

# Metabolic Acidosis Suppresses 25-Hydroxyvitamin D<sub>3</sub>-1 $\alpha$ -Hydroxylase in the Rat Kidney

## DISTINCT SITE AND MECHANISM OF ACTION

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**ABSTRACT** Effect of metabolic acidosis on two distinct 25-hydroxyvitamin D<sub>3</sub>-1 $\alpha$ -hydroxylase (1 $\alpha$ -hydroxylase) systems was studied in the kidneys of vitamin D-deficient rats; one is localized in the proximal convoluted tubule (PCT), is activated in vitamin D deficiency, and is regulated primarily by parathyroid hormone (PTH) via cyclic AMP; the other is localized in the proximal straight tubule (PST), is latent in vitamin D deficiency, and is selectively stimulated by calcitonin via a cyclic AMP-independent mechanism. The 1 $\alpha$ -hydroxylase activities were measured in the PCT and PST microdissected from the kidney of vitamin D-deficient rats with or without metabolic acidosis of varying duration. The 1 $\alpha$ -hydroxylase activity decreased in the PCT from 0.74 $\pm$ 0.07 fmol/mm per h to 0.24 $\pm$ 0.02 at day 3 of metabolic acidosis without a further decline at day 7. Neither metabolic acidosis of 16 h duration nor reduction of the incubation medium pH from 7.4 to 7.0 affected the enzyme activity in the PCT. To examine the underlying mechanism for the suppression of 1 $\alpha$ -hydroxylase activity, PTH, cyclic AMP, or calcitonin was given to rats with metabolic acidosis of 3 d duration. Although PTH failed to augment the suppressed 1 $\alpha$ -hydroxylase activity in the PCT, cyclic AMP restored it to the level of control rats. The 1 $\alpha$ -hydroxylase activity in the PST remained undetectable in control rats and in acidotic rats with

or without PTH or cyclic AMP treatments. However, calcitonin stimulated the 1 $\alpha$ -hydroxylase activity in the PST equally from undetectable to 0.75 $\pm$ 0.09 fmol/mm per h in control and to 0.78 $\pm$ 0.10 in acidotic rats. The data suggests that metabolic acidosis suppresses 1 $\alpha$ -hydroxylase only in the PCT by inhibiting PTH-dependent adenylate cyclase, and that cellular events beyond cyclic AMP in the PCT and the events responsive to calcitonin in the PST are unaffected. The results show the definite advantage of using defined single nephron segments to study the hormonal and ionic control of the 1 $\alpha$ -hydroxylase system in the kidney.

## INTRODUCTION

Metabolic acidosis has been associated with decreased activity of 25-hydroxy-vitamin D<sub>3</sub>-1 $\alpha$ -hydroxylase (1 $\alpha$ -hydroxylase)<sup>1</sup> as assessed in vivo in the vitamin D-deficient rat and chick (1, 2) and in vitro in kidneys from vitamin D-deficient chick (3). The mechanism whereby this suppression of 1 $\alpha$ -hydroxylase is affected, however, remains unclear. Because parathyroid hormone (PTH) is an important hormone stimulating 1 $\alpha$ -hydroxylase activity (4-6), and since metabolic acidosis has been shown to blunt certain renal responses to PTH, such as the tubular reabsorption of phosphate, by inhibiting PTH-sensitive adenylate cyclase (7), it is possible that metabolic acidosis depresses 1 $\alpha$ -hydroxylase activity by inhibiting PTH-sensitive adenylate cyclase.

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<sup>1</sup> Abbreviations used in this paper: 1,25(OH)<sub>2</sub>D<sub>3</sub>, 1 $\alpha$ ,25-dihydroxy-vitamin D<sub>3</sub>; 1 $\alpha$ -hydroxylase, 25-hydroxyvitamin D<sub>3</sub>-1 $\alpha$ -hydroxylase; 25(OH)D<sub>3</sub>, 25-hydroxyvitamin D<sub>3</sub>; PCT, proximal convoluted tubules; PST, proximal straight tubules; PTH, parathyroid hormone.

TABLE I  
Blood pH and Plasma Levels of HCO<sub>3</sub>, Calcium, and Inorganic Phosphate in Vitamin D-deficient Rats with Metabolic Acidosis of Varying Duration

Duration of acidosis	n	pH	HCO <sub>3</sub>	Ca	Pi
			mM	mg/dl	
0 or control	5	7.42±0.02	21.0±0.5	6.4±0.4	5.8±0.5
16 h	4	7.08±0.04*	7.3±1.0*†	6.3±0.4	4.3±0.7
3 d	5	7.20±0.06*	14.0±2.4*	6.1±0.5	3.3±1.1
7 d	4	7.10±0.01*	14.9±0.9*	6.3±1.0	6.1±0.4

Values are the mean±SEM.

\*  $P < 0.01$  vs. Control.

†  $P < 0.01$  vs. acidosis of 3 and 7 d.

Recent reports from our laboratory have demonstrated the presence of two distinct  $1\alpha$ -hydroxylase systems in the kidney: one is localized in the proximal convoluted tubule (PCT) and is regulated primarily by PTH via cyclic AMP (8); the other is localized in the proximal straight tubule (PST), is latent in vitamin D deficiency, is selectively stimulated by calcitonin via a cyclic AMP-independent mechanism, and is insensitive to PTH (9). If metabolic acidosis exerts its effects on  $1\alpha$ -hydroxylase activity by inhibiting PTH-sensitive adenylate cyclase, metabolic acidosis may have different effects on these two  $1\alpha$ -hydroxylase systems. To examine this possibility we studied the effects of metabolic acidosis on the  $1\alpha$ -hydroxylase activities in the PCT and PST of the rat kidney and on the responsiveness of the  $1\alpha$ -hydroxylase to the administration of PTH, calcitonin, and cyclic AMP.

## METHODS

**Preparation of animals.** Studies were carried out in weanling male Holtzman rats fed a vitamin D-deficient diet containing 0.45% calcium and 0.3% phosphorus for 4–6 wk (8). Metabolic acidosis was induced by adding 1.8% ammonium chloride to the drinking water for 16 h, 3 or 7 d. In experiments with acute acidosis rats were given the ammonium chloride containing drinking water for 16 h and given 5–7 ml of 1.8% ammonium chloride by gastric gavage on three occasions, 16, 12, and 8 h before the study. To evaluate the mechanism by which metabolic acidosis affects  $1\alpha$ -hydroxylase activity the effects of PTH, calcitonin, and cyclic AMP on the enzyme activity of the PCT and PST were examined in rats receiving ammonium chloride for 3 d. On the day of the study, either 50 U of synthetic bovine PTH (1–34 amino acids, 7,500 U/mg; Beckman Instruments, Inc., Palo Alto, CA), 10 U of synthetic salmon calcitonin (4,000 U/mg; Armour Laboratories, Scottsdale, AZ), or 20  $\mu$ mol of cyclic AMP (Sigma Chemical Co., St. Louis, MO) was injected subcutaneously at 8, 6, 4, and 2 h before killing. Calcium gluconate, 200 mg/kg, was given subcutaneously together with the calcitonin to prevent a further decline in serum calcium (9). Doses and the duration of PTH, cyclic AMP, and calcitonin were those maximally stimulating

the  $1\alpha$ -hydroxylase of the kidney of vitamin D-deficient rats (9–11).

**Preparation of the nephron segments.** The rats were anesthetized with pentobarbital, 40 mg/kg i.p.; arterial blood was obtained from the abdominal aorta using heparinized syringes for the measurement of pH, pCO<sub>2</sub>, calcium, and inorganic phosphorus. The abdominal aorta was then cannulated, and the left kidney was perfused with chilled Krebs-Ringer-bicarbonate buffer, pH 7.4, containing 8.3 mM glucose, 0.1% bovine serum albumin (fraction V, Sigma Chemical Co.) and 0.1% collagenase (type I, Sigma Chemical Co.). The slices of the kidney were prepared and incubated for 30 min at 30°C in the same buffer with a constant bubbling with a gas mixture of 95%:5% =O<sub>2</sub>:CO<sub>2</sub> (vol/vol). After rinsing the slices three times with 30–50 ml of ice-cold modified Hanks' solution, the PCT and PST, the nephron segments endowed with  $1\alpha$ -hydroxylase (8, 9) were dissected in the ice-cold Hanks' solution under a stereomicroscope. Details of perfusion and microdissection have been reported elsewhere (8, 12).

**Measurement of  $1\alpha$ -hydroxylase activity.** Each group of PCT or PST was incubated in 20  $\mu$ l of modified Hanks' solution containing 5 mM pyruvate, 8.3 mM glucose, and 10 mM Hepes, pH 7.4, with 0.5  $\mu$ M [23,24(n)<sup>3</sup>H] 25(OH)D<sub>3</sub> or [26,27-methyl-<sup>3</sup>H]25(OH)D<sub>3</sub> (sp act 102 and 22.3 Ci/mmol, respectively; both from Amersham Corp., Arlington Heights, IL). Each incubation contained the PCT or PST from one animal, and only one incubation was prepared for either segment from each animal. The incubation was carried out at 37°C for 60 min with room air as a gas phase and then terminated by the addition of 50  $\mu$ l of chloroform-methanol (1:2, vol/vol). Lipid extraction was performed according to the method of Bligh and Dyer (13). In an effort to determine the effect of acid pH on the  $1\alpha$ -hydroxylase, the PCT from other vitamin D-deficient rats with normal acid-base status were incubated in the modified Hanks' solution of pH 7.4 and 7.0 adjusted by adding hydrochloric acid. The metabolites of [<sup>3</sup>H]25(OH)D<sub>3</sub> were identified by sequential thin-layer chromatography and high performance liquid chromatography as described earlier (8). The metabolite 1,25(OH)<sub>2</sub>D<sub>3</sub> was identified by cochromatography with authentic 1,25(OH)<sub>2</sub>D<sub>3</sub>. The amount of 1,25(OH)<sub>2</sub>D<sub>3</sub> produced was calculated from results obtained by the thin-layer chromatography and were expressed in femtomoles per millimeter tubular length based on the specific activity of [<sup>3</sup>H]25(OH)D<sub>3</sub> after correcting for recovery (73–92%) and counting efficiency (43–52%) (5, 6).

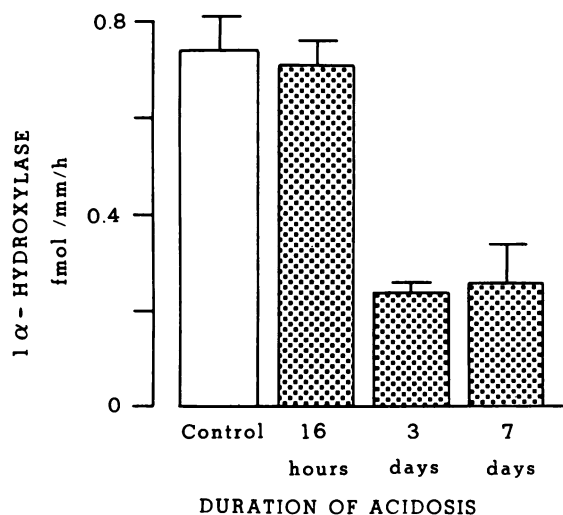
**Analytical methods for blood chemistries.** Blood pH and  $p\text{CO}_2$  were measured using the Blood Gas Analyzer 113 (Instrumentation Laboratory, Lexington, MA). Plasma bicarbonate concentration was calculated by the Henderson-Hasselbalch equation using a  $pK_1$  of 6.1 and solubility coefficient of 0.0301. Plasma calcium was measured by a model 303, Perkin-Elmer atomic absorption spectrophotometer (Perkin-Elmer Corp., Norwalk, CT) and plasma inorganic phosphate by the method of Fiske and Subbarow adapted for the Technicon Auto-Analyzer (Technicon Instruments Corp., Tarrytown, NY).

**Analysis of the data.** Results are expressed as the mean  $\pm$  SEM, and analyzed with one-way analysis of variance for multiple group comparison of Dunnett (14). Differences between groups were considered significant when  $P$  values were  $<0.01$ .

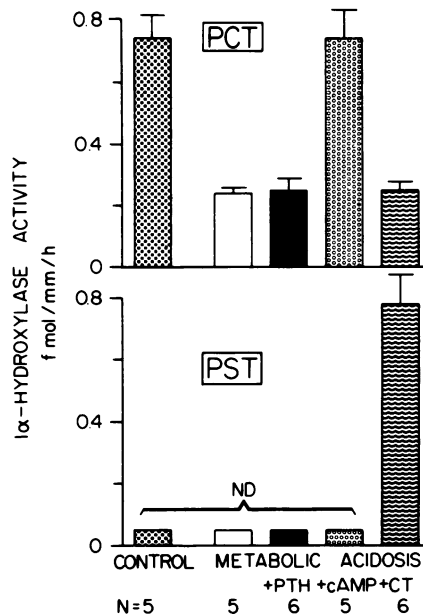
## RESULTS

Table I shows blood pH, plasma  $\text{HCO}_3^-$ , calcium, and inorganic phosphate in the acidotic and control vitamin D-deficient rats. Feeding of ammonium chloride led to a significant reduction in blood pH and plasma  $\text{HCO}_3^-$  concentration in all acidotic groups. However, plasma calcium and inorganic phosphate concentrations did not differ significantly among the groups.

**Effect of acidosis on  $1\alpha$ -hydroxylase activity in the nephron.** Fig. 1 depicts the effect of metabolic acidosis of varying duration on the  $1\alpha$ -hydroxylase activity in the PCT. 16 h of metabolic acidosis did not alter the enzyme activity, even though plasma bicarbonate was lower than values observed in rats of 3 and 7 d of



**FIGURE 1** Effect of metabolic acidosis of varying duration on  $25(\text{OH})$ - $1\alpha$ -hydroxylase in the PCT of rat kidney. The enzyme activities at day 3 and 7 are significantly ( $P < 0.001$ ) lower than that of control. Values are the means  $\pm$  SEM and the numbers of observations are 5, 4, 5, and 4 for control and 16 h, 3 and 7 d of metabolic acidosis, respectively. The tubule length per incubation ranged from 48 to 79 mm and the limit of the assay was 0.05 fmol/mm per h.



**FIGURE 2** Effects of PTH, cyclic AMP, and calcitonin on  $25(\text{OH})$ - $1\alpha$ -hydroxylase activities in the PCT and the PST of 3 d acidosis rat kidney. The enzyme activity in the PST in response to calcitonin in control rats with normal acid-base status was  $0.75 \pm 0.09$  fmol/mm per h. The tubule length per incubation ranged from 48 to 79 mm from PCT and 85 to 109 mm for PST. The assay limit was  $<0.05$  fmol/mm per h or not detectable (ND). Values are the means  $\pm$  SEM and the number of observations is indicated under each column. CT, calcitonin.

metabolic acidosis. In contrast, the  $1\alpha$ -hydroxylase was markedly reduced from  $0.74 \pm 0.07$  ( $n = 5$ ) to  $0.24 \pm 0.02$  fmol/mm per h ( $n = 5$ ) with acidosis of 3 d duration and acidosis of 7 d duration had no further effect. The  $1\alpha$ -hydroxylase activities in the PCT of vitamin D-deficient control rats with normal acid-base status incubated at pH 7.4 and 7.0 were  $0.89 \pm 0.09$  and  $0.96 \pm 0.22$  fmol/mm per h ( $n = 4$ ), respectively, an observation indicating the absence of direct inhibition by low pH of the  $1\alpha$ -hydroxylase activity in the PCT. The  $1\alpha$ -hydroxylase activity in vitamin D deficiency in the PST was undetectable in both acidotic and control rats. As reported earlier (8), no significant 24-hydroxylase activity was detectable in vitamin D-deficient rats, both in control and acidotic states.

**Effect of parathyroid hormone, cyclic AMP, and calcitonin on  $1\alpha$ -hydroxylase activity in metabolic acidosis.** The effect of PTH, cyclic AMP, and calcitonin on the  $1\alpha$ -hydroxylase activity in the PCT and PST in metabolic acidosis are summarized in Fig. 2. Even supraphysiological doses of PTH did not augment the  $1\alpha$ -hydroxylase activity in the PCT suppressed by acidosis. By contrast, the  $1\alpha$ -hydroxylase activity of acidotic rats was restored to the levels of

TABLE II  
*Blood pH and Plasma Levels of HCO<sub>3</sub>, Calcium, and Inorganic Phosphate in Acidotic Vitamin D-deficient Rats Treated with PTH, Cyclic AMP, and Calcitonin*

Treatment	n	pH	HCO <sub>3</sub>	Ca	Pi
			mM	mg/dl	
None	5	7.20±0.06	14.0±2.4	6.1±0.5	3.3±1.1
PTH	6	7.12±0.07	12.6±1.2	7.9±0.6	4.8±1.1
Cyclic AMP	5	7.11±0.05	9.3±2.1	5.7±0.5	4.0±1.1
Calcitonin	6	7.14±0.06	7.0±2.3	6.4±0.4	3.3±0.3

Vitamin D-deficient rats with metabolic acidosis of 3 d duration received injections at four 2-h intervals of either 50 U PTH, 20 μmol cyclic AMP, or 10 U calcitonin each.

Values are the mean±SEM. See text for details.

control rats with the administration of cyclic AMP. The 1α-hydroxylase activity in the PST remained undetectable with either PTH or cyclic AMP. However, calcitonin stimulated the 1α-hydroxylase equally in the PST from undetectable to 0.75±0.09 fmol/mm per h in control (n = 6) and to 0.78±0.10 in acidotic rats (n = 6); the enzyme activity in the PCT was unaffected.

Blood pH and plasma HCO<sub>3</sub>, calcium and inorganic phosphate levels in acidotic rats given PTH, cyclic AMP, and calcitonin were not different among the groups (Table II).

## DISCUSSION

The present study clearly demonstrates that metabolic acidosis suppresses 1α-hydroxylase activity in the PCT of the rat kidney when assessed in vitro. The data are consistent with previous studies examining the effects of metabolic acidosis on 1,25(OH)<sub>2</sub>D<sub>3</sub> production in the vitamin D-deficient rat in vivo (1) and in the chick in vivo and in vitro (2, 3). The present results also show that exposure to an acidotic milieu for longer than 16 h is necessary before the inhibition of 1α-hydroxylase becomes manifest. Thus, neither the reduction in the pH of the incubation medium nor the in vivo metabolic acidosis of 16 h duration decreased the 1α-hydroxylase activity in the PCT. On the other hand, metabolic acidosis of 3 or 7 d duration led to a marked reduction in the 1α-hydroxylase activity in the PCT. Our present data on the effect of acid pH on 1α-hydroxylase are consistent with those of Reddy et al. (15) but are at variance with those of Bickle and Rasmussen (16, 17). The reasons for these discrepancies are not clear at present.

In the present study, the 1α-hydroxylase activity was reduced by ~65% after metabolic acidosis for 3 d. In the earlier studies, the in vivo conversion of [<sup>3</sup>H]25(OH)D<sub>3</sub> to [<sup>3</sup>H]1,25(OH)<sub>2</sub>D<sub>3</sub> in vitamin D-

deficient rats was decreased by ~30% in metabolic acidosis of the same duration. The duration of acidosis, the degree of acidemia, and serum levels of calcium and inorganic phosphate were similar in this in vivo study (1) and in the present studies done in vitro. The reason for the difference in the magnitude of suppression of 1α-hydroxylase activity, assessed in vivo and in vitro, is not apparent. A change in the conversion of 25(OH)D<sub>3</sub> to 1,25(OH)<sub>2</sub>D<sub>3</sub>, as measured in vivo, can be modified by mechanisms other than by changes in the renal 1α-hydroxylase activity; there is a possibility that metabolic acidosis could affect the peripheral metabolism of 1,25(OH)<sub>2</sub>D<sub>3</sub> as has been suggested by others (18).

We did not measure plasma ionized calcium levels in the present study. One might argue that a higher ionized calcium in acidosis may be responsible for suppression of 1α-hydroxylase. Even though calcium ions may modulate 1α-hydroxylase activity independent of PTH, available data (16, 19, 20) do not suggest that a magnitude of difference in ionized calcium attended by acidosis would cause a marked reduction in the enzyme activity observed in the present study. Metabolic acidosis tends to decrease plasma inorganic phosphate concentrations, but the low plasma phosphate has been shown to stimulate rather than suppress 1α-hydroxylase (4-6). Thus, it is unlikely that changes in plasma phosphate levels in acidosis can account for the reduction of 1α-hydroxylase activity in acidosis.

It has been well established that PTH is an important hormone stimulating renal 1α-hydroxylase (4-6). Recent studies from our laboratory have shown that the elevated 1α-hydroxylase activity, seen only in the PCT in vitamin D-deficient rats, was markedly reduced by parathyroidectomy (8). Furthermore, other results indicate that the action of PTH on the renal 1α-hydroxylase is mediated by cyclic AMP (8, 10). Therefore, it is possible that the suppression of the 1α-hydroxylase activity in the PCT of acidotic rats may

be due either to inhibition of PTH secretion or to renal resistance to the action of PTH. Further, the renal resistance to PTH action in the PCT could be at the step of PTH-dependent cyclic AMP generation or at steps beyond cyclic AMP formation. Our findings that the excess PTH did not stimulate the suppressed  $1\alpha$ -hydroxylase activity in the PCT of acidotic rats, whereas cyclic AMP fully restored the enzyme activity to normal, strongly suggest that metabolic acidosis suppresses  $1\alpha$ -hydroxylase activity in the PCT by inhibiting PTH-dependent adenylate cyclase. Moreover, the data indicates that metabolic acidosis does not affect the intracellular processes necessary for  $1\alpha$ -hydroxylase stimulation after cyclic AMP generation.

The presence of another distinct  $1\alpha$ -hydroxylase system has recently been demonstrated in the PST: this  $1\alpha$ -hydroxylase is undetectable in vitamin D deficiency and insensitive to PTH, but it is stimulated by calcitonin via a mechanism independent of cyclic AMP (9). If the effect of metabolic acidosis is limited to the PTH-sensitive adenylate cyclase, one might expect that metabolic acidosis would not affect the calcitonin-sensitive  $1\alpha$ -hydroxylase in the PST. We found that calcitonin stimulates the  $1\alpha$ -hydroxylase activity of the PST of acidotic rats to levels similar to those observed in control nonacidotic rats treated with calcitonin. As might be expected from earlier findings that calcitonin stimulates  $1\alpha$ -hydroxylase only in the PST and not in the PCT (9), calcitonin was without effects on the enzyme activity in the PCT that was suppressed in metabolic acidosis. Since the PST is devoid of calcitonin-sensitive adenylate cyclase (9) and since cyclic AMP does not stimulate the  $1\alpha$ -hydroxylase in the PST, it is evident that cyclic AMP is not a mediator of the calcitonin action. Whatever the mechanism involved, the present results show that such mechanisms are not affected in metabolic acidosis.

The physiological significance of the present observations in vitamin D-deficient rats is not clear at present. Previous results from our laboratory showed that metabolic acidosis induced a modest rise in plasma  $1,25(\text{OH})_2\text{D}_3$  levels associated with a fall in plasma inorganic phosphate levels in vitamin D-replete rats (21). However, preliminary data by Bushinsky et al. (22) showed that metabolic acidosis caused a marked reduction in the plasma levels of  $1,25(\text{OH})_2\text{D}$  in vitamin D-replete rats fed a low calcium diet and supplemental phosphate to prevent the fall in plasma phosphate concentrations. These latter data, which are in accord with the present, indicate that metabolic acidosis can suppress  $1\alpha$ -hydroxylase and lower the plasma level of  $1,25(\text{OH})_2\text{D}$ , particularly when the  $1\alpha$ -hydroxylase is stimulated by maneuvers such as vitamin D deficiency or a low calcium diet.

In summary, our results demonstrate that metabolic

acidosis suppresses  $1\alpha$ -hydroxylase activity in the PCT by inhibiting PTH-dependent adenylate cyclase activation. The data suggest that the cellular events responsible for  $1\alpha$ -hydroxylase stimulation beyond cyclic AMP in the PCT or the events in the PST in response to calcitonin are unaffected in metabolic acidosis. Our results provided further insight into the site and mechanism of suppression by metabolic acidosis of the  $1\alpha$ -hydroxylase in the kidney and demonstrate the definite advantage of using well-defined single nephron segments to study the hormonal and ionic control of the  $1\alpha$ -hydroxylase system in the kidney.

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