High sodium intake increases HCO$_3^-$ absorption in medullary thick ascending limb through adaptations in basolateral and apical Na$^+/H^+$ exchangers

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Good DW, George T, Watts BA 3rd. High sodium intake increases HCO$_3^-$ absorption in medullary thick ascending limb through adaptations in basolateral and apical Na$^+/H^+$ exchangers. Am J Physiol Renal Physiol 301: F334–F343, 2011. First published May 25, 2011; doi:10.1152/ajprenal.00106.2011.—A high sodium intake increases the capacity of the medullary thick ascending limb (MTAL) to absorb HCO$_3^-$; here, we examined the role of the apical NHE3 and basolateral NHE1 Na$^+/H^+$ exchangers in this adaptation. Rats on a high sodium intake increased HCO$_3^-$ absorption rate by 60%. The increased HCO$_3^-$ absorptive capacity was mediated by an increase in apical NHE3 activity. Inhibiting basolateral NHE1 with bath amiloride eliminated 60% of the adaptive increase in HCO$_3^-$ absorption. Thus the majority of the increase in NHE3 activity was dependent on NHE3. High sodium intake increased basolateral Na$^+/H^+$ exchange activity by 89% in association with an increase in NHE1 expression. High sodium intake increased apical Na$^+/H^+$ exchange activity by 30% under conditions in which basolateral Na$^+/H^+$ exchange was inhibited but did not change NHE3 abundance. These results suggest that high sodium intake increases HCO$_3^-$ absorptive capacity in the MTAL through 1) an adaptive increase in basolateral NHE1 activity that results secondarily in an increase in apical NHE3 activity; and 2) an adaptive increase in NHE3 activity, independent of NHE1 activity. These studies support a role for NHE3 in the long-term regulation of renal tubule function and suggest that the regulatory interaction whereby NHE1 enhances the activity of NHE3 in the MTAL plays a role in the chronic regulation of HCO$_3^-$ absorption. The adaptive increases in Na$^+/H^+$ exchange activity and HCO$_3^-$ absorption in the MTAL may play a role in enabling the kidneys to regulate acid-base balance during changes in sodium and volume balance.

NHE1; NHE3; acid-base balance; kidney; salt-sensitive hypertension

THE MEDULLARY THICK ASCENDING LIMB (MTAL) OF THE MAMMALIAN KIDNEY PLAYS A ROLE IN MAINTAINING ACID-BASE BALANCE BY REABSORBING MOST OF THE FILTERED HCO$_3^-$ NOT REABSORBED BY THE PROXIMAL TUBULE (2, 26). ABSORPTION OF HCO$_3^-$ BY THE MTAL IS REGULATED ACUTE BY A VARIETY OF PHYSIOLOGICAL FACTORS, INCLUDING ALDOSTERONE, ANGIOTENSIN II, VASOPRESSIN, AND CHANGES IN OSMOLALITY (24, 26, 28, 31, 33, 65, 68, 69). IN ADDITION TO THIS SHORT-TERM REGULATION, A NUMBER OF CONDITIONS INDUCE LONG-TERM ADAPTIVE CHANGES IN THE MTAL THAT ALTER ITS REABSORPTION CAPACITY. CHRONIC METABOLIC ACIDOSIS INCREASES THE ABILITY OF THE RAT MTAL TO ABSORB HCO$_3^-$, WHEREAS CHRONIC CHLORIDE-DEPLETION METABOLIC ALKALOSIS REDUCES HCO$_3^-$ ABSORPTIVE CAPACITY (25, 26). THESE ADAPTATIONS WOULD CONTRIBUTE TO CHANGES IN RENAL NET ACID EXCRETION THAT MAINTAIN SYSTEMIC ACID-BASE BALANCE DURING THESE ACID-BASE DISORDERS. WE HAVE FOUND THAT DIETARY SODIUM INTAKE ALSO IS AN IMPORTANT DETERMINANT OF THE HCO$_3^-$ ABSORPTIVE CAPACITY OF THE MTAL. IN PARTICULAR, A HIGH SODIUM INTAKE INCREASES THE ABILITY OF THE RAT MTAL TO ABSORB HCO$_3^-$ IN VITRO (25). THIS ADAPTATION CORRELATES DIRECTLY WITH INCREASED REABSORPTION OF HCO$_3^-$ BY THE LOOP OF HENLE OF RATS ON A HIGH SODIUM INTAKE IN VIVO (12). CONVERSELY, DIETARY SODIUM RESTRICTION REDUCES THE CAPACITY OF THE MTAL TO ABSORB HCO$_3^-$ (25). THE ADAPTIVE INCREASE IN HCO$_3^-$ ABSORPTION IN THE MTAL IN RESPONSE TO INCREASED SODIUM INTAKE MAY BE PHYSIOLOGICALLY SIGNIFICANT BECAUSE IT OFFSETS EFFECTS OF VOLUME EXPANSION THAT DECREASE H+ SECRETION AND HCO$_3^-$ ABSORPTION IN OTHER SEGMENTS OF THE NEPHRON, THEREBY ENABLING THE KIDNEYS TO MAINTAIN ACID-BASE BALANCE DURING CHANGES IN SODIUM AND VOLUME BALANCE (25, 26). THE CELLULAR MECHANISMS RESPONSIBLE FOR THE ADAPTIVE CHANGES IN MTAL HCO$_3^-$ ABSORPTION IN RESPONSE TO CHANGES IN SODIUM INTAKE HAVE NOT BEEN DEFINED.

ABSORPTION OF HCO$_3^-$ BY THE MTAL DEPENDS ON H+ SECRETION MEDIATED BY THE AMIPICAL MEMBRANE Na$^+/H^+$ EXCHANGER NHE3 (6, 11, 34, 69), AND REGULATION OF NHE3 PLAYS A PRIMARY ROLE IN BOTH THE ACUTE AND CHRONIC REGULATION OF HCO$_3^-$ ABSORPTION (5, 26, 31, 34, 44, 45, 68, 69). THE ADAPTIVE INCREASE IN MTAL HCO$_3^-$ ABSORPTION IN RESPONSE TO CHRONIC METABOLIC ACIDOSIS IS MEDIATED BY AN INCREASE IN APICAL NHE3 ACTIVITY THAT IS ASSOCIATED WITH AN INCREASE IN NHE3 PROTEIN EXPRESSION (5, 34, 40, 44). IN CONTRAST, NO CHANGE IN NHE3 mRNA OR PROTEIN LEVEL IS OBSERVED IN THE MTAL OR INNER STRIPE OF OUTER MEDULLA OF RATS ON A HIGH SALT INTAKE (40, 45, 72). THE ROLE OF APICAL NHE3 IN MEDIATING THE ADAPTIVE INCREASE IN MTAL HCO$_3^-$ ABSORPTION INDUCED BY HIGH SODIUM INTAKE IS UNCLEAR.

THE BASOLATERAL Na$^+/H^+$ EXCHANGER NHE1 ALSO IS AN IMPORTANT DETERMINANT OF THE RATE OF HCO$_3^-$ ABSORPTION BY THE MTAL. INHIBITION OF NHE1 RESULTS SECONDARILY IN INHIBITION OF APICAL NHE3, THEREBY DECREASING HCO$_3^-$ ABSORPTION (29, 35, 65, 66). THIS MECHANISM PLAYS A ROLE IN THE ACUTE REGULATION OF MTAL HCO$_3^-$ ABSORPTION BY NERVE GROWTH FACTOR AND BACTERIAL LIPOLYSACCHARIDE (33, 35, 65, 66). WHETHER BASOLATERAL Na$^+/H^+$ EXCHANGE AND THE INTERACTION BETWEEN BASOLATERAL NHE1 AND APICAL NHE3 CONTRIBUTE TO CHRONIC REGULATION OF HCO$_3^-$ ABSORPTION IS NOT KNOWN. A HIGH DIETARY NaCl INTAKE HAS BEEN REPORTED TO INCREASE NHE1 ACTIVITY IN LYMPHOCYTES (23), AND RENAL SODIUM RETENTION MAY COINCIDE WITH INCREASED NHE1 ACTIVITY IN PATIENTS WITH ESSENTIAL HYPERTENSION (58). HOWEVER, STUDIES USING TRANSGENIC MICE SHOWED THAT CONSTITUTIVE OVEREXPRESSION OF NHE1 IS ASSOCIATED WITH INCREASED RENAL SODIUM REABSORPTION AND THE DEVELOPMENT OF ELEVATED BLOOD PRESSURE AFTER SALT LOADING (43). THESE FINDINGS SUGGEST THAT INCREASED NHE1 ACTIVITY MAY PROMOTE RENAL SODIUM RETENTION AND BE A CONTRIBUTING FACTOR IN THE PATHOGENESIS OF SALT-SENSITIVE HYPERTENSION. HOWEVER, TO OUR KNOWLEDGE THERE HAVE BEEN NO REPORTS
of the effects of dietary sodium intake on the activity, expression, or function of NHE1 in any segment of the nephron.

The aim of the present study was to determine the roles of basolateral NHE1 and apical NHE3 in mediating the adaptive increase in HCO$_3^-$ absorption induced in the MTAL by a high sodium intake. The results show that the increased HCO$_3^-$ absorptive capacity in MTALs from high sodium rats involves adaptive increases in the activity of both the basolateral NHE1 and apical NHE3 Na$^+$/H$^+$ exchangers.

**METHODS**

*Animals.* Male Sprague-Dawley rats weighing 60–100 g (Taconic, Germantown, NY) were allowed free access to standard rodent chow (NIH 31 diet, Ziegler Bros., Gardeners, PA) and drinking solution up to the time of the experiments. Control rats drank distilled H$_2$O, and rats on a high sodium intake drank 0.28 M NaCl for 5–7 days. This model was studied because 1) it induces an adaptive increase in HCO$_3^-$ absorption in the MTAL (25) that correlates directly with increased HCO$_3^-$ absorption by the loop of Henle in vivo (12); 2) it induces a similar increase in HCO$_3^-$ absorptive capacity in MTALs from rats drinking either NaCl or NaHCO$_3$, indicating that the increase in sodium intake rather than intake of the accompanying anion is responsible for the transport adaptation (25); and 3) it has been used extensively to investigate the chronic effects of high salt intake on the function of nephron segments, including the thick ascending limb, proximal tubule, and collecting duct (7, 12, 15–17, 25, 36, 40, 45, 56). Rats drinking 0.28 M NaCl have no differences in body weight, plasma Na$^+$, K$^+$, Cl$^-$, and HCO$_3^-$ concentrations, or arterial pH and PCO$_2$ compared with controls (7, 12, 17, 25, 45, 56). In each experimental series, tubules from NaCl-treated rats were studied concurrently with tubules from control rats obtained in the same shipment. All protocols in this study were approved by the Institutional Animal Care and Use Committee of The University of Texas Medical Branch.

**Tubule perfusion and measurement of net HCO$_3^-$ absorption.** MTALs were isolated and perfused in vitro as previously described (24, 25). Tubules were dissected from the inner stripe of the outer medulla at 10°C in bath solution (see below), transferred to a bath chamber on the stage of an inverted microscope, and mounted on concentric glass pipettes for perfusion at 37°C. The tubules were perfused and bathed under basal conditions in a 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). Experimental agents were added to the bath and lumen solutions as described in RESULTS. In one series of HCO$_3^-$ transport experiments (see Fig. 2D), Na$^+$ in the bath solution was replaced completely with N-methyl-D-glucammonium (NMDG$^+$) (29, 65).

The protocol for study of transepithelial HCO$_3^-$ absorption was as described (24, 25, 65). Tubules were equilibrated for 20–30 min at 37°C in the initial perfusion and bath solutions and the luminal flow rate (normalized per unit tubule length) was adjusted to 1.5–1.9 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 re-equilibrate for 5–10 min after an experimental agent was added to or removed from the bath or lumen solution. The absolute rate of HCO$_3^-$ absorption (JHCO$_3^-$, 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 (24). An average HCO$_3^-$ absorption rate was calculated for each period studied in a given tubule. 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 Figs. 1, 2, and 5. Mean values ± SE (n = no. of tubules) are presented in the text. The absolute decrease in HCO$_3^-$ absorption was calculated for individual tubules as the difference between absorption rates measured in the absence and presence of experimental agent (bath amiloride). The fractional decrease in HCO$_3^-$ absorption is the absolute decrease expressed as a percentage of the basal absorption rate measured in the same tubule.

**Measurement of intracellular pH and 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 (65, 68). 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. The protocols for determination of basolateral and apical Na$^+$/H$^+$ exchange rates were as previously described (32, 65, 68, 69). In brief, MTALs 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 rates were determined by measurement of the initial rate of pHi increase after addition of 145 mM Na$^+$ to the lumen solution (Na$^+$-replaced NMDG$^+$) (68, 69). Basolateral Na$^+$/H$^+$ exchange rates were determined by measuring the initial rate of pHi increase after addition of 145 mM Na$^+$ to the bath solution (32, 65, 70). Interruption of pHi recovery at various points along the recovery curve permits determination of Na$^+$/H$^+$ exchange rates over a range of pHi values, with appropriate corrections for a variable background acid loading rate (68). In experiments in which basolateral or apical Na$^+$/H$^+$ exchange activity was measured, EIPA (50 μM) was present on the opposite side of the tubule to eliminate any contribution of the contralateral exchanger to the Na$^+$-induced changes in pHi.

Net H$^+$ flux rates (JNa$^+$/H$^+$, pmol·min$^{-1}$·mm$^{-1}$) are calculated as (dH$^+$/dt) × βi × V, where dH$^+$/dt (pH units/min) is the initial slope of the record of pH, vs. time, βi is the intrinsic intracellular buffering power (mM/pH unit), and V is cell volume per unit tubule length (nl/mm), measured as previously described (65, 68, 69). βi was similar in MTALs from control and NaCl-treated rats. Similar to previous results (68), βi decreased with increasing pHi, averaging 52 ± 3 mM/pH unit at pH 6.70 and 40 ± 3 mM/pH unit at pH 7.15. V was determined from inner and outer tubule diameters measured under conditions identical to those used for measurement of initial rates of...
Na⁺-dependent pH, recovery (68, 69). V was 0.31 ± 0.01 nl/mm (n = 8) for control tubules and 0.48 ± 0.02 nl/mm (n = 10) for tubules from rats given NaCl (P < 0.001). The cell hypertrophy induced by high NaCl intake was observed in both the HCO₃⁻ transport and pH, protocols.

**Immunoblot analysis.** Immunoblotting of NHE1 and NHE3 was carried out as previously described (32, 35) on the outer stripe of the outer medulla dissected from kidneys of control rats and rats receiving NaCl. This tissue preparation is highly enriched in MTALs and exhibits regulatory changes in transport and signaling proteins that accurately reflect changes observed in the MTAL (15, 27, 32, 40, 63, 66, 67, 70, 72). The tissue samples were homogenized in ice-cold PBS and solubilized for 2 h at 4°C in RIPA buffer with protease inhibitors. Samples of equal protein content (50 μg/lane) were separated by SDS-PAGE using 8% gels and transferred to polyvinylidene difluoride membranes as described (32, 35). Membranes were blocked with 5% BSA in TBS/Tween and incubated overnight at 4°C with anti-NHE1 (1:1,000; Santa Cruz Biotechnology) or anti-NHE3 (1:1,000; Millipore) antibody. After washing in TBS, horseradish peroxidase-conjugated anti-rabbit (for NHE1) or anti-mouse (for NHE3) secondary antibody was applied and immunoreactive bands were detected by chemiluminescence (Luminol Reagent, Santa Cruz Biotechnology). Parallel gels stained with Coomassie blue were analyzed to confirm equal loading among lanes. Protein bands were quantified by densitometry (MetaMorph). Initial studies were carried out using gels loaded with a range of protein concentrations and using different exposure times to ensure a linear relationship between band density and NHE protein amount.

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

**RESULTS**

High sodium intake increases HCO₃⁻ absorption in the MTAL. HCO₃⁻ absorption rates were determined in isolated, perfused MTALs from rats given H₂O (control) or 0.28 M NaCl to drink for 5–7 days. The HCO₃⁻ absorption rate was increased by 60% (from 14.0 ± 0.8 to 22.4 ± 0.9 pmol·min⁻¹·mm⁻¹; P < 0.001) in MTALs from the NaCl-treated rats (Fig. 1). These data confirm previous results demonstrating that a high sodium intake causes an adaptive increase in HCO₃⁻ absorption in the MTAL (25).

**Effects of bath amiloride on HCO₃⁻ absorption.** Previously we demonstrated that the activity of basolateral NHE1 is an important determinant of the rate of HCO₃⁻ absorption in the MTAL (29, 32, 35, 65, 66). To assess the role of basolateral Na⁺/H⁺ exchange in the adaptation to a high sodium intake, we examined the effects of 10 μM bath amiloride, which inhibits HCO₃⁻ absorption in the MTAL through inhibition of NHE1 (29, 32, 35). Adding 10 μM amiloride to the bath decreased HCO₃⁻ absorption by 23% (from 15.8 ± 0.8 to 12.2 ± 0.7 pmol·min⁻¹·mm⁻¹; n = 8, P < 0.001) in MTALs from control rats compared with a decrease of 35% (from 23.2 ± 0.9 to 15.1 ± 0.6 pmol·min⁻¹·mm⁻¹; n = 9, P < 0.025) in MTALs from rats on a high sodium intake (Fig. 2A). Both the absolute and fractional decreases in HCO₃⁻ absorption induced by bath amiloride were significantly higher in tubules from the NaCl-treated rats (Fig. 2B). The decrease in HCO₃⁻ absorption induced by bath amiloride was more than doubled in tubules from the high-NaCl rats. As shown in Fig. 2C, the increase in
MTAL HCO₃⁻ absorption rate induced by a high sodium intake under basal conditions was greatly reduced in the presence of bath amiloride, with bath amiloride eliminating 60% of the adaptive increase in HCO₃⁻ absorption. In MTALs from control rats, the effect of bath amiloride to decrease HCO₃⁻ absorption through inhibition of NHE1 is eliminated in tubules studied in a Na⁺-free bath to inhibit basolateral Na⁺/H⁺ exchange activity (29, 35). As shown in Fig. 2D, a Na⁺-free bath also eliminated the inhibition of HCO₃⁻ absorption by bath amiloride in MTALs from NaCl-treated rats. These data support the view that the increased inhibition of HCO₃⁻ absorption by bath amiloride in MTALs from high sodium rats is mediated through inhibition of Na⁺/H⁺ exchange. Together, these findings support a major role for basolateral NHE1 in mediating the adaptive increase in HCO₃⁻ absorption induced by a high sodium intake in the MTAL.

Effect of high sodium intake on basolateral Na⁺/H⁺ exchange activity. Based on the preceding results, further experiments were carried out to examine directly the effect of a high sodium intake on basolateral Na⁺/H⁺ exchange. Basolateral Na⁺/H⁺ exchange activity was determined by measurement of the initial rate of pH₄ increase in response to the addition of bath Na⁺, as previously described (65, 70). A high NaCl intake increased basolateral Na⁺/H⁺ exchange activity at all pH₄ values studied (Fig. 3A). Overall, the basolateral Na⁺/H⁺ exchange rate was increased by 89% in MTALs from both control and NaCl-treated rats (65, 70). These results, together with those in Fig. 2, support the view that the increase in HCO₃⁻ absorptive capacity induced by a high sodium intake is mediated through an adaptive increase in basolateral Na⁺/H⁺ exchange activity.

Effect of high sodium intake on NHE1 protein level. To test whether the adaptive increase in basolateral Na⁺/H⁺ exchange induced by a high sodium intake involves a change in NHE1 protein level, NHE1 abundance was assessed by immunoblot analysis of the inner stripe of the outer medulla. Immunoblots of inner stripe homogenates show that NHE1 protein expression is increased in rats on a high NaCl intake compared with controls (Fig. 4). These results suggest that the adaptive increase in basolateral Na⁺/H⁺ exchange activity induced by a high sodium intake involves an increase in NHE1 expression.

Effects of lumen amiloride on HCO₃⁻ absorption. Absorption of HCO₃⁻ by the MTAL depends on H⁺ secretion mediated by the apical membrane Na⁺/H⁺ exchanger NHE3 (6, 11, 26, 69). To assess the functional role of this exchanger in the adaptation to a high sodium intake, we examined the effect on HCO₃⁻ absorption of 50 μM lumen EIPA, which inhibits apical NHE3 and HCO₃⁻ absorption in the MTAL (68, 69). As demonstrated previously in MTALs from control rats (69), addition of 50 μM EIPA to the lumen virtually eliminated HCO₃⁻ absorption in MTALs from NaCl-treated rats, reducing the HCO₃⁻ absorption rate from 22.1 ± 1.3 to 2.2 ± 0.5 pmol·min⁻¹·mm⁻¹ (P < 0.005; Fig. 5A). In contrast, lumen addition of 50 μM amiloride or 1 μM EIPA had no effect on HCO₃⁻ absorption (Fig. 5B). These results support the view that the adaptive increase in HCO₃⁻ absorption induced by a high sodium intake is mediated through an increase in apical NHE3 activity and
provide no evidence that an amiloride-sensitive apical Na+/H+ exchanger (NHE2) or an apical H+-ATPase contributes to HCO₃⁻ absorption in MTALs from high-NaCl rats (29, 53, 69). In addition, these results indicate that the NHE1-dependent increase in HCO₃⁻ absorptive capacity (Figs. 2–4) is mediated through an increase in apical Na⁺/H⁺ exchange activity.

Effect of high sodium intake on apical Na⁺/H⁺ exchange activity. The results presented above support the view that an effect of basolateral NHE1 to enhance the activity of apical NHE3 plays a major role in mediating the adaptive increase in HCO₃⁻ absorption in MTALs from high NaCl rats. However, the results in Fig. 2C show that a significant increase in HCO₃⁻ absorptive capacity persists in MTALs from NaCl-treated rats when basolateral Na⁺/H⁺ exchange is inhibited. Therefore, we examined whether a high sodium intake may increase apical Na⁺/H⁺ exchange activity independently of basolateral Na⁺/H⁺ exchange activity. Tubules were perfused and bathed in Na⁻/free solution, and apical Na⁺/H⁺ exchange activity was determined directly by measurement of initial rates of pH₄ increase following lumen Na⁺ addition (68, 69). As shown in Fig. 6, a high sodium intake increased apical Na⁺/H⁺ exchange activity by 30% over the range of pH₄ values studied. This adaptive increase in apical NHE3 activity may contribute to the increase in HCO₃⁻ absorptive capacity observed under conditions in which basolateral NHE1 is inhibited.

Effect of high sodium intake on NHE3 protein level. To determine whether the increase in apical Na⁺/H⁺ exchange activity induced by a high sodium intake involves an increase in NHE3 protein level, NHE3 abundance was examined in the inner stripe of the outer medulla by immunoblot analysis. As shown in Fig. 7, no difference in NHE3 protein expression was observed in inner stripe homogenates from control and NaCl-treated rats. This result is consistent with previous reports (40, 45, 72) and suggests that the adaptive increase in apical NHE3 activity involves a regulatory mechanism other than increased NHE3 protein expression.

DISCUSSION

Long-term adaptations in renal tubule transport play a role in the physiological regulation of systemic electrolyte and water balance and are important for the pathophysiology of disease. Chronic regulation of transport proteins occurs throughout the nephron and may involve changes in total transporter abundance, changes in the subcellular distribution of transporters, or changes in the state of activation of individual transporters within the membrane. Previously, we demonstrated that the ability of the MTAL to absorb HCO₃⁻ is increased by a high sodium intake (25). In the present study, we provide evidence that this increase in HCO₃⁻ absorptive capacity involves two mechanisms: 1) an adaptive increase in the activity of basolateral NHE1, which results secondarily in an increase in the activity of apical NHE3; and 2) an adaptive increase in NHE3 activity, independent of NHE1. The first mechanism accounts for a majority of the increase in HCO₃⁻ absorptive capacity and is associated with an increase in NHE3 protein expression. These studies provide new evidence of a role for basolateral Na⁺/H⁺ exchange in the chronic regulation of transepithelial H⁺ secretion by renal tubules and indicate that the effect of a high sodium intake to enhance HCO₃⁻ absorption in the MTAL involves adaptive upregulation of the NHE1 and NHE3 Na⁺/H⁺ exchangers.

NHE1 is expressed ubiquitously in the plasma membrane of nonpolarized cells and in the basolateral membrane of epithelial cells, where it plays a role in a variety of cell processes including maintenance of pH₄ and cell volume, growth and survival, cytoskeleton remodeling, and migration (48, 53, 55). We have identified a novel role for NHE1 in acute regulation...
of HCO$_3^-$ absorption in the MTAL. Specifically, decreasing basolateral NHE1 activity with nerve growth factor or amiloride, or by NHE1 knockout, results secondarily in a decrease in apical NHE3 activity, thereby reducing HCO$_3^-$ absorption (29, 32, 35, 65). NHE1 regulates NHE3 activity by altering the organization of the actin cytoskeleton (66). The rate of HCO$_3^-$ absorption in the MTAL thus depends on a regulatory interaction between the NHE1 and NHE3 Na$^+$/H$^+$ exchangers, whereby basolateral NHE1 enhances the activity of apical NHE3 (32, 35, 65). The results of the present study suggest that NHE1, through its interaction with NHE3, plays a major role in mediating the adaptive increase in HCO$_3^-$ absorption induced by a high sodium intake. This view is supported by several observations: 1) bath amiloride (10 μM), which inhibits HCO$_3^-$ absorption in the MTAL specifically through inhibition of NHE1 (32, 35), eliminates most of the adaptive increase in HCO$_3^-$ absorption; 2) the effect of bath amiloride to inhibit HCO$_3^-$ absorption is eliminated under conditions in which basolateral Na$^+$/H$^+$ exchange is inhibited; 3) high sodium intake induces a primary increase in basolateral Na$^+$/H$^+$ exchange activity; 4) the latter effect is associated with an increase in NHE1 protein expression; and 5) the NHE1-dependent increase in HCO$_3^-$ absorptive capacity is mediated through an increase in apical NHE3 activity. Taken together with our previous studies (29, 35, 65, 66), these results support a mechanism whereby a high sodium intake induces an adaptive increase in basolateral NHE1 activity that results secondarily in an increase in apical NHE3 activity. Thus the regulatory interaction between basolateral NHE1 and apical NHE3 in the MTAL appears to participate in the chronic regulation of HCO$_3^-$ absorption.

The mechanisms involved in upregulation of NHE1 by high sodium intake in the MTAL remain to be determined. NHE1 is a component of multiprotein complexes, where it binds and interacts with a variety of signaling molecules important in regulating NHE1 transport activity (48, 59). Acute regulation of NHE1 involves phosphorylation of its cytoplasmic domain by serine/threonine kinases such as ERK1/2-p90Rsk, Ca$^{2+}$/calmodulin-dependent kinase II, Rho-activated kinase (p160Rock), NcK-interacting kinase (NIK), and Akt as well as interactions with regulatory factors that include calmodulin, phosphatidylinositol 4,5-bisphosphate, calcineurin homologous protein (CHP), and ERM proteins that link NHE1 to the cytoskeleton (48, 49, 53, 55, 59). Whether these regulatory mechanisms are influenced by changes in dietary salt intake and may play a role in the long-term regulation of NHE1 in renal tubules is not known. NHE1 also is regulated through changes in gene transcription. We found that the increase in basolateral NHE1 activity induced in the MTAL by high sodium intake is associated with increased expression of NHE1 in the inner stripe of the outer medulla. This region of the kidney is highly enriched in MTALs and has been studied extensively to identify regulated changes in the expression of transport proteins and the activity of intracellular signaling molecules that accurately reflect changes in the MTAL (15, 27, 32, 40, 63, 66, 67, 70, 72). Consistent with our results in the inner stripe, a high sodium intake was reported recently to increase NHE1 mRNA and protein levels in isolated rat MTALs (54). Thus the adaptive increase in basolateral Na$^+$/H$^+$ exchange activity in the MTAL likely is attributable, at least in part, to an increase in NHE1 expression. Chronic acidosis increases NHE1 expression in the kidney and in renal epithelial cell lines (4, 41, 50), and activation of protein kinase C and increased expression of the transcription factor AP-1 may play a role in this response (38). Other transcription factors involved in regulation of the NHE1 gene include the c/EBP protein family, AP-2, and COUP-TF (19, 48). In addition, recent studies indicate that the NHE1 gene and NHE1 protein expression are responsive to reactive oxygen species (19), a finding of interest in view of studies showing that a high salt intake alters nitric oxide and superoxide levels in MTAL cells (36). Identification of the extra- and intracellular signals that regulate NHE1 activity and expression in the MTAL in response to elevated sodium intake will be important areas for future investigation.

The mechanisms responsible for the enhanced effect of NHE1 to increase apical NHE3 activity in MTALs from high-sodium rats also are undefined. Structural interactions of NHE1 with the cytoskeleton play a role in NHE1-dependent regulation of several cell functions, including actin cytoskeleton remodeling, cell survival, and cell migration (48). In the MTAL, the acute effects of NHE1 to regulate NHE3 activity are mediated through NHE1-dependent changes in the organization of the actin cytoskeleton (66). In the present study, we found that high sodium intake causes MTAL hypertrophy, indicating significant structural changes within the MTAL cells. How cytoskeletal structure may be altered in MTAL cells in response to high sodium intake, and whether actin cytoskeleton remodeling or other molecular mechanisms play a role in the chronic NHE1-dependent stimulation of NHE3 are unanswered questions. NHE1 has an established, permissive role in cell growth and proliferation and is involved in mediating myocardial hypertrophy (19, 53, 55). Thus an additional question raised by our studies is whether the increased NHE1 activity and the cell hypertrophy in MTALs from high sodium animals may be causally related, or if these represent unrelated adaptations to high sodium intake. Increased salt intake causes cell hypertrophy in other segments of the nephron, including the distal convoluted tubule and collecting duct (60), but whether this is associated with changes in NHE1 expression or activity has not been reported.

In addition to stimulation of NHE3 that is dependent on NHE1, a high sodium intake induces a primary increase in NHE3 activity. This adaptation could contribute to the increase in MTAL HCO$_3^-$ absorptive capacity that persists when NHE1 is inhibited (Fig. 2C). In agreement with previous studies (40, 45, 72), we did not see an effect of high sodium intake on NHE3 protein level. Thus the enhanced NHE3 activity is not the result of an increase in total transporter abundance. This finding is of interest in at least two respects. First, as detailed above, the increase in NHE1 activity induced by a high sodium intake is associated with an increase in NHE1 abundance. Thus the chronic regulation of NHE1 and NHE3 in the MTAL by high sodium intake involves different mechanisms. Second, chronic metabolic acidosis increases apical Na$^+$/H$^+$ exchange and HCO$_3^-$ absorption in the MTAL in association with an increase in NHE3 protein expression (5, 25, 34, 44). Thus, although chronic increases in sodium and acid intake cause similar adaptive increases in MTAL NHE3 activity and HCO$_3^-$ absorption, the molecular mechanisms underlying the NHE3 activation appear to be distinct.
In the absence of a change in NHE3 total protein level, a high sodium intake could regulate NHE3 in the MTAL by altering the distribution of the transporter between the apical membrane and subapical endomembrane compartments (1, 14, 47). In particular, a high sodium intake could induce net movement of NHE3 from a subapical compartment to the plasma membrane, thereby increasing the number of transporters in active apical domains and increasing NHE3 activity. In the proximal tubule, a high sodium intake induces a retraction of NHE3 from the top to the base of microvilli, decreasing the number of active transporters in the apical membrane and presumably reducing proximal sodium reabsorption (72). Membrane redistribution contributes to acute and chronic regulation of the apical Na\(^{+}\)-K\(^{+}\)-2Cl\(^{-}\) cotransporter NKCC2 in thick ascending limb cells, including an effect of high salt intake to induce a small shift of NKCC2 from low-density apical membrane fractions to higher-density intracellular membrane-enriched fractions in the rat renal cortex (8, 72). It is currently unknown whether subcellular redistribution plays a role in the acute or chronic regulation of NHE3 in the MTAL. An additional mechanism by which high sodium intake could increase NHE3 activity is through the chronic regulation of cell-signaling molecules that alter the intrinsic activity of individual transporters. Similar to NHE1, NHE3 is a component of multiprotein complexes where its activity is regulated through changes in phosphorylation and interactions with a variety of accessory and scaffolding proteins (1, 14). The abundance of phosphorylated NHE3 was increased in proximal tubule brush-border membrane fractions in response to a high sodium intake, which may have contributed to its redistribution within the microvilli (72). A high salt intake has been shown to alter the expression and/or activity of a number of signaling molecules in the thick ascending limb, including endothelial nitric oxide synthetase, cytochrome P-450 epoxygenase, cyclooxygenase-2, AMP-activated protein kinase, and connexin 37 (20, 36, 39, 61, 63, 73). Whether these or other signaling pathways may contribute to the adaptive increase in NHE3 activity and HCO\(_3\)\(^{-}\) absorption caused by high sodium intake in the MTAL remains to be determined.

Our experiments do not rule out the possibility that adaptations in transporters in addition to Na\(^{+}/\)H\(^{+}\) exchangers may contribute to the increase in MTAL HCO\(_3\)\(^{-}\) absorption. A high sodium intake has been reported to increase expression of the Na\(^{+}/\)K\(^{+}\) ATPase \(\alpha\)-subunit and the basolateral AE2 Cl\(^{-}/\)HCO\(_3\)\(^{-}\) exchanger in inner stripe of outer medulla and MTAL (40, 56). The latter observation suggests that the adaptive increase in HCO\(_3\)\(^{-}\) absorption may involve the coordinated upregulation of luminal H\(^{+}\) secretion via NHE3 and basolateral HCO\(_3\)\(^{-}\) efflux via AE2. Our results indicate, however, that NHE1 and NHE3 play a major role in the increased HCO\(_3\)\(^{-}\) absorptive capacity of the MTAL and that the increases in NHE1 and NHE3 activity are the result of primary adaptations that are observed in the absence of any differences in the driving force for the exchangers. In a previous study, a high sodium intake was reported to have no effect on NHE3 activity in rat MTALs studied in suspension (45). It is possible that the increase in NHE3 transport rate observed in the present study in microperfused tubules may have gone undetected in the tubule suspensions due to differences in the experimental methods: NHE3 activity in the tubule suspensions was estimated from increases in pH measured over 10 s following dilution of tubule fragments into Na\(^{+}\)-containing medium in the presence of NHE1 and Na\(^{+}/\)K\(^{+}\)-2Cl\(^{-}\) cotransport inhibitors (45).

The adaptive increases in Na\(^{+}/\)H\(^{+}\) exchange activity and HCO\(_3\)\(^{-}\) absorption in the MTAL may reflect a unique role of the MTAL in enabling the kidneys to maintain acid-base balance during changes in sodium and volume balance (25, 26). As detailed above, the adaptive increase in NHE3 activity in the MTAL is opposite to the downregulation of NHE3 induced by high salt intake and volume expansion in the proximal tubule (47, 72). The latter response contributes to the natriuresis that aids in maintaining extracellular fluid volume in response to increased NaCl intake but also results in decreased proximal tubule HCO\(_3\)\(^{-}\) reabsorption that would promote development of metabolic acidosis (3, 10, 13). A high salt intake and extracellular fluid volume expansion have additional effects that tend to reduce renal net acid excretion and systemic pH, including 1) a decrease in plasma angiotensin II, which results in decreased HCO\(_3\)\(^{-}\) absorption by proximal and distal tubules and reduced proximal tubule ammonium production (22, 46, 51, 64); 2) an increase in intrarenal dopamine, which decreases apical NHE3 and basolateral Na\(^{+}/\)HCO\(_3\)\(^{-}\) transport activity in the proximal tubule (9, 18, 42); and 3) a decrease in aldosterone, which reduces collecting duct acid secretion (2, 62). We propose that the adaptive increase in HCO\(_3\)\(^{-}\) absorption in the MTAL aids in offsetting these volume-related effects in the proximal and distal segments to maintain acid-base balance when salt intake is altered. This adaptive regulation would be augmented by acute responses of the MTAL to hormones that are opposite to those in other nephron segments: angiotensin II and aldosterone inhibit HCO\(_3\)\(^{-}\) absorption in the MTAL, compared with their effects to stimulate H\(^{+}\) secretion and HCO\(_3\)\(^{-}\) absorption in segments of the proximal and distal tubule and collecting duct (28, 30). Thus a high salt intake may act through an integrated combination of short-term and long-term regulatory mechanisms to reduce HCO\(_3\)\(^{-}\) absorption in the MTAL, thereby stabilizing acid-base balance while permitting regulated changes in sodium excretion that are necessary to control extracellular fluid volume and blood pressure. In the MTAL, the adaptive increase in apical NHE3 activity would not compromise the ability of this segment to participate in sodium and volume regulation because NaCl absorption is mediated by apical NKCC2 and can be regulated independently of NHE3-mediated NaHCO\(_3\)\(^{-}\) absorption.

Our finding that high sodium intake induces adaptive increases in Na\(^{+}/\)H\(^{+}\) exchange activity in the MTAL may have implications for physiological processes in addition to transcellular acid secretion. Increased NHE1 activity has been reported in cells of hypertensive patients and hypertensive animal models and enhanced activity of this exchanger has been implicated in salt sensitivity in humans (21, 52, 58, 71). Transgenic mice that overexpress NHE1 show impaired urinary excretion of sodium in association with elevated blood pressure after salt loading (43). These findings suggest that increased NHE1 activity can cause renal sodium retention and salt-sensitive hypertension (43). Our results show that NHE1 expression and transport activity are increased by high sodium intake in the MTAL, providing evidence of a causal relationship between high salt intake and elevated NHE1 activity in renal tubules. In addition, the sodium-induced increase in
NHE1 activity results secondarily in stimulation of apical NHE3, thereby increasing the capacity of the MTAL to absorb NaHCO3. These findings are noteworthy in view of data suggesting that increased NaHCO3 reabsorption may contribute to renal sodium retention in salt-sensitive humans (57). Thus understanding the systemic factors and molecular mechanisms through which sodium intake upregulates NHE1 and NHE3 in the MTAL in our experiments under normal conditions may provide insights into mechanisms that may be dysregulated in disease to contribute to salt-sensitive hypertension. In addition, the ability of NHE1 to contribute to adaptive regulation in renal tubules may take on broader significance as additional functions for NHE1 in vectorial transport are recognized. For example, recent studies suggest that NHE1 can interact with the Na+/K+-ATPase γ-subunit to modulate sodium pump activity in proximal tubule cell lines (37). Last, our studies provide a basis for future investigations into other physiological conditions and disease states that may alter renal tubule functions through adaptations in NHE1. As detailed above, chronic acidosis increases NHE1 activity and expression in renal epithelial cells, but what role NHE1 plays in the adaptive responses of renal tubules to systemic acid-base disorders has not been determined.

In summary, the results of this study suggest that a high sodium intake increases the capacity of the MTAL to absorb HCO3− through two mechanisms: 1) an adaptive increase in basolateral NHE1 activity that results secondarily in an increase in apical NHE3 activity; this mechanism accounts for the majority of the adaptive increase in HCO3− absorption and is associated with an increase in NHE1 protein expression; and 2) an adaptive increase in NHE3 activity, independent of the activity of NHE1. These studies support a role for NHE1 in the long-term regulation of renal tubule function and suggest that the regulatory interaction whereby basolateral NHE1 enhances the activity of apical NHE3 in the MTAL plays a role in the chronic regulation of HCO3− absorption. The adaptive increases in Na+/H+ exchange activity and HCO3− absorption in the MTAL would offset effects of high salt intake to reduce H+ secretion and HCO3− absorption in other segments of the nephron, thereby aiding the ability of the kidneys to maintain acid-base balance while regulating sodium balance and extracellular fluid volume. Understanding how a high sodium intake upregulates NHE1 and NHE3 in the MTAL may provide insights into mechanisms that may contribute to salt-sensitive hypertension.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

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