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Converting enzyme inhibition causes hypocitraturia independent of acidosis or hypokalemia

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Background. Angiotensin II stimulates the proximal tubular Na/H antiporter and increases proximal tubular cell pH. Because intracellular pH may affect urinary citrate excretion and enzymes responsible for renal citrate metabolism, the present studies examined the effect of enalapril, an angiotensin converting enzyme inhibitor, on the activity of renal cortical ATP citrate lyase and urinary citrate excretion.

Methods. Enalapril was given to rats (15 mg/kg/day) for seven days and to humans (10 mg twice daily) for 10 days. Blood and 24-hour urine samples were obtained in both groups. Renal cortical tissue from rats was analyzed for enzyme activity.

Results. In rats, enalapril decreased urinary citrate excretion by 88%. The change in urinary citrate was not associated with a difference in plasma pH, bicarbonate nor potassium concentration. However, similar to metabolic acidosis and hypokalemia, enalapril caused a 42% increase in renal cortical ATP citrate lyase activity. When given to humans, enalapril significantly decreased urinary citrate excretion and urine citrate concentration by 12% and 16%, respectively, without affecting plasma pH or electrolytes.

Conclusions. Enalapril decreases urinary citrate in rats and humans. This is due, at least in part, to increases in cytosolic citrate metabolism through ATP citrate lyase in rats similar to that seen with chronic metabolic acidosis and hypokalemia. The effects of enalapril on urinary citrate and renal cortical ATP citrate lyase occur independently of acidosis or hypokalemia but may be due to intracellular acidosis that is common to all three conditions.

Urinary citrate is freely filtered in the glomerulus and then reabsorbed in the proximal tubule [1]. Hypocitraturia, an important cause of kidney stones [2], occurs by an increase in proximal tubule reabsorption [3]. Chronic metabolic acidosis (CMA) [4] or hypokalemia [5] causes hypocitraturia with concomitant adaptive increases in proximal tubular apical membrane Na-citrate cotransporter (NaDC-1) activity [6, 7], and protein and mRNA abundance [8]. Once reabsorbed, citrate is metabolized either in the mitochondria through the tricarboxylic acid cycle [9] or in the cytosol through adenosine 5′-triphosphate (ATP) citrate lyase, which cleaves citrate to acetyl CoA and oxaloacetate [10]. The oxaloacetate enters the gluconeogenic pathway via an increase in phosphoenolpyruvate carboxykinase observed in CMA [11]. Since both CMA and hypokalemia decrease renal cortical intracellular pH (pH_i) [12], these adaptations are thought to occur through a change in pH_i [1, 3].

Angiotensin II (Ang II) increases pH_i by activating an amiloride-sensitive apical membrane Na/H antiporter in the proximal tubule [13]. Angiotensin converting enzyme inhibitors, by decreasing Ang II production, may decrease pH_i and cause hypocitraturia. To determine if inhibiting Ang II production could affect urinary citrate excretion, we administered enalapril first to rats and then humans. The results demonstrate that this therapy decreases urinary citrate excretion in both species. In rats, the change in urinary citrate excretion is associated with an increase in renal cortical ATP citrate lyase activity, similar to that seen in rats with CMA and hypokalemia [10].

METHODS

Animals

Male Sprague-Dawley rats, weighing 200 to 220 grams, were housed in individual metabolic cages and allowed to acclimate on a synthetic diet *ad libitum* for three days. The diet consisted of (in g) 180 casein (ICN Biomedicals, Inc., Costa Mesa, CA, USA), 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 Biomedicals, Inc.). Animals were allowed free access to water during the acclimation and experimental periods.

Key words: angiotensin converting enzyme inhibitor, citrate, rat, human, ATP citrate lyase.

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Experimental protocol

After three days of acclimation, all rats were weighed and experimental rats received powdered enalapril (gift from Merck & Co., West Point, PA, USA) 15 mg/kg mixed into the control diet. They were fed a maximum of 20 g diet daily for one week. Control rats were pair-fed with only the control diet. On the sixth day of the experimental period, a 24-hour urine collection for total volume, sodium, potassium, chloride, creatinine and citrate was obtained in the presence of thymol to prevent bacterial digestion of the citrate [14]. On the seventh day, rats were anesthetized with 100 mg/kg of Inactin (Promonta, Germany) intraperitoneally. Blood was obtained by aortic puncture for sodium, potassium, chloride, bicarbonate, pCO_2, pH, glucose and creatinine.

Tissue preparation

Kidneys were harvested, decapsulated and placed in ice-cold homogenization buffer containing (in mM), 300 sucrose, 5 K_2HPO_4, 1 EDTA, 10 NaCitrate at pH 7.4. Kidneys were then placed on a petri dish over ice and the medulla dissected away. Cortical tissue was homogenized at medium speed with a loose-fitting Potter-Elvehjem in 10 × volume homogenization solution with 1 mg/ml phenylmethylsulfonyl fluoride, 2 mg/ml aprotinin and 7 mM dithiotheitol added at harvest. Solid material was removed by centrifugation at 48,000 g and the supernatant was placed at −20°C overnight.

Measurement of ATP citrate lyase activity

Adenosine 5′-triphosphate (ATP) citrate lyase activity was measured using the hydroxamate assay in which hydroxylamine is deaminated and acetylated [15]. The assay was performed as previously published with generation of acetylhydroxamate measured spectrophotometrically [10]. Due to high intrinsic NADH oxidase activity in the kidney, a malate dehydrogenase-linked assay could not be used [10]. Protein concentration was determined using a bicinchoninic acid protein assay kit (Pierce Chemical Co., Rockford, IL, USA) with bovine serum albumin as a standard.

Human study

Ten normal adult volunteers (6 females and 4 males, ages 24 to 43) participated in the study after written consent was obtained. They were free of heart disease, hypertension, edema, hypokalemia, urolithiasis, chronic diarrheal states, diabetes mellitus, and renal disease. Normal subjects involved in daily physical exercise were excluded. Subjects receiving medication such as converting enzyme inhibitors, and non-steroidal anti-inflammatory drugs were also excluded.

The subjects participated in 13 days of study that consisted of two three-day phases. During those phases on days 1 to 3 (baseline) and 10 to 13 (enalapril), subjects ingested a frozen metabolic diet with a daily composition of 400 mg calcium, 800 mg phosphorus, 100 mEq sodium, 60 mEq potassium, and 3 liters of distilled water. For days 4 to 10, subjects were instructed by a dietician to receive a limited calcium and sodium diet. After completion of the baseline phase on day 4, subjects began oral enalapril 10 mg (Vasotec®; Merck & Co) twice a day for 10 days.

After two days of stabilization on the frozen metabolic diet in each phase of the study, the subjects collected urine for 24 hours under mineral oil for total volume, pH, sodium, potassium, ammonium, citrate, uric acid, oxalate, calcium and creatinine. On the morning of days 4 and 14, arterialized venous blood was obtained for sodium, potassium, chloride, glucose, total CO_2, pH, pCO_2, BUN, creatinine, uric acid, cholesterol, calcium and phosphorus [16].

Plasma and urine measurements

In the rat study, creatinine, plasma and urine electrolytes, were measured using a Beckman Astra-7 (Fullerton, CA, USA) analyzer. Plasma pH, pCO_2 and bicarbonate were determined using an Instrumental Laboratory (Lexington, MA, USA) blood gas analyzer. Citrate was measured using a commercially available kit (Boehringer Mannheim, Indianapolis, IN, USA).

For the human study, calcium was determined by atomic absorption spectrophotometry. Serum electrolytes, creatinine, BUN and glucose were measured using a Beckman Astra-7 analyzer. Serum pH and pCO_2 were analyzed by the CIBA-Corning (Medfield, MA, USA) blood gas system. Urinary sodium and potassium were determined by flame photometry, and creatinine and ammonium by colorimetry. Urinary pH was measured by electrode pH meter, uric acid by uricase method, oxalate by ion chromatography, and phosphorus by the method of Fiske and Subbarow [17]. Urinary citrate was determined by measuring the generation of phenylhydrazone from phenylhydrazine [18].

All results are expressed as mean ± SEM. Paired Student's *t*-test (Sigma Stat, Jandel Scientific, San Rafael, CA, USA) was used for comparison of results between control and experimental groups in the rat study and between baseline and enalapril in the human study. Sigma Chemical Co. (St. Louis, MO, USA) supplied all chemicals unless otherwise stated.

RESULTS

Twenty-six rats were studied (13 in each group) and consumed an average of 83% of the diet. Rats fed enalapril gained on average 30 grams with control rats gaining on average 25 grams during the experimental period (NS). Urine volume, electrolytes, and creatinine are listed in Table 1. Total sodium, chloride, and creatinine excretion were similar between control and experimental groups. Enalapril-fed rats excreted more urine (15.3 vs. 7.5 ml/day; $P < 0.005$) and potassium (0.60 vs. 0.43 mEq/day; $P < 0.05$). Plasma pH, electrolytes, glucose and creatinine are

Table 1. Urinary excretion in rats

	Control	Enalapril
Volume *ml*	7.5 ± 0.9	15.3 ± 2.0[a]
Na *mEq/day*	1.1 ± 0.1	1.3 ± 0.2
K *mEq/day*	0.43 ± 0.04	0.60 ± 0.06[b]
Cl *mEq/day*	1.3 ± 0.1	1.6 ± 0.2
Creatinine *mg/day*	6.1 ± 0.4	6.9 ± 0.8

Twenty-four-hour samples were obtained during day 7.
[a] $P < 0.005$
[b] $P < 0.05$

Table 2. Blood composition in rats

	Control	Enalapril
pH	7.43 ± 0.01	7.40 ± 0.01
pCO_2 *mm Hg*	34.3 ± 0.8	35.9 ± 1.0
[HCO_3]	23.7 ± 0.5	23.2 ± 1.1
[Na]	142 ± 1	141 ± 1
[K]	4.2 ± 0.1	4.3 ± 0.1
[Cl]	111 ± 1	112 ± 1
Creatinine *mg/dl*	0.38 ± 0.02	0.45 ± 0.04
Glucose *mg/dl*	191 ± 3	182 ± 6
Citrate *mg/liter*	49 ± 4	48 ± 6

Aortic blood punctures were performed on day 8.

listed in Table 2. All values were similar between the groups. Figure 1 reveals that both urinary citrate excretion and fractional excretion of citrate were decreased in rats given enalapril (0.34 vs. 2.80 mg/day and 0.66 vs. 4.10, respectively; $P < 0.005$ for both).

In animals with similar decreases in urinary citrate, 98% in CMA and 95% in hypokalemia, there is an increase in renal cortical citrate ATP citrate lyase [10]. To determine whether ATP citrate lyase activity increases in enalapril-induced hypocitraturia, we measured the activity of this enzyme in renal cortex. Figure 2 demonstrates that enalapril-fed rats exhibited a 42% increase in renal cortical ATP citrate lyase activity (in nmoles acetylhydroxamate formation/30 min/mg protein; 63.3 ± 7.2 vs. 44.6 ± 5.2; $P < 0.005$).

To examine the effect of enalapril on humans, we placed normal volunteers on a frozen metabolic diet and measured blood and urine samples before and after 10 days of enalapril. Results (Tables 3 and 4) reveal a small but consistent decrease in serum cholesterol following enalapril. Furthermore, as can be seen in Table 3, urinary sodium and potassium excretions matched the dietary composition, indicating the subjects were in balance and had good compliance with the prescribed diet. Figure 3 shows a small but significant decrease in both urinary citrate excretion (638 ± 82 vs. 560 ± 72 mg/day, $P < 0.1$) and concentration (327 ± 78 vs. 276 ± 61 mg/liter, $P < 0.05$) after enalapril.

DISCUSSION

Angiotensin converting enzyme inhibition by enalapril caused hypocitraturia in rats. In that plasma concentrations

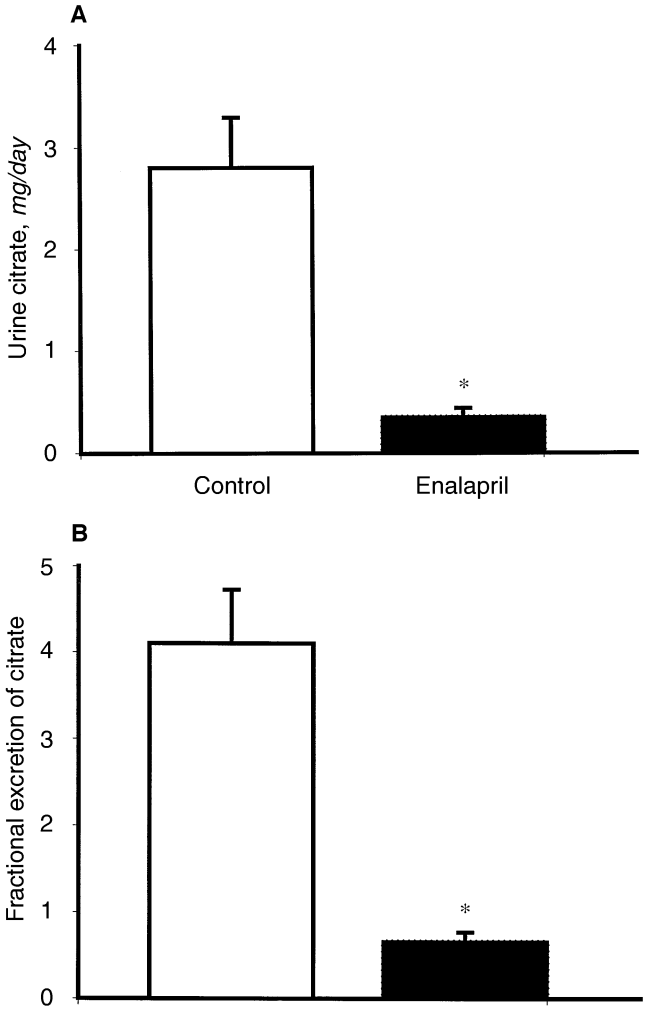

Fig. 1. Urinary citrate in rats. Twenty-four-hour urine collections were made from control and enalapril-treated animals on day 7. (*A*) Total urinary citrate excretion. (*B*) Fractional excretion of citrate. *$P < 0.005$.

of citrate and creatinine did not change, it is unlikely that the filtered load of citrate changed sufficiently to explain the hypocitraturia. Thus, in this model, as in acidosis and hypokalemia, enhanced tubular citrate reabsorption imputes the hypocitraturia. The degree of hypocitraturia with enalapril was similar to that seen in animals with metabolic acidosis or hypokalemia [10]. However, in contrast to these animals, enalapril-fed rats had no significant decrease in plasma pH, bicarbonate or potassium when compared with controls.

The higher urine volume seen in rats may be attributed to enalapril's inability to cross the blood-brain barrier and to block angiotensin II (Ang II) production in the central nervous system [19]. The reflexive increase in systemic renin levels, responding to systemic Ang II inhibition, stimulates Ang II generation within the central nervous system leading to increased thirst, polydipsia and polyuria [20]. This effect was not seen in humans. This may reflect differences in amount of drug given or different pathways for Ang II production in humans.

Table 3. Urinary excretion (24-hour urine samples) in humans

	Before enalapril	After enalapril
Volume *ml*	2323 ± 263	2419 ± 260
pH	6.33 ± 0.09	6.34 ± 0.08
Na *mEq/day*	95 ± 11	104 ± 16
K *mEq/day*	43 ± 6	42 ± 5
Creatinine *mg/day*	1513 ± 176	1434 ± 168
Sulfate *mg/day*	17.4 ± 1.4	18.8 ± 2.2
Uric acid *mg/day*	567 ± 41	576 ± 40
NH_4 *mmol/day*	34 ± 3	36 ± 4
Ca *mg/day*	150 ± 10	149 ± 13
Oxalate *mg/day*	29 ± 2	27 ± 2

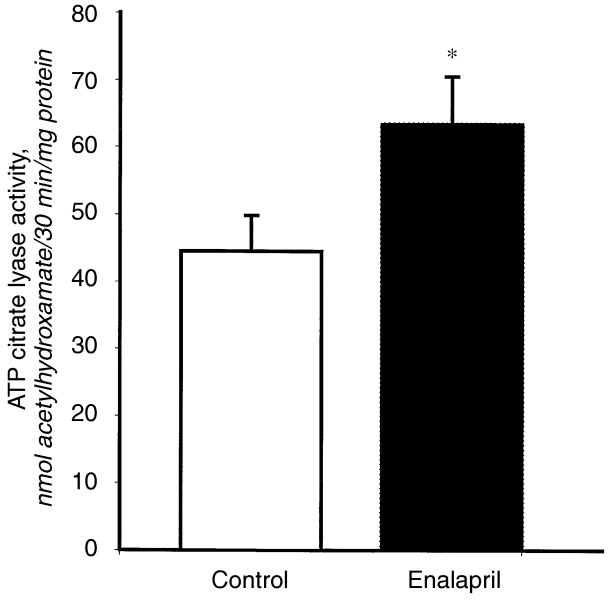

Fig. 2. Renal cortical ATP citrate lyase activity. Renal cortical tissue was harvested from control and enalapril-treated animals. Enzyme activity was measured spectrophotometrically (*$P < 0.005$).

An adaptive increase in ATP citrate lyase activity, similar to that seen in rats with metabolic acidosis and hypokalemia [10], likely contributes to the hypocitraturic effect of enalapril. This ATP-dependent cytosolic enzyme cleaves citrate to oxaloacetate and acetyl CoA. This provides the cytosol with acetyl CoA needed for fatty acid synthesis. The oxaloacetate generated enters the gluconeogenic pathway, also up-regulated in CMA and hypokalemia, through phosphoenolpyruvate carboxykinase [11].

The hypocitraturia seen with angiotensin converting enzyme inhibitors, CMA, and hypokalemia is likely due to a decrease in proximal tubule pH_i. In the case of converting enzyme inhibition, the decrease in pH_i is likely due to inhibition of Ang II-induced stimulation of the proximal tubule apical membrane Na/H antiporter. This decrease in pH_i then leads to a series of adaptations resulting in increased citrate absorption and hypocitraturia. Because Ang II directly stimulates proximal tubular bicarbonate absorption and ammoniagenesis, decreases in pH_i associated with converting enzyme inhibition may not stimulate these processes, as is seen in CMA and hypokalemia [21].

Table 4. Blood composition in humans

	Before enalapril	After enalapril
Glucose *mg/dl*	80 ± 3	82 ± 2
BUN *mg/dl*	10 ± 1	11 ± 1
Creatinine *mg/dl*	0.9 ± 0.04	1.0 ± 0.04
[Na]	140 ± 1	139 ± 1
[K]	4.1 ± 0.1	4.2 ± 0.1
[Cl]	105 ± 1	103 ± 1
[total CO_2]	26.4 ± 0.9	27.8 ± 1.1
Uric acid *mg/dl*	3.9 ± 0.4	3.6 ± 0.3
Phosphorus *mg/dl*	3.4 ± 0.1	3.3 ± 0.1
Cholesterol *mg/dl*	169 ± 7	161 ± 6[a]
iCalcium	4.0 ± 0.1	4.1 ± 0.1
pH	7.37 ± 0.01	7.36 ± 0.01
pCO_2 *mm Hg*	49 ± 1	50 ± 2

[a] $P < 0.05$

Administration of enalapril in typical pharmacological doses to normal human volunteers resulted in a small but significant decrease in both urine citrate excretion and concentration. In order to minimize the dietary influences on the urinary citrate excretion, we limited the dietary acid ash content. Both urinary uric acid and sulfate excretions were low and similar before and after enalapril, indicating low dietary consumption of purine and net acid production, respectively [22]. Similar to rats, the decrease in urinary citrate in humans occurred in the absence of a decrease in serum pH or potassium concentration. A number of possible explanations exist for the reason the effect on citrate excretion was smaller in humans than in rats. First, we used a smaller dose of enalapril in humans. Second, the difference in the amount and composition of protein in both diets may affect urinary citrate in control values that would be expected to modulate the magnitude of enalapril's effects [23]. Lastly, it is possible that Ang II has a smaller effect on proximal tubule cell pH_i in humans. In the proximal tubule, Ang II stimulates Na/H antiporter which increases pH_i, and the $Na/3HCO_3$ transporter which decreases pH_i [13]. The relative magnitude of the effects of these two transporters may be different between humans and rats, resulting in different effects on proximal tubule pH_i.

A decrease in urinary citrate excretion occurring with inhibition of Ang II synthesis may have significant clinical implications. These agents may increase the incidence of urolithiasis in certain patients. In addition, suppression of Ang II synthesis may be responsible for hypocitraturia seen in patients on a high salt diet [16], and Sakhaee et al showed that a high salt diet causes a 20% decrease in total urinary citrate excretion without affecting plasma pH or potassium. Thus, a high salt diet and enalapril administration may decrease urinary citrate through a similar mechanism, decreases in Ang II levels.

In summary, these studies reveal that enalapril decreases

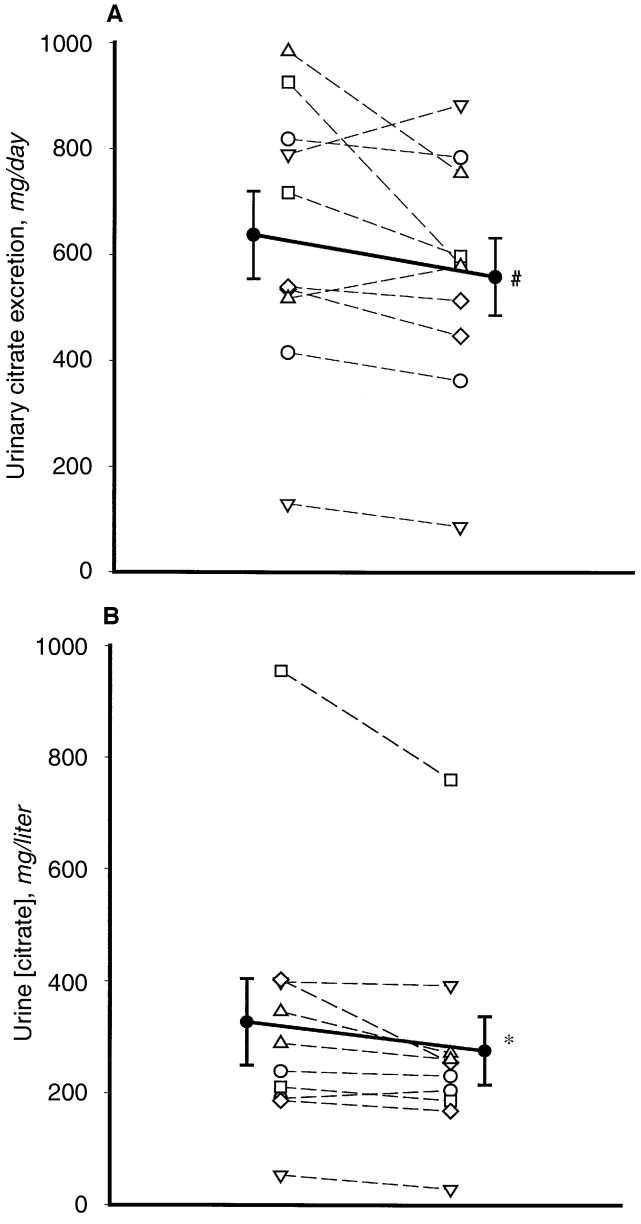

Fig. 3. Urinary citrate in humans. Symbols represent data pairs for each individual. (*A*) Total urinary citrate excretion and (*B*) urine citrate concentration in normal human volunteers before and after enalapril administration. Symbols represent individual patients before and after enalapril ($^{*}P < 0.1$; $^{\#}P < 0.05$).

urinary citrate excretion in rats and humans. The mechanism that mediates the hypocitraturia is similar to that observed in animals with CMA and hypokalemia, and may occur secondary to a decrease in renal proximal tubular pH_i. Furthermore, these studies suggest that angiotensin converting enzyme inhibitors should be used with caution in patients at risk for developing urolithiasis.

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