Effects of Parathyroid Hormone on Plasma and Urinary Adenosine 3′,5′-Monophosphate in Man

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ABSTRACT The effects of parathyroid hormone (PTH) on plasma and urinary adenosine 3′,5′-monophosphate (cyclic AMP) levels were studied in normal subjects. Under basal conditions normal adults have plasma concentrations of cyclic AMP ranging from 10 to 25 nmoles/liter and excrete from 1.5 to 5 μmoles of cyclic AMP per g of urinary creatinine. About one-half to two-thirds of the cyclic AMP excreted in the urine is derived from the plasma by glomerular filtration, and the remainder is produced by the kidney. Renal production of cyclic AMP is partly under the control of PTH. It can be suppressed by infusions of calcium and stimulated by infusions of the calcium chelating agent, EDTA. Infusions of PTH in doses up to 10 mU/kg per min were associated with dose-related increases both in urinary cyclic AMP and phosphate. Infusions of PTH in doses ranging from 20 to 80 mU/kg per min did not lead to any further increase in phosphaturia but did lead to further marked increases in urinary cyclic AMP. A modest increase in plasma cyclic AMP was noted when PTH was infused at 40 mU/kg per min. Anephric patients failed to show appreciable increases in plasma cyclic AMP in response to large doses of PTH but did show expected increases in response to glucagon. Surgical removal of parathyroid adenomas from nine patients with primary hyperparathyroidism was invariably followed by a decrease in urinary cyclic AMP. PTH, in large doses, and calcium infusion produced up to 2-fold increases in the other known naturally occurring cyclic nucleotide, guanosine 3′,5′-monophosphate (cyclic GMP).

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INTRODUCTION

With the rapid accumulation of evidence that adenosine 3′,5′-monophosphate (cyclic AMP) is the intracellular mediator of the action of several hormones (1, 2), it has seemed pertinent to inquire whether hormonally-induced increases in tissue cyclic AMP might be reflected in increases in plasma or urinary cyclic AMP. Parathyroid hormone (PTH) stimulates the accumulation of cyclic AMP in broken and unbroken cell preparations of bone and kidney and also increases the excretion of cyclic AMP in the urine (3–8). It has been shown in a companion paper (9) that urinary cyclic AMP is derived from two sources. A portion of it reaches the kidney via the circulation and is excreted by glomerular filtration. A smaller portion is released directly by the kidney. Theoretically, PTH could increase the amount of cyclic AMP in the urine from either or both of these sources. We have, therefore, undertaken a series of experiments to determine how plasma and urinary levels of this nucleotide might be altered by treatment with PTH, calcium infusion, and EDTA. Guanosine 3′,5′-monophosphate (cyclic GMP) excretion was evaluated simultaneously.

METHODS

Materials. Parathyroid hormone (control 2F R71 B) and glucagon (control 2Ex68A) were obtained from Eli Lilly & Co. (Indianapolis, Ind.). Dihydrate calcium chloride, as a 10% solution, was obtained from the Upjohn Co. (Kalamazoo, Mich.). Sodium EDTA (Endrate disodium) was purchased from Abbott Laboratories (Chicago, Ill.). Cyclic 3′,5′-GMP-^{3}H was purchased from Calbiochem (Los Angeles, Calif.), cyclic 3′,5′-AMP-^{3}H from Schwarz Bio Research,

Inc. (Orangeburg, N. Y.), and inulin from Warner-Chilcott Laboratories (Morris Plains, N. J.).

Subjects and procedures. PTH, calcium chloride, or sodium EDTA was infused intravenously into healthy men, 20–30 yr of age, who had neither eaten nor smoked for at least 6 hr before study. Hydration was accomplished by having the subjects drink water and by administering 0.45% sodium chloride by intravenous infusion. Urine output exceeded 7 ml/min, and voided urine collections were made with the subjects recumbent. Blood specimens for analysis were obtained through a catheter placed in the antecubital vein in the arm opposite the infusion site. Heparin was used as an anticoagulant. Blood and urine samples were obtained at 15-, 20-, or 30-min intervals. When inulin clearance was measured, a priming dose of 50 mg/kg was followed by an inulin infusion designed to produce a plasma concentration of 0.25 mg/ml. 30–60 min were allowed for equilibration before the beginning of the first control period. Urine specimens were frozen until analyzed. Blood specimens were centrifuged, and the plasma was separated and frozen for subsequent chemical analysis. Cyclic AMP, cyclic GMP, creatinine, and inulin were determined by methods outlined elsewhere (9, 10). Phosphate was analyzed by a modification of the method of Fiske and Subbarow (11) on a Technicon Auto-Analyzer (Technicon Co., Tarrytown, N. Y.), and calcium was measured by the method of Rudolph, Holder, and Ford (12).

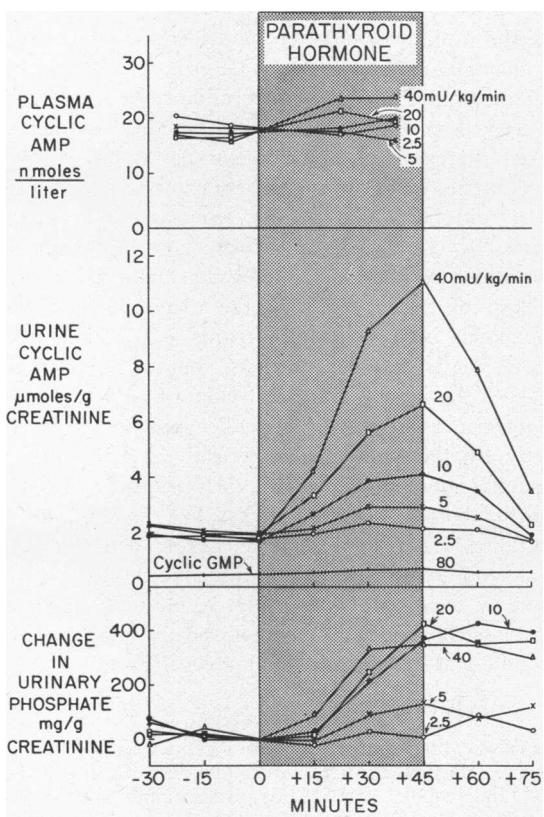

FIGURE 1 Responses of plasma and urinary cyclic AMP and urinary cyclic GMP and phosphate to graded doses of PTH in a single subject (H.He.). Infusions were given over 45-min periods.

RESULTS

Effects of PTH on plasma and urinary cyclic AMP, and urinary cyclic GMP and phosphate

PTH infusions into normal subjects produced increases in urinary cyclic AMP excretion and, to a lesser degree, in plasma cyclic AMP concentrations. The dose-response relationship in one subject studied on different occasions is illustrated in Fig. 1. The low dose of 2.5 mU/kg per min infused over a 45 min period induced at 25% increase and the high dose of 40 mU/kg per min caused a 430% increase in urinary cyclic AMP. In two additional subjects, the low dose of PTH increased urinary cyclic AMP 45% and 145% over control values, without affecting urinary cyclic GMP. Cyclic GMP excretion increased 50% in response to 80 mU/kg per min of PTH, a dose which increased urinary cyclic AMP by 1200% but did not change serum calcium detectably. In two additional subjects, doses of PTH sufficient to produce 15- and 50 fold increases in cyclic AMP excretion were associated with 25% and 115% increases, respectively, in urinary cyclic GMP. Plasma concentrations of cyclic AMP did not rise detectably with the small dose of PTH, but a 40% increase occurred in response to the 40 mU/kg per min dose. Still larger increases in plasma cyclic AMP were shown with larger doses of PTH.

Since it is probable that PTH-induced phosphaturia is mediated by renal cyclic AMP (3, 6–8), the relationship between cyclic AMP and phosphate in urine after treatment with PTH was also investigated (Fig. 1). The phosphaturic response to PTH reached a maximum at a dose of 10 mU/kg per min, but urinary cyclic AMP continued to rise in response to the maximum dose employed in this study, 80 mU/kg per min. With low doses of PTH the phosphaturic response was not noticeable until the second 15 min collection period after the beginning of the PTH infusion, whereas an increase in cyclic AMP excretion was apparent in the first collection period. With higher doses of PTH, however, both the phosphaturic response and the cyclic AMP response were present during the first 15 min of PTH infusion.

The source of PTH-induced increases in urinary cyclic AMP

In order to determine the relative importance of filtered versus nephrogenous cyclic AMP in response to PTH, it was necessary to measure renal clearances of cyclic AMP and inulin simultaneously. The results are depicted in Fig. 2. During control periods cyclic AMP clearance exceeded inulin clearance, the clearance ratio (cyclic AMP: inulin) being about 1.5. In related studies (9) it was determined that circulating cyclic AMP is cleared by glomerular filtration at the same rate as inu-

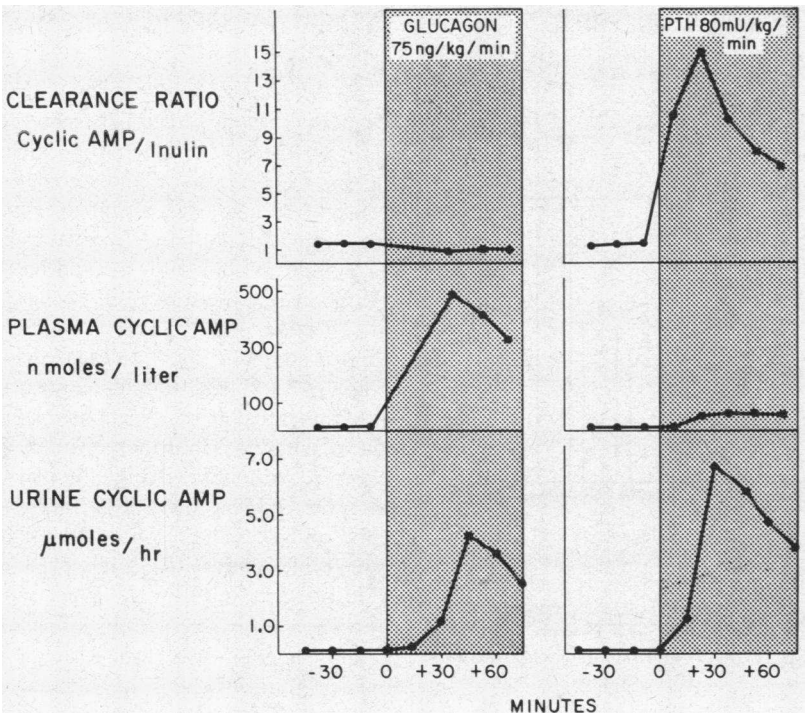

FIGURE 2 Comparison of the effects of glucagon and PTH on urinary cyclic AMP, plasma cyclic AMP, and clearance ratio in a single subject (H.He.). Urinary cyclic AMP determinations were made at the end of each 15 min collection period and the values expressed as micromoles per hour. Points representing plasma cyclic AMP and clearance ratio were obtained at the midpoint of each period.

lin; therefore, with a clearance ratio of 1.5 it may be inferred that about two-thirds of the urinary cyclic AMP was derived from the circulation and one-third was released directly into the urine by the kidney. In response to large doses of PTH, inulin clearance increased about 20% while cyclic AMP clearance rose approximately 1200%. The clearance ratio (cyclic AMP: inulin) increased to 15, indicating that some 94% of the urinary cyclic AMP was of renal origin. There was also a modest increase in plasma cyclic AMP, which will be considered in further detail later in this paper.

For purposes of comparison, the cyclic AMP response to PTH is shown contrasted with the response to glucagon (Fig. 2). Although PTH and glucagon both raised urinary cyclic AMP levels, it appeared that PTH did so principally by inducing an increase in nephrogenous cyclic AMP while glucagon did so principally by inducing an increase in plasma cyclic AMP (10).

When very large doses of PTH were infused (80–160 mU/kg per min), there was an initial peak in urinary cyclic AMP followed by a slightly lower plateau still well above control levels. This phenomenon was not observed with low doses of PTH, which induced 2-fold increases in cyclic AMP with a simple plateau configuration.

The source of PTH-induced increases in plasma cyclic AMP

When large doses of PTH were infused into normal subjects, there ensued not only a large increase in urinary cyclic AMP but a modest increase in plasma cyclic AMP as well. This increase in plasma cyclic AMP could have been due solely to increased release of cyclic AMP into the circulation by the kidney. However, PTH is also known to act on other tissues, e.g., bone and intestine; therefore, it was equally conceivable that the PTH-induced increase in plasma cyclic AMP might have been of extrarenal origin. In order to investigate these alternatives, comparative studies were performed in normal and anephric subjects (Fig. 3). In three normal subjects the infusion of 100 U of PTH over a period of 30 min induced approximately 4-fold increases in plasma cyclic AMP. In six anephric subjects similar infusions induced little or no increase in plasma cyclic AMP. Two of the anephric subjects were then given glucagon with resultant marked increases of cyclic AMP.

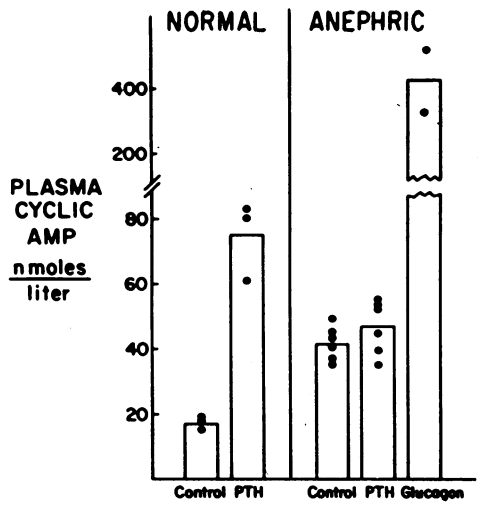

FIGURE 3 Plasma cyclic AMP responses to PTH infusions in normal and anephric subjects. PTH (200 U) was infused over 30 min. Two anephric subjects were subsequently given infusions of glucagon, 2.25 μg/kg intravenously over 30 min. Plasma specimens for cyclic AMP were obtained just before and 30 min after the beginning of each infusion.

It appears from these data that virtually all of the increase in plasma cyclic AMP that occurs during the infusion of PTH is attributable to the action of PTH on the kidney.

Studies designed to alter endogenous PTH secretion

EDTA-induced hypocalcemia. Since hypocalcemia is a potent stimulus to PTH secretion (13–15), studies were performed in which the calcium chelating agent, sodium EDTA, was administered to normal subjects in a dose of 40 mg/kg as an intravenous infusion over a 30 min period (Table I). Plasma calcium fell at least 2 mg/100 ml in both subjects, and both responded with increases in renal clearance of cyclic AMP. The clearance ratio (cyclic AMP: inulin) in H.H. increased 3-fold whereas that in W.C. increased only 40%. The response of W.C. was roughly comparable to that observed with the infusion of exogenous PTH at 5 mU/kg per min in which a small increment in urinary cyclic AMP occurred without a change in plasma cyclic AMP. In the other subject the 240% increase in urinary cyclic AMP and 40% increment in plasma cyclic AMP were comparable to the effects observed with the infusion of PTH at a rate of 40 mU/kg per min.

Calcium infusions. In an effort to suppress PTH secretion, calcium was infused over various periods of time. 15-min infusions of calcium were performed in four normal subjects on seven occasions, with doses of calcium ranging from 200 to 400 mg. Urinary cyclic AMP decreased 20–45%. More prolonged infusions of calcium were administered to two subjects with high initial cyclic AMP clearance rates and resulted in de-

TABLE I

Effects of Sodium EDTA, 40 mg/kg, given to Two Normal Subjects from Time Zero to +45 min as an Intravenous Infusion

Subject	Collection period	Urine cyclic AMP	Plasma cyclic AMP	Clearance cyclic AMP	Clearance cyclic AMP: clearance inulin	Plasma calcium
	min	*μmoles/g creatinine*	*nmoles/liter*	*ml/min*		*mg/100 ml*
W. C.	−60 to −30	3.02	24.2	174	1.44	9.5
	−30 to 0	2.90	23.1	160	1.48	9.3
	0 to +30	4.51	23.9	238	2.09	8.2
	+30 to +60	3.96	24.2	207	2.07	7.3
	+60 to +90	3.30	23.8	176	1.60	7.3
	+90 to +120	3.24	24.3	177	1.62	7.5
	+120 to +150	3.29	23.9	180	1.54	8.5
H. H.	−60 to −30	4.06	—*	—	—	9.1
	−30 to 0	3.73	19.4	304	2.27	8.9
	0 to +30	13.0	19.9	990	7.28	8.2
	+30 to +60	12.6	27.1	657	5.86	6.6
	+60 to +90	8.0	22.0	536	4.55	7.3
	+90 to +120	7.72	23.1	474	3.94	6.9

* Dashes indicate points not determined.

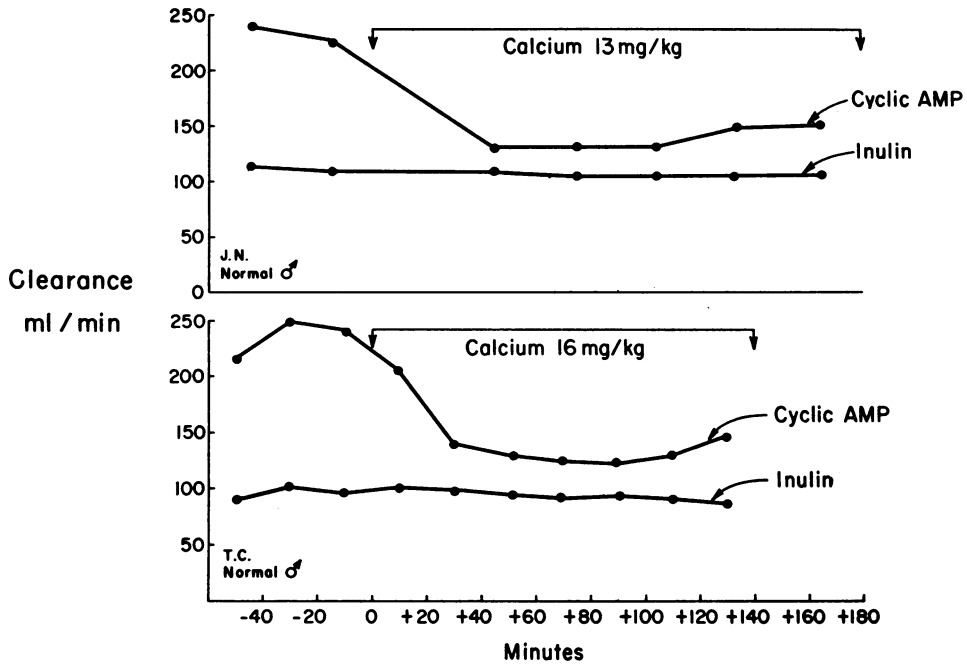

FIGURE 4 Cyclic AMP response to calcium infusion. In two subjects, cyclic AMP clearance and inulin clearance were measured before and during calcium chloride infusion. T.C. received 0.47 mg/kg per min of Ca^{++} over 15 min then 0.07 mg/kg per min over 125 min. J.N. received 0.17 mg/kg per min of Ca^{++} over 15 min then 0.06 mg/kg per min over 165 min.

creases in urinary cyclic AMP of about 50% and decreases in plasam cyclic AMP of 10–20%. Cyclic AMP clearance (Fig. 4) and the clearance ratio (cyclic AMP: inulin) dropped about 50%, indicating a great reduction in the nephrogenous contribution to urinary cyclic AMP. Cyclic GMP excretion was also measured in one of these two subjects (T.C.), and in three additional subjects both plasma and urinary cyclic GMP were determined. In contrast to cyclic AMP, plasma and urinary cyclic GMP rose during calcium infusion (Fig. 5). The variability in the plasma cyclic GMP determinations was such that it was not possible to be certain whether or not all of the increment in urinary cyclic GMP was derived from plasma.

Correction of hyperparathyroidism. Nine patients with primary hyperparathyroidism had urinary cyclic AMP determinations performed before and after the surgical removal of their parathyroid adenomas. In normal subjects cyclic AMP excretion is significantly correlated with creatinine excretion, so that there is somewhat less scatter of normal cyclic AMP values when these are expressed as micromoles per gram of creatinine than when expressed as micromoles per day. Before operation, patients with hyperparathyroidism excreted more cyclic AMP than did normal subjects, but after operation their urinary cyclic AMP values fell within the normal range in every case (Fig. 6). Lowest values were usually observed within 1 day after the operation, after which cyclic AMP excretion gradually rose, but never to preoperative values. In three patients, plasma cyclic AMP determinations were obtained, and the preoperative clearance ratios (cyclic AMP: creatinine) were considerably higher (4.96, 2.80, 2.76) than those seen in normal subjects (9). Studies in patients undergoing other surgical procedures have indicated no consistent decrease in cyclic AMP excretion during the postoperative period. It is inferred that the decrease observed in the nine patients with hyperparathyroidism was a consequence of a decrease in their secretion of PTH. All nine patients were apparently cured of their hyperparathyroidism, judging from the fact that their serum calcium concentrations fell to normal. An additional patient (No. 10) had hypercalcemia in association with a carcinoma of the lung. His urinary cyclic AMP was elevated. Irradiation of the tumor resulted in a marked decrease in the size of the tumor, and the serum calcium and urinary cyclic AMP values both fell to normal.

DISCUSSION

In 1962 Butcher and Sutherland demonstrated the presence of cyclic AMP in human urine (16). 5 yr later Chase and Aurbach found that urinary cyclic AMP in rat and man rose in response to PTH (6). They also demonstrated a PTH-sensitive adenyl cyclase in the

cortical portion of the rat kidney (8), while Rasmussen and Tenenhouse were able to demonstrate cyclic AMP accumulation in the rat kidney after PTH infusion (17). It was then shown that the dibutyryl derivative of cyclic AMP mimics the actions of PTH on serum calcium and on the urinary excretion of phosphate (18, 19) and hydroxyproline (18). Vaes demonstrated that dibutyryl cyclic AMP had an effect similar to that of PTH on bone explants (20). Recently it has been shown by Chase, Fedak, and Aurbach that PTH stimulates bone adenyl cyclase and the accumulation of cyclic AMP in fetal rat calvaria (4, 5). These observations are consistent with the view that a principal action of PTH is to stimulate adenyl cyclase in its target tissues and that the cyclic AMP that is formed in response to this action is responsible for the various metabolic effects of PTH.

It has been suggested that PTH might be the major determinant of urinary cyclic AMP in the rat (6), but this suggestion has not been supported by other studies (21). The evidence developed in the present paper and a companion paper (9) indicates that most of the cyclic AMP that appears in the urine is derived from the plasma by glomerular filtration and that this process is not under the control of physiologic levels of PTH. On the other hand, that portion of the urinary cyclic AMP that is added directly by the kidney may be in part under the control of PTH. Simultaneous inulin and cyclic AMP clearance studies indicate that the prolonged infusion of calcium diminishes the excretion of nephrogenous cyclic AMP, suggesting that some of the cyclic AMP which is formed by the kidney and excreted directly into the urine is under the regulatory control of PTH.

Although it is possible to decrease nephrogenous cyclic AMP by infusing calcium, it has not been possible to bring the cyclic AMP: inulin clearance ratio to unity. Thus, even during prolonged calcium infusions, the kidney still makes a small contribution to urinary cyclic AMP. Perhaps this is attributable to difficulty in suppressing endogenous PTH with calcium, but another possibility is that the kidney forms some cyclic AMP through a mechanism that is not PTH dependent. Our studies in hypoparathyroid patients have been insufficient in number to determine with certainty whether or not there is a measureable nephrogenous component of uri-

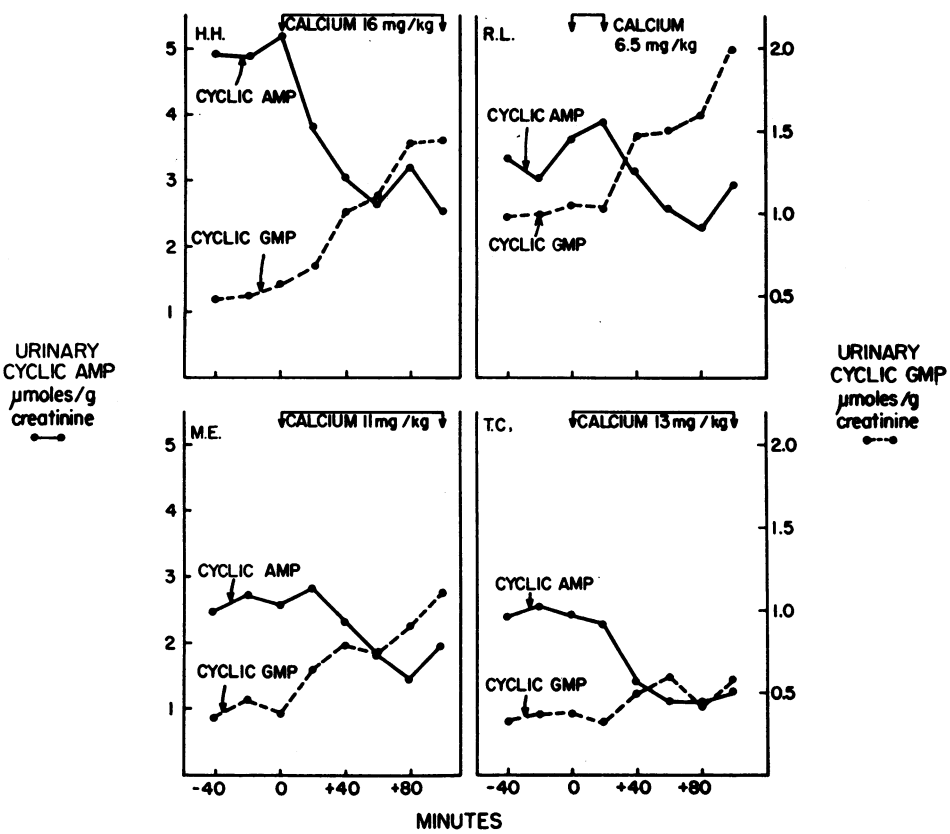

FIGURE 5 Comparison of urinary cyclic AMP and cyclic GMP in response to calcium infusion. Subjects received between 0.33 and 0.50 mg/kg per min of Ca^{++} over the first 15 min of the infusion.

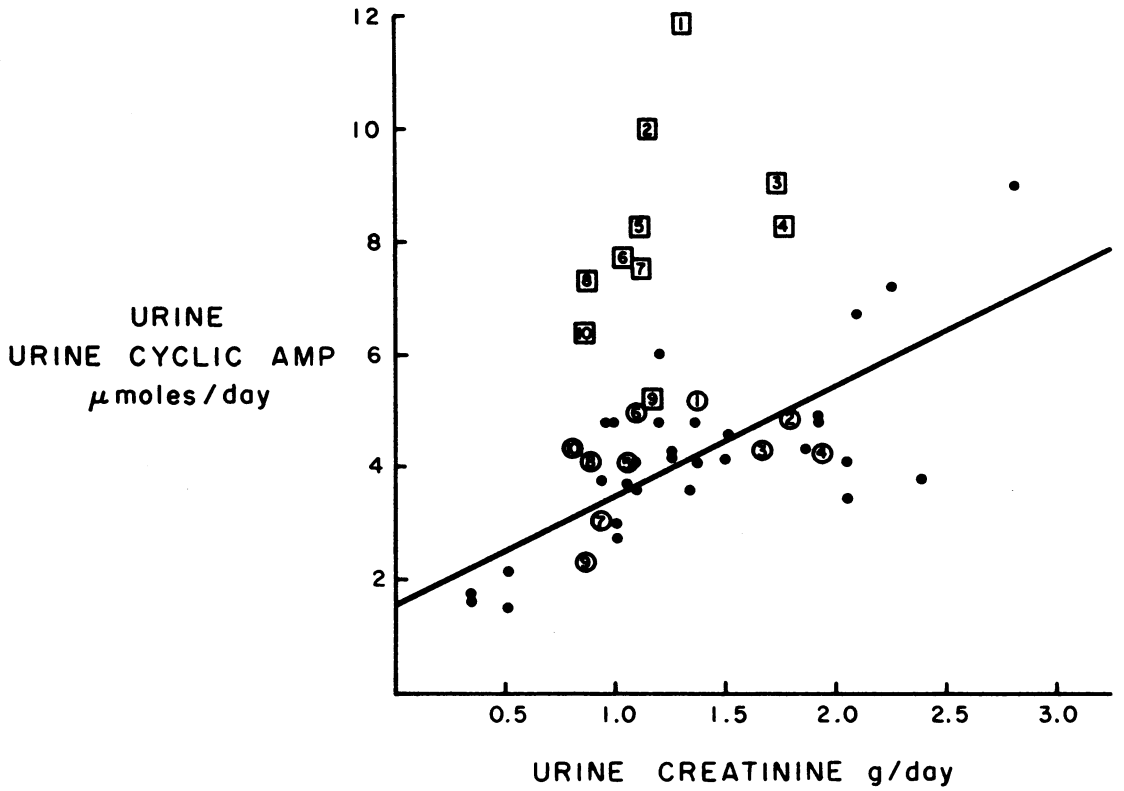

FIGURE 6 Preoperative and postoperative urinary cyclic AMP values in subjects with hyperparathyroidism compared to urinary cyclic AMP in a group of normal subjects. Each patient is represented by a number; the square represents the preoperative value, and the circle represents the postoperative value. The values for patient 10 were obtained before and after irradiation of a "PTH"-producing lung carcinoma. Regression line was fitted by the method of least squares.

nary cyclic AMP in these patients. Whether calcium has a direct effect on nephrogenous cyclic AMP unrelated to parathyroid hormone suppression is undergoing evaluation. Antidiuretic hormone (ADH)-dependent adenyl cyclase has been demonstrated in the renal medulla (8), and Takahashi, Kamimura, Shinko, and Tsuji have suggested that urinary cyclic AMP is under the control of ADH (22). Under our study conditions, in which the subjects were very well hydrated and experiencing a brisk diuresis, it is quite unlikely that an ADH effect was present. Furthermore, unpublished studies in this laboratory have failed to show an appreciable rise in urinary cyclic AMP after the infusion of ADH. We are, therefore, inclined to discount ADH as an important determinant of urinary cyclic AMP. It has yet to be determined what controls that minor portion of the urinary cyclic AMP which is produced by the kidney but not suppressed by calcium.

In normal subjects the PTH-dependent nephrogenous fraction usually represents from 20 to 50% of the total urinary cyclic AMP but has occasionally been noted to be as high as 60%. In primary hyperparathyroidism and in normal subjects undergoing experimental stimulation of PTH secretion by EDTA, the fraction contributed by the kidneys is higher. Supraphysiologic doses of PTH can induce very impressive increases in nephrogenous cyclic AMP; the 200 U dose commonly used to test phosphaturic responsiveness to PTH induces approximately a 3-fold rise in plasma cyclic AMP and a 30-fold rise in urinary cyclic AMP. Under these conditions the cyclic AMP: inulin clearance ratio can be as high as 15.

Even the increase in *plasma* cyclic AMP which occurs with the administration of very large doses of PTH is apparently of renal origin, since little or no increase occurs in anephric patients. This does not preclude the possibility that PTH might stimulate cyclic AMP formation in certain extrarenal tissues such as bone or intestine, but it does indicate that relatively little PTH-induced cyclic AMP of extrarenal origin is released into the circulation.

A generally recognized phenomenon in hormone assay work is the "plateauing" of dose-response curves. With the administration of progressively larger doses of a hormone, a point is reached in the dose-response curve beyond which further increments in dosage do not include further increments in target cell response. What it is that limits the responsiveness of a target cell to a hormone has never been clearly defined. In the present studies a plateau in the phosphaturic response curve was reached with a PTH dose of 10 mU/kg per min. By contrast, the cyclic AMP response curve was still rising steeply with a PTH dose of 80 mU/kg per min. This would suggest that the limiting factor in the phosphaturic response of PTH was beyond the formation of cyclic AMP. Analogous observations have been made in studies of other tissues (23, 24).

Another phenomenon encountered during these studies which also is of general interest was the character of the time-course response to large doses of PTH. An initial high peak in cyclic AMP excretion was followed by a somewhat lower plateau. This pattern of peak followed by plateau has also been observed in other studies of cyclic AMP (10, 25, 26).

It is possible that measurements of plasma and urinary cyclic AMP might provide useful diagnostic information in certain clinical disorders. In our experience, hyperparathyroidism has been associated with an abnormally high rate of cyclic AMP excretion. The utility of this finding is now undergoing evaluation, and no firm conclusions are justified at this time.

The possibility that cyclic GMP may be involved with calcium balance is raised by the observation that calcium infusions caused urinary and plasma cyclic GMP to rise. Cyclic GMP excretion was increased slightly by very large doses of PTH, but not by small doses of PTH. Whether or not the rise in cyclic GMP excretion that occurred in response to PTH could have been secondary to changes in calcium seems open to question since plasma calcium was not detectably elevated. The hypothesis that the cyclic GMP response might have been mediated by an increase in thyrocalcitonin production was investigated in an ancillary study and was shown to be untenable (27).

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