JCI The Journal of Clinical Investigation

Effect of adenosine 3′,5′-monophosphate on production of glucose and ammonia by renal cortex

Anthony S. Pagliara, A. David Goodman

J Clin Invest. 1969;48(8):1408-1412. https://doi.org/10.1172/JCI106106.

Research Article

In studies employing rat renal cortical slices, the addition of adenosine 3′,5′-monophosphate (cyclic AMP) to the incubation medium caused an increase in production of glucose from glutamine, glutamate, α-ketoglutarate, fumarate, malate, and oxalacetate, but not from glycerol and fructose. These observations suggest that cyclic AMP accelerates a rate-limiting gluconeogenic reaction between oxalacetate and the triose phosphates. The addition to the medium of parathyroid hormone, which is known to increase renal cortical cyclic AMP, also stimulated glucose production from glutamine.

When renal cortical slices were incubated in the presence of glutamine, the addition of cyclic AMP caused a fall in tissue glutamate concentration and a rise in ammonia production, as well as an increase in gluconeogenesis. These changes are similar to those observed in renal cortex of rats with induced metabolic acidosis. The present observations are consistent with a previously advanced hypothesis that cortical gluconeogenesis, ammonia production, and glutamate concentration may be interdependent.

Effect of Adenosine 3′,5′-Monophosphate on Production of Glucose and Ammonia by Renal Cortex

ANTHONY S. PAGLIARA and A. DAVID GOODMAN

From the Division of Endocrinology and Metabolism, Department of Medicine, Albany Medical College, Albany, New York 12208

ABSTRACT In studies employing rat renal cortical slices, the addition of adenosine 3′,5′-monophosphate (cyclic AMP) to the incubation medium caused an increase in production of glucose from glutamine, glutamate, α-ketoglutarate, fumarate, malate, and oxalacetate, but not from glycerol and fructose. These observations suggest that cyclic AMP accelerates a rate-limiting gluconeogenic reaction between oxalacetate and the triose phosphates. The addition to the medium of parathyroid hormone, which is known to increase renal cortical cyclic AMP, also stimulated glucose production from glutamine.

When renal cortical slices were incubated in the presence of glutamine, the addition of cyclic AMP caused a fall in tissue glutamate concentration and a rise in ammonia production, as well as an increase in gluconeogenesis. These changes are similar to those observed in renal cortex of rats with induced metabolic acidosis. The present observations are consistent with a previously advanced hypothesis that cortical gluconeogenesis, ammonia production, and glutamate concentration may be interdependent.

INTRODUCTION

Renal cortex and liver are the two mammalian tissues which can produce glucose from noncarbohydrate precursors (1, 2). It has been demonstrated that adenosine 3′,5′-monophosphate (cyclic AMP) stimulates gluconeogenesis by the perfused liver, and the stimulatory effects of glucagon and epinephrine on hepatic gluconeogenesis are thought to be mediated by an increase in the cellular concentration of cyclic AMP (3). In the present study we have examined the effect of cyclic AMP on glucose production by renal cortical slices, and found that it stimulates glucose production from glutamine, glutamate, and other substrates. As parathyroid hormone is known to increase renal cyclic AMP (4), we have studied the effect of this hormone on renal cortical slices and observed that it enhances glucose production.

Renal cortex from rats with metabolic acidosis has an enhanced capacity to produce glucose from glutamine and glutamate (5), a decrease in glutamate content (6), and an increased capacity to release ammonia from glutamine (7). The decrease in glutamate concentration has been attributed to accelerated conversion of glutamate to glucose (5). Further, it has been suggested that since glutamate is an inhibitor of phosphate-dependent glutaminase (6), the fall in cortical glutamate causes activation of glutaminase, thus increasing ammonia production from glutamine (Fig. 1) (5, 6). To test these hypotheses, we have examined the possibility that the increase in cortical gluconeogenesis evoked by cyclic AMP might be accompanied by changes in cortical glutamate content and ammonia production. Cyclic AMP was found to cause a decrease in the glutamate content of cortical slices, and a rise in ammoniagenesis.

METHODS

In vitro studies of renal cortical production of glucose and ammonia. Sprague-Dawley male rats,[1] weighing 250–350 g, were used in all of the studies except the experiment on the effect of parathyroid hormone. For the latter experiment, 200- to 250-g Sprague-Dawley male rats were obtained[2] which had been parathyroidectomized by electrocautery 2 wk before study, and were hypocalcemic at the time of study. In all experiments the rats were deprived of food and water for 16 hr before sacrifice.

The rats were killed by decapitation and renal cortical slices were prepared with a Stadie-Riggs microtome (8). 150-mg portions of sliced tissue were added to 50-ml flasks containing 8 ml of modified Krebs-Ringer bicarbonate solution (pH 7.4), to which had been added individual gluconeogenic substrates in a concentration of 10 mmoles/

This work was presented at a meeting of the Eastern Section of the American Federation for Clinical Research, Boston, Mass., 7 December 1968.

Received for publication 30 December 1968 and in revised form 17 March 1969.

[1] Holtzman Company, Madison, Wis.

[2] Charles River Breeding Laboratories, North Wilmington, Mass.

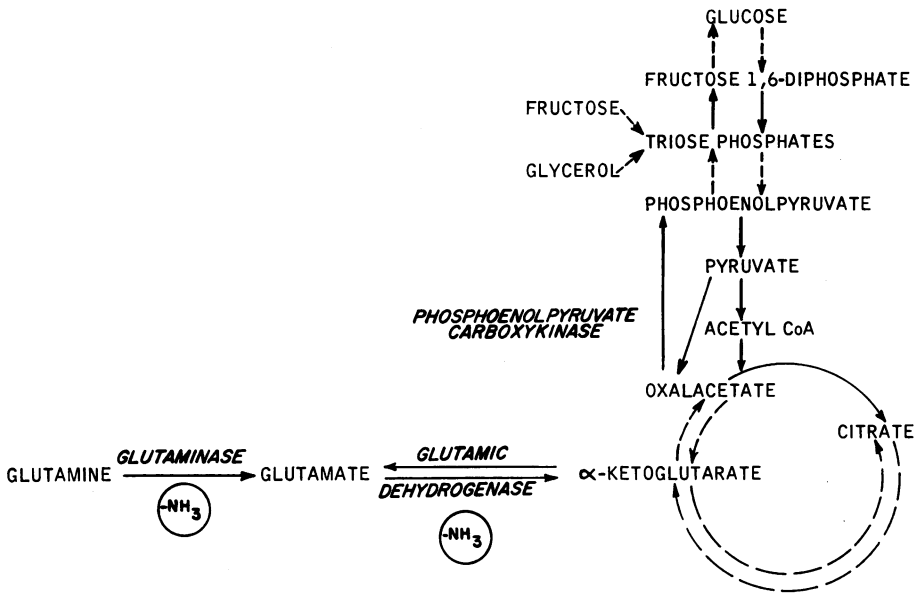

FIGURE 1 Schematic diagram of the gluconeogenic pathway.

liter. The flasks were then incubated at 37°C for 90 min, except in the parathyroid hormone experiment where the incubation was for 60 min. The electrolyte composition of the modified Krebs-Ringer solution and the details of the incubation methods have been published elsewhere (5). When glutamate, α-ketoglutarate (α-KG), fumarate, malate, and oxalacetate were employed, these were added to the medium as neutral sodium salts. Cyclic AMP (free acid)[3] or dibutyryl cyclic AMP (monopotassium salt)[4] were added to some of the flasks.

In the studies in which parathyroid hormone was used, 16 mg of trichloroacetic acid parathyroid powder (9),[5] containing approximately 300 USP units/mg, was dissolved in 2 ml of 0.1 N acetic acid, and the solution made up to 16 ml with 0.15 M saline. The acetic acid and saline were equilibrated with nitrogen before addition of the parathyroid powder, to minimize oxidation of the hormone. A "control solution" containing 2 ml of 0.1 N acetic acid and 14 ml of 0.15 M saline was also prepared. 0.1 ml of the parathyroid hormone solution was injected into half of the incubation flasks through the rubber stoppers just before incubation of the cortical slices, and every 10 min during the incubation; 0.1 ml of the "control solution" was injected similarly into the remaining flasks. The parathyroid hormone was added at 10-min intervals because of the possibility that it might undergo rapid degradation during the incubation.

In all experiments, after incubation the glucose content of the medium was determined as previously described (5), and in some experiments the ammonia content of the medium was measured by the microdiffusion method of Seligson and Hirahara (10). Glucose and ammonia production were calculated as micromoles per gram dry weight of tissue per 90 min. In calculating glucose and ammonia production from each substrate, we did not subtract production by slices incubated in medium free of substrate from production by slices incubated in the presence of substrate, in contrast with the procedure in an earlier publication (5). Changes in glucose and glycogen content of the slices during the incubation were not taken into account in the determination of glucose production, since the glucose and glycogen content of cortical slices is very small before incubation and does not change significantly after incubation in either medium with substrate or medium without substrate (5).

In vitro studies of renal cortical glutamate concentration. Cortical slices were incubated for 90 min in the presence of either 10 mM glutamine or 10 mM glutamine plus 0.5 mM cyclic AMP. At the end of the incubation, the medium in the flasks was decanted and the cortical slices frozen rapidly in liquid nitrogen. The frozen tissue was homogenized

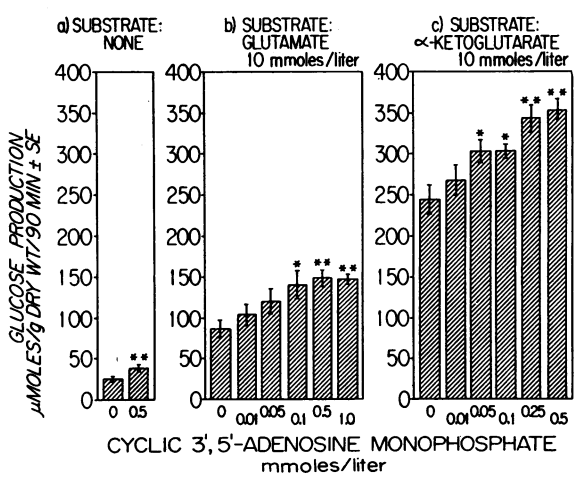

FIGURE 2 Effect of various concentrations of cyclic AMP on renal cortical glucose production. Each point represents the mean of six flasks. (* indicates $P < 0.05$; ** indicates $P < 0.01$.)

[3] Sigma Chemical Co., St. Louis, Mo.
[4] Boehringer Mannheim Corp., New York.
[5] The Wilson Laboratories, Chicago, Ill.

TABLE I

Effect of Cyclic AMP on Gluconeogenesis by Rat Renal Cortical Slices

Substrate (10 mmoles/liter)	Glucose production: Control Mean ±SE	No. of flasks	Cyclic AMP (0.5 mmoles/liter) Mean ±SE	No. of flasks	Per cent change	P
	μmoles/g dry wt per 90 min		*μmoles/g dry wt per 90 min*		%	
None	25 ±1	12	36 ±2	12	+44	<0.001
Glutamine	112 ±3	24	179 ±5	24	+60	<0.001
Glutamate	94 ±5	24	153 ±5	24	+63	<0.001
α-ketoglutarate	198 ±5	36	282 ±6	36	+42	<0.001
Fumarate	174 ±9	6	222 ±9	6	+28	<0.01
Malate	73 ±5	6	99 ±6	6	+36	<0.01
Oxalacetate	112 ±3	30	135 ±5	30	+20	<0.001
Glycerol	78 ±3	12	81 ±2	12	+4	>0.3
Fructose	546 ±15	24	485 ±11	24	−11	<0.01

in ice-cold 5% trichloroacetic acid, and the supernatant separated by centrifugation. The glutamate concentration of the supernatant was determined by a modification of the enzymatic fluorometric technique of Graham, Werman, and Aprison (11). The modifications consisted of diluting the glycine-hydrazine buffer 1:4, omitting H_2SO_4 from the buffer, and increasing the concentration of NAD in the assay tubes to 20 mg/100 ml. The intracellular glutamate concentration was calculated as micromoles per gram wet weight of tissue.

RESULTS

Effect of various concentrations of cyclic AMP and dibutyryl cyclic AMP on renal cortical glucose production from glutamate and α-KG. 0.1 mM cyclic AMP caused a significant increase in glucose production from glutamate, and the effect was not substantially greater at 0.5 mmoles/liter (Fig. 2). 0.05 mM cyclic AMP increased glucose production from α-KG with maximal stimulation when cyclic AMP was in a concentration of 0.25 mmoles/liter (Fig. 2). 0.1 mM dibutyryl cyclic AMP did not enhance production of glucose from glutamate, but in a concentration of 0.3 mmoles/liter it increased glucose production from glutamate from 91 ±5 to 120 ±10 μmoles/g dry weight per 90 min ($P < 0.05$).

Effect of cyclic AMP on renal glucose production from various gluconeogenic substrates. 0.5 mM cyclic AMP increased renal glucose production from glutamine, glutamate, α-KG, fumarate, malate, and oxalacetate, as well as in the absence of exogenous substrate (Table I). However, glucose production from glycerol was not increased, and the production of glucose from fructose was significantly decreased. The decrease in glucose production when fructose was used as a substrate, may be due to the fact that cyclic AMP inhibits fructose 1,6-diphosphatase (12), and stimulates phosphofructokinase (13).

Effect of cyclic AMP on cortical NH_3 production. 0.5 mM cyclic AMP increased the capacity of cortical slices to produce ammonia from glutamine and from glutamate (Table II). Ammonia production in the absence of exogenous substrate also was increased by cyclic AMP. Theoretically, some of the increase in ammonia production in the presence of cyclic AMP might have been due to liberation by the cortical tissue of the amine group of cyclic AMP. However, the addition of cyclic AMP to the media containing glutamine or glutamate resulted in

TABLE II

Effect of Cyclic AMP on Ammonia Production by Rat Renal Cortical Slices

Substrate (10 mmoles/liter)	Ammonia production: Control Mean ±SE	No. of flasks	Cyclic AMP (0.5 mmoles/liter) Mean ±SE	No. of flasks	Per cent change	P
	μmoles/g dry wt per 90 min		*μmoles/g dry wt per 90 min*		%	
None	85 ±5	6	171 ±14	6	+100	<0.001
Glutamine	1075 ±36	19	1400 ±35	19	+30	<0.001
Glutamate	277 ±21	6	448 ±29	6	+62	<0.001

TABLE III

Effect of Cyclic AMP on Glutamate Content of Rat Renal Cortical Slices

Substrate (10 mmoles/liter)	Glutamate content					
	Control		Cyclic AMP (0.5 mmoles/liter)			
	Mean ±SE	No. of flasks	Mean ±SE	No. of flasks	Per cent change	*P*
	μmoles/g wet wt		*μmoles/g wet wt*		%	
Glutamine	6.49 ±0.16	12	4.57 ±0.16	12	−30	<0.001

a considerably greater increase in ammonia production than when cyclic AMP was added to medium in which there was no exogenous amino acid. This indicates that much of the increase in ammonia production effected by cyclic AMP in the presence of glutamine and glutamate must have derived from these amino acids rather than from the cyclic AMP itself.

Effect of 0.5 mM cyclic AMP on renal cortical glutamate. The glutamate content of slices incubated in the presence of 0.5 mM cyclic AMP and 10 mM glutamine was significantly decreased as compared with that of slices incubated in the presence of glutamine alone (Table III).

Effect of parathyroid hormone on renal cortical glucose production. Parathyroid hormone significantly increased the capacity of renal cortical slices to produce glucose in the presence of 10 mM glutamine (Table IV).

DISCUSSION

In the present study it was observed that cyclic AMP stimulates renal cortical gluconeogenesis in vitro. Consistent with this observation was the finding that parathyroid hormone, which is known to increase renal cyclic AMP, also stimulates cortical glucose production.

The concentration of cyclic AMP required to produce a significant increase in renal gluconeogenesis in our experiments was 0.05–0.1 mmoles/liter, which is similar to that required for stimulation of hepatic gluconeogenesis and glycogenolysis (3). This is a much greater concentration than that found in kidney and liver (4, 14). Similarly, the concentration of cyclic AMP required to stimulate lipolysis in isolated fat cells (15), and the concentration required for stimulation of steroidogenesis in adrenal cortex (16), are much greater than the intracellular concentration of cyclic AMP in these tissues (15, 17). The relatively poor potency of exogenous cyclic AMP is thought to be due to the fact that it penetrates poorly into intact cells and is rapidly degraded by a phosphodiesterase (18). Consequently, the fact that a large concentration of exogenous cyclic AMP is required to affect cortical gluconeogenesis does not rule out the possibility that cyclic AMP plays a role in the physiologic regulation of this process.

In this study it was noted that cyclic AMP increases glucose production from oxalacetate and its precursors, but not from glycerol and fructose. As the latter two compounds enter the gluconeogenic pathway primarily at the level of the triose phosphates (19, 20), it appears that cyclic AMP accelerates a rate-limiting step in the pathway at some point between oxalacetate and the triose phosphates. The rate-limiting reaction between oxalacetate and the triose phosphates probably is the conversion of oxalacetate to phosphoenolpyruvate by the enzyme phosphoenolpyruvate carboxykinase (PEPCK) (Fig. 1) (21), so it is likely that it is this reaction which is accelerated by cyclic AMP. It is of interest in this regard that cyclic AMP has been shown to enhance the synthesis of PEPCK in fetal rat liver (22).

The effect of cyclic AMP on cortical gluconeogenesis is similar to that of metabolic acidosis, for cortical slices from acidotic rats have an increased capacity to produce glucose from glutamine, glutamate, α-ketoglutarate, and oxalacetate, but not from fructose and glycerol (5), apparently owing to an increase in activity of cortical

TABLE IV

Effect of Parathyroid Hormone on Gluconeogenesis by Rat Renal Cortical Slices

Substrate (10 mmoles/liter)	Glucose production					
	Control		Parathyroid hormone			
	Mean ±SE	No. of flasks	Mean ±SE	No. of flasks	Per cent change	*P*
	μmoles/g dry wt per 60 min		*μmoles/g dry wt per 60 min*		%	
Glutamine	63±3	24	73 ±3	24	+16	<0.02

PEPCK (23, 24). There are at least four possible explanations for the similarity between the effects of cyclic AMP and metabolic acidosis on renal gluconeogenesis. First, the effect of metabolic acidosis may be mediated by an increase in the concentration or effectiveness of renal cortical cyclic AMP.[6] Second, the effect of cyclic AMP may be mediated by a decrease in intracellular pH. Third, cyclic AMP and metabolic acidosis may stimulate gluconeogenesis independently, but through a common mechanism. Fourth, cyclic AMP and metabolic acidosis may stimulate gluconeogenesis by different mechanisms.

Renal cortex from rats with metabolic acidosis, has a decreased glutamate content (6) and an increased capacity to produce ammonia from glutamine (7). It has been hypothesized that the decrease in glutamate concentration is due to accelerated conversion of glutamate to glucose (5). As glutamate is known to be an inhibitor of phosphate-dependent glutaminase (6), it has been suggested that the fall in glutamate causes activation of this glutaminase, thereby increasing release of ammonia from glutamine (5, 6). The observation in this study that cyclic AMP, which stimulates glucose production from glutamine and glutamate, also decreases cortical glutamate content and increases ammonia production, is consistent with these hypotheses.

ACKNOWLEDGMENTS

We gratefully acknowledge the excellent technical assistance of Miss Judith Igler and Miss Elizabeth Sheldon.

This investigation was supported by U. S. Public Health Service Research Grant AM-09232. Dr. Pagliara performed this work during the tenure of a Daland Fellowship of the American Philosophical Society.

REFERENCES

1. Krebs, H. A., D. A. H. Bennett, P. DeGasquet, T. Gascoyne, and T. Yoshida. 1963. Renal gluconeogenesis. The effect of diet on the gluconeogenic capacity of rat kidney cortex slices. *Biochem. J.* **86**: 22.
2. Hems, R., B. D. Ross, M. N. Berry, and H. A. Krebs. 1966. Gluconeogenesis in the perfused rat liver. *Biochem. J.* **101**: 284.
3. Exton, J. H., and C. R. Park. 1968. Control of gloconeogenesis in liver. II. Effects of glucagon, catecholamines and adenosine 3′,5′-monophosphate on gluconeogenesis in the perfused rat liver. *J. Biol. Chem.* **243**: 4189.
4. Nagata, N., and H. Rasmussen. 1968. Parathyroid hormone and renal cell metabolism. *Biochemistry.* **7**: 3728.
5. Goodman, A. D., R. E. Fuisz, and G. F. Cahill, Jr. 1966. Renal gluconeogenesis in acidosis, alkalosis, and potassium deficiency: its possible role in regulation of renal ammonia production. *J. Clin. Invest.* **45**: 612.
6. Goldstein, L. 1966. Relation of glutamate to ammonia production in the rat kidney. *Amer. J. Physiol.* **210**: 661.
7. Davies, B. M. A., and J. Yudkin. 1952. Studies in biochemical adaptation. The origin of urinary ammonia as indicated by the effect of chronic acidosis and alkalosis on some renal enzymes in the rat. *Biochem. J.* **52**: 407.
8. Stadie, W. C., and B. C. Riggs. 1944. Microtome for the preparation of tissue slices for metabolic studies of surviving tissue *in vitro*. *J. Biol. Chem.* **154**: 687.
9. Rasmussen, H., Y.-L. Sze, and R. Young. 1964. Further studies of the isolation and characterization of parathyroid polypeptides. *J. Biol. Chem.* **239**: 2852.
10. Seligson, D., and K. Hirahara. 1957. The measurement of ammonia in whole blood, erythrocytes, and plasma. *J. Lab. Clin. Med.* **49**: 962.
11. Graham, L. T., Jr., R. Werman, and M. H. Aprison. 1965. Microdetermination of glutamate in single cat spinal roots. *Life Sci.* **4**: 1085.
12. Mendicino, J., C. Beaudreau, and R. N. Bhattacharyya. 1966. Reversible inactivation of D-fructose 1,6-diphosphatase by adenosine triphosphate and cyclic 3′,5′-adenosine monophosphate. *Arch. Biochem. Biophys.* **116**: 436.
13. Mansour, T. E. 1963. Studies on heart phosphofructokinase: purification, inhibition and activation. *J. Biol. Chem.* **238**: 2285.
14. Exton, J. H., L. S. Jefferson, Jr., R. W. Butcher, and C. R. Park. 1966. Gluconeogenesis in perfused liver: the effects of fasting, alloxan diabetes, glucagon, epinephrine, adenosine 3′,5′-monophosphate and insulin. *Amer. J. Med.* **40**: 709.
15. Brodie, B. B., J. I. Davies, S. Hynie, G. Krishna, and B. Weiss. 1966. Interrelationships of catecholamines with other endocrine systems. *Pharmacol. Rev.* **18**: 273.
16. Haynes, R. C., Jr., S. B. Koritz, and F. G. Peron. 1959. Influence of adenosine 3′,5′-monophosphate on corticoid production by rat adrenal glands. *J. Biol. Chem.* **234**: 1421.
17. Haynes, R. C., Jr. 1958. The activation of adrenal phosphorylase by the adrenocorticotropic hormone. *J. Biol. Chem.* **233**: 1220.
18. Robison, G. A., R. W. Butcher, and E. W. Sutherland. 1968. Cyclic AMP. *Annu. Rev. Biochem.* **37**: 149.
19. Teng, C.-T., M. L. Karnovsky, B. R. Landau, A. B. Hastings, and F. B. Nesbett. 1953. Metabolism of C^{14}-labeled glycerol and pyruvate by liver *in vitro*. *J. Biol. Chem.* **202**: 705.
20. Hers, H. G. 1955. The conversion of fructose-1-C^{14} and sorbitol-1-C^{14} to liver and muscle glycogen in the rat. *J. Biol. Chem.* **214**: 373.
21. Shrago, E., H. A. Lardy, R. G. Nordlie, and D. O. Foster. 1963. Metabolic and hormonal control of phosphoenolpyruvate carboxykinase and malic enzyme in rat liver. *J. Biol. Chem.* **238**: 3188.
22. Yeung, D., and I. T. Oliver. 1968. Induction of phosphopyruvate carboxylase in neonatal rat liver by adenosine 3′,5′-cyclic monophosphate. *Biochemistry.* **7**: 3231.
23. Alleyne, G. A., and G. H. Scullard. 1969. Renal metabolic response to acid-base changes. I. Enzymatic control of ammoniagenesis in the rat. *J. Clin. Invest.* **48**: 364.
24. Rosenzweig, S., and D. Frascella. 1968. Effects of metabolic acidosis on kidney and liver phosphoenolpyruvate carboxykinase activity. *Bull. N. J. Acad. Sci.* **13**: 17.

[6] It has been observed that the intravenous administration of hydrochloric acid to parathyroidectomized rats does not cause a change in renal cyclic AMP (cortex and medulla combined) within 5 min (4). This short-term study in parathyroidectomized animals does not exclude the possibility that metabolic acidosis may increase cortical cyclic AMP under different experimental conditions. Study of the effect of more prolonged infusions of hydrochloric acid on cortical cyclic AMP in intact animals should help to clarify the issue.