Adenosine Triphosphate Citrate Lyase Mediates Hypocitraturia in Rats

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Abstract

Chronic metabolic acidosis increases proximal tubular citrate uptake and metabolism. The present study addressed the effect of chronic metabolic acidosis on a cytosolic enzyme of citrate metabolism, ATP citrate lyase. Chronic metabolic acidosis caused hypocitraturia in rats and increased renal cortical ATP citrate lyase activity by 67% after 7 d. Renal cortical ATP citrate lyase protein abundance increased by 29% after 3 d and by 141% after 7 d of acid diet. No significant change in mRNA abundance could be detected. Hypokalemia, which causes only intracellular acidosis, caused hypocitraturia and increased renal cortical ATP citrate lyase activity by 28%. Conversely, the hypercitraturia of chronic alkali feeding was associated with no change in ATP citrate lyase activity. Inhibition of ATP citrate lyase with the competitive inhibitor, 4S-hydroxycitrate, significantly abated hypocitraturia and increased urinary citrate excretion fourfold in chronic metabolic acidosis and threefold in K^+-depletion. In summary, the hypocitraturia of chronic metabolic acidosis is associated with an increase in ATP citrate lyase activity and protein abundance, and is partly reversed by inhibition of this enzyme. These results suggest an important role for ATP citrate lyase in proximal tubular citrate metabolism. (*J. Clin. Invest.* 1996. 98:2381–2387.) Key words: urolithiasis • acidosis • citrate • hydroxycitrate • hypokalemia

Introduction

Urinary citrate plays an important role in preventing formation of calcium-containing kidney stones by chelating calcium, directly inhibiting the crystallization and precipitation of calcium, and interacting with Tamm-Horsfal proteins to inhibit Ca-oxalate crystallization (1, 2). Low urinary citrate occurs in approximately half of patients with renal stone disease (3). In many patients, this hypocitraturia can be ascribed to some form of metabolic acidosis (4, 5), excess acid generation (6, 7), or hypokalemia (decreased intracellular pH, pH_i[1]) (8, 9).

Citrate is freely filtered by the glomerulus and reabsorbed in the proximal tubule (10). Because the filtered load of citrate remains relatively constant, the rate of proximal tubular citrate reabsorption is the major determinant of urinary citrate excretion. The renal proximal tubule reabsorbs citrate through an apical membrane Na/citrate cotransporter (11, 12). Once absorbed across the apical membrane, it is believed that cytosolic citrate is transported into mitochondria and metabolized in the tricarboxylic acid cycle (13). However, in other tissues, an alternate pathway for citrate metabolism occurs in the cytoplasm through ATP citrate lyase, which cleaves citrate to oxaloacetate and acetyl CoA (14).

The purpose of the present study was to determine the role of ATP citrate lyase in renal citrate metabolism. The results demonstrate that the hypocitraturia of chronic metabolic acidosis and hypokalemia is associated with adaptive increases in ATP citrate lyase activity. In addition, inhibiting ATP citrate lyase with 4S-hydroxycitrate (15) attenuates the hypocitraturia of chronic metabolic acidosis and hypokalemia, demonstrating a role for this enzyme in mediating hypocitraturia.

Methods

Experimental design

Male Sprague-Dawley rats (180–200 g; Harlan Sprague-Dawley Inc., Indianapolis, IN) were treated with the following protocols.

Chronic acid feeding. The basic diet consisted of ground 4%-fat rat/mouse chow (Teklad Premier Laboratory Diets, Madison, WI). Animals were fed ad lib for 2 d to allow them to acclimate to the metabolic cages. After 2 d, control rats were pair fed to rats receiving the same diet, but with 0.28 M NH_4Cl in their drinking water.

Chronic alkali feeding. The animals were placed in metabolic cages and allowed to acclimate on a synthetic diet consisting of (in g/kg) 180 casein, 200 cornstarch, 500 sucrose, 35 corn oil, 35 peanut oil, 10 $CaHPO_4$, 6 $MgSO_4$, 6 NaCl, 8.3 K_2HPO_4, and 10-vitamin fortification mixture (ICN Nutritional Biochemicals, Cleveland, OH) for 2 d. Subsequently, control rats receiving the synthetic diet plus 6 mmol/kg body wt per d NaCl were pair fed to rats receiving the synthetic diet plus 6 mmol/kg body wt per day $NaHCO_3$.

Chronic K^+-deficiency. The animals were placed in metabolic cages and allowed to acclimate on a synthetic diet consisting of (in g/kg) 180 casein, 200 cornstarch, 500 sucrose, 35 corn oil, 35 peanut oil, 10 $CaHPO_4$, 6 $MgSO_4$, 6.8 Na_2HPO_4, 7.1 KCl, and 10-vitamin fortification mixture. After 2 d, control rats continued on this diet and were pair fed to rats receiving the same diet with NaCl substituted for KCl.

For the above groups, 24-h urine collections were obtained on day 7 in the presence of thymol to prevent bacterial digestion of citrate (16). On day 8, the animals were anesthetized with 100 mg/kg body wt Inactin (Promonta, Germany) by intraperitoneal injection. Arterial blood gases were obtained by aortic puncture through an abdominal incision. Kidneys then were harvested immediately, the outer capsule removed, and the kidney placed in ice cold PBS.

In vivo inhibition of ATP citrate lyase. 4S-hydroxycitrate inhibits the function of ATP citrate lyase in vivo (17). After 7 d of control, acid, or K^+-free diet, rats received either 2 mmol/kg body wt per day i.p. of 4S-hydroxycitrate (kindly provided by Hoffman-LaRoche, Nutley, NJ; neutralized with NaOH to pH 7.4) or an equal volume and concentration of saline. The injections were given at 0800 h for an

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1. *Abbreviation used in this paper:* pH_i, intracellular pH.

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additional 2 d of dietary protocol. During the second additional day, a 24-h urine sample was collected. Rats then were anesthetized and aortic blood was collected and analyzed.

Blood gas determinations were performed using a CMS System 1304 blood gas analyzer (Instrumentation Laboratory, Inc., Lexington, MA); electrolytes and creatinine were measured on an Astra 7 electrolyte analyzer (Beckman Instruments Inc., Fullerton, CA). Citrate measurements were performed using a citric acid enzymatic kit (Boehringer Mannheim Biochemicals, Indianapolis, IN) on the Cobas Fara II chemistry system (Roche Diagnostic Systems, Somerville, NJ).

Measurement of renal cortical apical membrane Na/citrate cotransporter

Kidneys were placed in ice cold PBS. The renal cortex was dissected away and minced on an iced petri dish. Cortical tissue was placed in 15 ml of buffer (300 mM mannitol, 16 mM Hepes/20 mM Tris, pH 7.4, 5 mM EGTA, and 100 μg/ml PMSF) and homogenized with a Polytron (Brinkman Instruments, Inc., Westbury, NY). Brush border membrane vesicles were isolated by Mg^{2+} precipitation and differential centrifugation as previously described (18). The final pellet was resuspended in 15 ml of intravesicular buffer consisting of (in mM) 200 mannitol, 50 KCl, 16 Hepes/12 Tris, pH 7.4, and incubated at 4°C for 1 h. Membranes were pelleted and resuspended in 0.5 ml of intravesicular buffer for transport studies.

Na-dependent citrate transport was determined in brush border membrane vesicles as previously described (18) using the difference between 1,5-^{14}C-citrate (New England Nuclear, Boston, MA) uptake in Na-free (consisting of [in mM] 100 choline Cl, 50 KCl, 16 Hepes/12 Tris, pH 7.4) and Na-containing ([in mM] 100 NaCl, 50 KCl, 16 Hepes/12 Tris, pH 7.4) extravesicular solutions. Increasing concentrations of 4S-hydroxycitrate, from 10^{-7} to 10^{-2} M, were added to the extravesicular fluids. We measured uptake over 10 s at room temperature using the Millipore filtration technique with 0.65-μm pore size cellulose nitrate filters (DAWP 2500; Millipore Corp., Bedford, MA).

Measurement of ATP citrate lyase activity

1 g renal cortical tissue was homogenized in 10 ml ice cold homogenization solution (300 mM sucrose, 5 mM KH_2PO_4, 1 mM EGTA, 0.1% BSA, 10 mM Na_3citrate, 5 mM Mops, pH 7.4, 50 μg/ml PMSF, and 4 μg/ml aprotinin) with 4 strokes of a Potter-Elvehjem. The homogenate was centrifuged at 48,000 *g* for 20 min at 4°C. The supernatant was placed at −20°C overnight in the presence of 6.5 mM dithiothreitol to reduce the sulfhydryl groups of the enzyme (19).

ATP citrate lyase activity was measured using the hydroxamate assay (20). Attempts to use the malate dehydrogenase coupled assay (21) were unsuccessful because of high native renal NADH dehydrogenase activity. The assay solution contained (in mM) 20 Na_3 citrate, 10 $MgCl_2$, 200 hydroxylamine, and 20 Tris-HCl, pH 8.4. Coenzyme A–dependent acetylation was determined by performing the assay with and without 0.1 mM CoA. 100 μg of cytosolic protein was added to the assay solution in a total volume of 475 μl. The reaction was started by adding 25 μl of 0.1 M ATP and allowed to continue for 30 min at 37°C. 1 ml of 12.5% trichloroacetic acid was added to stop the reaction. The amount of acetylhydroxamate generated was quantitated spectrophotometrically at 520 nm in the presence of $FeCl_3$ (0.2 M final concentration). The difference in absorbance between the reaction performed in the presence and absence of CoA determined the ATP citrate lyase specific generation of acetylhydroxamate.

Immunoblotting

Cortex was placed in TBS (150 mM NaCl/20 mM Tris-HCl, pH 7.4) containing protease inhibitors (100 μg/ml PMSF, 2 μg/ml aprotinin, 2 μg/ml leupeptin, 2 μg/ml pepstatin A, and 2 mM EDTA), and homogenized for four strokes of a Potter-Elvehjem. The homogenate was centrifuged at 10,000 *g* for 30 min at 4°C. The supernatant was size fractionated by SDS/PAGE on a 6% gel and electrophoretically transferred to nitrocellulose membranes. After blocking with 5% powdered milk and 0.1% Tween 20 in TBS for 1 h, blots were probed with a 1:300 dilution of a rabbit anti–rat ATP citrate lyase antibody (22) for 1 h, and washed three times with TBS containing 5% powdered milk and 0.5% Tween 20, and then once in PBS. Blots then were incubated in TBS with 0.5% Tween 20 and a 1:5,000 dilution of horseradish peroxidase–labeled anti–rabbit IgG and washed three times with TBS containing 0.5% Tween 20, and then once in PBS. Binding was detected by enhanced chemiluminescence (Amersham International, Little Chalfont, UK) and quantitated by densitometry.

Northern blotting

Renal cortical tissue was placed in a guanidium thiocyanate solution containing 4 M guanidium thiocyanate (Fisher Scientific Co., Fair Lawn, NJ), 0.75 M sodium citrate, 17 mM *N*-lauroyl-sarcosine, 0.1 M β-mercaptoethanol, and homogenized using a Polytron. Total RNA was isolated by acid phenol extraction, size fractionated by formaldehyde gel electrophoresis, and transferred to nylon membranes. Probing was performed as previously described (23) using a random-primed uniformly ^{32}P-labeled full length ATP citrate lyase cDNA (22). 18S cDNA (American Type Culture Collection, Rockville, MD) was used in sequential probing to control for loading. Densitometry was performed and results were normalized for 18S mRNA abundance.

Sigma Chemical Co. (St. Louis, MO) supplied all chemicals unless otherwise noted. All protein concentrations were determined using the bicinchoninic acid assay method (Pierce Chemical Co., Rockford, IL). Data are expressed as mean±SEM. All comparisons were made using the paired *t* test (Sigma Stat; Jandel Science, San Rafael, CA).

Table I. Plasma Values for Control and Experimental Groups on Respective Diets

	Acid feeding		Alkali feeding		K^+-deficiency	
	Control	Experimental	Control	Experimental	Control	Experimental
pH	7.41±0.02	7.35±0.02	7.42±0.02	7.41±0.01	7.41±0.01	7.37±0.01*
pCO_2 (mmHg)	38.9±1.1	39.0±1.1	39.1±1.3	39.3±1.1	40±2	43±2*
HCO_3 (meq/liter)	24.4±0.4	21.6±0.7*	25.6±0.3	25.4±0.7	25.4±0.4	25.4±0.3
Na (meq/liter)	142±1	145±2	140±1	140±1	142±1	143±1
K (meq/liter)	3.9±0.1	3.8±0.3	4.0±0.1	3.9±0.1	3.6±0.1	2.5±0.01*
Cl (meq/liter)	106±1	110±1*	110±1	109±1	107±1	107±1
Creatinine (mg/dl)	0.4±0.03	0.4±0.02	0.4±0.02	0.4±0.02	0.4±0.02	0.5±0.03*
Glucose (mg/dl)	165±5	159±5	198±3	202±3	187±5	174±8*
Citrate (mg/liter)	68±3	50±4*	41±3	38±1	36±2	40±2

$n = 6$ for acid and alkali groups, $n = 7$ for K^+-deficient group. $*P < 0.05$ vs. control.

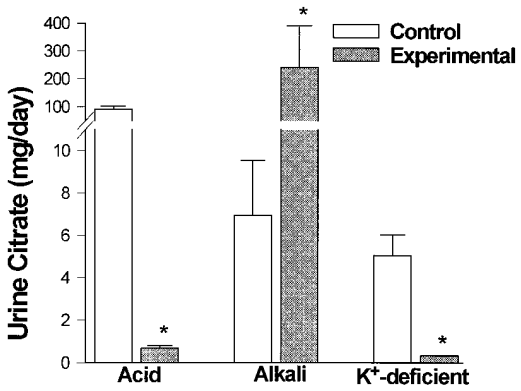

Figure 1. Urinary citrate excretion. Absolute urine citrate excretion during day 7 of either acid, alkali, or K^+-deficient diet protocols. $^*P < 0.05$ vs. control.

Results

Chronic metabolic acidosis increases renal cortical ATP citrate lyase activity. We reasoned that if ATP citrate lyase plays an important role in proximal tubular citrate metabolism, chronic metabolic acidosis may cause a chronic adaptation in its activity. To examine this, rats were pair fed either control or acid diets for 7 d. Control and experimental animals gained a similar amount of weight (in grams; control, 14±4; acid, 20±3, NS). As shown in Table I, acidotic animals developed a small but significant metabolic acidosis as evidenced by a decrease in their plasma (HCO_3^-) and blood pH, and increase in plasma (Cl^-). The absence of a compensatory respiratory alkalosis in the acidotic animals may have been due to the fact that blood gases were measured immediately after administration of anesthesia. Acid feeding also caused a significant decrease in plasma [citrate]. Although the magnitude of the metabolic acidosis was small, acid feeding caused a large decrease in absolute urinary citrate excretion (Fig. 1). As shown in Fig. 2, renal cortical ATP citrate lyase activity was increased by 67% in acid-fed rats.

Since the abundance of ATP citrate lyase is highest in liver (24), we examined whether acid feeding caused an adaptation in hepatic ATP citrate lyase activity. Liver homogenates were prepared and the assay was performed as in kidney cortex. While ATP citrate lyase activity was 50-fold higher in liver compared with kidney cortex, chronic metabolic acidosis did not affect enzyme activity (in micromoles acetylhydroxamate/milligram protein per 30 min; control, 1.30±0.07; acid, 1.22±0.07, $n = 6$).

To determine if alkali feeding had an opposite effect on renal cortical ATP citrate lyase activity, rats were administered $NaHCO_3$ for 7 d. Alkali-fed rats gained a similar amount of weight as control rats (in grams; control, 32±3; alkali, 38±3, NS) and, as shown in Table I, displayed no change in plasma composition. This is likely due to the kidneys' ability to correct metabolic alkalosis. Nevertheless, these animals did demonstrate an increase in absolute urinary citrate excretion (Fig. 1). In spite of the development of hypercitraturia, these animals demonstrated no change in renal cortical ATP citrate lyase activity (Fig. 2). This observation is analogous to that found with the apical membrane Na/citrate cotransporter, where chronic acid feeding caused an increase in transporter activity, but chronic alkali feeding had no effect (25).

Chronic metabolic acidosis increases renal cortical ATP citrate lyase protein abundance. To determine if changes in ATP citrate lyase protein abundance could explain the effect of acid feeding on ATP citrate lyase activity, we performed immunoblotting. As shown in Fig. 3 (lanes *4* and *5*), rabbit anti–rat ATP citrate lyase antibodies labeled a number of bands in kidney and liver homogenates in addition to the predicted 120-kD band (*arrow*, Fig. 3) (26). Three findings in Fig. 3 support the specificity of the 120-kD band. First, it is the only band labeled in both kidney and liver homogenates (lanes *4* and *5*). Second, purified ATP citrate lyase protein ran at 120 kD (lane *6*). Third, preincubation of the antibody with excess purified rat liver ATP citrate lyase protein prevented labeling of the pure enzyme, and the 120-kD band in liver and kidney (lanes *1–3*).

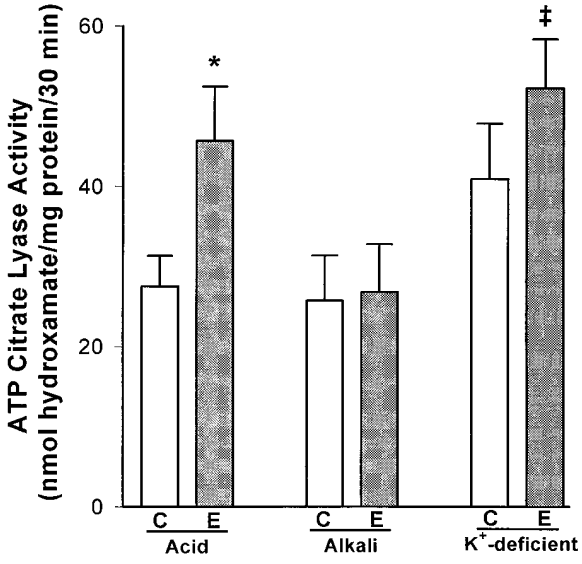

Figure 2. Chronic metabolic acidosis or K^+-deficiency increases ATP citrate lyase activity. Alkali feeding did not affect enzyme activity. ATP citrate lyase activity was determined as the generation of acetylhydroxamate in animals receiving either control (*C*) or experimental (*E*) diets for 7 d on chronic acid, alkali, or K^+-deficent protocols, $n = 6$. $^*P < 0.01$ and $^‡P < 0.05$ vs. control.

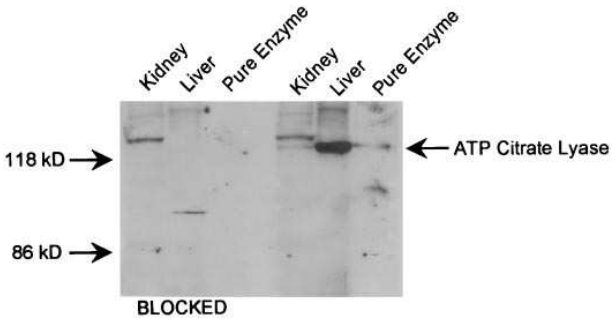

Figure 3. Specificity of anti–ATP citrate lyase antibody. Immunoblot with anti–ATP citrate lyase antibody (1:300 dilution) performed in the presence and absence of blocking with excess purified ATP citrate lyase (10 μg). Lanes were loaded with 50 μg renal cortical protein, 10 μg liver protein, or 0.5 μg purified rat liver ATP citrate lyase protein as indicated. The antibody labeled a 120-kD band in all three lanes, which was blocked by preincubation of antibody with antigen.

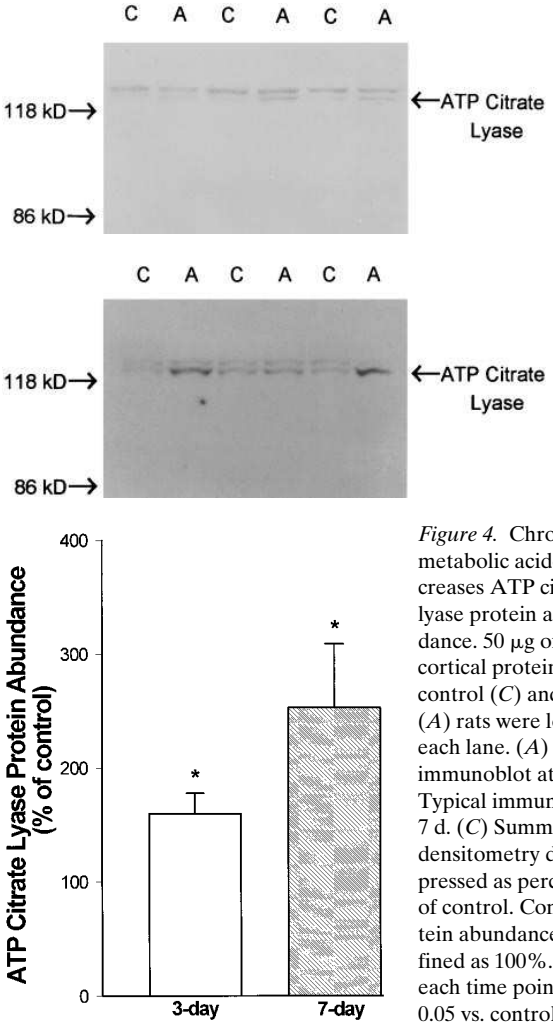

Figure 4. Chronic metabolic acidosis increases ATP citrate lyase protein abundance. 50 μg of renal cortical protein from control (*C*) and acid (*A*) rats were loaded in each lane. (*A*) Typical immunoblot at 3 d. (*B*) Typical immunoblot at 7 d. (*C*) Summary of densitometry data expressed as percentage of control. Control protein abundance is defined as 100%. $n = 6$ at each time point. $^*P <$ 0.05 vs. control.

Immunoblotting of renal cortex demonstrated an increase in ATP citrate lyase protein abundance by 29% after 3 d of acid and by 141% after 7 d ($n = 6$ at each time point, $P < 0.05$ for both, Fig. 4).

To examine whether the increase in ATP citrate lyase protein abundance was accompanied by a change in mRNA abundance, RNA blotting was performed on renal cortical RNA. The ATP citrate lyase probe labeled an appropriately sized band of 4.4 kb in total RNA. Representative blots are shown in Fig. 5. After 3 d, acid feeding caused an increase in mRNA abundance in four of six animals with a mean increase of 23% for the group. After 7 d, acid feeding was associated with an increase in mRNA abundance in three of six animals for a mean increase of 5%. Neither time point reached statistical significance.

Chronic K^+-deficiency increases ATP citrate lyase activity. Hypocitraturia is also observed in chronic K^+-deficiency (8, 27). Because chronic K^+-deficiency is associated with extracellular alkalosis and intracellular acidosis (9), it has been inferred that pH_i is the key regulator of proximal tubular citrate reabsorption (28). To address whether intra- or extracellular pH is the key regulator of ATP citrate lyase activity, we examined the effect of chronic K^+-deficiency on ATP citrate lyase activity. Rats fed a control diet were compared with rats pair fed a K^+-free diet for 7 d. A high sodium, K^+-free diet was used to prevent the development of metabolic alkalosis (29). As shown in Table I, rats fed a K^+-free diet developed hypokalemia. There was no change in plasma bicarbonate concentration. The K^+-deficient animals demonstrated a decrease in absolute urinary citrate excretion (Fig. 1). As shown in Fig. 2, K^+-deficient animals displayed a 28% increase in renal cortical ATP citrate lyase activity. Thus, these studies suggest that intracellular, rather than extracellular, acidosis is responsible for the increase in ATP citrate lyase activity.

Inhibition of ATP citrate lyase attenuates hypocitraturia of chronic metabolic acidosis and K^+-depletion. The above studies demonstrate that acidosis and K^+-deficiency increase ATP citrate lyase activity, but do not address whether this adaptation contributes to the hypocitraturia in these conditions. To address this, we examined the effect of the competitive inhibitor of ATP citrate lyase, 4S-hydroxycitrate, on urinary citrate excretion in rats on control, acid, and K^+-free diets. 4S-hydroxycitrate has been shown to inhibit ATP citrate lyase selectively without affecting citrate synthase, isocitrate dehydrogenase, aconitase, or the mitochondrial citrate/malate exchanger (30). However, it is not known whether this inhibitor affects the Na/citrate cotransporter in the renal proximal tubular apical mem-

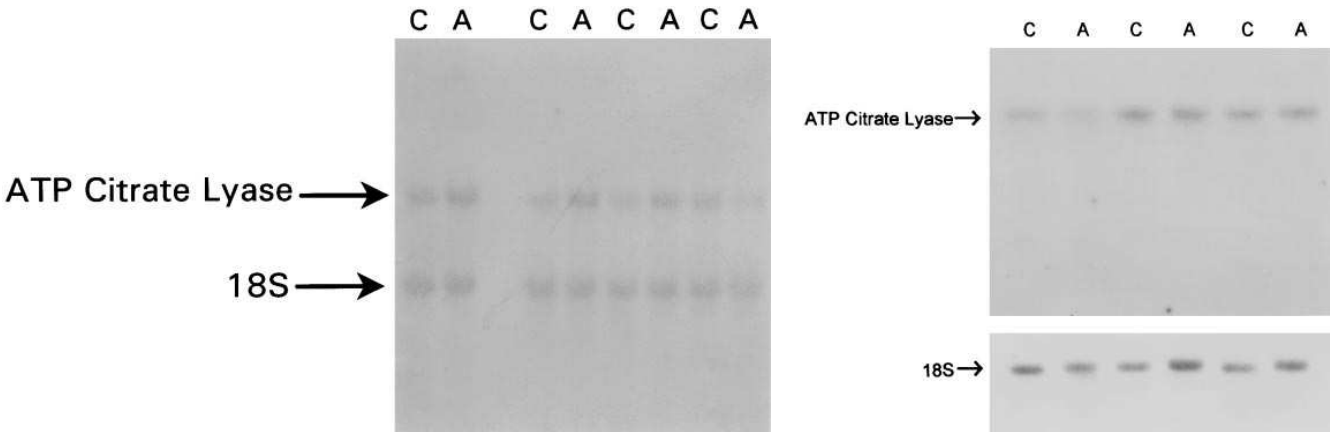

Figure 5. Chronic metabolic acidosis does not affect ATP citrate lyase mRNA abundance. 10 μg of renal cortical total RNA from control (*C*) and acid (*A*) rats were loaded in each lane. (*A*) Typical RNA blot at 3 d. (*B*) Typical RNA blot at 7 d. Blots were probed for ATP citrate lyase and 18S.

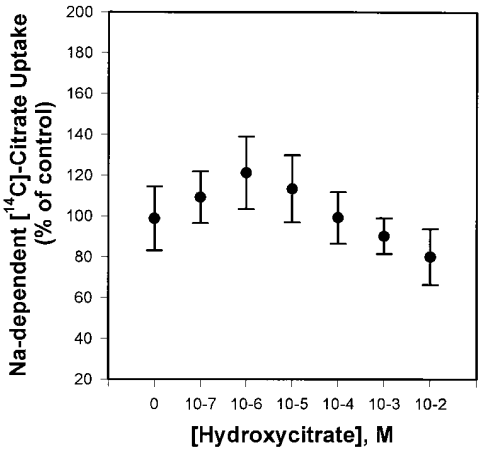

Figure 6. 4S-hydroxycitrate does not inhibit the proximal tubular apical membrane Na/citrate cotransporter. Na-dependent ^{14}C-citrate uptake measured in the presence of increasing concentrations of the ATP citrate lyase inhibitor, 4S-hydroxycitrate, is plotted. Uptake is expressed as percentage of control (in the absence of 4S-hydroxycitrate).

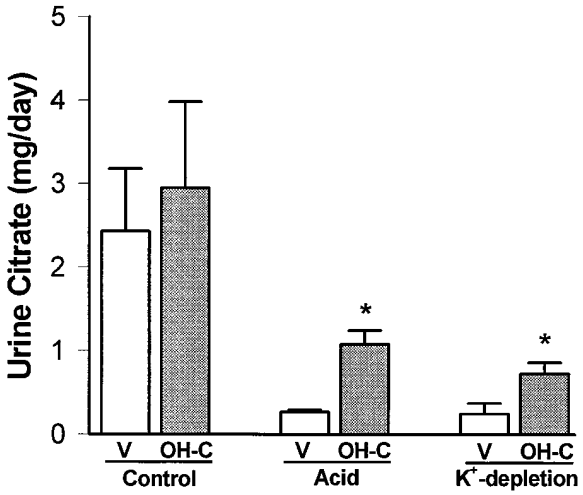

Figure 7. 4S-hydroxycitrate inhibits acidosis- and K^+-depletion–induced hypocitraturia. Rats ingested either control, acid, or K^+-free diets for 9 d, and received either vehicle (*V*) or 2 mmol/kg per d of 4S-hydroxycitrate (*OH-C*) on the last 2 d. 24-h urine samples were collected during day 2 of either vehicle or hydroxycitrate, $n = 6$ for control and acid, $n = 4$ for K^+-depletion. $^*P < 0.05$ vs. vehicle.

brane. Fig. 6 shows that at concentrations from 10^{-7} to 10^{-2} M, 4S-hydroxycitrate has no effect on Na-dependent citrate uptake in renal cortical brush border membrane vesicles when compared with control studies performed in the absence of inhibitor.

Rats were fed control, acid, or K^+-free diets for 9 d, and on the last 2 d received 2 mmol/kg per d 4S-hydroxycitrate (OH-citrate) or vehicle. Plasma values are shown in Table II. 4S-hydroxycitrate caused no significant changes in plasma composition. As shown in Fig. 7, acid feeding and K^+-depletion caused hypocitraturia. 4S-hydroxycitrate had a small, insignificant effect on urinary citrate excretion in control rats. In both acid-fed and K^+-depleted rats, 4S-hydroxycitrate significantly increased absolute citrate excretion four- and threefold, respectively. Thus, 4S-hydroxycitrate partially inhibits hypocitraturia induced by metabolic acidosis or K^+-depletion.

Discussion

The proximal tubule serves as the principal site of renal tubular citrate reabsorption and provides the major site for the regulation of renal citrate absorption and excretion (10). The first step in proximal tubular citrate absorption is citrate transport across the apical membrane on the Na/citrate cotransporter. This transporter is acutely stimulated by decreases in luminal pH, which modulate the relative concentrations of citrate^{-2} (the transported substrate) and citrate^{-3} (31). In addition, chronic acidosis and K^+-depletion cause an adaptive increase in transporter activity that persists when transporter activity is measured in vitro (18, 25). After citrate enters the cell, it can be transported into and metabolized by mitochondria in the tricarboxylic acid cycle (13). Simpson and Hager (32) have shown that decreases in extramitochondrial [HCO_3^-] acutely stimulate mitochondrial citrate uptake. However, it is not clear

Table II. Plasma Values for Animals Receiving Control, Acid, or K^+-free Diets Receiving Vehicle or the Competitive Inhibitor of ATP Citrate Lyase, 4S-hydroxycitrate (OH-citrate)

	Control		Acid		K^+-depletion	
	Vehicle	OH-citrate	Vehicle	OH-citrate	Vehicle	OH-citrate
pH	7.47±0.01	7.48±0.02	7.45±0.01	7.42±0.03	7.49±0.01	7.50±0.02
pCO_2 (mmHg)	34.7±1.3	31.3±1.2	35.5±0.8	35.3±2.2	32.6±0.3	32.3±1.0
HCO_3 (meq/liter)	25.2±0.3	23.2±0.8	25.2±0.6	23.2±0.8	24.9±0.3	25.3±0.4
Na (meq/liter)	142±1	144±1*	143±1	144±1	144±1	141±1*
K (meq/liter)	3.2±0.2	3.2±0.1	3.1±0.2	2.9±0.1	2.1±0.1	2.1±0.1
Cl (meq/liter)	110±3	111±2	111±1	113±1*	108±1	105±1*
Creatinine (mg/dl)	0.4±0.04	0.4±0.04	0.5±0.03	0.5±0.02	0.6±0.03	0.7±0.03
Glucose (mg/dl)	188±7	198±6	186±6	186±5	187±11	192±17
Citrate (mg/liter)	46±2	41±4	49±2	39±2*	44±3	39±3

$n = 6$ for control and acid, $n = 4$ for K^+-depletion. $^*P < 0.05$ vs. vehicle group.

whether in vivo changes in pH_i and cytosolic [HCO_3^-] are sufficient to elicit this effect.

In other tissues, mitochondria produce and export citrate to the cytosol. In the cytosol, ATP citrate lyase catalyzes the conversion of citrate and CoA to oxaloacetate and acetyl CoA. The acetyl CoA is used in various acetylation processes and for the synthesis of fatty acids and cholesterol, while oxaloacetate enters the gluconeogenic pathway through phosphoenolpyruvate carboxykinase. In liver tissue, citrate is transported to the cytosol and cleaved by ATP citrate lyase for the production of fat (33), cholesterol (34), and glucose (35). Cytosolic citrate in the brain is catabolized by ATP citrate lyase for the formation of acetylcholine and myelin (36).

Kidney tissue homogenates possess the third highest activity of ATP citrate lyase, with brain and liver tissue having higher activities (24). Because of this high rate of activity, we hypothesized that this pathway could serve an important role in citrate metabolism in the proximal tubule. The most commonly used assay to assess ATP citrate lyase activity determines the formation of oxaloacetate by measuring consumption of NADH by malate dehydrogenase (21). However, when we attempted to employ this method, the magnitude of native renal NADH dehydrogenase activity interfered with that of malate dehydrogenase. We therefore turned to the hydroxamate assay. This assay measures the generation of acetylhydroxamate from acetyl CoA and hydroxylamine, and is useful in tissues with high intrinsic NADH dehydrogenase activity (21).

We found that chronic metabolic acidosis caused an increase in renal cortical ATP citrate lyase activity. However, this adaptation is tissue selective. Chronic metabolic acidosis did not affect hepatic ATP citrate lyase activity. This finding is similar to the effect of acidosis on phosphoenolpyruvate carboxykinase activity where it is also increased in the kidney and unaffected in liver (37).

Both chronic metabolic acidosis and K^+-deficiency are associated with hypocitraturia (13). As these conditions have differential effects on extracellular pH but exhibit a similar decrease in pH_i, it has been inferred that the key determinant of hypocitraturia is intracellular acidosis (13). A similar increase in renal cortical ATP citrate lyase activity was elicited with chronic K^+-deficiency as with chronic metabolic acidosis, again suggesting that decreases in pH_i mediate this effect. In addition, converting enzyme inhibition, which may be expected to lower proximal tubular pH_i (38), causes hypocitraturia and is also associated with an increase in ATP citrate lyase activity (39). These results demonstrate a strong correlation between hypocitraturia and increased ATP citrate lyase activity, suggesting that regulation of ATP citrate lyase activity may contribute to hypocitraturia.

To demonstrate this further, we examined the effect of the specific inhibitor of ATP citrate lyase, 4S-hydroxycitrate, on urinary citrate excretion. This compound increased urinary citrate excretion fourfold in acid-fed rats and threefold in K^+-depleted rats, significantly blunting the hypocitraturia in these two states. Together these studies suggest an important role for a proximal tubular cytoplasmic pathway involving ATP citrate lyase in citrate metabolism. In addition, the upregulation of this pathway plays an important role in hypocitraturia. The lack of complete reparation of the hypocitraturia by 4S-hydroxycitrate may be due to a lack of complete inhibition of the enzyme in vivo or may be due to the existence of other regulated pathways.

At least one component of the increase in renal cortical ATP citrate lyase activity associated with chronic metabolic acidosis is an increase in ATP citrate lyase protein abundance. In our studies, we found small but statistically insignificant changes in ATP citrate lyase mRNA abundance. These results are similar to those we found when we examined the effect of chronic metabolic acidosis on the brush border membrane Na/H antiporter, Na/H exchanger–3 (40). Chronic metabolic acidosis caused an increase in Na/H antiporter activity and Na/H exchanger–3 protein abundance, but no significant change in Na/H exchanger–3 mRNA abundance. It is possible that while the increase in ATP citrate lyase mRNA after 3 d was not statistically significant, this change in mRNA is sufficient to lead to cumulative changes in ATP citrate lyase protein synthesis and abundance. Alternatively, the results suggest that metabolic acidosis may have a direct effect on ATP citrate lyase protein synthesis or degradation.

Chronic metabolic acidosis causes an increase in proximal tubular apical membrane uptake and metabolism of citrate. The present study shows that chronic metabolic acidosis increases the activity and protein abundance of renal cortical ATP citrate lyase and that upregulation of this enzyme plays an important role in the generation of hypocitraturia. ATP citrate lyase metabolizes citrate to oxaloacetate and acetyl CoA. The oxaloacetate generated is metabolized through phosphoenolpyruvate carboxykinase, the activity of which is also increased in chronic metabolic acidosis (37). Overactivity of ATP citrate lyase may provide an etiology for hypocitraturia, and inhibition of this enzyme may provide a target for treatment of hypocitraturia.

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