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### Research Article

The mammalian proximal tubule is an important mediator of the renal adaptive response to systemic acidosis. In chronic metabolic and respiratory acidosis the bicarbonate reabsorptive (or proton secretory) capacity is increased. This increase is mediated, at least in part, by an increase in  $V_{max}$  of the luminal Na/H antiporter. To determine whether this adaptation involves increased mRNA expression, Na/H antiporter mRNA levels were measured by Northern analysis in renal cortex of rats with metabolic (6 mmol/kg body wt NH<sub>4</sub>Cl for 2 or 5 d) and respiratory (10% CO<sub>2</sub>/air balanced for 2 or 5 d) acidosis and of normal, pair-fed rats. Na/H antiporter mRNA levels were unchanged after 2 d of both metabolic and respiratory acidosis. After 5 d, however, Na/H antiporter mRNA expression was increased 1.76  $\pm$  0.12-fold in response to metabolic acidosis ( $P$  less than 0.005,  $n = 8$ ), but was not different from normal in response to respiratory acidosis: 1.1  $\pm$  0.2 (NS,  $n = 8$ ). Thus, the renal adaptive response to metabolic acidosis involves increased cortical Na/H antiporter mRNA levels. In contrast, the enhanced proximal tubule Na/H antiporter activity and bicarbonate reabsorption in respiratory acidosis seem to involve mechanisms other than increased Na/H antiporter gene expression.

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## Expression of Rat Renal Na/H Antiporter mRNA Levels in Response to Respiratory and Metabolic Acidosis

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### Abstract

The mammalian proximal tubule is an important mediator of the renal adaptive response to systemic acidosis. In chronic metabolic and respiratory acidosis the bicarbonate reabsorptive (or proton secretory) capacity is increased. This increase is mediated, at least in part, by an increase in  $V_{\max}$  of the luminal Na/H antiporter.

To determine whether this adaptation involves increased mRNA expression, Na/H antiporter mRNA levels were measured by Northern analysis in renal cortex of rats with metabolic (6 mmol/kg body wt  $\text{NH}_4\text{Cl}$  for 2 or 5 d) and respiratory (10%  $\text{CO}_2$ /air balanced for 2 or 5 d) acidosis and of normal, pair-fed rats.

Na/H antiporter mRNA levels were unchanged after 2 d of both metabolic and respiratory acidosis. After 5 d, however, Na/H antiporter mRNA expression was increased  $1.76 \pm 0.12$ -fold in response to metabolic acidosis ( $P < 0.005$ ,  $n = 8$ ), but was not different from normal in response to respiratory acidosis:  $1.1 \pm 0.2$  (NS,  $n = 8$ ).

Thus, the renal adaptive response to metabolic acidosis involves increased cortical Na/H antiporter mRNA levels. In contrast, the enhanced proximal tubule Na/H antiporter activity and bicarbonate reabsorption in respiratory acidosis seem to involve mechanisms other than increased Na/H antiporter gene expression. (*J. Clin. Invest.* 1991. 87:747–751.) **Key words:** Na/H antiporter • respiratory acidosis • metabolic acidosis • gene expression • acidification

### Introduction

The mammalian proximal tubule reabsorbs 80–90% of bicarbonate filtered through the glomerulus and assumes an outstanding role in the metabolic regulation of acid-base balance. As much as two-thirds of this proximal tubule bicarbonate

reabsorption (or equivalent proton secretion) are mediated by the Na/H antiporter (1) located exclusively on the apical (luminal) membrane (2, 3). Interestingly, systemic acid-base disturbances have been shown to affect both the rates of proximal tubule bicarbonate reabsorption (or proton secretion) and the activity of the proximal tubule Na/H antiporter, suggesting an important role for the Na/H antiporter in the homeostatic renal response to these conditions. In metabolic acidosis, proximal tubule Na/H antiporter activity has been shown to be stimulated potently via an increase in the maximal transport capacity or  $V_{\max}$  (4–8). Conceivably, this increased transport activity could mediate the increased (fractional) proximal tubule bicarbonate reabsorption (9–11) and/or the enhanced  $\text{NH}_4^+$  secretion in response to metabolic acidosis (12–14). In respiratory acidosis, proximal tubule Na/H antiporter activity has also been shown to be increased both in the isolated proximal tubule perfused in vitro (15) and in proximal tubule cells grown in primary culture (16). Results from brush border vesicle studies have been less consistent than in metabolic acidosis (17–19). However, one study did find an increase in maximal transport capacity or  $V_{\max}$  (17). An increased Na/H antiporter activity in response to chronic respiratory acidosis could mediate the increased proximal tubule bicarbonate reabsorption (16, 20) that maintains the hyperbicarbonatemia characteristic of this disorder.

The increase in maximal transport capacity or  $V_{\max}$  of the renal cortical Na/H antiporter in response to both metabolic and respiratory acidosis would be expected to be associated with an increase in number of active transporter proteins. Such an increase can be due to many different mechanisms, including increased specific mRNA expression (by either transcriptional or posttranscriptional mechanisms), altered balance between insertion and retrieval of transporter proteins, or recruitment of silent transporters within the membrane (i.e., by phosphorylation or activation by regulatory proteins).

The recent cloning and sequencing of the human growth factor-activatable Na/H antiporter (21) now allows investigation of the molecular physiology of the Na/H antiporter. In this study, we examined whether the increased renal cortical (proximal tubule) Na/H antiporter activity and consequently the enhanced transepithelial bicarbonate reabsorption in response to metabolic and respiratory acidosis could be due to altered expression of Na/H antiporter mRNA.

### Methods

**Animal model.** 6 different groups consisting of 8 male Sprague-Dawley rats each (250–300 g, Batin-King, Fremont, CA) underwent the follow-

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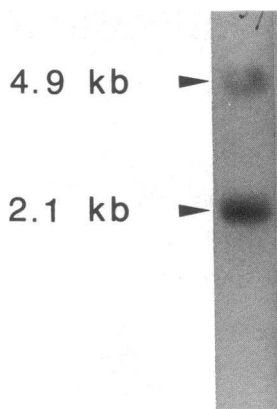
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**Figure 1.** Cross-hybridization of human DNA probe to rat Na/H antiporter mRNA.  $^{32}\text{P}$ -labeled DNA generated by random primed labeling using the human cDNA as template detects one single band at 4.9 kb corresponding to Na/H antiporter mRNA on a Northern blot loaded with 10  $\mu\text{g}$  of rat renal cortical poly (A<sup>+</sup>) RNA. The beta actin band at 2.1 kb is shown for comparison.

ing in vivo experiments: induction of respiratory acidosis (RA)<sup>1</sup> for 2 and 5 d, respectively, by exposure to 10% CO<sub>2</sub>/90% air in an environmental chamber. Metabolic acidosis (MA) was induced in two other groups by feeding 6 mmol/kg body wt (BW) of NH<sub>4</sub>Cl for 2 and 5 d, respectively. Normal control rats (C) for each period were pair-fed and were exposed to room air. Rats were fed 40 ml/d of a standard electrolyte-deficient diet (22) supplemented with 1.5 mmol KCl/d and 6.0 mmol NaCl/d. Rats had free access to tap water. All rats studied ate all of the diet daily. On the study days, rats were anesthetized using pentobarbital intraperitoneally and an arterial blood sample was obtained from the abdominal aorta (about 300  $\mu\text{l}$  with a heparinized syringe). Animals with RA were removed from the hypercapnic environment after anesthesia. Arterial pH and CO<sub>2</sub> tension were measured on a blood gas analyzer (model 278; Ciba-Corning, Zurich, Switzerland). [HCO<sub>3</sub><sup>-</sup>] was then calculated using the Henderson-Hasselbalch equation ( $\text{pK}' = 6.1$ , CO<sub>2</sub> solubility coefficient = 0.0301).

**mRNA analysis.** After anesthesia, both kidneys were excised and cut into sagittal slices. Obtaining the blood gas sample and removal of the kidney were completed within 5–10 min. The renal medulla was totally removed and the renal cortex was snap-frozen in liquid nitrogen. About 90% of the tissue mass in renal cortex consists of proximal tubules. Total RNA was isolated after tissue homogenization using guanidinium isothiocyanate (4 M) and cesium chloride (5.7 M) gradient centrifugation. Poly (A<sup>+</sup>) selection of RNA was performed by passing total RNA through oligo (dT) columns (Sigma Chemical Co., St. Louis, MO). mRNA (10  $\mu\text{g}$  per lane) was electrophoresed on a 1.2% agarose/19% formaldehyde gel and then transferred to zeta-probe blotting membrane (Bio-Rad Laboratories, Richmond, CA).

Blotted mRNA was hybridized to  $^{32}\text{P}$  DNA probes synthesized using the random primer method. To measure Na/H antiporter mRNA, a 1.9-kb template from the human antiporter cDNA (Bam HI digested) was used (21). The human probe thus synthesized cross-hybridized to rat renal cortical mRNA yielding one single band at 4.9 kb on Northern analysis (Fig. 1). During the performance of these studies, the template was changed because a partial length rat cDNA (0.9-kb fragment with ~95% homology to the human sequence, 23) became available. Results were essentially the same using either of the templates although less background occurred on the blots when the rat probe was used.

Hybridization was performed overnight at 42°C in a solution containing 50% formamide, 5 × SSPE, 5 × Denhardt's solution, 0.25% SDS, and salmon sperm DNA (200  $\mu\text{g}/\text{ml}$ ). The filters were then washed to a stringency of 0.1 × SSC, 0.1% SDS at 42°C. The filters were exposed to x-ray films (XOMat AR 50; Eastman Kodak Co., Rochester, NY) for 24 h. The intensity of the bands on the autoradiogram was determined by laser densitometry.

The blots were washed free of antiporter probe (65°C, 0.1 × SSC, 0.1% SDS) and reprobbed with rat cytoplasmic beta-actin (courtesy of

1. **Abbreviations used in this paper:** BW, body weight; C, control; MA, metabolic acidosis; RA, respiratory acidosis.

Dr. Lawrence C. Kedes; Hind III digested, 1.86-kb template). Hybridization and washing conditions were the same as with the Na/H antiporter probes, but film exposure time was reduced to 4 h. Na/H antiporter mRNA concentration was then corrected for the beta-actin signal obtained on the same blot. Results are expressed as the ratio of the densitometric signals obtained with the Na/H antiporter probe and the beta-actin probe.

All chemicals were purchased from Sigma and were of molecular biology grade quality.

**Statistics.** The data were analyzed using Student's *t* test for paired data. Results are reported as means ± SEM.

## Results

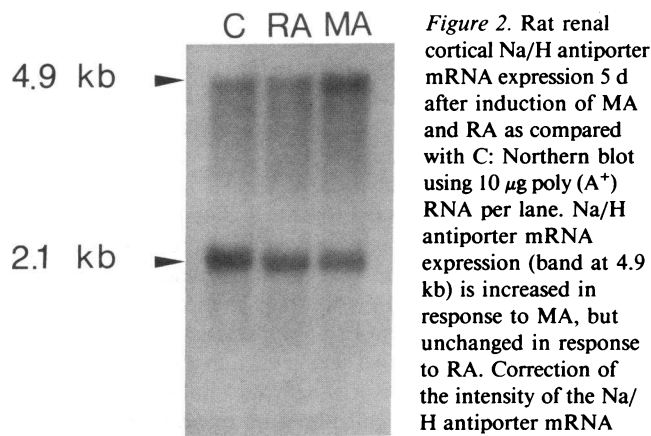
**In vivo animal data.** All animals tolerated the in vivo experiments well. The weight gain was not significantly different among rats in the different groups at either day 2 or 5. The following blood gas results were obtained after 2 d (*P* values obtained by comparison with control): C rats: pH = 7.41 ± 0.02, PCO<sub>2</sub> = 40.7 ± 0.8 Torr, HCO<sub>3</sub><sup>-</sup> = 23.2 ± 0.6 mmol/liter; MA rats: pH = 7.34 ± 0.03 (*P* < 0.01), PCO<sub>2</sub> = 35.9 ± 1.5 Torr (*P* < 0.01), HCO<sub>3</sub><sup>-</sup> = 21.4 ± 1.1 mmol/liter (*P* < 0.05); RA rats: pH = 7.31 ± 0.03 (*P* < 0.01), PCO<sub>2</sub> = 76.0 ± 2.3 Torr (*P* < 0.01), HCO<sub>3</sub><sup>-</sup> = 38.5 ± 0.8 mmol/liter (*P* < 0.01). After 5 d the arterial blood gases were as follows: C rats: pH = 7.45 ± 0.03, PCO<sub>2</sub> = 40.4 ± 0.7 Torr, HCO<sub>3</sub><sup>-</sup> = 27.1 ± 1.2 mmol/liter; MA rats: pH = 7.34 ± 0.04 (*P* < 0.01), PCO<sub>2</sub> = 34.6 ± 1.6 Torr (*P* < 0.01), HCO<sub>3</sub><sup>-</sup> = 18.3 mmol/liter ± 0.8 mmol/liter (*P* < 0.01); RA rats: pH = 7.43 ± 0.03 (ns), PCO<sub>2</sub> = 65.1 ± 3.4 Torr (*P* < 0.01), HCO<sub>3</sub><sup>-</sup> = 41.8 ± 1.8 mmol/liter (*P* < 0.01). (The fact that animals had to be removed from the hypercapnic environment during several minutes for blood gas sampling may have resulted in an acute fall in arterial PaCO<sub>2</sub> levels in some animals. We assume, however, that these last minute changes did not affect critically steady-state mRNA expression.)

**Na/H antiporter mRNA levels 5 d after induction of metabolic and respiratory acidosis.** Fig. 2 shows a representative Northern blot comparing Na/H antiporter mRNA from a C, RA, and MA rat, respectively. The figure illustrates that there is an increase in the renal cortical Na/H antiporter mRNA levels in the animal with MA, while there is no increase in the animal with RA as compared with control.

Fig. 3 summarizes the results of mRNA measurements in renal cortices from eight different animals in each group. The results represent the ratio of the Na/H antiporter mRNA and the beta-actin mRNA signals as read by laser densitometry. The results are given in percent change from control. As illustrated by the figure, MA rats exhibited a significant increase in specific Na/H antiporter mRNA expression. mRNA levels increased 1.76 ± 0.13-fold (*P* < 0.005). In contrast, Na/H antiporter mRNA levels in the renal cortex from RA rats increased only 1.13 ± 0.17-fold. This increase was not significantly different from controls.

MA, but not RA, induced a small increase in beta-actin mRNA expression compared with C (+22 ± 11%, *P* < 0.025). Thus, the increase in Na/H antiporter mRNA levels was over and above the associated increase in actin mRNA.

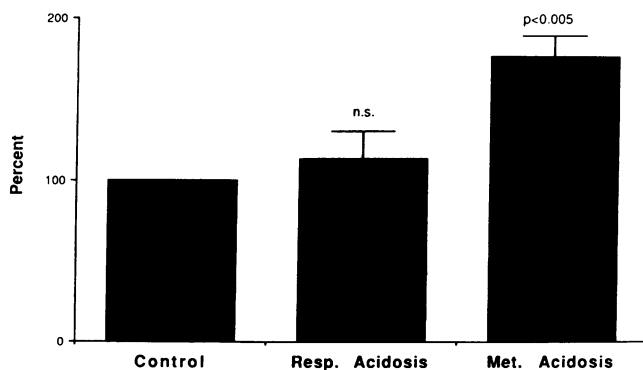
**Na/H antiporter mRNA expression in rat renal cortex 2 d after induction of metabolic or respiratory acidosis.** The next experiments were designed to investigate whether Na/H antiporter mRNA expression in rat renal cortex is affected earlier in the adaptation to metabolic acidosis or respiratory acidosis. In metabolic acidosis, functional adaptation of the proximal



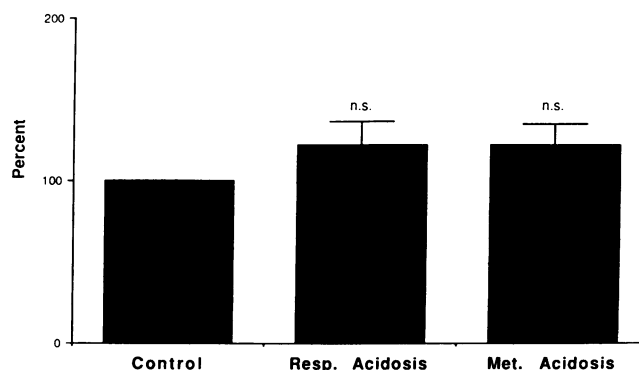
**Figure 2.** Rat renal cortical Na/H antiporter mRNA expression 5 d after induction of MA and RA as compared with C: Northern blot using 10  $\mu$ g poly (A<sup>+</sup>) RNA per lane. Na/H antiporter mRNA expression (band at 4.9 kb) is increased in response to MA, but unchanged in response to RA. Correction of the intensity of the Na/H antiporter mRNA band by the

corresponding beta-actin signal (band at 2.1 kb) shows that the increase in Na/H antiporter mRNA is specific. The blots were first analyzed with the antiporter probe, then washed and reanalyzed using the beta-actin probe. When total RNA was used, cross-hybridization of the DNA probe with the 28 S ribosomal band overwhelmed the antiporter signal. Only after poly (A<sup>+</sup>) selection the specific Na/H antiporter mRNA signal became visible as a distinct band. As shown in Fig. 2, there was sometimes a faint band at about 4.7 kb (rat 28 S ribosomal RNA). Analysis of this band by laser densitometry showed that the intensity of this band did not differ among the different groups of animals studied. Based on these observations, it is concluded that this band represents nonspecific cross-hybridization of the Na/H antiporter probe with ribosomal RNA.

tubule Na/H antiporter is detectable after 24–72 h of induction both in vivo and in cultured cells in vitro (8, 17) and it is possible that Na/H antiporter mRNA expression is also stimulated at this time point. In the case of respiratory acidosis, it is interesting to note, that the renal adaptation involves a transient (2–3 d) increase in net acid excretion (24). In this context, it is important to stress that increased proximal tubule Na/H antiporter activity in response to respiratory acidosis was found after about 48 h (15, 16). Based on these functional observa-



**Figure 3.** Renal cortical Na/H antiporter mRNA expression 5 d after induction of MA and RA as compared with C: summary of the results of eight animals in each group. Results are expressed in percent change from normal and represent the ratio of Na/H antiporter mRNA signal as detected by laser densitometry divided by the corresponding beta-actin mRNA signal. The ratio increases by a factor of  $1.76 \pm 0.12$  in response to metabolic acidosis, but does not change significantly in response to respiratory acidosis.



**Figure 4.** Renal cortical Na/H antiporter mRNA expression 2 d after induction of MA and RA as compared with C: summary of the results of eight animals in each group. Results are expressed in percent change from normal and represent the ratio of Na/H antiporter mRNA signal as detected by laser densitometry divided by the corresponding beta-actin mRNA signal. Both metabolic and respiratory acidosis do not affect Na/H antiporter mRNA levels significantly.

tions it is possible that the adaptation to chronic respiratory acidosis involves only a transient increase in renal cortical Na/H antiporter mRNA expression.

To test these hypotheses, renal cortical Na/H antiporter mRNA levels were measured 48 h after induction of metabolic or respiratory acidosis. Eight rats in each group were studied. Fig. 4 illustrates that there was no significant increase in renal cortical Na/H antiporter mRNA levels both in respiratory and metabolic acidosis. The results again represent the ratio of the Na/H antiporter mRNA and the beta-actin mRNA and are expressed as percentage of control. Although there was a slight increase in Na/H antiporter mRNA levels in metabolic ( $1.22 \pm 0.13$ -fold) and respiratory ( $1.22 \pm 0.14$ -fold) acidosis, these changes did not reach statistical significance.

## Discussion

The activity of the luminal Na/H antiporter of the mammalian proximal tubule has been shown to be regulated by in vivo metabolic and respiratory acidosis (4–8, 15–17). This transporter could therefore mediate, at least in part, the increased proximal tubule bicarbonate reabsorption (or possibly increased  $\text{NH}_4^+$  secretion in the case of metabolic acidosis) that characterizes the adaptive response of the proximal tubule to these disturbances. These studies examined whether the increased proximal tubule Na/H antiporter activity could be secondary to increased Na/H antiporter mRNA expression. The key findings were: (a) 5 d after induction, there was a significant increase in Na/H antiporter mRNA levels in response to metabolic acidosis while there was no increase in response to respiratory acidosis (Figs. 2 and 3); and (b) Na/H antiporter mRNA levels were unchanged in response to both metabolic and respiratory acidosis 2 d after induction (Figs. 2 and 4).

Metabolic acidosis involves an increase in the maximal transport capacity ( $V_{\text{max}}$ ) of the proximal tubule Na/H antiporter (4–8). This kinetic adaptation can be assumed to be associated with an increase in number of Na/H antiporter proteins in the luminal membrane of the proximal tubule. Our results indicate that this increase could be mediated, at least in

part, by increased expression of Na/H antiporter mRNA. It remains to be examined whether this increased expression involves transcriptional and/or posttranscriptional mechanisms.

The increase in proximal tubule Na/H antiporter activity is detectable anywhere from 24 to 72 h after induction of metabolic acidosis (4–8, 17). Our observations that Na/H antiporter mRNA expression was unchanged after 48 h, but increased after 5 d therefore raises several possibilities. First, it is possible though speculative that the functional adaptation of the proximal tubule Na/H antiporter to metabolic acidosis involves two different mechanisms: an early mechanism independent of changes in steady-state Na/H antiporter mRNA expression and a late mechanism that involves increased specific mRNA expression. Theoretically, the early mechanism(s) could involve increased insertion of presynthesized cytoplasmic transporters or activation of silent membrane transporters by phosphorylation or a regulatory protein(s). Thus, increased transcription and/or enhanced transporter protein synthesis would be needed only after the intracellular pool of inactive transporters is depleted. Alternatively, it is conceivable that the observed slow time course of the functional adaptation of the renal Na/H antiporter is caused by a similarly sluggish stimulation of Na/H antiporter mRNA expression. Thus, initially, changes in mRNA levels may be below the sensitivity of Northern analysis. This would be compatible with the observation that even 5 d of moderate to severe metabolic acidosis increased Na/H antiporter mRNA levels only moderately. To investigate the possibility that a more severe degree of metabolic acidosis could increase mRNA levels after 2 d, preliminary studies were performed by feeding rats 8 instead of 6 mmol/kg BW of  $\text{NH}_4\text{Cl}$  per d. However, no change in mRNA levels in comparison with controls was demonstrated.

The Na/H antiporter probe used in this study is known to localize a basolateral Na/H antiporter in the membranes of polarized intestinal cell lines  $\text{CaCO}_2$  and HT 29 (Huet, C., D. Louvard, C. Sardet, and J. Pouyssegur, unpublished results). Therefore, if we postulate the existence of a distinct apical Na/H antiporter not recognized by the basolateral probe, it is possible that more pronounced changes in mRNA levels of the apical form could exist.

Increased proximal tubule Na/H antiporter activity in response to respiratory acidosis in vivo has been demonstrated in the isolated rabbit S2 proximal tubule perfused in vitro, in rabbit proximal tubule cells grown in primary culture, and in rabbit brush border vesicles harvested from animals with respiratory acidosis (15–17). The reason(s) for the discrepant results in the different studies using rat and rabbit brush border vesicles (17–19) is not clear at this time. It is possible, however, that differences in species as well as the time and technique of harvesting and processing the vesicles may critically affect the results of the in vitro analysis in this preparation. The increased proximal tubule Na/H antiporter activity in response to respiratory acidosis may also be characterized by an increase in the maximal transport capacity ( $V_{\text{max}}$ , 17). These studies show that this functional adaptation involves mechanisms other than increased Na/H antiporter mRNA expression. In particular, increased Na/H antiporter mRNA expression can be excluded as a mechanism that mediates increased proximal tubule bicarbonate reabsorption via increased Na/H antiporter activity both in the adaptation phase (where a transient increase in net acid excretion occurs and Na/H antiporter activity has been found to be stimulated) and in the maintenance phase of respiratory acidosis.

To investigate whether differences in species could partly account for the lack of change in renal cortical Na/H antiporter mRNA expression, similar studies in rabbits and/or analysis of the Na/H antiporter activity of the rat proximal tubule Na/H antiporter in response to chronic respiratory acidosis by in vivo microperfusion would be interesting.

The differential stimulation of renal cortical Na/H antiporter mRNA expression in response to metabolic and respiratory acidosis raises interesting possibilities about the nature of the extra- and intracellular signal(s) that regulate(s) steady-state Na/H antiporter mRNA levels. Acute changes in extracellular bicarbonate concentration or  $\text{CO}_2$  tension yielding the same change in ambient pH have been shown to affect cell pH similarly (25). In addition, decreasing extracellular pH either by a decrease in bicarbonate concentration or an increase in  $\text{CO}_2$  tension enhances Na/H antiporter activity (16). This is consistent with the observation that Na/H antiporter activity is regulated allosterically by acute changes in intracellular proton concentration (26). If the aforementioned in vitro results (25) reflect the in vivo conditions of this investigation, the results from this study suggest that differential changes in cell pH probably are not the reason for the differential changes of Na/H antiporter mRNA expression in response to chronic metabolic and respiratory acidosis. In addition, cell pH in the mammalian proximal tubule has been shown to be similarly depressed in the early phase of metabolic (4, 27) and respiratory acidosis (15, 27, 28). Changes in cell bicarbonate concentration and cell  $\text{CO}_2$  tension and/or the respective secondary intracellular events that they elicit must therefore be considered as potential regulators of renal Na/H antiporter mRNA expression.

In conclusion, renal cortical Na/H antiporter mRNA expression is enhanced 5 d after induction of metabolic acidosis. This mechanism may be responsible, at least in part, for the increased Na/H antiporter activity in the proximal tubule in the new steady state. It remains to be determined whether the observed increases in steady-state mRNA levels are due to transcriptional and/or posttranscriptional regulatory events. In contrast, the increased proximal tubule Na/H antiporter activity and increased proximal tubule bicarbonate reabsorption in response to respiratory acidosis have to be attributed to mechanisms other than increased mRNA expression both in the adaptation and maintenance phase of chronic respiratory acidosis.

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