demonstrated that aldosterone inhibits HCO\(_3^-\) absorption in the MTAL via a nongenomic pathway; however, the mechanism of this regulation has not been defined. The finding that NHE1 is a target for nongenomic regulation by aldosterone in other cell systems raises the possibility that aldosterone could inhibit...
HCO$_3^-$ absorption in the MTAL through a primary effect on this exchanger. Such a mechanism would require that aldosterone inhibit NHE1 in the MTAL (16, 19, 35, 36), an effect opposite to the stimulation of Na$^+/H^+$ exchange by aldosterone in other cell types.

The purpose of the present study was to determine the transport mechanism responsible for nongenomic inhibition of HCO$_3^-$ absorption by aldosterone in the MTAL. The results show that the inhibition of HCO$_3^-$ absorption does not involve basolateral NHE1 but instead is mediated by a direct action of aldosterone to inhibit apical NHE3. These studies identify NHE3 as a target for nongenomic regulation by aldosterone and show that aldosterone can control the absorptive function of epithelial tissues through regulation of this exchanger.

METHODS

Tubule perfusion. MTALs from male Sprague-Dawley rats (60–90 g; Taconic, Germantown, NY) were isolated and perfused in vitro as previously described (17, 35, 37). In brief, the tubules were dissected from the inner stripe of the outer medulla, transferred to a bath chamber on the stage of an inverted microscope, and mounted on micropipettes for perfusion at 37°C. The composition of the perfusion and bath solutions for individual protocols is given below. Solutions were prepared as described (16, 17, 35). In one series of experiments (Fig. 3), HCO$_3^-$ absorption was studied in isolated, perfused MTALs from wild-type (129Sv/J/Black Swiss) and NHE1 null mutant (NHE1$^{-/-}$) mice between 6 and 8 wk old, as previously described (19). There was no difference in results using wild-type littermates or age-matched wild-type mice from concurrent litters as NHE1$^{-/-}$ controls.

Measurement of net HCO$_3^-$ absorption. To measure transepithelial HCO$_3^-$ absorption, tubules were perfused and bathed in control solution that contained (in mM) 146 Na$^+$, 4 K$^+$, 122 Cl$^-$, 25 HCO$_3^-$, 2.0 Ca$^{2+}$, 1.5 Mg$^{2+}$, 2.0 phosphate, 1.2 SO$_4^{2-}$, 1.0 citrate, 2.0 lactate, and 5.5 glucose (equilibrated with 95% O$_2$, 5% CO$_2$; pH 7.45, at 37°C). Bath solutions also contained 0.2 g/100 ml fatty acid-free bovine albumin. In one series of HCO$_3^-$ transport experiments (Fig. 2B), Na$^+$ in the bath solution was replaced completely with N-methyl-d-glucammonium (NMDG$^+$) (16, 35). Experimental agents were added to the bath or lumen solutions, as described in RESULTS.

The protocol for study of transepithelial HCO$_3^-$ absorption was as described (11, 17). The tubules were equilibrated for 20–30 min at 37°C in the initial perfusion and bath solutions and the luminal flow rate was adjusted to 1.5–2.0 nl/min$^{-1}$mm$^{-1}$. One to three 10-min tubule fluid samples were then collected for each period (initial, experimental, and recovery). The tubules were allowed to reequilibrate for 5–10 min after aldosterone was added to or removed from the bath solution. The absolute rate of HCO$_3^-$ absorption (J$_{HCO_3^-}$, pmol/min$^{-1}$mm$^{-1}$) was calculated from the luminal flow rate and the difference between total CO$_2$ concentrations measured in perfused and collected fluids (11). When repeat measurements were made at the beginning and end of an experiment (initial and recovery periods), the values were averaged. Single tubule values are presented in the figures. Mean values ± SE (n = number of tubules) are presented in the text.

Measurement of intracellular pH and apical Na$^+/H^+$ exchange activity. Intracellular pH (pHi) was measured in isolated, perfused MTALs by use of the pH-sensitive dye BCECF and a computer-controlled spectrofluorometer (CM-X, SPEX Industries) coupled to the perfusion apparatus, as previously described (35, 37). The tubules were perfused in the same manner used for HCO$_3^-$ transport experiments except that the lumen and bath solutions were delivered via rapid flow systems that permit complete exchange of the solutions in <2 s (37). Two previously described methods were used to determine apical membrane Na$^+/H^+$ exchange activity (16, 35, 37). In the first method, MTAL were perfused and bathed in the control solution used for HCO$_3^-$ transport experiments and apical Na$^+/H^+$ exchange activity was determined by measuring the initial rate of cell acidification in response to rapid addition of 50 μM ethylisopropyl amiloride (EIPA) to the tubule lumen (16, 35). The basis for this approach is that before EIPA addition, H$^+$ extrusion via the apical Na$^+/H^+$ exchanger balances background acid loading to maintain pH$_i$ constant. When the apical exchanger is inhibited, the initial rate of pH$_i$ decrease estimates the steady-state rate of apical NHE that balances background acid loading (16, 35). In the second method, tubules were perfused and bathed in Na$^+$-free, HEPES-buffered solution that contained (in mM) 145 NMDG$^+$, 4 K$^+$, 147 Cl$^-$, 2.0 Ca$^{2+}$, 1.5 Mg$^{2+}$, 1.0 phosphate, 1.0 SO$_4^{2-}$, 1.0 citrate, 2.0 lactate, 5.5 glucose, and 5 HEPES (equilibrated with 100% O$_2$; titrated to pH 7.4). The lumen solution also contained furosemide to block Na$^+-K^+$-2Cl$^-$ cotransport activity. Apical Na$^+/H^+$ exchange activity was determined by measurement of the initial rate of pH$_i$ increase after the addition of 145 mM Na$^+$ to the lumen solution (Na$^+$ replaced NMDG$^+$) (37, 38). Interception of pH$_i$ recovery at various points along the recovery curve permits determination of the apical Na$^+/H^+$ exchange rate over a broad range of pH$_i$ values (6.4 to 7.7), with appropriate corrections for a variable background acid loading rate (37). The Na$^+$-dependent pH$_i$ recovery was inhibited ∼90% by lumen EIPA (50 μM) under all experimental conditions. In both methods, net H$^+$ flux rates (J$_{Na^+/H^+}$, pmol/min$^{-1}$mm$^{-1}$) were calculated as (dpH/dt) × β × V, where dpH/dt (pH U/min) is the initial slope of the record of pH$_i$ vs. time, β is the appropriate intracellular buffering power (mM·pH$^{-1}$), and V is cell volume per unit tubule length (nl/mm), measured as previously described (16, 35, 37, 38). Use of the two methods in combination permits apical Na$^+/H^+$ exchange to be assessed under the same conditions used to measure HCO$_3^-$ absorption along with direct study of the transport properties of the exchanger, independent of other transporters.

Statistical analysis. Results are presented as means ± SE. Differences between means were evaluated using Student’s t-test for paired or unpaired data, as appropriate. P < 0.05 was considered statistically significant.

RESULTS

Aldosterone inhibits HCO$_3^-$ absorption. The addition of 1 nM aldosterone to the bath decreased HCO$_3^-$ absorption by 30%, from 14.7 ± 0.3 to 10.3 ± 0.3 pmol·min$^{-1}$·mm$^{-1}$ (P < 0.001; Fig. 1). This inhibition is complete within 15 min and is reversible. These data confirm previous results demonstrating that aldosterone inhibits HCO$_3^-$ absorption in the MTAL via a nongenomic pathway (17).

Inhibition by aldosterone does not involve basolateral Na$^+/H^+$ exchange. Inhibiting the basolateral NHE1 Na$^+/H^+$ exchanger decreases HCO$_3^-$ absorption in the MTAL (16, 19, 35, 36). Because NHE1 is a target for nongenomic regulation by aldosterone in other systems (8, 9, 20, 24, 40), we considered whether basolateral Na$^+/H^+$ exchange was involved in mediating aldosterone-induced inhibition of HCO$_3^-$ absorption in the MTAL. The effect of aldosterone was tested in the presence of 1 μM bath EIPA, in the absence of bath Na$^+$, and in the presence of 0.7 mM nerve growth factor, three conditions that inhibit basolateral Na$^+/H^+$ exchange (16, 19, 35, 36). The results in Fig. 2 show that aldosterone decreased HCO$_3^-$ absorption by 30–40% under all three conditions. Thus the inhibition of HCO$_3^-$ absorption by aldosterone is not mediated through basolateral Na$^+/H^+$ exchange.
To examine further the possible role of NHE1, MTALs from wild-type and NHE1 knockout (NHE1−/−) mice were perfused in vitro using the same methods as described for rat MTALs (19). The addition of 1 nM aldosterone to the bath decreased HCO₃⁻ absorption by 28% (from 14.8 ± 0.6 to 10.7 ± 0.6 pmol·mm⁻¹·min⁻¹; \( P = 0.005 \)) in MTALs from wild-type mice and this inhibition was preserved in MTALs lacking NHE1 (Fig. 3, A and B). These results demonstrate that aldosterone inhibits HCO₃⁻ absorption by a similar amount in mouse and rat MTALs, and confirm that this inhibition does not involve basolateral NHE1.

Aldosterone inhibits apical Na⁺/H⁺ exchange. Two experimental approaches were used to determine whether aldosterone decreases HCO₃⁻ absorption in the MTAL through inhibition of apical Na⁺/H⁺ exchange, which is mediated by NHE3. In the first approach, MTALs were perfused and bathed with the control solution used for transepithelial HCO₃⁻ transport experiments (146 mM Na⁺; 25 mM HCO₃⁻; pH 7.4) and apical Na⁺/H⁺ exchange activity was determined by measurement of the initial rate of cell acidification in response to lumen EIPA addition (see METHODS). A typical experiment is shown in Fig. 4A. The tubule was bathed with 1 nM aldosterone for 15 min before the addition of EIPA. The addition of 50 μM EIPA to the tubule lumen caused a rapid decrease in pHᵢ due to inhibition of apical Na⁺/H⁺ exchange. Removal of EIPA caused pHᵢ to recover to its initial value (≈7.30). Aldosterone was then removed from the bath solution and the pHᵢ response to lumen EIPA was repeated. Aldosterone decreased the initial rate of cell acidification (broken lines), indicating a decrease in the steady-state rate of apical Na⁺/H⁺ exchange (16, 35). Similar results were obtained when the order of the experimental conditions was reversed. For a total of six experiments,

Fig. 1. Aldosterone (Aldo) inhibits HCO₃⁻ absorption in the medullary thick ascending limb (MTAL). Rat MTALs were isolated and perfused in vitro. The absolute rate of HCO₃⁻ absorption (\( J_{\text{HCO}_3^-} \)) was measured in control solution, and then 1 nM Aldo was added to and removed from the bath solution. Data points are average values for single tubules. Lines connect paired measurements made in the same tubule. \( P \) value is for paired \( t \)-test. Mean values are given in RESULTS.

Fig. 2. Inhibition of HCO₃⁻ absorption by Aldo does not involve basolateral Na⁺/H⁺ exchange. Rat MTALs were studied with 1 mM ethylisopropylamiloride (EIPA) in the bath (A), in a Na⁺-free bath (B), or with 0.7 nM nerve growth factor (NGF) in the bath (C), conditions that inhibit basolateral Na⁺/H⁺ exchange (16, 19, 35, 36). Aldo (1 nM) was then added to and removed from the bath solution. In B, Na⁺ in the bath was replaced completely with N-methyl-D-glucammonium (NMDG); the lumen was perfused with control solution containing 146 mM Na⁺ (16, 35). \( J_{\text{HCO}_3^-} \), data points, lines, and \( P \) values are the same as in Fig. 1.

Fig. 3. Aldosterone inhibits HCO₃⁻ absorption in MTAL from NHE1 knockout (NHE1−/−) mice. MTALs from wild-type (A) and NHE1−/− (B) mice were studied in control solution and then 1 nM Aldo was added to the bath. \( J_{\text{HCO}_3^-} \), data points, lines, and \( P \) values are the same as in Fig. 1.
Fig. 4. Aldosterone decreases apical Na\(^+\)/H\(^+\) exchange activity in the MTAL. Rat MTALs were perfused and bathed in control (cont) solution (146 mM Na\(^+\), 25 mM HCO\(_3\)\(^-\)) and apical Na\(^+\)/H\(^+\) exchange activity was determined from the initial rate of intracellular pH (pHi) decrease after lumen addition of 50 μM EIPA (see METHODS). A: trace shows response of pHi, to lumen EIPA addition, first in the presence of bath Aldo (1 nM for 15 min) and then after Aldo removal. Aldo decreased the initial rate of cell acidification (broken lines), indicating a decrease in the steady-state rate of apical Na\(^+\)/H\(^+\) exchange (16, 35). B: effect of Aldo on apical Na\(^+\)/H\(^+\) exchange activity (JNa\(^+\)/H\(^+\)) in 6 experiments similar to A. Data points are values for single tubules. Lines and P values are the same as in Fig. 1. Mean values are given in the text.

Fig. 5. Aldosterone inhibits apical Na\(^+\)/H\(^+\) exchange activity. In this approach, MTALs were perfused and bathed in Na\(^+\)-free solution and apical Na\(^+\)/H\(^+\) exchange activity was determined by measurement of initial rates of cell pH increase in response to lumen Na\(^+\) addition (see METHODS). Tubules were maintained in the absence or presence of 1 nM bath aldosterone for 15–20 min before lumen Na\(^+\) addition. As shown in Fig. 5A and B, aldosterone decreased apical Na\(^+\)/H\(^+\) exchange activity over the range of pHi values studied (6.4 to 7.7). The exchanger exhibited a sigmoidal dependence on pHi, as described previously (35, 37, 38). Kinetic analysis showed that the inhibition of apical Na\(^+\)/H\(^+\) exchange by aldosterone was due to a 30% decrease in V\(_{\text{max}}\) (Fig. 5A). The apparent affinity for intracellular H\(^+\) was similar in the absence and presence of aldosterone. These results demonstrate that aldosterone decreases apical Na\(^+\)/H\(^+\) exchange activity in the MTAL by decreasing its maximal rate of exchange.

Inhibition by aldosterone does not involve an H\(^+\) conductance. In a renal epithelial cell line (Madin-Darby canine kidney), nongenomic stimulation of Na\(^+\)/H\(^+\) exchange by aldosterone required aldosterone-induced activation of a Zn\(^{2+}\)-sensitive H\(^+\) conductance (9, 10). To determine whether an H\(^+\) conductance was involved in regulation of Na\(^+\)/H\(^+\) exchange by aldosterone in the MTAL, HCO\(_3\)\(^-\) absorption was examined in tubules perfused with 0.1 mM Zn\(^{2+}\), a concentration that blocked aldosterone-induced H\(^+\) conductance and Na\(^+\)/H\(^+\) exchange stimulation in Madin-Darby canine kidney cells (9, 10). In tubules studied with Zn\(^{2+}\) in the tubule lumen, the addition of 1 nM aldosterone to the bath decreased HCO\(_3\)\(^-\) absorption by 30%, from 15.2 ± 1.1 to 10.7 ± 1.5 pmol·min\(^{-1}\)·mm\(^{-1}\) (P < 0.025) (Fig. 6). Increasing the lumen Zn\(^{2+}\) concentration to 0.5 mM or adding Zn\(^{2+}\) to the bath also did not affect the inhibition of HCO\(_3\)\(^-\) absorption (not shown). Thus we found no evidence that an H\(^+\) conductance is involved in mediating inhibition of apical Na\(^+\)/H\(^+\) exchange by aldosterone in the MTAL.

Inhibition of HCO\(_3\)\(^-\) absorption by aldosterone is not dependent on apical K\(^+\)-dependent HCO\(_3\)\(^-\) transport. Recently we identified an apical K\(^+\)-dependent HCO\(_3\)\(^-\) transport pathway in the rat MTAL (39). This pathway is inhibited by DIDS and opposes net HCO\(_3\)\(^-\) absorption by mediating the coupled transport of K\(^+\) and HCO\(_3\)\(^-\) from cell to tubule lumen. To determine whether this pathway is involved in inhibition of HCO\(_3\)\(^-\) absorption by aldosterone, we examined the effect of aldosterone in the presence of luminal DIDS. In MTAL studied with 1 mM DIDS in the tubule lumen, the addition of 1 nM aldosterone to the bath decreased HCO\(_3\)\(^-\) absorption from 22.7 ± 2.3 to 17.7 ± 2.0 pmol·min\(^{-1}\)·mm\(^{-1}\) (P < 0.005) (Fig.
DISCUSSION

In addition to its classic regulatory actions mediated through changes in gene expression, aldosterone influences a variety of cellular processes through rapid, nongenomic pathways. The cellular targets for nongenomic regulation include several ion transport proteins; however, the relevance of nongenomic pathways to aldosterone-induced regulation of epithelial function is poorly understood. Recently, we demonstrated that aldosterone inhibits HCO$_3^-$ absorption in the MTAL via a pathway that is rapid, not dependent on transcription or translation, and not mediated through the classic mineralocorticoid receptor (17). This regulation is observed with physiological concentrations of aldosterone and is highly selective for aldosterone over cortisol and other corticosteroids (17). These findings establish a role for nongenomic pathways in mediating aldosterone-induced regulation of transepithelial transport in mammalian renal tubules. In the present study, we demonstrate that aldosterone decreases HCO$_3^-$ absorption in the MTAL through inhibition of the apical membrane NHE3 Na$^+$/H$^+$ exchanger. These results identify NHE3 as a previously unrecognized target for nongenomic regulation by aldosterone, and show that aldosterone can influence epithelial acid secretion and Na$^+$ absorption through direct regulation of this exchanger.

Na$^+$/H$^+$ exchange has been identified previously as a target for nongenomic regulation by aldosterone. Aldosterone rapidly increases Na$^+$/H$^+$ exchange activity in a variety of epithelial and nonpolarized cells, effects that are attributed to stimulation of the ubiquitously expressed NHE1 isoform (7–9, 20, 24, 40). The physiological significance of this stimulation is unclear, but may involve pH$_i$ regulatory processes important for subsequent nuclear events and protein processing, or aldosterone-induced changes in pH$_i$ that regulate the activity of K$^+$ channels important for transepithelial Na$^+$ absorption or K$^+$ secretion (9, 10, 20, 21, 24, 40). In the MTAL, basolateral NHE1 is an important determinant of the rate of transepithelial HCO$_3^-$ absorption (16, 19, 35, 36). However, two series of experiments demonstrate that NHE1 is not involved in mediating the aldosterone-induced inhibition of HCO$_3^-$ absorption: 1) inhibiting basolateral Na$^+$/H$^+$ exchange by three different maneuvers does not prevent inhibition of HCO$_3^-$ absorption (Fig. 2); 2) inhibition by aldosterone is preserved in MTALs from NHE1 knockout mice (Fig. 3). We found instead that the nongenomic inhibition of HCO$_3^-$ absorption by aldosterone is mediated through primary inhibition of apical NHE3. The inhibition of NHE3 is opposite to the rapid stimulation of Na$^+$/H$^+$ exchange by aldosterone in other cells (7, 9, 20, 24, 40). Whether this reflects differential regulation by aldosterone of the NHE3 and NHE1 isoforms, or whether inhibition by aldosterone may be a cell-type specific property of NHE3 in the MTAL, remains to be determined.

Several lines of evidence indicate that apical NHE3 is responsible for H$^+$ secretion and HCO$_3^-$ absorption in the rat MTAL, including immunocytochemical localization (3, 4), functional studies (2, 12, 18), inhibitor sensitivity (2, 16, 35, 38), acute regulatory properties (13, 14, 36–38), and chronic adaptation, in which increased transport activity parallels increased NHE3 expression (18, 22). In the present study, the inhibition of NHE3 by aldosterone is sufficient to account for the observed decrease in HCO$_3^-$ absorption and is observed both when tubules are studied in physiological solutions (Fig. 4) and when apical exchange activity is studied independently of other transporters (Fig. 5). The latter experiment shows that

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1 The percent decrease in HCO$_3^-$ absorption induced by aldosterone in the presence of DIDS (22 ± 2%; Fig. 7) is slightly less than that observed in the absence of DIDS (30 ± 2%; Fig. 1) (P < 0.05). This small difference in fractional inhibition is attributable to the higher rate of basal HCO$_3^-$ absorption in the presence of luminal DIDS (39); however, our results do not exclude a small role for apical K$^+$/HCO$_3^-$ cotransport in the aldosterone-induced regulation (see DISCUSSION).
the aldosterone-induced signaling pathway is coupled directly to inhibition of NHE3. Aldosterone inhibited NHE3 via a decrease in \( V_{\text{max}} \), indicating that the hormone decreases the intrinsic activity of individual transporters, the number of functional transporters in the apical membrane, or both. Regulation of NHE3 in other cell systems involves its redistribution between the cell membrane and intracellular vesicles (4, 25, 26, 42). However, whether trafficking is involved in regulation of NHE3 in the MTAL, and whether this process may be influenced by aldosterone, remains to be determined. The effect of aldosterone to inhibit NHE3 through \( V_{\text{max}} \) differs from the kinetic mechanism reported for aldosterone-induced stimulation of NHE1, which involves an increase in \( H^+ \) affinity (8). A wide variety of signaling mechanisms have been reported to play a role in the rapid stimulation of NHE1 by aldosterone, including protein kinase C, ERK, intracellular \([Ca^{2+}]\), pertussis toxin-sensitive G proteins, and arachidonic acid pathways (7–10, 20, 24, 40). The possible role of these and other pathways in mediating inhibition of NHE3 by aldosterone in the MTAL is currently under investigation.

In addition to NHE3, the MTAL contains an apical membrane \( K^+ \)-dependent \( HCO_3^- \) transport mechanism (39). This mechanism, presumably a \( K^+\text{-}HCO_3^- \) cotransporter, opposes net \( HCO_3^- \) absorption by mediating the coupled transfer of \( K^+ \) and \( HCO_3^- \) from cell to tubule lumen (39). Aldosterone decreased \( HCO_3^- \) absorption by a similar amount when this transporter was inhibited with luminal DIDS. Thus, the aldosterone-induced regulation is not dependent on apical \( K^+\text{-}HCO_3^- \) cotransport, consistent with the finding that decreased NHE3 activity can account for the decrease in \( HCO_3^- \) absorption. Although the fractional inhibition by aldosterone was slightly lower in the presence of luminal DIDS, we are hesitant to ascribe any physiological significance to this because it is the result of an increase in basol \( HCO_3^- \) absorption rate observed when apical \( K^+\text{-}HCO_3^- \) cotransport is inhibited (39). Nevertheless, our results do not conclusively rule out that an effect of aldosterone to increase apical \( K^+ \)-dependent \( HCO_3^- \) transport may contribute minimally to the decrease in \( HCO_3^- \) absorption. Direct studies of apical \( K^+ \)-dependent \( HCO_3^- \) transport will be required to address this. Although inhibition of NHE3 is the primary mechanism responsible for the aldosterone-induced inhibition of \( HCO_3^- \) absorption, our results do not rule out the possibility that aldosterone also may influence \( HCO_3^- \) efflux via basolateral \( Cl^-\text{-}HCO_3^- \) exchange (1, 5) or other basolateral \( H^+\text{-}HCO_3^- \) transport pathways (5, 6, 31).

In keeping with its central role in the maintenance of sodium, volume, and acid-base balance, NHE3 is regulated acutely by several important factors, including catecholamines, angiotensin II, endothelin, dopamine, and growth factors (2, 25, 32, 42). The results of the present study identify aldosterone as an additional factor involved in the acute regulation of NHE3. The effect of aldosterone to inhibit NHE3 and \( HCO_3^- \) absorption in the MTAL may play a role in enabling the kidney to maintain acid-base balance during changes in \( Na^+ \) balance. As discussed previously (12, 15), activation of the renin-angiotensin-aldosterone system by \( Na^+ \) and volume depletion results in multiple transport effects that tend to increase renal acid excretion and promote metabolic alkalosis, including stimulation of \( HCO_3^- \) absorption by angiotensin II in proximal and distal tubules, and stimulation of \( H^+ \) secretion by aldosterone in segments of the collecting duct (2, 12, 15, 33). We suggest that these effects are opposed by the direct action of aldosterone to inhibit NHE3 and \( HCO_3^- \) absorption in the MTAL, thereby minimizing changes in acid excretion and preserving acid-base balance while permitting regulated changes in \( Na^+ \) excretion that control plasma volume and blood pressure. Nongenomic regulation by aldosterone also could contribute to changes in NHE3 activity in pathophysiological conditions. For example, \( K^+ \) depletion increases renal net acid excretion and promotes metabolic alkalosis in part through stimulation of NHE3. Our results suggest that increased NHE3 activity in the MTAL in response to decreased aldosterone levels could contribute to the increased renal \( HCO_3^- \) absorptive capacity induced by hypokalemia (2).

Our finding that aldosterone regulates NHE3 directly through nongenomic mechanisms may be relevant to other epithelia. NHE3 is the major absorptive \( Na^+\text{/}H^+ \) exchanger in both kidney and intestine. In addition to mediating \( HCO_3^- \) absorption in the MTAL, NHE3 is primarily responsible for mediating \( NaCl \), water, and/or \( NaHCO_3 \) absorption by the renal proximal tubule and segments of the intestinal tract (2, 25, 29, 30, 32, 34, 42). The results of the present study raise the possibility that these transport processes could be regulated directly by aldosterone through nongenomic pathways. Thus in addition to its regulation of \( Na^+ \) absorption through classic genomic targets, such as the epithelial \( Na^+ \) channel and \( Na^+\text{-}K^+\text{-}ATPase \), aldosterone may influence sodium, volume, and acid-base balance through nongenomic regulation of NHE3. Whether aldosterone can influence NHE3 directly in epithelia other than the MTAL remains to be determined. However, aldosterone infusion was shown recently to induce a rapid increase in urinary \( Na^+ \) excretion in the rat (28), an effect that could be mediated by aldosterone-induced inhibition of NHE3. Our finding that the regulation of NHE3 by aldosterone is nongenomic raises the possibility that aldosterone could specifically control NHE3-mediated absorptive functions in epithelia such as the proximal tubule that do not express the mineralocorticoid receptor.

In summary, our results identify the epithelial NHE3 \( Na^+\text{/}H^+ \) exchanger as a target for direct regulation by aldosterone. Aldosterone inhibits NHE3 in the MTAL via a nongenomic pathway, which results in a decrease in transepithelial \( H^+ \) secretion and \( HCO_3^- \) absorption. These findings suggest that aldosterone could influence a broad range of epithelial transport functions important for extracellular fluid volume and acid-base homeostasis through nongenomic regulation of NHE3.

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