

Differential Regulation of Na/H Antiporter by Acid in Renal Epithelial Cells and Fibroblasts

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Abstract

Increased Na/H antiporter activity has been demonstrated after *in vivo* chronic metabolic acidosis as well as *in vitro* acid preincubation of cultured rabbit renal tubule cells. To study the underlying molecular mechanisms of this adaptive increase in Na/H antiporter activity, the present studies examined the effect of low pH media on Na/H antiporter activity and mRNA abundance in cultured renal tubule cells. Na/H antiporter activity was increased by 60% in a mouse renal cortical tubule cell line (MCT), and by 90% in an opossum kidney cell line (OKP) after 24 h of preincubation in acid (low [HCO₃]) media. The ethylisopropylamiloride sensitivity of the Na/H antiporters were different in these two cell lines (MCT IC₅₀ = 65 nM; OKP IC₅₀ = 4.5 μM). In MCT cells, Na/H antiporter mRNA abundance measured by RNA blots increased by two- to fivefold after 24 h in low [HCO₃] media. Na/H antiporter mRNA abundance was also increased in MCT cells with high CO₂ preincubation as well as in rat renal cortex with *in vivo* chronic acid feeding. In contrast to renal epithelia, acid preincubation of NIH 3T3 fibroblasts led to suppression of Na/H antiporter activity. RNA blots of 3T3 fibroblasts revealed the same size Na/H antiporter transcript as in MCT cells. However, Na/H antiporter mRNA levels were suppressed by acid preincubation. These studies demonstrate differential regulation of Na/H antiporter activity and mRNA abundance in renal epithelial cells and fibroblasts in response to an acidotic environment. (*J. Clin. Invest.* 1991. 88:1703–1708.) Key words: metabolic acidosis • adaptation • epithelia • H transport • mRNA

Introduction

Chronic metabolic acidosis causes an increase in proximal tubule Na/H antiporter activity which persists when the transporter is removed from the acidotic environment (1–6). Similarly, preincubation of cultured proximal tubule cells in acid medium leads to an increase in Na/H antiporter activity that persists when the cells are removed from the acid milieu (7, 8). This adaptation occurs in primary proximal tubule cell cultures, but does not occur in primary fibroblast cultures (7).

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Although this increase in Na/H antiporter activity was dependent on protein synthesis (7), the molecular nature of this adaptation has not been defined. Sardet et al. have cloned a human Na/H antiporter cDNA that predicts an 815–amino acid glycoprotein with 10 putative transmembrane domains and a cytoplasmic COOH-terminus (9). The purpose of the present studies was to utilize the cloned cDNA to examine whether preincubation in acid extracellular pH *in vitro* and chronic acid feeding *in vivo* lead to increased abundance of Na/H antiporter mRNA, and whether a similar adaptation occurs in nonepithelial cells. The results demonstrate that acid preincubation leads to increased Na/H antiporter activity and mRNA abundance in renal cells. Acid preincubation of fibroblasts led to a decrease in Na/H antiporter activity and mRNA abundance. *In vivo* acid feeding also causes an increase in renal cortical Na/H antiporter mRNA abundance.

Methods

Cell culture. MCT cells (gift from Dr. Tom Haverty) were grown in RPMI medium with 5% FBS in a humidified atmosphere with 5% CO₂ at 37°C and subcultured upon reaching confluence. For acid preincubation experiments, cells at passage 26–50 were subcultured in DMEM/Ham's F12 medium (1:1) supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin (pen/strep) and 10% FBS. FBS was removed after 12–24 h of plating, and cells were grown to confluence in 3–4 d and then split to acid and control preincubations for defined periods of time. Control medium was DMEM/Ham's F12 with pen/strep. Acid medium was prepared by titrating control medium with HCl to lower pH by 0.3 U (and [HCO₃] by 50%). The high PCO₂ acid preincubation was performed with control medium in 10% ambient CO₂ yielding a similar 0.3-U drop in medium pH. All experiments were conducted in a paired fashion with simultaneous acid and control preincubations performed in parallel.

NIH 3T3 fibroblasts were grown in DMEM with 4.5 mg/ml glucose, pen/strep, and 7% calf serum (CS) to confluence and rendered quiescent by incubation in DMEM/Ham's F12 (1:1) with pen/strep and 0.5% CS for 24 h. Acid and control preincubation protocols were similar to that of MCT cells except that 0.5% CS was included in both the acid and control preincubation medium.

OKP cells (gift from Dr. K. Hruska) are a clonal subline of the opossum kidney (OK) cell line originally described by Cole et al. (10). OKP cells were maintained in DMEM with 4.5 mg/ml glucose supplemented with pen/strep and 7% FBS. For acid preincubation experiments, OKP cells between passages 16–18 were grown to confluence, rendered quiescent by serum deprivation for 48 h, and subjected to acid (low [HCO₃]) and control preincubation as described for MCT cells.

Measurement of intracellular pH and Na/H antiporter activity. After 24 h of acid or control preincubation, cells were removed from the experimental environment and Na/H antiporter activity was assayed. Intracellular pH was measured with the pH-sensitive fluorescent dye, 2',7'-bis(2-carboxyethyl)-5-(and-6)carboxy-fluorescein (BCECF) as described previously (7). Confluent monolayers on glass cover slips were loaded with 10 μM of the acetoxymethyl derivative of BCECF and studied at 37°C under continuous perfusion in a computer controlled spectrofluorimeter (λ_{ex} alternating between 450 and 500 nm; λ_{em} 530

nm). Intracellular pH was estimated from the fluorescence intensity ratio (λ_{ex} 500/450) corrected for background, and calibrated with the nigericin technique at the end of each day at pH 6.40, 7.00, and 7.60 (11). Na/H antiporter activity was assayed as the initial rate of Na-dependent pHi recovery (dpHi/dt, pH units per minute) after an acid load imposed by NH_3/NH_4 prepulse in the absence of $\text{CO}_2/\text{HCO}_3^-$ as described previously (7). For the inhibitor sensitivity studies, 10^{-9} to 10^{-4} M ethylisopropylamiloride (EIPA)¹ was added to the extracellular fluid before and during Na-induced alkalization. Inhibitor sensitivity was expressed as the IC_{50} of the inhibition of dpHi/dt by EIPA. Intracellular buffer capacity was calculated as previously described (7). Results are expressed as mean \pm SE and statistical significance was evaluated by the unpaired *t* test.

RNA preparation and analysis. Total cellular RNA was extracted using a modification of the method of Chirgwin et al. (12). After preincubation in acid or control medium, cells were homogenized in 4 M guanidinium thiocyanate, 0.5% (wt/vol) *N*-lauroylsarcosine, 0.1 M 2-mercaptoethanol, and 25 mM sodium citrate (pH 7.0), pelleted by ultracentrifugation through a 5.7 M CsCl cushion, further purified by phenol/chloroform extraction and ethanol precipitation, and poly-A selected by oligo-dT column chromatography. Poly-A⁺ RNA samples were fractionated by agarose-formaldehyde gel electrophoresis and transferred to nylon filters (GenescreenPlus; New England Nuclear, Boston, MA). After 4 h of prehybridization at 45°C with 5 \times Denhardt's (1 mg/ml each of Ficoll, PVP, BSA), 5 \times SSC (0.75 M NaCl; 75 mM Na₂ citrate), 50% formamide, 0.5% SDS, and 0.5 mg/ml sheared salmon sperm DNA, the nylon filters were hybridized in the above solution plus 20–40 \times 10⁶ cpm of a single-stranded cDNA probe (see below) for 16 h at 45°C. After hybridization, membranes were washed for 15 min in 2 \times SSC and 0.1% SDS at room temperature and for 60 min in 0.1 \times SSC and 2% SDS at 55°C. The membranes were then exposed to Kodak X-Omat film with intensifying screens at –70°C for 24–36 h when probing for the Na/H antiporter. The nylon filters were then stripped and reprobed for β -actin mRNA under similar hybridization and washing conditions and exposed to film for 1–2 h. For blots where Na/H antiporter and β -actin probes were hybridized simultaneously, the specific activity of the β -actin probe was made to be 10–15% of that of the antiporter probe to obtain comparable signals on autoradiography. For blots where 18s ribosomal RNA was probed, hybridization condition was similar except that washes were performed at 65°C instead of 55°C. Autoradiograms were scanned with a Hoefer laser densitometer and signals were quantified with the GS-365-W software program (Hoefer Scientific Instruments, San Francisco, CA). All Na/H antiporter signals were normalized to β -actin unless indicated otherwise. All comparisons between acid and control incubations were made from paired experiments.

Probe synthesis. For Na/H antiporter probe synthesis, a 713-bp Bam HI/Sac I restriction fragment of the human c28 clone (n325-1038) (9) was subcloned into the vector pTZ18. Single-stranded sense DNA was prepared and used as template for specific synthetic oligonucleotide (n634-651) primed synthesis of a uniformly ³²P-labeled single-stranded antisense probe (n325-651) with specific activities of $\sim 10^9$ cpm/ μ g using a modification of the method of Church and Gilbert (13, 14). A uniformly labeled single stranded β -actin probe was synthesized in a similar fashion from a β -actin cDNA subcloned in bacteriophage M13 (gift from Dr. Helen Hobbs). The 18S rRNA probe (gift from Dr. Chris Newgard) was a synthetic 50-mer that was end-labeled with λ -³²P-ATP and T₄ kinase to 10⁹ cpm/ μ g, and purified by cetylpyridium bromide precipitation (15).

Animal studies. Experiments were performed on male Sprague-Dawley rats using established acid feeding protocols (6). Animals weighing 200–300 g were housed in individual metabolic cages and

individually fed 20 g of diet daily consisting of (in grams per kilogram diet): casein, 180 g; cornstarch, 200 g; sucrose, 500 g; corn oil, 35 ml; peanut oil, 35 ml, CaHPO₄, 10 g; MgSO₄, 6 g; NaCl, 6 g; K₂PO₄, 8.3 g; and vitamin fortification mixture (ICN Pharmaceuticals, Cleveland, OH). The control animals were fed the above diet for 2–6 d and the acidotic rats were fed the same diet to which 20 mmol/kg body weight HCl was added daily. Animals were allowed water ad lib and all animals consumed all of the diet. At the end of the experimental period, animals were anesthetized with 100 mg/kg i.p. Inactin, arterial blood samples drawn, and kidneys harvested. For RNA preparation, cortices were immediately dissected from the kidneys, and homogenized in the guanidinium thiocyanate solution (1 g tissue/10 ml) described above. The remainder of the RNA preparation and Northern analysis was performed as described above. One lane of poly-A⁺ RNA was derived from a single animal.

Results

Acid preincubation (low [HCO₃]) increases Na/H antiporter activity in two renal cell lines with different EIPA sensitivities: MCT cells and OKP cells. We have previously shown that preincubation of proximal cells in primary culture in low [HCO₃]⁻ media leads to a persistent increase in Na/H antiporter activity (7). In the present study we examined the effect of low [HCO₃]⁻ acid preincubation on Na/H antiporter activity in two renal cell lines with characteristics of the proximal tubule, MCT and OKP cells. 24 hours of acid preincubation caused a 60% and a 90% increase in Na/H antiporter activity in MCT and OKP cells, respectively (Fig. 1). There was no difference in buffer capacity (mean \pm SE; MCT control vs. acid: 12.97 \pm 0.32 vs. 11.97 \pm 0.41 mM/pH units; OKP control vs. acid: 15.41 \pm 0.49 vs. 16.14 \pm 0.40 mM/pH units) or pHi at the time of Na addition (mean \pm SE; MCT control vs. acid: 6.40 \pm 0.02 vs. 6.39 \pm 0.01; OKP control vs. acid: 6.65 \pm 0.04 vs. 6.69 \pm 0.04) that could account for the augmented Na/H antiporter activity in cells preincubated in acid.

Based on different inhibitor kinetics, Haggerty et al. have postulated two pharmacological isoforms of the Na/H antiporter (16), one form that is EIPA-sensitive (IC_{50} in nanomolar range), and one form that is relatively EIPA-insensitive (IC_{50} in the micromolar range). In the present study, the Na-dependent alkalization was inhibited by EIPA in a dose-dependent fashion in both MCT and OKP cells (Fig. 2). Na/H antiporter activity was EIPA-sensitive in MCT cells with an IC_{50} of 65 nM,

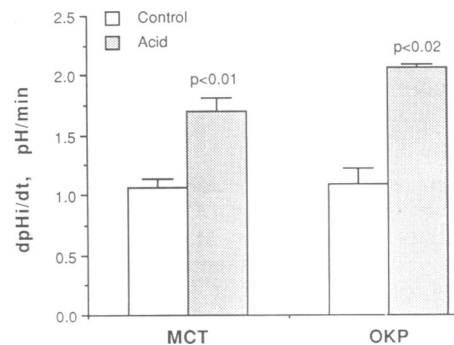


Figure 1. Effect of 24 h of low [HCO₃]⁻ acid preincubation on Na/H antiporter activity in MCT and OKP cells. Results are expressed as rate of Na-dependent pHi recovery after an acid load (dpHi/dt). Bars and error bars represent mean \pm SE. (MCT: control, *n* = 8; acid, *n* = 8. OKP: control, *n* = 10; acid, *n* = 10).

1. **Abbreviations used in this paper:** EIPA, ethylisopropylamiloride; PDG, phosphate-dependent glutaminase; PEPCK, phosphoenolpyruvate carboxykinase.

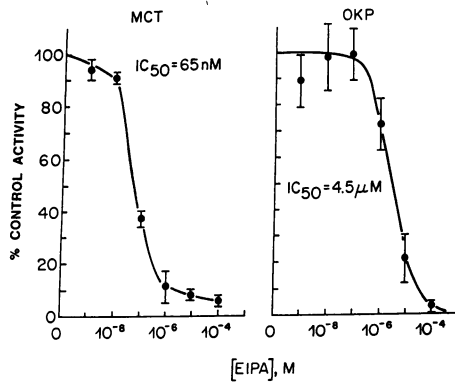


Figure 2. Dose-response curve for inhibition of Na/H antiporter activity by EIPA measured as the percent inhibition of Na-dependent pHi recovery in MCT and OKP cells. Each data point represents the mean \pm SE of five experiments. (MCT cells, $IC_{50} = 65$ nM; OKP cells, $IC_{50} = 4.5$ μ M).

whereas in OKP cells Na/H antiporter activity was relatively EIPA-insensitive, with an IC_{50} of 4.5 μ M.

Acid preincubation (low $[HCO_3^-]$) increases Na/H antiporter mRNA abundance in MCT cells. The adaptation of Na/H antiporter activity to low $[HCO_3^-]$ acid preincubation in proximal tubule cells in primary culture has previously been shown to depend on intact protein synthesis (7). We now examine whether the increase in Na/H antiporter activity seen with acid preincubation in a renal cell line is accompanied by increased levels of Na/H antiporter mRNA. We have demonstrated that Na/H antiporter activity is EIPA-sensitive in MCT cells and is EIPA-insensitive in OKP cells. Because the cloned cDNA of Sardet et al. codes for an EIPA-sensitive Na/H antiporter (Pouyssegur, J., personal communication), we proceeded to examine regulation of Na/H antiporter mRNA abundance by acid preincubation in MCT cells. Our probe did not detect a specific transcript in the 4.8–5.0-kb range in poly-A⁺ from OKP cells.

Under high stringency conditions, the cDNA probe hybridized to a single 4.8–4.9-kb transcript in MCT cells. Lowering of hybridization stringency ($5\times$ SSC, 30% formamide, 37°C) did not reveal additional bands. Fig. 3 shows a representative RNA blot of MCT cells preincubated in control or acid medium for 24 h. In this experiment, acid preincubation led to a 4.1-fold increase in Na/H antiporter mRNA abundance with no effect on β -actin mRNA. To study the time course of this increase in Na/H antiporter mRNA, we subjected MCT cells to 4, 12, and 24 h of acid preincubation and Na/H antiporter mRNA was quantified and normalized to that of β -actin. Acid preincubation for 4 h (relative abundance: acid/control in three experiments; 1.12, 0.93, 1.02), and 12 h (relative abundance: acid/control in three experiments; 1.42, 1.06, 1.28), did not lead to significant increases in Na/H antiporter mRNA abundance. After 24 h of acid preincubation, Na/H antiporter mRNA was increased from two- to fivefold in five independent experiments (relative abundance: acid/control; 4.07, 5.23, 3.00, 2.10, 2.56). This time course is depicted graphically in Fig. 4.

Acid preincubation (high P_{CO_2}) increases Na/H antiporter mRNA abundance in MCT cells. In proximal tubule cells in primary culture, we have demonstrated that Na/H antiporter activity was increased by preincubation with low $[HCO_3^-]$ or

high P_{CO_2} acid media. We next examined the effect of preincubation in high P_{CO_2} acid medium on Na/H antiporter mRNA levels in MCT cells. Fig. 5 is an RNA blot of an experiment where preincubation in high P_{CO_2} media led to an increase in Na/H antiporter mRNA by 2.4-fold when normalized to β -actin. In three other independent experiments, Na/H antiporter mRNA abundance was increased by 2.0-, 1.6-, and 3.2-fold after 24 h of high P_{CO_2} preincubation.

Acid preincubation decreases Na/H antiporter activity and mRNA abundance in NIH 3T3 fibroblasts. In contrast to proximal tubule cells in primary culture, Na/H antiporter activity was suppressed by acid preincubation in human foreskin fibroblasts in primary culture in our previous study (7). In the present study we examined the effect of acid preincubation on Na/H antiporter activity and mRNA levels in NIH 3T3 fibroblasts. The protocol was similar to that of MCT acid preincubation except that 0.5% CS was maintained in both acid and control media throughout the experiment. Na/H antiporter activity was suppressed to 60% of control after 24 h of acid preincubation (Fig. 6). Acid preincubation did not affect intracellular buffer capacity (control vs. acid: 9.05 ± 0.88 vs. 7.95 ± 0.59 mM/pH units) or pHi at the time of Na addition (control vs. acid: 6.87 ± 0.04 vs. 6.93 ± 0.04).

We next examined Na/H antiporter mRNA abundance by RNA blots. As shown in Fig. 7 A, a single \sim 4.9-kb transcript similar to that of MCT cells was detected in NIH 3T3 cells under high stringency conditions. In this experiment, 24 h of acid preincubation led to a decrease in Na/H antiporter mRNA levels to 20% of control and in β -actin mRNA to 60% of control when normalized to the total amount of poly-A⁺ RNA per lane (Fig. 7 A). This suppression of β -actin mRNA by acid preincubation was confirmed by quantifying the β -actin message in total cellular RNA using the 18S rRNA as a loading control (Fig. 7 B). Two other independent experiments were performed showing acid-induced suppression of Na/H antiporter mRNA to 30 and 22% of control and β -actin mRNA to 65 and 55% of control.

In vivo chronic acid feeding increases Na/H antiporter mRNA abundance in kidney cortex. To examine whether Na/H antiporter mRNA levels are modulated in whole cortical tissue in *in vivo* metabolic acidosis, we used a model of chronic acid feeding previously shown to induce adaptations of Na/H antiporter activity (6). Rats fed an acid diet had a slightly lower $[HCO_3^-]$ compared to controls after 2 d (control: pH = 7.35, $HCO_3^- = 24.9$, $P_{CO_2} = 45.6$; acid: pH = 7.26, $HCO_3^- = 11.8$, $P_{CO_2} = 26.1$), but $[HCO_3^-]$ returned to normal by 6 d (controls: pH = 7.42, $HCO_3^- = 22.8$, $P_{CO_2} = 35$; acid: pH = 7.31, $HCO_3^- = 23.0$, $P_{CO_2} = 45.6$). Fig. 8 is an RNA blot of renal cortical

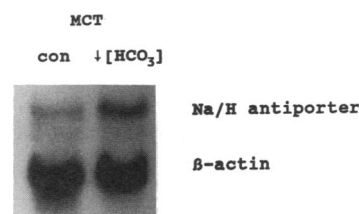


Figure 3. RNA blot from one representative experiment showing Na/H antiporter and β -actin transcript levels in MCT cells after 24 h of preincubation in low $[HCO_3^-]$ media. 15 μ g of poly-A⁺ RNA prepared as described in Methods were loaded per lane. Four other experiments showed similar results.

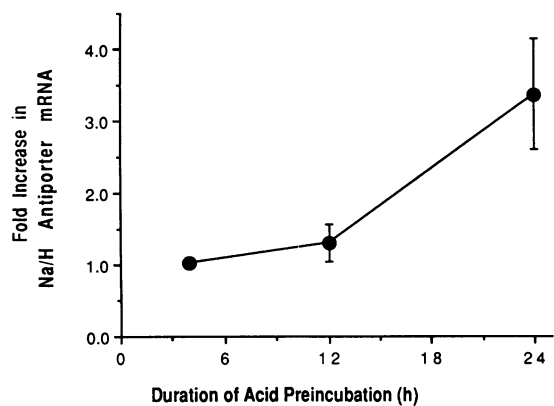


Figure 4. Time course of changes in Na/H antiporter mRNA level in response to 4, 12, or 24 h of acid preincubation. *y*-Axis shows the ratio of Na/H antiporter mRNA abundance of acid to control cells in paired experiments. Data points are mean \pm SE (2 h, *n* = 3; 12 h, *n* = 3; 24 h, *n* = 5).

poly-A⁺ RNA from rats fed either acid or control diet for 6 d. A similar 4.9-kb transcript was detected in rat cortex. When normalized to β -actin, Na/H antiporter mRNA abundance was 75 and 60% higher in animals fed an acid diet in two experiments. RNA blots performed after 2 and 4 d of acid feeding failed to detect a significant change in Na/H antiporter mRNA levels (relative abundance acid/control in four experiments: 2 d, 0.96, 1.05; 4 d, 1.06, 1.11).

Discussion

Chronic metabolic acidosis causes an increase in proximal tubule apical membrane Na/H antiporter activity which persists when the transporter is removed from the acidotic milieu (1–6). We found that acidic pH alone in vitro can induce a persistent increase in Na/H antiporter activity in proximal tubule cells in primary culture (7). This adaptation is thus independent of changes in hemodynamics, hormone levels, and renal nerve activity, all of which can modulate Na/H antiporter activity in the in vivo acidosis model. While the molecular nature of this increased activity is not known, previous kinetic studies have consistently revealed it to be a V_{max} effect (2, 4, 5), and we have shown in cultured cells that this adaptation is dependent on protein synthesis (7). We now demonstrate that acid preincubation leads to an increase in Na/H antiporter activity in MCT and OKP cells and an increase in Na/H antiporter

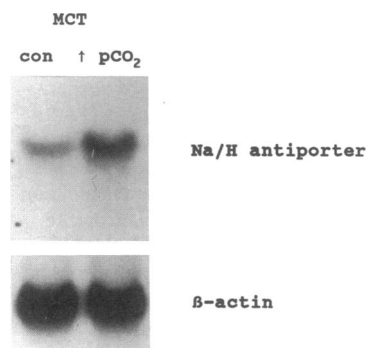


Figure 5. RNA blot from one experiment showing Na/H antiporter and β -actin transcript levels in MCT cells after 24 h of incubation in high PCO_2 media. 17 μ g of poly-A⁺ RNA were loaded per lane. Three other experiments showed similar results.

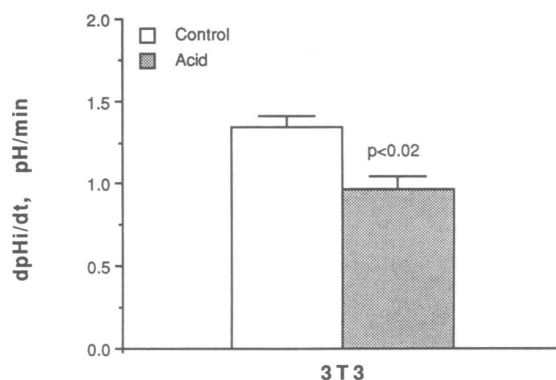


Figure 6. Effect of 24 h of low $[HCO_3^-]$ acid preincubation on Na/H antiporter activity in NIH 3T3 fibroblasts. Results are expressed as rate of Na-dependent pHi recovery (dpHi/dt) after an acid load and presented as mean \pm SE (control, *n* = 5; acid, *n* = 5).

mRNA levels in MCT cells. In a preliminary report, Igarashi et al. described similar results in LLC-PK₁/Cl₄ cells, a porcine renal epithelial cell line, where acid preincubation led to increased Na/H antiporter activity and mRNA levels (8). In our model, the increase in Na/H antiporter mRNA was demonstrable only after 24 h of acid preincubation. In growth-arrested vascular smooth muscle cells, serum addition induced a detectable elevation in Na/H antiporter mRNA after 2 h with peak elevation occurring at 24 h (17). The different time courses most likely reflect different cellular regulatory cascades initiated by growth factors as compared to acid.

In the in vivo acid feeding studies, the duration of acidosis required is considerably longer and the degree of increase in Na/H antiporter mRNA in renal cortex is much smaller than in the in vitro model. Our finding is similar to that reported by Krapf et al. (18). It is somewhat difficult to draw direct comparisons between the in vitro and in vivo studies because these systems are vastly different. One possible explanation is that the degree of acidosis in acid-fed rats is mild, whereas the extracellular acidosis in the cell culture model is more extreme. An-

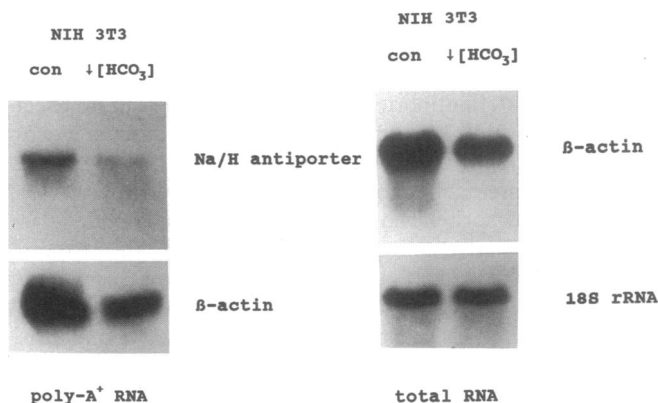


Figure 7. NIH 3T3 cells were preincubated in acid vs. control media for 24 h in low $[HCO_3^-]$ acid media and 19 μ g/lane of poly-A⁺ RNA was prepared and probed for the Na/H antiporter and β -actin transcript (A); 19 μ g/lane of total cellular RNA was prepared and probed for β -actin mRNA and the 18s rRNA (B). Two other experiments showed similar results.

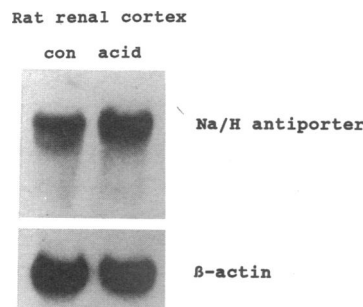


Figure 8. RNA blot of 15 μg of rat renal cortex poly-A⁺ RNA from animals fed either acid or control diet for 6 d and probed for Na/H antiporter and β -actin. Two independent experiments showed similar results.

other possibility is that other effects secondary to acidosis such as hemodynamic, neural, and hormonal alterations, modulate the response in vivo.

Whereas some investigators have demonstrated increased Na/H antiporter activity in chronic respiratory acidosis (19, 20), others have not (21, 22). In cultured proximal tubule cells, we and others have demonstrated increased Na/H antiporter activity after preincubation in high ambient CO₂ (7, 23). Using the human c28 cDNA, Krampf et al. found no increase in renal cortical Na/H antiporter mRNA in rats with hypercapnia (18). In the present study, we found that Na/H antiporter mRNA is increased in MCT cells exposed to a high PCO₂ for 24 h. The disparity between the in vivo results and the present study might be due to the smaller degree of acidosis achieved in vivo compared to in vitro hypercapnia, or due to compounding systemic alterations secondary to hypercapnia in vivo. Even in our cell culture model of hypercapnia, the magnitude of the increase in Na/H antiporter mRNA is smaller compared to that seen with low [HCO₃]⁻ acid media. This finding is similar to our previous studies in proximal tubule cells where high PCO₂ caused a smaller increase in Na/H antiporter activity compared to low [HCO₃]⁻ (7). The reason for this difference may be due to a smaller degree of intracellular acidosis seen in high PCO₂ compared to low [HCO₃]⁻ acid preincubation (7).

A major finding in this study is the differential regulation of the Na/H antiporter to acid preincubation in renal epithelial cells and fibroblasts. Thus far, acid preincubation has been shown to increase Na/H antiporter activity in several renal cell culture systems: primary culture of proximal tubule cells (7), LLC-PK₁ cells (8), MCT cells, and OKP cells. In contrast, Na/H antiporter activity was suppressed by acid in both fibroblasts in primary culture (7) and NIH 3T3 fibroblasts. One principal question is what cellular elements confer this differential regulation. One possibility is that specificity is due to different Na/H antiporter isoforms in the two types of tissue. Based on differential EIPA sensitivities, Haggerty et al. postulated the existence of both an apical EIPA-insensitive (IC₅₀ = 13 μM) and a basolateral EIPA-sensitive (IC₅₀ = 44 nM) pharmacological isoform of the Na/H antiporter in LLC-PK₁/Cl₄ cells (16). In a preliminary report by Igarashi et al., Na/H antiporter activities on both membranes in LLC-PK₁/Cl₄ cells adapted to acid preincubation (8). In the present study, we found that despite different EIPA sensitivities in MCT and OKP cells, activities of both isoforms responded to acid preincubation in a similar fashion. Thus, if two forms of the Na/H antiporter exist in renal epithelia, they both appear to respond similarly to acid, irrespective of their EIPA sensitivities.

We also observed that the same size Na/H antiporter transcript was increased with acid treatment in MCT cells, whereas

a decrease was seen in NIH 3T3 fibroblasts. Although the possibility exists that related yet distinct genes can give rise to partially homologous identical size transcripts that cross hybridize to the same probe at high stringency, it is more likely that the differential regulation observed stems from differential cellular regulatory mechanisms rather than different Na/H antiporter isoforms. The nature of this differential regulation is presently unclear.

While chronic acid feeding causes renal hypertrophy (24), acid incubation suppresses growth in fibroblasts. In the present study, acid preincubation suppressed β -actin mRNA levels in fibroblasts but did not affect that in MCT cells. This represents another gene that is differentially regulated in the two types of cells and may be related to the differential growth response.

Chronic metabolic acidosis causes increased activities of the proximal tubule apical membrane Na/H antiporter and Na/citrate cotransporter (24), the basolateral membrane Na/3HCO₃ cotransporter (5, 6), phosphate-dependent glutaminase (PDG), and phosphoenolpyruvate carboxykinase (PEPCK) (25). All of these transporters and enzymes have in common that they contribute to renal acidification, either by H-equivalent transport or by ammonia synthesis (25). Increased renal PDG transcript levels are detected after 12 h of acute metabolic acidosis (27) and are maximally increased after 7 d of metabolic acidosis (28). A similar increase in PDG mRNA is also seen with acid preincubation of renal cells in culture (29). This increase is primarily due to increased mRNA stability rather than increased transcription (27, 29). In the case of PEPCK, increases in both renal PEPCK mRNA and immunoreactive protein precede the increase in enzyme activity in vivo (27, 29–32). Increased transcription of the PEPCK gene has been demonstrated in chronic in vivo acidosis (27) and the 5'-flanking region of the PEPCK gene has been shown to increase transcription of a reporter gene in response to acid extracellular pH when expressed in a hepatoma and a renal epithelial cell line (33). In addition, increased PEPCK mRNA stability also accounts for some of the increased mRNA levels in a cell culture model of acidosis (29). At present, it is not known whether the increased Na/H antiporter mRNA abundance in acidosis is due to increased transcription, or decreased transcript degradation.

The possibility exists that the regulatory region of all of the above genes and/or mRNAs possess a common *cis* element which binds a *trans*-acting factor that is activated or synthesized in acidosis, resulting in increased rates of transcription and/or decreased rates of transcript degradation. The delayed time course suggests that synthesis of an intermediate factor (or factors) may be required before the activation of Na/H antiporter gene transcription. Systemic acid-base alterations cause markedly different responses of PEPCK mRNA in the liver and kidney in vivo, and acidosis increases PDG activity in kidney but not in brain and small intestine (34). We now find opposite effects of acid on Na/H antiporter mRNA in NIH 3T3 fibroblasts and MCT cells in vitro. These findings suggest that a key component of the "acid" signaling pathway is tissue specific.

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References

1. Cohn, D. E., S. Klahr, and M. R. Hammerman. 1983. Metabolic acidosis and parathyroidectomy increase Na/H exchange in brush border vesicles. *Am. J. Physiol.* 245:F217-F222.
2. Tsai, C.-J., H. E. Ives, R. J. Alpern, V. J. Yee, D. G. Warnock, and F. C. Rector, Jr. 1984. Increased V_{max} for Na/H antiporter in proximal tubule brush border vesicles from rabbits with metabolic acidosis. *Am. J. Physiol.* 247:F339-F343.
3. Kinsella, J. L., L. T. Cujdik, and B. Sacktor. 1984. Na/H exchange activity in renal brush border membrane vesicles in response to metabolic acidosis: the role of glucocorticoids. *Proc. Natl. Acad. Sci. USA.* 81:630-634.
4. Kinsella, J. L., T. Cujdik, and B. Sacktor. 1984. Na/H exchange in isolated renal brush border membrane vesicles in response to metabolic acidosis: kinetic effects. *J. Biol. Chem.* 259:13224-13227.
5. Akiba, T., V. K. Rocco, and D. G. Warnock. 1987. Parallel adaptation of the rabbit renal cortical sodium/proton antiporter and sodium/bicarbonate co-transporter in metabolic acidosis and alkalosis. *J. Clin. Invest.* 80:308-315.
6. Preisig, P. A., and R. J. Alpern. 1988. Chronic metabolic acidosis causes an adaptation in the apical membrane Na/H antiporter and basolateral membrane $\text{Na}(\text{HCO}_3)_2$ symporter in the rat proximal convoluted tubule. *J. Clin. Invest.* 82:1445-1453.
7. Horie, S., O. Moe, A. Tejedor, and R. J. Alpern. 1990. Preincubation in acid medium increases Na/H antiporter activity in cultured renal proximal tubule cells. *Proc. Natl. Acad. Sci. USA.* 87:4742-4745.
8. Igarashi, P., M. Ganz, and R. F. Reilly. 1990. Metabolic acidosis stimulates transport activity and transcript levels of the basolateral Na/H exchanger in LLC-PK1 cells. *J. Am. Soc. Nephrol.* 1:721. (Abstr.)
9. Sardet, C., A. Franchi, and J. Pouyssegur. 1989. Molecular cloning, primary structure, and expression of the human growth factor-activatable Na/H antiporter. *Cell.* 56:271-280.
10. Cole, J. A., L. R. Forter, W. J. Krause, and P. K. Thorne. 1989. Clonal sublines that are morphologically distinct from parental OK cells. *Am. J. Physiol.* 256:F672-F679.
11. Alpern, R. J. 1985. Mechanism of basolateral membrane H/OH/ HCO_3 transport in the rat proximal convoluted tubule: a sodium-coupled electrogenic process. *J. Gen. Physiol.* 86:613-636.
12. Chirgwin, J. J., A. E. Przybyla, R. J. MacDonald, and W. J. Rutter. 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry.* 18:5294-5299.
13. Hobbs, H. H., M. S. Brown, J. L. Goldstein, and D. W. Russell. 1986. Deletion of exon encoding cysteine-rich repeat of low density lipoprotein receptor alters its binding specificity in a subject with familial hypercholesterolemia. *J. Biol. Chem.* 261:13114-13120.
14. Church, G. M., and W. Gilbert. 1981. Genomic sequencing. *Proc. Natl. Acad. Sci. USA.* 81:1991-1995.
15. Geck, P., and I. Nasz. 1983. Concentrated, digestible DNA after hydroxyapatite chromatography and cetylpyridium bromide precipitation. *Anal. Biochem.* 135:264-268.
16. Haggerty, J. G., N. Agarwal, R. F. Reilly, E. A. Adelberg, and C. W. Slayman. 1988. Pharmacologically different Na/H antiporters on the apical and basolateral surfaces of cultured porcine kidney cells (LLC-PK1). *Proc. Natl. Acad. Sci. USA.* 85:6797-6801.
17. Rao, G. N., C. Sardet, J. Pouyssegur, and B. Berk. 1990. Differential regulation of Na/H antiporter gene expression in vascular smooth muscle cells by hypertrophic and hyperplastic stimuli. *J. Biol. Chem.* 265:19393-19396.
18. Krapf, R., D. Pearce, L. Lynch, X.-P. Xi, T. L. Reudelhuber, J. Pouyssegur, and F. C. Rector, Jr. 1991. Expression of rat renal Na/H antiporter mRNA levels in response to respiratory and metabolic acidosis. *J. Clin. Invest.* 87:747-751.
19. Talor, Z., W.-C. Yang, J. Schuffield, E. Sack, and J. A. L. Arruda. 1987. Chronic hypercapnia enhances V_{max} of Na/H antiporter of renal brush-border membranes. *Am. J. Physiol.* 253:F394-F400.
20. Krapf, R. 1989. Mechanisms of adaptation to chronic respiratory acidosis in the proximal tubule. *J. Clin. Invest.* 84:890-896.
21. Zeidel, M. L., and J. L. Siefter. 1988. Regulation of Na/H exchange in renal microvillus vesicles in chronic hypercapnia. *Kidney Int.* 34:60-66.
22. Northrup, T. E., S. Garella, E. Perticucci, and J. J. Cohen. 1988. Acidemias alone does not stimulate rat Na/H antiporter activity. *Am. J. Physiol.* 255:F237-F243.
23. Cowell, G., P. Pahlavan, L. Wang, and J. A. L. Arruda. 1990. Adaptive increase in Na/H antiporter activity induced by hypercapnia in primary cultures of rabbit proximal tubule cells. *J. Am. Soc. Nephrol.* 1:648. (Abstr.)
24. Fine, L. 1986. The biology of renal hypertrophy. *Kidney Int.* 29:619-634.
25. Jenkins, A. D., T. P. Dousa, and L. H. Smith. 1985. Transport of citrate across renal brush border membrane: effects of dietary acid and alkali loading. *Am. J. Physiol.* 249:F590-F595.
26. Alpern, R. J., D. K. Stone, and F. C. Rector, Jr. 1990. Renal acidification mechanisms. In *The Kidney*, 4th ed. B. M. Brenner and F. C. Rector, Jr., editors. W. B. Saunders Co., Philadelphia.
27. Hwang, J.-J., and N. P. Curthoys. 1991. Effect of acute alterations in acid-base balance on rat renal glutaminase and phosphoenolpyruvate carboxykinase gene expression. *J. Biol. Chem.* 266:9392-9396.
28. Tong, J., R. A. Shapiro, and N. P. Curthoys. 1987. Changes in the levels of translatable glutaminase mRNA during onset and recovery from metabolic acidosis. *Biochemistry.* 26:2773-2777.
29. Kaiser, S., and N. P. Curthoys. 1991. Effect of pH and bicarbonate on phosphoenolpyruvate carboxykinase and glutaminase mRNA levels in cultured renal epithelial cells. *J. Biol. Chem.* 266:9397-9402.
30. Iynedjian, P. B., F. J. Ballard, and R. W. Hansen. 1975. The regulation of phosphoenolpyruvate carboxykinase synthesis in rat kidney cortex. *J. Biol. Chem.* 250:5596-5603.
31. Cimbala, M. A., W. H. Lamers, K. Nelson, J. E. Monahan, H. Yoo-Waren, and R. W. Hanson. 1982. Rapid changes in the concentration of phosphoenolpyruvate carboxykinase mRNA in rat liver and kidney. *J. Biol. Chem.* 257:7629-7636.
32. Pollock, A. S. 1989. Induction of renal phosphoenolpyruvate carboxykinase mRNA: suppressive effect of glucose. *Am. J. Physiol.* 257:F145-F151.
33. Pollock, A. S., and J. A. Long. 1989. The 5' region of the rat phosphoenolpyruvate carboxykinase gene confers pH sensitivity to chimeric genes expressed in renal and liver cell lines capable of expressing PEPCK. *Biochem. Biophys. Res. Commun.* 164:81-87.
34. Tong, J., G. Harrison, and N. P. Curthoys. 1986. The effect of metabolic acidosis on the synthesis and turnover of rat renal phosphate-dependent glutaminase. *Biochem. J.* 233:139-144.