Aldosterone stimulates intestinal Na\(^{+}\) absorption in rats by increasing NHE3 expression of the proximal colon

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Aldosterone stimulates intestinal Na\(^{+}\) absorption in rats by increasing NHE3 expression of the proximal colon. Am. J. Physiol. 274 (Cell. Physiol. 43): C586–C594, 1998.—Na\(^{+}\) retention by the colon in response to salt deprivation is mediated in part by the resulting secondary hyperaldosteronism. We show that experimental hyperaldosteronism, to levels seen with salt deprivation, causes an increase in the selective expression and activity of NHE3, an apically located isoform of the Na\(^{+}\)/H\(^{+}\) exchange family that functions in transepithelial Na\(^{+}\) absorption. The effect of aldosterone on NHE3 expression is tissue specific, occurring in intestine and not in kidney. Within the intestine, these effects are regional, being observed only in proximal colon, and different in distribution from that observed with glucocorticoids, where the predominant effect occurs in ileum. Although glucocorticoids are well known to exert many effects via regulation of transcript levels, the present study demonstrates that aldosterone stimulates intestinal Na\(^{+}\) absorption by increasing cellular NHE3 expression, a response that is tissue and region specific.

MATERIALS AND METHODS

Animal Model of Hyperaldosteronism

The subcutaneous insertion of osmotic minipumps was performed under halothane anesthesia. Animals were treated with either aldosterone at a dose of 70 µg·100 g\(^{-1}\)·day\(^{-1}\) or vehicle (50% dimethyl sulfoxide) for 3 days. Animals were given free access to chow and water during the infusion. After the 3-day infusion, animals were killed, plasma aldosterone levels were measured by radioimmunoassay (Diagnostic Products), and tissues were harvested. Control rats had a plasma aldosterone level of 12.7 ± 3.1 ng/dl compared with 715.1 ± 54.7 ng/dl for aldosterone-treated rats. The latter value is slightly lower than that previously reported for Na\(^{+}\)-depleted rats (929.3 ± 100.6) at 72 h (18).

Northern Blot Hybridization of NHE Isoforms

Intestinal scrapings were obtained of proximal jejunum, distal ileum, proximal colon (5 cm of colon harvested immediately distal to the ileocecal valve), and control (5 cm of colon harvested immediately above the peritoneal reflection), and kidney. Tissues were homogenized in guanidium isothiocyanate buffer and ultracentrifuged (32,000 g) over a 5.7 M cesium chloride cushion. Total RNA was purified with an oligo(dT) cellulose column. RNA was obtained from poly(A)\(^{+}\) enrichment and micromolar programs of poly(A)\(^{+}\) RNA were run on a 1% denaturing agarose gel. After transfer to Hybond-N membrane by capillary action, the membrane was cross-linked by ultraviolet irradiation. Probes for NHE1, NHE3 (full-length cDNAs), and NHE2 (Pst I–Pst I) were labeled with \(\alpha\)-\(^{32}\)PdCTP (NEN, 3,000 mCi/mmol) by random-prime labeling (15). After purification of radiolabeled probes by Nick column (Pharmacia), \(10^{6}\) counts·min\(^{-1}\) (cpm)·ml\(^{-1}\) of hybridization buffer was used to hybridize the membranes.

Immunoblotting

Intestinal scrapings were obtained from rat jejunal, ileal, and proximal colon from which membranes were iso-
lated by differential centrifugation (11). Sixty micrograms of membrane were loaded on a 7.5% sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis. After transfer to a polyvinylidene fluoride membrane (Immobilon, Millipore, Medford, MA), Western blotting was performed. After 5% milk in phosphate-buffered saline (PBS)-0.2% Nonidet P-40 was used for blocking, the membranes were incubated with a polyclonal glutathione S-transferase fusion protein antibody incorporating amino acids 528–648 of the NHE3 sequence at a dilution of 1:500 (7). Control blotting with preimmune serum was performed on intestinal samples for comparative purposes in all instances. Secondary antibody [donkey anti-rabbit immunoglobulin-horseradish peroxidase conjugated F(ab)2 fragment; Amersham] was incubated at 1:5,000, and detection was performed using enhanced chemiluminescence (Amersham).

In Situ Hybridization With NHE Isoforms

Tissue preparation. Freshly frozen tissue mounted in OCT freezing compound (Baxter) was sectioned (10 µm) and adhered to gelatin-coated (EM Sciences) and poly-L-lysine-coated slides (Sigma). Mounted tissue was fixed with ice-cold 4% paraformaldehyde in 1× PBS for 2 min and then in ice-cold 70% ethanol for 10 min. Slides were rehydrated in a series of 15-s incubations in 70, 50, and 30% ethanol and 80% water. Slides were equilibrated in 0.1 M triethanolamine (pH 8; Sigma) for 15 s, followed by a 10-min incubation in 0.1 M tetraethylammonium and 0.25% acetic anhydride to block partial positive charges on the slide. After a 15-s rinse in water and a graded dehydration of the slides in 60 and 80% ethanol (15 s each) and two changes of 100% ethanol (2 min), the tissue sections were hybridized.

Probe preparation. Sense and antisense strand riboprobes were generated from linearized gel-purified pGEM4Z plasmid (Promega) containing the NHE sequences. Transcription with T7 (sense) and SP6 (antisense) RNA polymerase (2,000 Ci/mmol [3P]UTP; NEN) was performed for 2 h at 37°C. The mixture was placed on ice for 10 min. The Golgi, endoplasmic reticulum, and basolateral membrane fractions were pelleted by centrifuging at 8,000 g for 10 min. The supernatant was centrifuged at 42,000 g for 45 min. The pellet containing brush-border membrane vesicles (BBMV) was resuspended in uptake buffer of pH 6.1 (10 mM NaCl, 10 mM 2-N-morpholinol)ethanesulfonic acid (pH 6.1), 3 mM EDTA, and protease inhibitors. Protein concentrations were measured by the Bradford method. A pH gradient was developed to enhance apical Na+/H+ exchange activity by placing 5 µl of saline solution (pH 6.1) in 45 µl of a 22Na-containing uptake buffer at a pH of 7.4 (1 mM 22NaCl at 55,000 cpm/mmol, 80 mM mannitol, 10 mM N-2-hydroxylpipеразине-N′-2-ethanesulfonic acid (HEPES), pH 7.4, 3 mM EDTA, and protease inhibitors in the presence and absence of 100 µM ethylisopropylamiloride).

22Na+ Uptake in Brush-Border Membrane Vesicles in Control and Aldosterone-Treated Rats

Light intestinal scrapings were placed in 20 ml of homogenization buffer. After homogenization, the solution was centrifuged at 500 g for 5 min to remove nuclei and cell debris and 10,000 g for 10 min to remove mitochondria. Magnesium sulfate was added to the supernatant to a final concentration of 15 mM. The mixture was placed on ice for 15 min. The supernatant was centrifuged at 42,000 g for 45 min. The pellet containing brush-border membrane vesicles (BBMV) was resuspended in uptake buffer of pH 6.1 (10 mM NaCl, 10 mM 2-N-morpholinol)ethanesulfonic acid (pH 6.1), 3 mM EDTA, and protease inhibitors. After filters were rinsed with stop solution, they were

Unidirectional apical membrane Na+ uptake (lumen to cell) was determined in flux buffer (composition in mmol/l: 130 choline chloride, 5 KCl, 1 MgCl2, 2 CaCl2, 1 ouabain, 15 HEPES, pH 7.4, 20 NaCl with 1 µCi/ml 22NaCl and 1 µCi/ml [3H]mannitol, to mark extracellular space) for 10 min (the linear uptake phase for Na+ influx in these cells is longer than 10 min) in the presence and absence of 500 µM dimethylamiloride (DMA; to inhibit both NHE2 and NHE3) or 30 µM of HOE-694 (to inhibit NHE2 only). Uptake was terminated by four washes in cold wash buffer [140 NaCl, 5 KCl, 15 HEPES (pH 7.4), and 1 sodium phosphate]. Cells were extracted in 1% SDS, and radioactivity was measured by liquid scintillation spectroscopy.

Comparisons of 22Na+ uptake in BBMV from control and aldosterone-treated rats measured in the presence and absence of 500 µM DMA and 30 µM HOE-694 (14). This dose of HOE-694 inhibits 90% of NHE2 PS-120 transfectants but only ~10% of NHE3 activity (data not shown). The reaction was stopped at 0 and 10 s with 4 ml of stop solution (100 mM mannitol) and placed on a 0.65-µm cell filter (DAWP, Millipore). After filters were rinsed with stop solution, they were
RESULTS

Regional Effects on Expression of NHE3 in Models of Mineralocorticoid Excess and Deficiency

Figure 1 demonstrates a representative Northern blot hybridization with an isoform-specific NHE3 probe of proximal and distal colon mucosal scrapings in control and aldosterone-treated rats. Average densitometric measurements of NHE3 transcripts in three experiments normalized for glyceraldehyde-3-phosphate dehydrogenase expression were obtained. A three-fold increased expression of NHE3 in proximal colon was observed in aldosterone-treated compared with control rats. No significant change in NHE3 expression was observed in distal colonic mucosa (Fig. 1). These results correlate with previously reported effects of aldosterone on colonic transport as measured by transmucosal, voltage-clamp flux techniques. An increase in electroneutral NaCl absorption was observed in proximal colon with 3- and 7-day aldosterone infusions with no significant increase in short-circuit current (34). In contrast, a decrease in electroneutral NaCl absorption was observed in rat distal colon, with a concomitant increase in electrogenic Na⁺ absorption at 7 days (33).

Aldosterone’s effects on intestinal transport were limited to the colon. Although we have previously noted an increase in NHE3 expression with dexamethasone administration in rat ileum (12), we did not observe any change in NHE3 expression with aldosterone infusion in the ileum, jejunum, or kidney (Fig. 2).

Similar changes in NHE3 protein expression were observed when Western blotting with a polyclonal antibody specific to the NHE3 isoform was used, with an increase in protein observed in proximal colon comparable to that observed at the mRNA level. No increase in NHE3 protein expression was observed in the distal colon (Fig. 3).

Effect of Aldosterone on NHE Expression Is Specific to the NHE3 Isoform

NHE3 is located on the apical domain of intestinal epithelial cells and participates in the vectorial transport of Na⁺ from the lumen, resulting in salt absorption (4, 7, 19). Three other NHE isoforms have been cloned and have varying tissue distributions and functions. NHE1 is ubiquitously expressed and maintains intracellular pH and volume (28). NHE1 is located on the basolateral domain of polarized epithelia (32). Like NHE3, NHE2 is located on the apical domain and functions to transport Na⁺ across epithelia (19). Although previously reported to be present in the intestine, isoform-specific probes differentiating NHE2 from NHE4 have shown that NHE4 is not present in rat intestine (8).

Aldosterone infusion does not affect mRNA expression of NHE1 or NHE2 (Fig. 4). It has been previously...
reported that dexamethasone, a synthetic glucocorticoid, increases NHE3 expression (12, 37) but similarly did not affect expression of NHE1 or NHE2. Together, these findings indicate that NHE3 functions as the corticosteroid-responsive NHE isoform that can respond to conditions of stress or Na\(^+\) depletion by increasing mRNA and protein expression.

Aldosterone Affects Apical Na\(^+\)/H\(^+\) Exchange in Proximal Colon: Measurement of \(^{22}\)Na\(^+\) Uptake in BBMV

Amiloride-sensitive \(^{22}\)Na\(^+\) uptake of BBMV is a measure of apical Na\(^+\)/H\(^+\) activity. Isolation of ileum, proximal colon, and kidney BBMV was accomplished by MgSO\(_4\) precipitation and differential centrifugation. Confirmation of comparable membrane enrichments in control and aldosterone-treated rats was obtained by measurement of alkaline phosphatase and Na\(^+\)-K\(^+\)-ATPase activity (Table 1). The proximal colon enrichments (10) were comparable to previously reported enrichments obtained by differential centrifugation (4- to 5-fold compared with 3-fold), and the ileal enrichments (29) were lower (6-fold compared with 10- to 11-fold) than reported previously. \(^{22}\)Na\(^+\) uptake in the proximal colon was 694.6 ± 37.7 pmol \(^{22}\)Na\(^+\) · 10\(^{-2}\) s\(^{-1}\) · mg

In colons of control rats, NHE3 mRNA is present primarily in mature, surface absorptive cells, with some expression found in the uppermost crypt regions by in situ hybridization (Fig. 5). Alterations in expression of NHE3 in rat proximal colon mucosal scrapings could result from an increase in the mRNA abundance in cells normally expressing it, or alternatively, from recruitment of less mature cells deeper within the intestinal crypts as has been demonstrated for hexose transporters in response to insulinopenic states (11). Hyperaldosteronism does not affect the distribution of NHE3 mRNA within intestinal crypts (Fig. 5); therefore, increased levels of NHE3 result from either transcriptional activation of NHE3 by aldosterone or transcript stabilization solely in cells normally expressing this isoform.

Measurement of NHE3 mRNA levels in whole kidney homogenates could obscure the region-specific changes, which are shown by in situ hybridization. However, control and aldosterone-treated rats demonstrated no difference in the distribution of NHE3 mRNA in the kidney (Fig. 6, A and B). In both groups, NHE3 mRNA is very abundant in the outer medulla (specifically, the thick ascending limb of Henle, Fig. 6, C and D), with no message observed in inner medulla. Intermediate levels of NHE3 mRNA were observed in kidney cortex.

Aldosterone Does Not Affect the Distribution of NHE3 mRNA Along the Crypt-Villus Axis in Rat Colon or in Kidney

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protein$^{-1}$ in aldosterone-treated rats compared with $214 \pm 10.9$ pmol $^{22}$Na$^+\cdot 10^3$ s$^{-1}\cdot$mg protein$^{-1}$ in controls ($P < 0.001$) (Fig. 7). No significant changes in activity were observed in BBMV prepared from ileum. Although NHE2 also localizes to the apical domain (19), the amount of increase in activity with aldosterone paralleled the amount of increase observed in NHE3 expression.

Validation of HOE Compounds as Specific Inhibitors of NHE2 in Polarized Cell Systems Expressing Both NHE2 and NHE3

To investigate the contribution of NHE2 and NHE3 to increased BBMV flux in aldosterone-treated rats, we utilized a cell culture system to validate the use of the novel amiloride analogs from Hoechst (HOE-694) (14) as specific inhibitors of NHE2 in cell systems containing both isoforms. In PS-120 transfectants (NHE-deficient fibroblast cell line, gift from J. Pouyssegur, University de Nice), HOE-694 significantly inhibited NHE1 at very low concentrations (<1 µM), NHE2 at slightly higher concentrations (50% inhibitory concentration of ~5 µM), and NHE3 poorly (<10% inhibition at 30 µM). Because of the differential sensitivities of NHE2 (HOE-694 sensitive) and NHE3 (HOE-694 insensitive) to HOE-694 inhibition, this compound can be used to discriminate between the activities of the two isoforms.

The C2 cell line provides an ideal model system to assess brush-border exchanger activity and inhibition (as measured by unidirectional apical Na$^+$ uptake, lumen to cell) in a polarized cell system. Apical Na$^+$/H$^+$ exchange in endogenous C2 cells is mediated by NHE2, as evidenced by comparable inhibition of apical Na$^+$ influx with DMA (isoform-nonspecific inhibition) and HOE-694 (30 µM, specific to NHE2) (Fig. 8) as well as Western analysis (data not shown). In NHE3 transfectants, total (DMA-inhibitable) Na$^+$ influx is greatly increased (reflecting increased NHE3 apical activity), but HOE-sensitive Na$^+$ influx is unchanged from the endogenous state. This suggests an absence of NHE2 and NHE3 interaction with respect to apical Na$^+$ influx and validates the use of HOE compounds to discriminate between NHE2 and NHE3 activity in polarized cell systems containing both isoforms.

Increased $^{22}$Na$^+$ Influx in Aldosterone-Treated BBMV Results From Increased NHE3 and Not NHE2 Activity

To confirm that increased $^{22}$Na$^+$ uptake in aldosterone-treated rats results from increased NHE3 and not NHE2 activity, DMA-dependent BBMV $^{22}$Na$^+$ uptake was compared with HOE-694-inhibitable uptake (Fig. 9). There was no difference in control and aldosterone-treated rats in HOE-694-dependent $^{22}$Na$^+$ uptake, indicating that aldosterone does not increase NHE2 activity. Therefore, the observed increase in DMA-dependent uptake in aldosterone-treated rats can be attributed to increased expression and function of NHE3.

Fig. 5. In situ hybridization of control and aldosterone-treated rat proximal colon with the NHE3 antisense probe. Freshly frozen tissues from control (A and C) and aldosterone-treated (B and D) rats were fixed to coated slides and hybridized with an NHE3-specific antisense probe. After they were washed, slides were stained with Richardson's stain and viewed (~20 magnification) with darkfield (A and B) to visualize silver grains and brightfield (C and D) to visualize morphology. SA, surface absorptive cells; C, crypt regions.
22Na\(^+\) uptake with aldosterone reflects increased activity of NHE3, in parallel with increased NHE3 expression. Furthermore, comparison of HOE-694-inhibitable (310 ± 64 pmol·10\(^{-2}\)·mg protein\(^{-1}\)) and DMA-inhibitable (1,005 ± 32 pmol·10\(^{-2}\)·mg protein\(^{-1}\)) 22Na\(^+\) uptake in control proximal colon indicates the relative contribution of NHE2 to total apical Na\(^+\)/H\(^+\) exchange in this region.

### DISCUSSION

It has long been known that aldosterone is a major physiological regulator of Na\(^+\) homeostasis, particularly by the kidney and intestine. However, the molecular mechanisms behind its effects are incompletely defined. In this study, we induced levels of hyperaldosteronism comparable to those observed with salt deprivation (17, 23) to observe effects on NHE expression and activity. We demonstrate that the increase in Na\(^+\) absorption in rat proximal colon observed in conditions of hyperaldosteronism results from an increase in mRNA transcript abundance and functional apical Na\(^+\)/H\(^+\) exchange. The observed increase in 22Na\(^+\) uptake with aldosterone administration does not result from increased NHE2 activity, as demonstrated by studies using HOE-694, a guanidine derivative that specifically inhibits NHE2 (14) in cell systems expressing both NHE2 and NHE3. Furthermore, results from DMA- and HOE-inhibitable Na\(^+\) uptake experiments in C2 transfectants do not demonstrate any interactive effect of NHE2 and NHE3 on apical Na\(^+\) uptake.

### Table 1. Enzyme enrichments of brush-border membranes

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<th>Alkaline Phosphatase</th>
<th>Na(^+)-K(^+)-ATPase</th>
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<tr>
<td></td>
<td>CH BBMV Enrichment</td>
<td>CH BBMV Enrichment</td>
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<tr>
<td><strong>Ileum</strong></td>
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<tr>
<td>Control</td>
<td>0.153 ± 0.008</td>
<td>0.084 ± 0.011</td>
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<tr>
<td>Aldosterone</td>
<td>0.156 ± 0.008</td>
<td>0.167 ± 0.015</td>
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<tr>
<td><strong>Proximal colon</strong></td>
<td></td>
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<tr>
<td>Control</td>
<td>0.049 ± 0.008</td>
<td>0.041 ± 0.004</td>
</tr>
<tr>
<td>Aldosterone</td>
<td>0.049 ± 0.004</td>
<td>0.091 ± 0.012</td>
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Values are means ± SE; n = 4 rats/group. Marker enzyme activities were measured in whole cell homogenates (CH) and in brush-border membrane vesicles (BBMV) and are given in µmol·min\(^{-1}\)·mg protein\(^{-1}\). Multiples of enrichment were calculated as specific activity in brush-border membranes divided by activity in homogenate. Alkaline phosphatase was measured as a marker for brush border and Na\(^+\)-K\(^+\)-ATPase as a basolateral marker.
Although increased Na$^+$ absorption has also been observed with aldosterone in distal colon, this increase results from electrogenic Na$^+$ absorption through Na$^+$ channels and not through increased Na$^+/H^+$ exchange (33). Similarly, despite the presence by Northern blot of mineralocorticoid receptors in the small intestine (17), no effects on NHE expression with aldosterone were observed. This correlates with the previously described effects of aldosterone as being confined to the large intestine (27). In contrast, it has been previously shown that NHE3 expression and activity are increased in rat ileum in response to glucocorticoid administration (12, 37).

The regional differences in glucocorticoid and mineralocorticoid effects on NHE3 expression in intestine illustrate the unique transport domains present along the intestinal longitudinal axis. The present results in proximal colon correlate with the previously observed increase in transmembrane electroneutral Na$^+$/Cl$^-$ flux observed with aldosterone administration (34). We observed no change in mRNA or protein levels of NHE3 in distal colon, whereas Turnamian and Binder (33) demonstrated an increase in electroneutral NaCl absorption after 1 day and an inhibition of NaCl absorption after 7 days. This difference could result from time course requirements, as the inhibition of electroneutral NaCl absorption in distal colon with aldosterone administration requires at least 48–72 h to be observed, with maximal inhibition observed with 12 days of aldosterone administration (18). Alternatively, the difference may result from the greater length of distal colon used in the present study (5.0 cm) compared with the previous study (2.3 cm) (33). Because clear gradient differences in expression and regulation of transporters through the colon exist, the inclusion of the additional length of colon may well be the major underlying factor.

Although many glucocorticoid effects are mediated through regulation of mRNA levels, it is less well established that mineralocorticoid effects are similarly mediated (21). Although the mineralocorticoid receptor bears significant structural homology to the glucocorticoid receptor and has been shown to activate transcription in vitro (2, 26), the in vivo significance of transcriptional regulation by aldosterone is largely unknown. Thus our findings that transcript levels of NHE3 increase with aldosterone administration represents the first in vivo demonstration that aldosterone acts via regulation of transcript levels. It has previously been
demonstrated that aldosterone increases mRNA levels of Na\(^+\)-K\(^+\)-ATPase in cultured cells (35). Na\(^+\)-K\(^+\)-ATPase is involved in the vectorial transport of Na\(^+\) across polarized epithelia, specifically, by transporting Na\(^+\) across the basolateral domain. However, the critical mechanism of aldosterone control of Na\(^+\) transport in rat proximal colon is likely mediated by regulation of NHE3 expression, as transport across the apical domain is the rate-limiting step in transepithelial Na\(^+\) transport (5).

The mineralocorticoid receptor bears a high degree of homology (94%) to the glucocorticoid receptor in the DNA binding domain, thus specificity of glucocorticoid vs. mineralocorticoid effects is unlikely to be mediated by disparate DNA binding elements (2). The ligand binding domain is 67% homologous, and overlap of mineralocorticoid and glucocorticoid effects is a well-recognized phenomenon (2). Various glucocorticoids are known to bind the mineralocorticoid receptor and thus produce mineralocorticoid effects. The converse, however, does not occur: aldosterone is not known to bind the glucocorticoid receptor (18). This is confirmed by our present finding that hyperaldosteronism does not increase NHE3 mRNA in ileum despite the previously reported fivefold increase in NHE3 expression with dexamethasone administration (12, 37).

The regional differences between mineralocorticoid and glucocorticoid effects may result from regional variation in receptor expression or activity. However, the mineralocorticoid receptor is present throughout the intestinal tract by Northern hybridization (17). We speculate that the observed differences result from regional variation in transcription factors acting in concert with the activated corticosteroid receptor. Because the NH\(_2\)-terminal domains of the mineralocorticoid and glucocorticoid receptors (which are known to bind and be regulated by other transactors) bear relatively little homology (47%) to each other (2), these additional transactors may well be different for the mineralocorticoid and glucocorticoid receptors. Also possible would be factors affecting mRNA stability in a region-specific manner, which has been demonstrated with glucocorticoids but not aldosterone (25).

We did not observe a difference in expression or activity of NHE3 in whole kidney homogenates nor alterations in parenchymal distribution by in situ hybridization. It is known that NHE3 localizes to the apical domain in kidney epithelia and functions in NaCl and NaHCO\(_3\) absorption, with highest expression in proximal tubule and thick ascending limb (1, 9). Apical Na\(^+\)/H\(^+\) exchange in distal tubule must be mediated by other isoforms (1). Given this distribution, it is not surprising that aldosterone does not affect NHE3 expression in the kidney, as mineralocorticoid effects are exerted primarily in distal tubule and collecting duct (21). We did not observe an increase in electroneutral Na\(^+\) absorption in kidney, suggesting that the increase in Na\(^+\) absorption with aldosterone is mediated through increased electrogenic Na\(^+\) absorption through Na\(^+\) channels. Alternatively, aldosterone-induced activation of a minority of Na\(^+\)/H\(^+\) exchangers could be obscured in measurements of whole kidney homogenates.

In summary, we have demonstrated that one mechanism of salt conservation occurring with hyperaldosteronism to levels observed with salt deprivation is a commensurate increase in the expression and activity of an apical Na\(^+\)/H\(^+\) exchanger, NHE3, in rat proximal colon. The regional effect is different from that observed with glucocorticoid treatment, in which the predominant effect is observed in ileum. Aldosterone does not affect the distribution of NHE3 mRNA in the proximal colon, where expression is limited to more differentiated enterocytes in surface absorptive and upper crypt regions in both control and aldosterone-treated rats. Although glucocorticoids are well known to exert many effects via regulation of transcript levels, the present study on NHE3 expression demonstrates that aldosterone stimulates intestinal Na\(^+\) absorption through similar, but regionally distinct, mechanisms.

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REFERENCES


