Adaptation of NHE-3 in the rat thick ascending limb: effects of high sodium intake and metabolic alkalosis

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Laghmani, Kamel, Régine Chambrey, Marc Froissart, Maurice Bichara, Michel Paillard, and Pascale Borensztein. Adaptation of NHE-3 in the rat thick ascending limb: effects of high sodium intake and metabolic alkalosis. Am. J. Physiol. 276 (Renal Physiol. 45): F18–F26, 1999.—The present studies examined the effects of chronic NaCl administration and metabolic alkalosis on NHE-3, an apical Na+/H+ exchanger of the rat medullary thick ascending limb of Henle (MTAL). NaCl administration had no effect on NHE-3 mRNA abundance as assessed by competitive RT-PCR, as well as on NHE-3 transport activity estimated from the Na+-dependent cell pH recovery of Na+-depleted acidified MTAL cells, in the presence of 50 µM Hoe-694, which specifically blocks NHE-1 and NHE-2. Two models of metabolic alkalosis were studied, one associated with high sodium intake, i.e., NaHCO3 administration, and one not associated with high sodium intake, i.e., chloride depletion alkalosis (CDA). In both cases, the treatment induced a significant metabolic alkalosis that was associated with a decrease in NHE-3 transport activity (~27% and ~25%, respectively). Negative linear relationships were observed between NHE-3 activity and plasma pH or bicarbonate concentration. NHE-3 mRNA abundance and NHE-3 protein abundance, assessed by Western blot analysis, also decreased by 35% and 25%, respectively, during NaHCO3-induced alkalosis, and at 47 and 33%, respectively, during CDA. These studies demonstrate that high sodium intake has a positive effect on MTAL NHE-3. In contrast, chronic metabolic alkalosis, regardless of whether it is associated with high sodium intake or not, leads to an appropriate adaptation of NHE-3 activity, which involves a decrease in NHE-3 protein and mRNA abundance.

DURING CHRONIC METABOLIC acidosis, the capacity of the proximal tubule and thick ascending limb (TAL) of Henle to reabsorb bicarbonate is appropriately increased (21, 25), which contributes to the defense against metabolic acidosis. Current evidence strongly suggests that NHE-3, which encodes an amiloride-resistant Na+/H+ exchanger (NHE), is an apical isoform of the NHE family and plays a significant role in bicarbonate absorption in both the proximal tubule and the medullary TAL (MTAL) (3, 5, 8, 11, 31, 33). Recent studies have demonstrated that enhanced NHE-3 mRNA (26) protein abundance and activity (4, 26, 40) are involved in the adaptation of bicarbonate reabsorption during chronic metabolic acidosis in the proximal tubule and MTAL.

In contrast with chronic metabolic acidosis, the effects of metabolic alkalosis on NHE-3 have not been previously studied. In the proximal tubule, acute metabolic alkalosis induced by NaHCO3 administration results in a decreased bicarbonate absorption that is probably due to both alkalemia (2) and extracellular fluid volume expansion (10). Chronic extracellular fluid volume expansion is also associated with a decreased activity of the apical Na+/H+ exchanger (29). Thus chronic NaHCO3 load is expected to reduce HCO3 absorption in the proximal tubule, which may explain at least in part the mild alkalosis observed in this situation. In contrast, in chronic metabolic alkalosis associated with chloride depletion (CDA), absolute proximal HCO3 absorption is enhanced (28, 39), an effect attributed to the HCO3 load-dependent reabsorption indistinguishable from that seen in normal rats (28, 39). Furthermore, in CDA, apical Na+/H+ exchange activity is only slightly decreased if anything (1) despite alkalemia. This may be the result of potassium depletion, which has been shown to increase the activity of the Na+/H+ exchanger (32). Thus the response to alkalemia of the proximal tubule depends on the experimental model of metabolic alkalosis.

The response of MTAL to chronic metabolic alkalosis also depends on the experimental model but is different from the response of the proximal tubule. The ability of the rat MTAL to absorb bicarbonate is decreased in CDA (22), but increased in NaHCO3-induced alkalosis (20). The latter effect of NaHCO3 administration has been interpreted to result from the high sodium intake, since NaCl administration also induces an increase in MTAL bicarbonate absorptive ability (20). The question arises as to whether the differences observed in MTAL HCO3 absorption capacity in models of chronic metabolic alkalosis are related to regulation of the apical Na+/H+ exchanger independently of alkalemia or to the occurrence of additional factors that could override an appropriate alkalosis adaptation of the apical Na+/H+ exchanger. The aim of this study was then to examine, in the rat MTAL, the regulation of the main apical Na+/H+ exchanger, NHE-3, by chronic high sodium intake and metabolic alkalosis associated or unassociated with high sodium intake. To this purpose, NHE-3 mRNA was quantified by a competitive RT-PCR in freshly isolated MTAL cells. NHE-3 transport activity and protein abundance were also determined in the same preparations. The results show that, in the rat...
MTAL, chronic high sodium intake has no effect per se on NHE-3. By contrast, chronic metabolic alkalosis caused by both NaHCO₃ administration and chloride depletion decreases NHE-3 transport activity, an effect related to a decrease in NHE-3 mRNA and protein abundance. From these studies, we suggest that chronic metabolic alkalosis per se is responsible for an intrinsic and appropriate reduction of the apical NHE-3 activity of the MTAL.

METHODS
Animal Treatment

Experiments were performed on male Sprague-Dawley rats (200–300 g body wt). The animals were allowed free access to food and drinking solution up to the time of the experiments. In each series, a group of experimental animals was compared directly with controls that were obtained from the same shipment and studied during the same period of time. The specific treatments were as follows.

Chronic NaCl administration. Experimental animals drank 0.28 M NaCl for 6 days. Control rats received distilled water. Both groups were allowed free access to standard rat chow.

Chronic NaHCO₃ administration. Experimental animals drank 0.28 M NaHCO₃ for 6 days. Control rats received distilled water. Both groups were allowed free access to standard rat chow.

Chloride depletion metabolic alkalosis. CDA was generated by peritoneal dialysis (16, 19). The rats were anesthetized by intramuscular injection of 7 and 0.3 mg/100 g body wt of ketamine chlorohydrate and chlorpromazine, respectively. Warmed dialysate (15 ml/100 g body wt) was infused into the peritoneal cavity through an 18-gauge catheter. Control rats were dialyzed against a solution composed of 125 mM NaCl, 20 mM NaHCO₃, 4 mM KCl, and 25 g/l glucose. In CDA rats, chloride depletion alkalosis was produced by a dialysate composed of 150 mM NaHCO₃, 4 mM KHCO₃, and 25 g/l glucose. After 30 min, the dialysate was removed (more than 90% of the initial volume). The rats were then submitted for 6 days to a Cl⁻, Na⁺-, and K⁺-deficient diet (in mg/kg: 4.3 Cl⁻, 53 Na⁺, and 61 K⁺). Control rats drank a solution containing 110 mM sodium acetate, 40 mM NaHCO₃, 50 mM potassium acetate, and 25 g/l glucose.

On the day of the experiment, rats were anesthetized with pentobarbital sodium. In most animals, blood was collected by aortic puncture for arterial blood gas and plasma electrolyte analyses, and the kidneys were rapidly removed and immersed into ice-cold Hanks’ solution containing (in mM) 140 NaCl, 3 KCl, 1 CaCl₂, 1 MgCl₂, 0.2 KH₂PO₄, 0.8 K₂HPO₄, 10 HEPES, 5 glucose, and 1 leucine, as well as 0.1 mg/ml BSA, pH 7.4, bubbled with 100% O₂ and containing 5 μM of the acetoxymethyl ester of BCECF (BCECF-AM) dissolved in DMSO and stored at −20°C. The BCECF-loaded tubules were then washed by gentle centrifugation to remove the extracellular dye and resuspended in the same medium. Just before each run, aliquots of the MTAL tubules were Na⁺ depleted and acidified by three washings and gentle centrifugation in a CO₂-free and Na⁺-free medium containing (in mM) 140 N-methyl-D-glucamine chloride (NMDG-Cl), 3 KCl, 1 CaCl₂, 1 MgCl₂, 0.2 KH₂PO₄, 0.8 K₂HPO₄, 10 HEPES, 5 glucose, and 1 leucine, as well as 0.1 mg/ml BSA, pH 7.4. The MTAL cells were then diluted into cuvettes containing 2 ml of the experimental medium to reach a final cytocrit of ~0.1% (vol/vol). BCECF fluorescence was monitored by use of a Shimadzu RF-5000 spectrofluorometer equipped with water-jacketed temperature-controlled cuvette holder and magnetic stirrer. Fluorescence intensity was recorded at one emission wavelength, 530 nm, whereas the excitation wavelengths alternated automatically at 2-s intervals between two wavelengths, 500 and 450 nm. The values of the 500- to 450-nm fluorescence ratio were converted into intracellular pH (pHᵢ) values with use of calibration curves that were established after each experimental run; cells were permeabilized with Triton X-100R (0.025 g/100 ml), and the relationship between extracellular BCECF fluorescence and medium pH was established.

To quantitate Na⁺/H⁺ exchange activity, proton flux rates (JH⁺ = dpHᵢ/dt × β × V; where dpHᵢ/dt is the Na⁺-dependent initial rate of pHᵢ recovery (pH units/min), β is intrinsic intracellular buffering power (mmol·l⁻¹·pH unit⁻¹), and V is cell volume (nl/mm tubule length)). To initiate a Na⁺-dependent pHᵢ recovery, Na⁺-depleted acidified MTAL cells were abruptly added into an isosmotic CO₂-free medium containing 30 mM Na⁺ (30 mM NMDG-Cl replaced with 30 mM NaCl). This Na⁺ concentration is clearly higher than the apparent Na⁺ affinity constant for NHE-3 (5 to 10 mM) and has been shown to support NHE-3 activity close to Vmax (30). Initial pHᵢ values were not different between control and experimental (data not shown), and the first 10 s of time course of the Na⁺-dependent pHᵢ recovery was fitted to a linear equation to calculate the initial rate of change of pHᵢ (dpHᵢ/dt); correlation coefficients for these linear fits were >0.96. As previously described (26), to specifically study NHE-3 transport activity, NHE-1 and NHE-2 activities were inhibited by 50 μM Hoe-694. Bumetanide, 0.1 mM, and amiloride, 1 μM (that had per se no significant effect on NHE-3 activity), were also present to block Na-K-2Cl cotransport and possible Na⁺ conductance, respectively (7). The value for β was determined from immediate alkalization of Na⁺-depleted acidified MTAL cells caused by addition of 15 mM NH₄Cl in the Na⁺-free medium containing 10 mM barium and 0.5 mM amiloride. These conditions were chosen to inhibit transport pathways for NH₄⁺ and H⁺ (6). Indeed, the stable pHᵢ observed during the plateau phase following the rapid initial increase in pHᵢ confirmed that NH₄⁺ or other acid-base transports were effectively blocked and could not...
influence the determination of β. Cell volume was determined by measuring tubule diameters under light microscopy as described (26, 35).

**RNA Extraction, Reverse Transcription, and Polymerase Chain Reaction**

Total RNA was extracted from MTAL cells using the method of Chomczynski and Sacchi (13) as previously described and quantified by the measure of its optical density at 260 nm. cDNA was synthesized from RNA samples with 200 U Moloney murine leukemia virus (MMLV) reverse transcriptase, 20 pmol of downstream primer, 4 µg of yeast tRNA, 2.5 mM of each deoxyribonucleotide (dNTP), 10 mM dithiothreitol, and 2 U of RNase inhibitor in 22 µl reaction buffer containing 50 mM Tris·HCl, 75 mM KCl, and 3 mM MgCl2 at 37°C for 60 min. Each reaction was performed in parallel with an otherwise identical one that contained no reverse transcriptase.

For PCR, 10 µl of the cDNA solution was supplemented with 5 µl of 10× PCR buffer, 5 µl of a 25 mM MgCl2 solution, 10 pmol of each primer, 1 µl of a 25 mM dNTP solution, and 1.25 U Taq polymerase in a final volume of 50 µl. Samples were overlaid with mineral oil, denatured at 94°C for 4 min followed by 30 cycles consisting of denaturation at 94°C (1 min), annealing at 60°C (1 min), and extension at 72°C (1.5 min). PCR was completed by a final extension step of 10 min at 72°C. The PCR products were size-fractionated on 1.5% agarose gels stained with ethidium bromide.

**Quantification of NHE-3 mRNA**

The method used has been previously described in detail (26). Briefly, NHE-3 mRNA abundance was quantified by a competitive RT-PCR, using an internal standard of cRNA that differed from the wild-type NHE-3 mRNA by an 80-bp deletion. Both cRNA and wild-type NHE-3 mRNA were reverse transcribed and amplified using primers 5'-GGAAAGCAGAGGCGGAGGAGCAT-3' (antisense, bp 2186–2205) and 5'-GAAGTTGTGTGTCGACCGATTCT-3' (sense, bp 1885–2005). These NHE-3 primers yielded a product of 321 and 241 bases for wild-type mRNA and cRNA, respectively (26). For each determination, eight to seven competitive RT-PCR reaction points were performed and resolved on 1.5% agarose gels stained with ethidium bromide. The ratio of the fluorescence intensity of each PCR product, which is proportional to the amount of cRNA, was performed on NIH-image 1.51. Then, NHE-3 mRNA abundance was calculated (Fig. 1B) as previously described. Results are expressed in attomoles of NHE-3 mRNA per nanogram of total RNA.

**Dot-Blot Analysis**

Dot-blot analyses were performed as previously described (26). Serial dilutions from 130 to 12.5 ng of MTAL total RNA were denatured in 2.2 M formaldehyde and 15× SSC (1× SSC is 0.15 M NaCl and 0.015 M sodium citrate, pH 7.0). dot-blotted onto Biodyne nylon membranes using a commercial vacuum-driven dot-blot (GIBCO-BRL) apparatus and fixed by ultraviolet cross-linking. Filters were prehybridized in 4× SSC, 50 mM NaPO4, and 1× Denhardt's solution for 2 h at 42°C, hybridized in the same solution containing 25 × 106 cpmp of 32P-radiolabeled oligo(dT) at 42°C overnight, and washed once in 2× SSC with 0.1% SDS at room temperature for 10 min, then twice in the same solution at 37°C for 20 min. Filters were exposed to film for 1 h at −80°C, and labeling was quantified by densitometry (Fig. 1, C and D).

**Preparation of MTAL Membranes and Detection of NHE-3 Protein Abundance by Western Blot Analysis**

MTAL tubule suspensions were homogenized in membrane buffer (250 mM sucrose and 20 mM Tris-HEPES, pH 7.4, containing 50 µg/ml 4-(2-aminoethyl)-benzenesulfonyl fluoride, 4 µg/ml leupeptin, and 4 µg/ml aprotinin) and centrifuged at 1,000 g for 10 min at 4°C. The supernatants were then centrifuged at 450,000 g for 20 min, and the pellets were resuspended in the same buffer. Protein content was assessed by the method of Lowry.

Equal amounts of solubilized proteins (30 µg) of either control or treated crude membrane fractions were separated by SDS-PAGE using 7.5% gels according to Laemmli. Proteins were transferred to nitrocellulose membrane at 100 V for 1 h at 4°C with a mini-Transblot cell electrophoresis unit (Bio-Rad Laboratories, Hercules, CA), and equal loading of the lanes was controlled by staining with 0.5% Ponceau S in acetic acid. Immunoblotting was performed as follows: nitrocellulose membrane was incubated overnight at 4°C in 10% nonfat dry milk containing PBS, pH 7.4, to block nonspecific binding of antibody, followed by 2-h incubation with a 1:300 dilution of affinity-purified anti-rat NHE-3 antibody as previously described (9). The strips were then washed twice in 1% nonfat dry milk containing PBS and 0.05% Tween and then once in PBS for 15 min each. The strips were then incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG diluted 1:3,000 in 10% nonfat dry milk containing PBS for 2 h.
RESULTS

Systemic measurements made at the end of the treatment period are shown in Table 1. Animals tolerated each of the treatment regimens well. For each experiment in this study, experimental and control rats were studied in parallel. Measurements of $\beta_i$, cell volume and Na$^+$-dependent dpH/dt were all performed on each MTAL tubule suspension of control and experimental rats.

Effects of Chronic NaCl Administration

Effect on NHE-3 mRNA abundance. The effect of chronic NaCl administration on the MTAL NHE-3 mRNA abundance, measured by a competitive RT-PCR, as previously described (26), is shown in Fig. 2A; NHE-3 mRNA abundance was not different in NaCl-treated rats vs. control (0.031 ± 0.007 vs. 0.031 ± 0.002 amol/ng total RNA, n = 4, not significant (NS)).

Effect on NHE-3 transport activity. Results of four independent experiments showed that the dpH/dt values were not different in the NaCl-treated vs. control groups (0.953 ± 0.062 vs. 0.880 ± 0.057 pH unit/min, n = 4, NS). These dpH/dt values were not different in the presence or absence of 10$^{-3}$M ouabain (1.03 ± 0.23 vs. 1.02 ± 0.15 pH unit/min, n = 4, NS), which demonstrates that the first 10 s of time course of Na$^+$-dependent pH recovery was not dependent on Na-K-ATPase activity. The $\beta_i$ and cell volume were also not different between NaCl-treated vs. control groups (42.6 ± 8.4 vs. 47.6 ± 5.7 mmol H$^+ \cdot l^{-1} \cdot pH$ unit$^{-1}$, n = 4, NS; and 0.41 ± 0.01 vs. 0.37 ± 0.01 nl/mm tubule length, n = 4, NS). Finally, as shown in Fig. 2B, the Na$^+$-dependent $J_{\text{H+}}$ values were not different in the NaCl-treated vs. control groups (16.6 ± 1.7 vs. 15.4 ± 0.8 pmol·min$^{-1}$·mm$^{-1}$, n = 4, NS).

Effects of Chronic Metabolic Alkalosis

The next step of this study was to determine whether NHE-3 is affected by chronic metabolic alkalosis in-
duced either by NaHCO₃ administration or chloride depletion. As shown in Table 1, plasma pH and bicarbonate concentration were significantly increased in NaHCO₃-treated and CDA rats compared with controls.

Effects on NHE-3 transport activity. After NaHCO₃ administration, there was no change in dpH/dt (0.794 ± 0.012 vs. 0.840 ± 0.027 pH unit/min, NaHCO₃ vs. control groups; n = 5, NS) and cell volume (0.40 ± 0.03 vs. 0.40 ± 0.03 nl/mm tubule length, NaHCO₃ vs. control; n = 5, NS); however, β was significantly lower in treated animals (41.1 ± 3.9 vs. 53.9 ± 4.4 mmol·l⁻¹·pH unit⁻¹, NaHCO₃ vs. control; n = 5, P < 0.05). The Na⁺-dependent Jₜ was thus significantly decreased by 27% (Fig. 3A) in NaHCO₃-treated vs. control animals (12.8 ± 0.9 vs. 17.5 ± 0.5 pmol·min⁻¹·mm⁻¹, n = 5, P < 0.05). In the presence of 1 mM amiloride, the Na⁺-dependent Jₜ was abolished in both control and alkalosis conditions (1.3 ± 1.9 vs. 1.6 ± 3.0 pmol·min⁻¹·mm⁻¹, alkalosis vs. control; n = 4, NS), which demonstrates that in the presence of 50 μM Hoe-694, the Na⁺-dependent Jₜ was due to NHE-3 activity.

After CDA, dpH/dt was also not different between experimental and control rats (0.813 ± 0.058 vs. 0.755 ± 0.028 pH unit/min, CDA vs. control; n = 4, NS). However, both cell volume (0.31 ± 0.01 vs. 0.38 ± 0.01 nl/mm tubule length, CDA vs. control; n = 4, P < 0.001) and β (53.7 ± 2.1 vs. 66.6 ± 5.6 mmol·l⁻¹·pH unit⁻¹, CDA vs. control; n = 4, P < 0.05) were significantly decreased by CDA. Thus, during CDA, the Na⁺-dependent Jₜ was also significantly decreased (13.6 ± 0.7 vs. 18.0 ± 0.8 pmol·min⁻¹·mm⁻¹, n = 4, P < 0.01) (Fig. 3B).

Since these results show that both NaHCO₃-induced metabolic alkalosis and CDA decrease NHE-3 activity in the MTAL, the next step of this study was to determine the mechanisms involved in this regulation. To this purpose, quantification of NHE-3 mRNA and protein abundance was performed in both models of metabolic alkalosis.

Effect of NaHCO₃ administration on NHE-3 mRNA abundance and protein abundance. The effect of NaHCO₃-induced metabolic alkalosis on the MTAL NHE-3 mRNA abundance is shown in Fig. 4A. Results showed a 35% decrease in NHE-3 mRNA abundance in alkalosis vs. control groups (0.020 ± 0.003 vs. 0.031 ± 0.004 amol/ng total RNA; n = 6, P < 0.05). To appreciate the specificity of the alkalosis-induced decrease in NHE-3 mRNA, we quantified the amount of mRNAs present in MTAL total RNA samples by dot-blot analysis using radiolabeled oligo(dT), as previously described (26). Results showed that, for a fixed amount of total RNA, the amount of mRNAs is not significantly affected by chronic NaHCO₃ administration (1,259 ± 169 vs. 1,105 ± 137 integrated optical density units, NaHCO₃-treated vs. control groups; n = 6, NS). Thus the decrease in NHE-3 mRNA abundance observed during chronic NaHCO₃ administration cannot be explained by a global decrease of mRNAs.

NHE-3 protein abundance was determined by Western blot analysis using an affinity-purified antibody raised against rat NHE-3 peptide (9). Figure 5A shows the results of three representative experiments from a total of seven. This antibody recognized an ~82-kDa protein in crude membranes isolated from MTAL cells, which corresponded to the NHE-3 protein (8). For each experiment, immunoblotting was performed at least twice and showed that NHE-3 protein amount was decreased during alkalosis. Results of seven experiments showed a mean 25 ± 5% decrease in NHE-3 protein abundance (P < 0.02) in rats given NaHCO₃ compared with control animals (Fig. 5B).

Effect of CDA on NHE-3 mRNA abundance and protein abundance. To determine whether the mechanisms by which NHE-3 activity is reduced in CDA are the same as in NaHCO₃-induced metabolic alkalosis, we performed further experiments to quantify NHE-3 mRNA and protein abundance during CDA in the same MTAL suspensions. As shown in Fig. 4B, NHE-3 mRNA...
abundance was decreased by 47% in CDA vs. control groups (0.018 ± 0.001 vs. 0.034 ± 0.003 amol/ng total RNA, n = 3, P < 0.001). Figure 6 shows the results of Western blot analysis. NHE-3 protein abundance was also decreased by 33 ± 4% (P < 0.05, n = 3) compared with control (when the results were normalized for β-actin, the decrease of NHE-3 protein abundance was 45 ± 6%, P < 0.05).

Relationships Between Plasma pH or HCO₃⁻ Concentration and NHE-3 Transport Activity in Alkalosis and Acidosis Series

We have previously shown that NHE-3 transport activity in MTAL cells is increased during chronic metabolic acidosis (26). Pooling the results of the present study and those of acidosis series, we expressed the values of NHE-3 transport activity as a function of plasma pH or bicarbonate concentration in each experiment (for control and experimental group). A negative linear relationship was observed between NHE-3 transport activity and plasma bicarbonate concentration (n = 34, r = 0.68, P < 0.001). The best negative linear relationship was observed between NHE-3 transport activity and plasma pH (n = 34, r = 0.81, P < 0.001) (Fig. 7).

**DISCUSSION**

We have previously shown that, in MTAL cells, chronic metabolic acidosis increases NHE-3 mRNA associated with an increase in abundance and functional activity of the protein (26). These adaptations can explain the increased bicarbonate transport capacity observed in in vitro microperfused MTAL harvested from chronically acidic rats (20). Since the bicarbonate transport changes observed in the rat MTAL in association with chronic metabolic alkalosis depend on the model being studied, the present experiments were designed to determine whether chronic metabolic alkalosis is also associated with an intrinsic adaptation of NHE-3. Our results show that, in the rat MTAL, 1) chronic high sodium intake has no effect per se on NHE-3 mRNA and transport activity, 2) chronic metabolic alkalosis induced by NaHCO₃ administration or CDA decreases the functional activity of NHE-3, and 3) this effect is associated with a decrease of NHE-3 mRNA and protein abundance.

Since it has been previously shown that chronic changes in dietary sodium intake alter the capacity of the MTAL to absorb bicarbonate (20), we first studied the effects of chronic NaCl administration on NHE-3 mRNA and transport activity. Our results show that chronic NaCl administration had no effect on NHE-3 mRNA abundance. Furthermore, NaCl administration had no effect on NHE-3 intrinsic transport activity.
estimated by the Na⁺-dependent proton flux rate measured in the presence of 50 µM Hoe-694 to block NHE-1 and NHE-2 but not NHE-3 (14, 26). We then examined the effects of chronic metabolic alkalosis on NHE-3 transport activity in two models of alkalosis, one associated with high sodium intake, i.e., NaHCO₃ administration, and one without high sodium intake, i.e., CDA. The present results show that, in both models, NHE-3 transport activity is decreased. This reduced MTAL NHE-3 intrinsic activity is likely related to chronic alkalemia, because other factors expected to chronically affect NHE-3 may not have accounted for the reduced MTAL NHE-3 intrinsic activity observed in the present study. First, chronic increase in extracellular fluid volume has been shown to reduce apical Na⁺/H⁺ exchange activity (i.e., NHE-3) in the proximal tubule, whereas a chronic decrease has the opposite effect (29).

In the present study, MTAL NHE-3 intrinsic activity was unchanged in chronic NaCl administration and reduced in chronic NaHCO₃ administration, although extracellular fluid volume was probably increased in both situations. Furthermore, MTAL NHE-3 activity was also reduced in CDA in which extracellular fluid volume is not increased (19). Second, potassium depletion has been shown to be associated with an increased apical Na⁺/H⁺ exchanger activity in the proximal tubule (32). It is thus unlikely that the decreased NHE-3 activity we observed in the MTAL results from potassium depletion, when present. Finally, the negative relationship we observed between NHE-3 intrinsic activity and plasma pH strongly suggests that acid-base status is a major determinant of NHE-3 activity in the rat MTAL. However, whether MTAL NHE-3 activity was similarly reduced in both models of metabolic alkalosis despite higher plasma bicarbonate concentration in CDA is less clear. It should be noted that, in the present study, blood pH was similarly increased in both models, which suggests that cellular alkalosis was also similar. Furthermore, NHE-3 activity was correlated better to blood pH than plasma bicarbonate concentration. Thus it is possible that cellular pH is one of the major signals that regulate NHE-3. Support for an important role of cell pH derives also from recent study showing that a decrease in cell pH activates protein tyrosine kinase pathways, which likely contributes to acid-induced increase in NHE-3 activity (41). Furthermore, potassium depletion, which is present only in CDA, could have limited the inhibition of NHE-3 activity in CDA. Indeed, as already discussed, potassium depletion has been shown to stimulate the apical Na⁺/H⁺ exchanger of the proximal tubule (32).

We further investigated which cellular mechanisms could be responsible for this alkalosis-induced decrease in NHE-3 activity. Our results showed that both NaHCO₃ administration and CDA resulted in a decrease in NHE-3 mRNA abundance. NHE-3 protein abundance, estimated on MTAL crude membranes by immunoblot, was also decreased during the two models of metabolic alkalosis. Thus this study demonstrates that in vivo chronic metabolic alkalosis decreases the level of NHE-3 transcript, which is associated with decreases in NHE-3 protein abundance and intrinsic transport activity in freshly isolated MTAL cells. Whether the adaptation of NHE-3 mRNA abundance is related to adaptations of NHE-3 gene transcription and/or changes at the posttranscriptional level remains to be determined.

Chronic increase in sodium intake has been shown to enhance the ability of the rat MTAL to absorb bicarbonate (20). This increase was observed when sodium was administered as either sodium chloride or sodium bicarbonate. One factor previously suggested to mediate the Na⁺-dependent stimulation of HCO₃⁻ absorption in the MTAL is cell sodium uptake (20). Indeed, chronic high NaCl intake results in an increased sodium delivery to the loop of Henle, which is associated with a marked increase in NaCl absorption by the loop of Henle, presumably due at least in part to MTAL NaCl absorption (15, 27). Thus it was suggested that chronic increase in sodium and fluid delivery to MTAL may enhance cell sodium uptake via apical Na⁺ transporters, which results in a stimulation of basolateral Na-K-ATPase activity. Indeed, an increased capacity of NH₄⁺ absorption (20) and an upregulation of BSC1 protein expression (17), which implies an increased activity of the Na⁺-K⁺-(NH₄)₂-2Cl⁻ cotransporter, have been reported in rats with high sodium intake. Increased Na-K-ATPase activity has also been reported under this condition (38). However, in the present study, NHE-3 intrinsic activity and mRNA abundance were not modified by sodium chloride administration, whereas NaHCO₃ administration was associated with decreased NHE-3 intrinsic activity and mRNA and protein abundance. These results suggest that other mechanisms are involved in the increased HCO₃⁻ absorptive ability of the rat MTAL during high sodium intake. One hypothesis could be an indirect stimulation of NHE-3 activity. First, chronic increase in cell sodium intake may be associated with MTAL cell hypertrophy as described in proximal and distal tubules (34). However, MTAL cell hypertrophy was not observed in the present study. Second, undefined factors related to increased sodium intake might stimulate basolateral HCO₃⁻ transport, Na-K-ATPase activity, and/or basolateral Na⁺/H⁺ exchanger, with a secondary stimulation of apical NHE-3 activity (23). Another possibility could be the stimulation of apical H⁺ secretion independently of NHE-3. It should be noted that N-ethylmaleimide (NEM)-sensitive ATPase is decreased in the rat MTAL during NaHCO₃ administration (24), which seems to exclude a possible role of the NEM-sensitive H⁺-ATPase in this increased bicarbonate absorption. NHE-2, another isoform of the Na/H antipporter, has been recently demonstrated to be expressed in the apical membrane of the rat MTAL (12, 36). It could be possible that NHE-2 activity, which was not studied in our experiments, mediates the increased bicarbonate absorption observed during an increase in sodium intake.

In the dialysis-generated model of metabolic alkalosis, in which sodium intake was not increased, chronic alkalosis was associated with an adaptive decrease in
MTAL bicarbonate absorptive capacity (22), which is in agreement with the CDA-induced decrease in intrinsic NHE-3 transport activity observed in the present study. In conclusion, chronic NaCl administration has no effect on MTAL NHE-3 intrinsic activity. Chronic metabolic alkalosis, in both NaHCO₃-induced and CDA, is responsible for an appropriate reduction of the apical intrinsic NHE-3 activity of the MTAL, probably via a decrease in mRNA transcript and protein abundance. However, the bicarbonate absorptive capacity of the MTAL is likely dependent on the sodium delivery to the loop of Henle. During high NaCl intake, undefined mechanisms enhance HCO₃ absorption capacity of the loop of Henle. During high NaHCO₃ intake, in which sodium delivery to MTAL is also increased, the same mechanisms probably overcome the inhibitory effect of alkalemia on MTAL bicarbonate absorptive capacity (22), which is in agreement with the reduction in NHE-3 activity.

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