Effects of Acetazolamide on the Urinary Excretion of Cyclic AMP and on the Activity of Renal Adenyl Cyclase

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ABSTRACT Acetazolamide, an inhibitor of the enzyme carbonic anhydrase, increased the urinary excretion of cyclic AMP in normal and parathyroidectomized rats. The increase was greater in rats with intact parathyroid glands than in parathyroidectomized rats. This rise in the urinary excretion of cyclic AMP was not due to an increase in urine flow or a change in urine pH. Furosemide caused an increase in urine flow, but did not affect the excretion of cyclic AMP or phosphate. Alkalinization of the urine with bicarbonate did not increase the urinary excretion of phosphate or cyclic AMP. Acetazolamide increased the production of cyclic AMP by rat renal cortical slices in vitro. This effect was dose-dependent. Acetazolamide also stimulated the activity of renal cortical adenyl cyclase in a dose-dependent manner but had no effect on the activity of cyclic nucleotide phosphodiesterase. The pattern of urinary excretion of cyclic AMP and phosphate after administration of acetazolamide was similar to that observed in rats given parathyroid hormone. It is suggested that acetazolamide stimulates the renal production of cyclic AMP by activating adenyl cyclase and that this may be the mechanism by which this inhibitor of carbonic anhydrase produces phosphaturia.

INTRODUCTION

The administration of acetazolamide, a potent inhibitor of the enzyme carbonic anhydrase, results in marked phosphaturia and bicarbonaturia, but only modest natriuresis (1, 2). These effects are strikingly similar to those observed after administration of parathyroid hormone (3–5). It is now widely accepted that cyclic AMP is the mediator of the hormonal effects of parathyroid hormone in bone (6) and kidney (7). Recently, carbonic anhydrase inhibitors have been found to antagonize the calcemic response observed after administration of parathyroid hormone to nephrectomized rats (8) and the resorption of bone induced by parathyroid hormone in tissue cultures (9). These findings suggest an interaction of carbonic anhydrase inhibitors with the adenyl cyclase system of bone activated by parathyroid hormone.

These considerations prompted us to study the effects of acetazolamide on the urinary excretion of cyclic AMP and on the adenyl cyclase system of the renal cortex. Our results indicate that acetazolamide increases the urinary excretion of cyclic AMP in normal or parathyroidectomized rats. The temporal relationship observed between the urinary excretion of cyclic AMP and phosphate suggests that the phosphaturia associated with the administration of acetazolamide may be mediated by cyclic AMP. Studies in vitro indicated that acetazolamide directly stimulates the adenyl cyclase system of the renal cortex in the rat.

METHODS

In vivo experiments

Experiments were performed in female Sprague-Dawley rats weighing 180 to 240 g. The rats were allowed food and water ad lib until 12 h before study, when food was withheld. The following studies were performed:

Administration of acetazolamide to normal rats. Polyethylene catheters were inserted, under light ether anesthesia, into the right femoral artery to obtain blood samples and into the left jugular vein for the infusion of solutions. A Silastic catheter was placed in the bladder. The rats were then placed in Plexiglas holders and allowed to recover completely from the effects of the anesthetic. A priming dose of inulin, 12.5 mg in 1 ml of normal saline, was administered, followed by a sustaining solution of inulin (12.5 mg/ml) in normal saline, infused at a rate of 50 μl/min. After an equilibration period of 60 min, three 30–40-min clearance periods were obtained. Acetazolamide, 3 mg in 0.3 ml of normal saline, was then administered, followed by a sustaining infusion which delivered 3 mg

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of acetazolamide/h, and three additional clearance periods (each of 10 min duration) were obtained. Urine was collected under mineral oil into previously weighed tubes.

Administration of acetazolamide to parathyroidectomized rats. Surgical parathyroidectomy was carried out under light ether anesthesia 24 h before study. The animals had free access to water, but no food after surgery. Serum calcium was less than 7.5 mg/100 ml at the time of study. The experimental protocol for clearance studies was identical to that used in normal rats.

Administration of furosemide to normal rats. The experimental protocol was identical to that used in normal rats receiving acetazolamide. After obtaining three control clearance periods, 1.5 mg of furosemide was administered, followed by a sustaining infusion delivering 1.5 mg of furosemide/h, and three additional clearance periods were obtained.

Administration of bicarbonate to normal rats. Three normal rats were prepared in the manner described above. After obtaining two to three control clearance periods, a sodium bicarbonate solution containing 120 meq/liter of HCO_3 was given at a rate of 50 μl/min. After the urine pH reached or exceeded 7.0, three additional clearance periods were obtained.

In vitro experiments

Production of cyclic AMP by renal cortical slices. Female Sprague-Dawley rats weighing 200–300 g were sacrificed and their kidneys quickly removed, decapsulated, and placed in ice-cold Krebs-Ringer phosphate. Cortical slices were obtained using a Stadie-Riggs microtome. Slices were preincubated for 1 h in Krebs-Ringer phosphate containing 10^{-3} M theophylline, 10^{-3} M glucose, and 0.25% bovine serum albumin, and gassed with a mixture of 95% O_2 and 5% CO_2 in a Dubnoff metabolic shaker at 37°C. The slices were then transferred to individual flasks containing 1 ml of the same Ringer's solution, to which acetazolamide, parathyroid hormone, or both were added. In another series of experiments, the effects of 10^{-4} M furosemide and 10^{-4} M ethacrynic acid on the concentration of cyclic AMP in rat renal cortical slices were studied. Incubations were carried out at 37°C for 20 min, after which the slices were quickly frozen in Freon (E. I. duPont & Co., Inc., Wilmington, Del.), then boiled in 1 ml of 0.05 M Na-acetate-acetic acid buffer, pH 4, for 10 min, and finally centrifuged at 6,000 *g* for 20 min. This extraction procedure did not result in significant loss of cyclic AMP (10). A sample for determination of protein was obtained before centrifugation. Cyclic AMP was measured in duplicate in both the clear supernate and the incubation medium by a modification (10) of the protein-binding assay of Gilman (11). A standard curve was run with each assay. The curve was linear between 0.5 and 20 pmol of cyclic AMP when plotted in a semilogarithmic scale and the concentration of cyclic AMP in all samples tested was within this range. Neither acetazolamide nor the phosphate buffer interfered with the binding of cyclic AMP to the protein.

Activity of adenyl cyclase from renal cortex. Adenyl cyclase activity was determined by a modification of the method of Marcus and Aurbach (12). The cortex from two kidneys was homogenized in 0.02 M Tris-HCl buffer, pH 7.4, containing 10% vol/vol dimethylsulfoxide, and the homogenate was centrifuged at 2,000 *g* for 10 min. The precipitate was resuspended in the same buffer and centrifuged again. The particulate enzyme preparation was then suspended in the Tris-HCl-dimethylsulfoxide buffer to give a protein concentration of approximately 15–20 mg/ml and was stored at −75°C. The activity of adenyl cyclase was assayed by determining the formation of cyclic AMP from ATP with the addition of KCl and an ATP-regenerating system. The reaction mixture contained: Tris-HCl, 0.02 M, pH 7.4; $MgCl_2$, 4.5×10^{-3} M; theophylline, 9×10^{-3} M; bovine serum albumin, 0.013%; KCl, 3×10^{-2} M; phosphoenolpyruvate, 5.4×10^{-3} M; ATP, 1.1×10^{-3} M; pyruvate kinase, 0.07 mg/ml; approximately 600–800 μg of enzyme suspension and acetazolamide in a total volume of 200 μl. Incubations were carried out in a Dubnoff metabolic shaker at 37°C for 10 min, at the end of which the reaction was stopped by boiling for 3 min. The content of the tubes was diluted with 1 ml of 0.05 M Na-acetate-acetic acid buffer, pH 4, and the precipitated protein was separated by centrifuging at 5,900*g* for 15 min. Cyclic AMP then was measured in the clear supernate. In each experiment, a blank containing the whole reaction mixture but no enzyme suspension was run in triplicate and its average value was subtracted from the value in each tube.

Activity of phosphodiesterase from renal cortex. Cyclic nucleotide phosphodiesterase was prepared from rat renal cortex as described by Cheung for rat brain (13). The renal cortex was homogenized in 5 vol of distilled water and the homogenate was centrifuged for 30 min at 30,000 *g*. The supernatant fluid was dialyzed for 16 h against 200 vol of 0.02 M Tris-HCl, pH 7.4. Phosphodiesterase activity was assayed by incubating the enzyme in 200 μl of 0.02 M Tris-HCl, pH 7.4, containing cyclic AMP, 2×10^{-6} M; $MgCl_2$, 1.8×10^{-3} M; and either theophylline or acetazolamide. Incubations were carried out at 37°C for 30 min. The reaction was stopped by boiling for 3 min. The content of the tubes was diluted with 1 ml of 0.05 M Na-acetate-acetic acid buffer (pH 4) and the protein separated by centrifuging at 5,900*g* for 15 min. The clear supernate was assayed for cyclic AMP. The recovery of cyclic AMP added to the adenyl cyclase and phosphodiesterase assays was 95–100%.

Analytical procedures. Inulin was determined by the microanthrone method (14), and sodium and potassium, with a flame photometer (Model 43, Instrumentation Laboratory, Inc., Lexington, Mass.). Plasma and urine pH and P_{CO_2} were measured on an Instrumentation Laboratory gas analyzer, model 123, and the plasma bicarbonate was calculated by the Henderson-Hasselbach equation, with a pK value of 6.1 and a solubility value of 0.03 for plasma. For urine, the solubility value used was 0.0309, and the pK values were calculated for each sample by the formula $pK = 6.33 - 0.5 \times \sqrt{B}$, where B represents the total cation concentration estimated in the urine as the sum of sodium and potassium concentrations. Plasma and urinary phosphate were measured by the method of Chen, Toribara, and Warner (15). For the determination of urinary cyclic AMP, samples were diluted appropriately with 0.05 M Na-acetate-acetic acid buffer, pH 4, to bring the concentration of the nucleotide within the range of the standard curve, and the pH (which was found to be critical for binding) to the optimal values. Serum calcium was measured by atomic absorption spectrophotometry with an Instrumentation Laboratory Model 153. Protein concentration was measured by the method of Lowry, Rosebrough, Farr, and Randall (16).

Cyclic AMP and acetazolamide were purchased from Sigma Chemical Co. (St. Louis, Mo.) and [^{3}H]cyclic AMP (sp act 24.1 Ci/mol) from New England Nuclear (Boston, Mass.) For the in vivo experiments, the sodium salt of acetazolamide (Diamox, Lederle Laboratories, Div. of

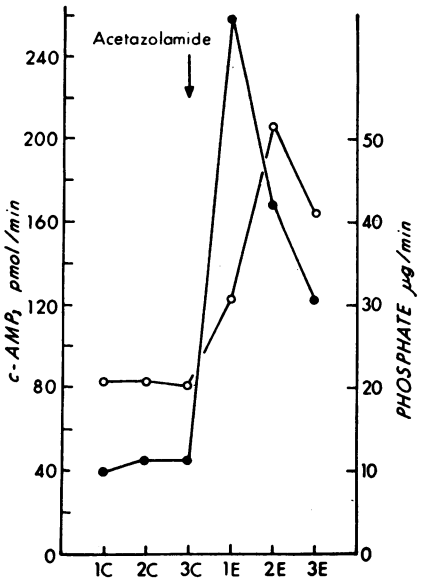

FIGURE 1 Representative experiment on the effects of acetazolamide (3 mg in 0.3 ml of normal saline followed by an infusion delivering 3 mg/h) on the urinary excretion of cyclic AMP (c-AMP) (closed circles) and phosphate (open circles) in a normal rat. The points correspond to three control (C) and three experimental (E) periods before and after acetazolamide administration.

American Cyanamid Co., Pearl River, N. Y.) was used. The parathyroid hormone used in these studies was the synthetic, 1–34, tetratriacontapeptide (sp act 3760 IU/mg), purchased from Beckman Instruments, Inc., Spinco Div. (Palo Alto, Calif).

RESULTS

Effects of administration of acetazolamide on urinary excretion of phosphate, bicarbonate, and cyclic AMP in normal rats. A representative experiment is depicted in Fig. 1. After infusion of acetazolamide, there was a marked increase in the urinary excretion of cyclic AMP during the first experimental period. Thereafter, the excretion rate decreased progressively over the ensuing two periods. However, even at the end of 30 min, the excretion of cyclic AMP was substantially greater than in control periods. The excretion of phosphate followed a similar pattern, but the peak excretion of phosphate occurred some time after the peak increase in cyclic AMP.

Table I summarizes clearance data in four normal rats before and after administration of acetazolamide. Each value represents the mean of three control and three experimental periods, with the exception of the value for cyclic AMP during the experimental periods, which represents the peak response. This invariably occurred during the first 10-min period after infusion of acetazolamide. Inulin clearance was not significantly different in control and experimental periods, whereas urine flow, sodium, bicarbonate, phosphate, and cyclic AMP excretion rates were significantly greater after the administration of acetazolamide.

Effects of administration of acetazolamide on urinary excretion of phosphate, bicarbonate, and cyclic AMP in parathyroidectomized rats. A representative experiment is shown in Fig. 2. Excretion rates for cyclic AMP and phosphate in the control periods were less than those observed in normal rats. After administration of acetazolamide, there was a brisk and marked increase in the urinary excretion of cyclic AMP during the first experimental period, similar to that observed in normal animals. However, unlike the pattern observed in the normal rats, the excretion rates of cyclic AMP returned to control values quite rapidly. The peak excretion of phosphate occurred in the second 10 min after the peak excretion in cyclic AMP. This temporal relationship in the excretion of cyclic AMP and phosphate is strikingly similar to that seen in parathyroidectomized rats infused with parathyroid hormone (7).

Table II summarizes clearance data in parathyroidectomized rats before and after the administration of acetazolamide. Each value represents the mean of three control or three experimental periods. The values for cyclic AMP in the experimental periods represent the peak response observed in the first period after infusion of acetazolamide. The differences for all parameters, except for clearance of inulin, were statistically significant between control and experimental values. The degree of phosphaturia and the increase in the urinary excretion of cyclic AMP were less in parathyroidectomized than

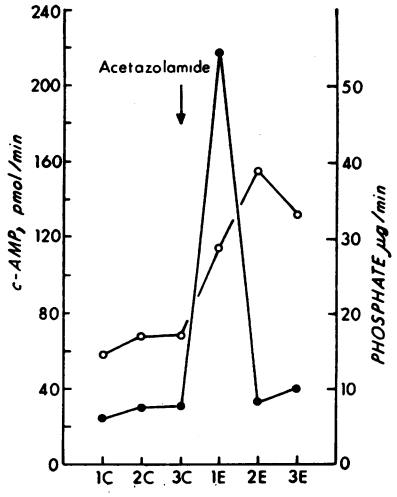

FIGURE 2 Effect of acetazolamide on the urinary excretion of cyclic AMP (c-AMP) (closed circles) and phosphate (open circles) in a parathyroidectomized rat. The points correspond to three control (C) and three experimental (E) periods before and after acetazolamide administration.

TABLE I

Effects of Acetazolamide on Urinary Excretion of Sodium, Bicarbonate, Phosphate, and Cyclic AMP in Normal Rats

Experiment	Periods	V	C_{In}	$U_{Na}V$	$U_{HCO_3}V$	$U_{PO_4}V$	FE_{Na}	cAMP
		μl/min	*ml/min*	*μeq/min*	*μeq/min*	*μg/min*	%	*pmol/min*
1	Control	45.4	2.30	5.07	0.84	25.83	1.5	113.1
	Experimental	121.7	2.34	15.47	28.49	33.74	4.5	220.7
2	Control	34.8	2.57	1.98	0.37	21.97	0.5	45.2
	Experimental	122.2	2.05	14.84	32.96	38.93	4.9	157.3
3	Control	51.5	2.80	5.87	0.76	23.06	1.5	43.0
	Experimental	151.5	2.17	19.56	36.18	44.31	6.3	277.9
4	Control	28.2	2.13	1.57	0.01	25.76	0.5	32.9
	Experimental	113.2	2.23	13.80	28.06	42.45	4.0	156.3
	Control							
	Mean	40.0	2.45	3.62	0.49	24.35	0.9	58.5
	SEM	±5.2	±0.11	±0.60	±0.11	±1.09	±0.1	±10.6
	Experimental							
	Mean	118.7	2.20	15.92	31.35	39.86	4.9	203.1
	SEM	±14.9	0.14	0.95	1.60	2.71	0.4	29.1
	P	<0.001	NS	<0.001	<0.001	<0.001	<0.001	<0.02

C_{In}, inulin clearance; FE_{Na}, fraction of the filtered sodium excreted; $U_{HCO_3}V$, urinary excretion rate of bicarbonate; $U_{Na}V$, urinary excretion rate of sodium; $U_{PO_4}V$, urinary excretion rate of phosphate; V, urine flow. In these experiments, 3 mg of acetazolamide in normal saline were given, followed by a sustaining solution delivering 3 mg of acetazolamide/h.

in normal rats, but the rise in urine flow, natriuresis, and bicarbonaturia was comparable in the two groups of rats.

Fig. 3 summarizes the urinary excretion of cyclic AMP in four normal and four parathyroidectomized rats before and after administration of acetazolamide. Control values for excretion of cyclic AMP were consistently lower in the parathyroidectomized rats. After infusion of acetazolamide, the peak response in the excretion of cyclic AMP in both groups of rats occurred in the first 10 min, but whereas the excretion rate returned

TABLE II

Effects of Acetazolamide on Urinary Excretion of Sodium, Bicarbonate, Phosphate, and Cyclic AMP In Parathyroidectomized Rats

Experiment	Periods	V	C_{In}	$U_{Na}V$	$U_{HCO_3}V$	$U_{PO_4}V$	FE_{Na}	cAMP
		μl/min	*ml/min*	*μeq/min*	*μeq/min*	*μg/min*	%	*pmol/min*
1	Control	60.8	2.51	4.17	0.15	16.63	1.1	27.9
	Experimental	184.1	2.36	23.08	41.26	33.19	6.9	217.7
2	Control	51.1	3.03	5.98	1.04	13.94	1.4	23.9
	Experimental	102.0	2.32	17.26	37.11	25.47	4.3	110.4
3	Control	61.3	2.69	7.45	2.16	12.88	1.9	27.2
	Experimental	149.5	2.45	20.64	39.56	25.84	6.1	113.9
4	Control	29.4	2.15	2.68	0.01	10.44	0.9	31.7
	Experimental	76.6	1.52	14.53	29.05	26.17	4.9	192.9
	Control							
	Mean	50.6	2.59	4.85	0.72	13.53	1.3	26.9
	SEM	±5.4	±0.15	±0.63	±0.26	±1.59	±0.1	±1.6
	Experimental							
	Mean	128.0	2.16	18.88	36.75	27.67	5.6	158.7
	SEM	±17.4	±0.16	±1.46	±2.14	±2.30	±0.5	±27.4
	P	<0.005	NS	<0.001	<0.001	<0.001	<0.001	<0.02

For abbreviations, see Table I.

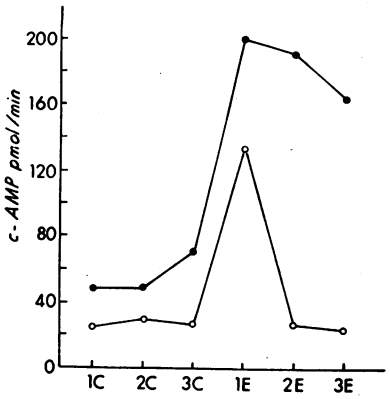

FIGURE 3 Mean urinary excretion of cyclic AMP in four normal (closed circles) and four parathyroidectomized (open circles) rats, before and after administration of acetazolamide. Each point represents the mean value for three control (C) and three experimental (E) periods of duplicate or triplicate determinations in each animal.

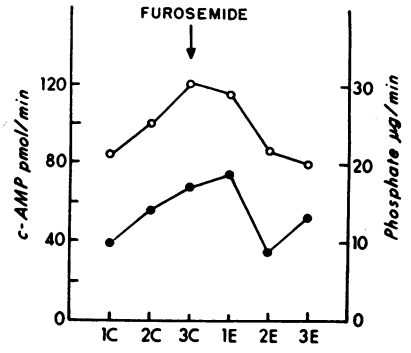

FIGURE 4 Effects of furosemide (1.5 mg followed by an infusion delivering 1.5 mg/h) on the urinary excretion of cyclic AMP (c-AMP) (closed circles) and phosphate (open circles). See Fig. 1 for other details.

rapidly to control values in the parathyroidectomized rats, it remained substantially elevated in the normal rats.

Effects of furosemide on the urinary excretion of cyclic AMP in normal rats. To explore the role of a marked diuresis on the observed increase in the urinary excretion of cyclic AMP, experiments were conducted in four normal rats, in which diuresis was induced by administration of furosemide. After administration of furosemide to a normal rat, the urinary excretion of cyclic AMP and phosphate did not change, despite a marked and sustained diuresis (Fig. 4). Clearance data in rats before and after administration of furosemide are summarized in Table III. After administration of furosemide there was a significant decrease in clearance of inulin. A marked natriuresis occurred but there was no increase in the excretion of bicarbonate, phosphate, or cyclic AMP.

TABLE III

Effects of Furosemide on Urinary Excretion of Sodium, Bicarbonate, Phosphate, and Cyclic AMP in Normal Rats

Experiment	Period	V	C_{In}	$U_{Na}V$	$U_{HCO_3}V$	$U_{PO_4}V$	FE_{Na}	cAMP	Peak response in cAMP	cAMP/C_{in}
		µl/min	*ml/min*	*µeq/min*	*µeq/min*	*µg/min*	%	*pmol/min*	*pmol/min*	
1	Control	57.4	2.62	7.02	0.34	21.93	1.8	148.7	148.7	56.8
	Experimental	319.5	1.90	40.00	0.72	12.57	14.6	95.3	116.0	50.2
2	Control	30.9	1.53	4.06	0.04	20.46	1.8	95.7	95.7	62.5
	Experimental	260.1	1.09	32.99	1.04	9.87	21.0	111.0	105.0	101.8
3	Control	50.7	2.48	7.71	1.41	25.60	2.2	54.4	54.4	21.9
	Experimental	260.7	1.54	42.70	0.68	16.89	18.5	54.5	53.7	35.4
4	Control	69.8	3.18	9.96	1.49	35.98	2.2	44.3	44.3	13.9
	Experimental	230.4	1.96	30.44	0.51	34.43	11.2	12.0	15.2	6.1
	Control									
	Mean	52.2	2.46	7.19	0.82	26.00	2.0	85.8	85.8	38.8
	SEM	±5.3	±0.19	±0.66	±0.20	±2.17	±0.07	±14.5	±14.5	±12.2
	Experimental									
	Mean	267.7	1.62	36.53	0.74	21.17	16.3	68.2	72.5	48.4
	SEM	±28.3	±0.16	±4.06	±0.20	±5.81	±1.3	±12.6	±23.4	±20.0
	P	<0.001	<0.01	<0.001	NS	NS	<0.001	NS	NS	NS

For details of abbreviations see Table I. In these experiments 1.5 mg of furosemide were given followed by a sustaining solution delivering 5.5 mg of furosemide/h.

Excretion of cyclic AMP in Experimental is expressed as the mean value of three experimental periods, or as the peak response (highest experimental value). In addition, the excretion of cyclic AMP has been expressed per milliliter of inulin clearance (c-AMP/C_{In}).

To study the possibility that the observed increase in the urinary excretion of cyclic AMP after acetazolamide administration was the result of alkalinization of the intratubular fluid, three normal rats were infused with sodium bicarbonate as outlined under Methods. As the urinary pH increased from 6.26±0.07 to 7.23±0.11, the excretion rate of cyclic AMP fell from 106.3±8.9 pmol/min to 52.4±15.8 pmol/min, and phosphate excretion remained unchanged 19.5±2.1 vs. 20.5±1.5 μg/min.

Effects of acetazolamide on production of cyclic AMP by renal cortical slices. Acetazolamide, 10^{-4} M, increased cyclic AMP concentration in cortical slices from 20.6±1.9 pmol/mg protein to 44.9±5.2 pmol/mg protein. Synthetic parathyroid hormone (0.03 U/ml) increased cyclic AMP concentration to 33.6±2.2 pmol/mg protein. When both 10^{-4} M acetazolamide and 0.03 U/ml of parathyroid hormone were used, cyclic AMP increased to 70.0±9.0 pmol/mg protein. All these values are significantly different from the control ($P < 0.01$). The results of these experiments are summarized in Table IV. From these data, an additive effect of acetazolamide and PTH is apparent; however, when higher concentrations of parathyroid hormone were used an additive effect could not be demonstrated.

Fig. 5 shows the effects of increasing concentrations of acetazolamide on the production of cyclic AMP. An increase in the production of cyclic AMP was detected at a concentration of acetazolamide of 10^{-6} M and the peak response occurred at a concentration of acetazolamide of 5×10^{-4} M. Higher concentrations did not produce a further increase in cyclic AMP, but this could reflect the fact that acetazolamide is sparingly soluble at concentrations greater than 10^{-3} M. The activity measured was entirely due to cyclic AMP, since in some experiments, incubation of a sample of the tissue-homogenate supernate with phosphodiesterase resulted in no measurable cyclic AMP.

TABLE IV

Effects of 10^{-4}M Acetazolamide and 0.03 U/ml Parathyroid Hormone on the Production of Cyclic-AMP by Rat Renal Cortical Slices

Experiment	cAMP			
	Control	Acetazolamide	PTH	PTH & Acetazolamide
1	17.9	35.2	27.5	65.2
2	22.7	58.5	37.5	—
3	23.9	53.6	38.6	—
4	13.8	26.6	29.8	52.4
5	18.4	40.3	28.8	67.1
6	26.7	55.2	39.5	95.3
Mean	20.6	44.9	33.6	70.0
SEM	±1.9	±5.2	±2.2	±9.0
P	—	<0.01	<0.01	<0.01

PTH = parathyroid hormone. The *P* values refer to the differences of each of the three experimental groups and the control. Cortical slices from the same rat were used for the control and experimental conditions. Each experiment represents the values for one animal.

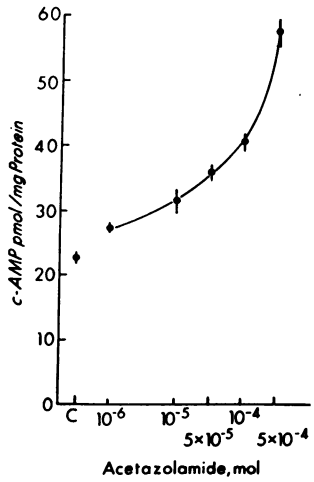

FIGURE 5 Cyclic AMP (c-AMP) production by rat renal cortical slices, in picomoles per milligram of protein, as a function of increasing concentrations of acetazolamide. The points represent the means±1 SEM ($n = 3$). No cyclic AMP was detected in portions of the same samples treated with phosphodiesterase.

Effects of acetazolamide on renal cortical adenyl cyclase. As shown in Fig. 6, acetazolamide, 10^{-4} M, increased the activity of rat renal cortical adenyl cyclase from 122.3±SEM 3.0 pmol/mg protein per h to 299±5.3 pmol/mg protein per h ($P < 0.001$). Synthetic para-

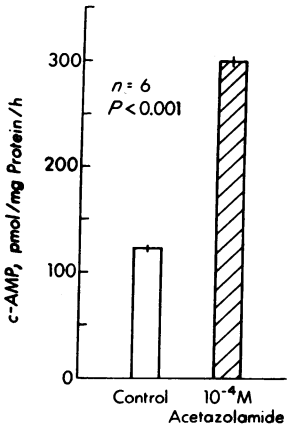

FIGURE 6 Activity of rat renal cortical adenyl cyclase in picomoles of cyclic AMP produced per milligram of protein per hour. The open bar shows the mean control value and the cross-hatched bar the value obtained in the presence of 10^{-4} M acetazolamide. The bars represent the means ±1 SEM ($n = 6$). The difference between control and acetazolamide is statistically significant ($P < 0.001$).

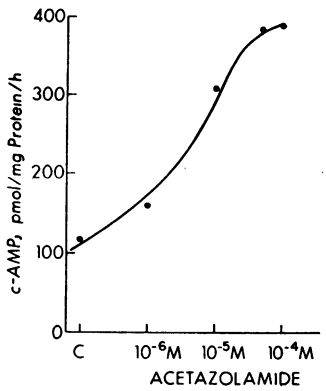

FIGURE 7 Effects of increasing concentrations of acetazolamide on the activity of rat renal cortical adenyl cyclase. Adenyl cyclase activity is expressed as cyclic AMP produced in picomoles per milligram protein per hour. The points are the mean of triplicate determinations.

thyroid hormone at a concentration of 10^{-6} M (approximately 1 U/ml) increased cyclic AMP production to 354.0±7.0 pmol/mg protein per h. The values for control and parathyroid hormone-stimulated adenyl cyclase are similar to those reported by others (12, 17).

As shown in Fig. 7, the stimulation of rat renal cortical adenyl cyclase activity by acetazolamide was dose-dependent. The maximum effect was observed at a concentration of acetazolamide of 10^{-4} M.

Effects of acetazolamide and theophylline on rat renal cortical phosphodiesterase. Theophylline is a known inhibitor of the enzyme phosphodiesterase (18). As shown in Fig. 8, 10^{-2} M theophylline decreased the activity of rat renal cortical phosphodiesterase from 332.5±SEM 5.4 pmol cyclic AMP converted to 5'-AMP/mg protein per 30 min to 227.5±22.0 pmol/mg protein per 30 min

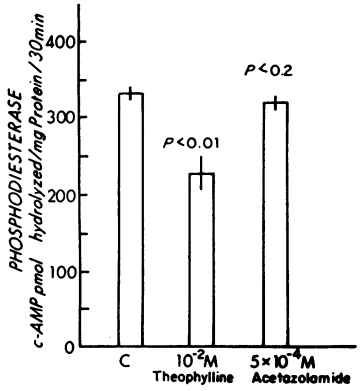

FIGURE 8 Effects of 10^{-2} M theophylline and 5×10^{-4} acetazolamide on the activity of rat renal cortical phosphodiesterase. Enzyme activity is expressed as picomoles of cyclic AMP converted to 5'-AMP per milligram protein per 30 min. The bars represent the means±SEM ($n = 6$) C, control.

($P < 0.01$). Acetazolamide 5×10^{-4} M did not decrease the activity of the enzyme. The value of 319.5±6.8 pmol of cyclic AMP hydrolyzed/mg protein per 30 min observed with acetazolamide was not statistically different from the control.

Effects of other diuretics on cyclic AMP production by cortical slices. The effects of 10^{-4} M furosemide and 10^{-4} M ethacrynic acid on the concentration of cyclic AMP by renal cortical slices were also studied. The mean value for cyclic AMP concentration in control slices from four rats was 23.4±4.4 pmol/mg protein. In the presence of furosemide the mean value was 25.8±2.5 and with ethacrynic acid 21.5±3.1 pmol/mg protein. These latter two values are not significantly different from the control value, indicating that neither diuretic had any effect on cyclic AMP production in vitro.

DISCUSSION

The results of this study clearly indicate that acetazolamide increases the urinary excretion of cyclic AMP in both normal and parathyroidectomized rats. This effect is not due to an increase in sodium and water excretion since furosemide failed to augment the urinary excretion of cyclic AMP, despite an increase in the urinary excretion of sodium and water comparable with that seen with acetazolamide. The increased excretion of cyclic AMP does not seem to be the consequence of an increase in urinary pH, since alkalinization of the urine by the infusion of sodium bicarbonate did not increase the excretion of the nucleotide. Although the present studies do not completely rule out the possibility of an increased clearance of cyclic AMP after acetazolamide administration, this appears most unlikely in view of the results of our in vitro studies. Acetazolamide markedly increased the concentration of cyclic AMP in vitro in rat renal cortical slices. There was a direct stimulation of the renal cortical adenyl cyclase system by acetazolamide, but no inhibition of phosphodiesterase, the enzyme responsible for degradation of cyclic AMP. Thus, it seems reasonable to conclude that the observed increase in urinary excretion of cyclic AMP after acetazolamide administration reflects an increase in the renal production of the nucleotide.

The data in parathyroidectomized rats clearly indicate that acetazolamide directly stimulates the urinary excretion of phosphate and that this effect is not mediated via parathyroid hormone. This is in agreement with recent micropuncture studies (5). The marked increase in the urinary excretion of cyclic AMP observed with acetazolamide closely resembles that seen in parathyroidectomized rats infused with parathyroid hormone (7), and suggests that the mechanism of the phosphaturia induced by acetazolamide is probably mediated through stimulation of the renal production of cyclic AMP.

The fact that normal rats responded to the administration of acetazolamide with a larger increment in urinary phosphate excretion and with an enhanced response in cyclic AMP production suggests that acetazolamide may either potentiate the renal effects of endogenous parathyroid hormone or stimulate release of the hormone from the parathyroid glands, directly or indirectly. Although the first possibility seems to receive support from the results of our in vitro studies, we have not excluded the latter. In the in vitro studies, when low doses of parathyroid hormone were used in conjunction with maximum doses of acetazolamide an additive effect was observed. However, we were unable to demonstrate such additive effect with maximum doses of parathyroid hormone. This casts doubt about the possible existence of two separate receptor sites for the hormone and acetazolamide.

The mechanism of the phosphaturia after administration of acetazolamide has not been elucidated. It has been postulated that the phosphaturia is secondary to the increase in the urinary excretion of bicarbonate (1, 19, 20), but data from both clearance (21) and micropuncture studies (22) do not support this theory. The clear stimulation of the renal cortical adenyl cyclase system by acetazolamide as well as the temporal relationship between the urinary excretion of cyclic AMP and phosphate in the rat strongly suggest that the phosphaturia induced by acetazolamide is mediated by an increase in the renal production of cyclic AMP. Acetazolamide may produce phosphaturia not only by directly inhibiting phosphate reabsorption due to increased levels of cyclic AMP but also by decreasing salt and water reabsorption in the proximal tubule (5, 23). Phosphate reabsorption in the proximal tubule is coupled to fluid reabsorption (5, 24) and a decrease in fluid reabsorption due to either carbonic anhydrase inhibition or cyclic AMP production, or both (3), by acetazolamide may contribute to the phosphaturia.

Furosemide, in contrast to the data reported in man (25), did not produce phosphaturia in the rat. Furosemide has carbonic anhydrase-inhibitory capacity, and its lack of effect on cyclic AMP production may indicate either that not all carbonic anhydrase inhibitors activate adenyl cyclase or that the dose of furosemide used in the in vitro studies was less than that required for carbonic anhydrase inhibition and adenyl cyclase activation. It has been reported that the concentrations of furosemide required to produce 50% inhibition of carbonic anhydrase in vitro are about 50 times greater than those of acetazolamide (25).

In conclusion, stimulation of the renal adenyl cyclase system and increased production of cyclic AMP by both acetazolamide and parathyroid hormone seem to be the best explanation for the striking and puzzling similarities in the renal effects of these two chemically unrelated molecules.

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