Rapid Development of Renal Resistance to Low Doses of Synthetic Bovine Parathyroid Hormone Fragment 1–34

DISSOCIATION OF URINARY CYCLIC ADENOSINE MONOPHOSPHATE, PHOSPHATURIC, AND CALCIURIC RESPONSES

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ABSTRACT The designing of parathyroid hormone (PTH)-renal dose-response studies in human beings is complicated by the possibility of rapid homologous receptor down-regulation, a phenomenon that is clearly shown to occur in vitro. Large amounts of PTH given to human subjects as serial injections or prolonged infusions cause decreased urinary 3',5'-cyclic adenosine monophosphate (cAMP) responses to subsequent PTH doses, but it is uncertain whether lower doses given over shorter periods similarly cause renal tachyphylaxis to PTH action. Thus, in seven water-loaded adults, we infused in ascending order 10, 30, 75, 150, and 300 U of synthetic bovine PTH fragment 1–34 (bPTH 1–34) per 70 kg body wt over 15 min on widely separated days ("separate day administration"). On another day, each subject received all five 15-min doses in ascending order at 75-min intervals ("single day administration"). Urine collection intervals were control, 0–30 min (including the PTH infusion), and 30–60 min. Peak nephrogenous cAMP (NcAMP, nmol/100 ml glomerular filtrate) response was linearly related to the dose of PTH (separate day study, $r = 0.94$, $P < 0.001$; single day study, $r = 0.88$, $P < 0.001$). However, the slope of NcAMP responses plotted against PTH dose for the single day study was only 36% of that derived from separate day administration of the same PTH doses ($P < 0.001$). After only 40 U (10 + 30) of bPTH 1–34/70 kg, the NcAMP response to 75 U was reduced 44%, and the effect of 300 U/70 kg, when given as the last of the sequential single day infusions, was 64% less than the response to 300 U of bPTH 1–34 given alone ($P < 0.001$). The phosphaturic response (fractional excretion of phosphorus, FEP [percent]) was also linearly related to bPTH 1–34 dose, but combined administration of the PTH infusions on one day increased FEP at each dose identically with the effects of separate day administration. A transient, dose-related, early hypercalciuric response to bPTH 1–34 also occurred, and was of equal magnitude in both protocols. These studies demonstrate that significant blunting of the NcAMP response to bPTH 1–34 occurs rapidly and follows brief exposure to relatively low doses of hormone. In contrast, there is no effect of recent PTH administration on the phosphaturic and early hypercalciuric actions of bPTH 1–34. This seeming dissociation of PTH effects makes unclear the physiologic importance of PTH-induced cAMP tachyphylaxis in the regulation of final PTH actions. In any case, studies of NcAMP responses in which the occurrence of tachyphylaxis would be undesirable should be designed to avoid prolonged or closely spaced administrations of the hormone.

INTRODUCTION

The acute administration of exogenous parathyroid hormone (PTH)[1] causes increased urinary excretion of

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[1] *Abbreviations used in this paper:* bPTH 1–34, synthetic bovine parathyroid hormone fragment 1–34; bPTH 1–84, purified intact bovine parathyroid hormone fragment 1–84; C_{Ca}/C_{Cr}, ratio of calcium clearance to creatinine clearance; FEP, fractional excretion of phosphorus; HPT, hyperparathyroidism; NcAMP, nephrogenous cyclic AMP; PTH, parathyroid hormone.

phosphate and cyclic 3',5'-adenosine monophosphate (cAMP) (1). "Resistance" or tachyphylaxis to the effects of PTH follows sustained or repetitive exposure to the hormone in animal studies in vitro (2, 3) and in vivo (4). For human beings, chronic endogenous PTH excess (primary or secondary) is associated with reduction of the urinary cAMP response to stimulation by exogenous PTH in some patients (5–11).

In normal human volunteers, Tomlinson et al. found that the infusion of 1,000 U of purified intact bovine PTH (bPTH 1–84) over 2 h caused an initial increase in plasma and urinary cAMP, which was followed by a progressive decline in their levels during the second hour of the infusion (9). They also noted marked blunting of the plasma and urinary cAMP responses to a 200-U dose of bPTH 1–84 given immediately after the 1,000-U infusion (9), as well as a progressive decrease in the responses to serial, 200 U, hourly doses (11). These results are consistent with in vitro data that show rapid homologous down-regulation of peptide hormone receptors (12). It is not known whether smaller amounts of PTH, given over shorter periods, similarly engender resistance to subsequent PTH administration in man, but such considerations are obviously significant in assessing the physiologic importance of homologous desensitization as a modulator of PTH action.

We wished to study PTH-renal dose-response relationships in man. Practical considerations made it desirable to give multiple doses on a single day rather than separately over several weeks. Because dose-dose interactions and tachyphylaxis might alter responses to closely spaced PTH doses, we designed a series of experiments to answer the following questions: (*a*) How rapidly does resistance to the renal action of PTH develop in man? (*b*) Does such tachyphylaxis occur after doses smaller than those customarily used to test PTH action on the kidney? (*c*) Do the various renal effects of PTH differ in the degree of resistance caused by prior administration of the hormone?

These studies demonstrate that significant blunting of the nephrogenous cAMP (NcAMP) response to synthetic bovine PTH fragment 1-34 (bPTH 1–34) occurs rapidly and after brief exposure to relatively low doses of hormone. In contrast, there is no blunting of either phosphaturic or early calciuric responses. The results have both theoretical and practical implications.

METHODS

Study subjects. We recruited seven healthy volunteers (three male, four female) with a mean age of 43 yr (range 26–69 yr). They had no known chronic medical problems and were taking no medications. Laboratory evaluations excluded hepatic and renal disease. The experimental protocols were approved by the Mayo Human Studies Committee and informed consent was obtained from each individual. Studies were conducted under the authority of Investigational New Drug exemption No. 19067 (H. Heath).

Hormone. bPTH 1–34 from a single batch (lot B10946) was purchased from Beckman Instruments, Inc. (Palo Alto, CA) as a lyophilized powder that was 79.4% peptide as assessed by disk gel electrophoresis. The biologic potency of this preparation, based on in vitro stimulation of adenyl cyclase activity in the chick renal cortex (13), was 7,000 IU per milligram when compared to natural bPTH standard 67/342 from the National Institutes of Biologic Standards and Control (London). The hormone was shipped in a single vial that contained sufficient peptide for all of the studies.

Sterilization of bPTH 1–34 for human use. Since bPTH 1–34 is a labile peptide, the most practical means of sterilization is by filtration in solution. The notorious adsorptive properties of PTH (14) raised the possibility of significant peptide loss during this procedure, and we have investigated this question (15). Using electrolytically labeled ^{125}I-bPTH 1–34 as a tracer (16), we found that adsorptive losses during filtration through a Millex-GV 0.22-μm filter (Millipore Corp., Bedford, MA) were consistently <5% using a concentration of 20 μg bPTH 1–34/ml in 0.1 M acetic acid containing 1% human serum albumin (HSA).

Because all patient studies were accomplished during an 8-wk period, adequate amounts of bPTH 1–34 for each week were weighed at one sitting, placed into sterile vials, and frozen at −70°C. Each Monday, we prepared fresh 0.1 M acetic acid containing 1% HSA, and added the amount of diluent necessary to achieve a concentration of 20 μg bPTH 1–34/ml to that week's vial. This solution was gently swirled until the powder had completely dissolved, and then it was aspirated into a plastic syringe. A Millex-GV filter was attached to this syringe under a standard laminar flow pharmaceutical hood and a sterile 20-gauge needle was attached to the filter. The entire contents of the syringe were then injected through the rubber stopper into a sterile pyrogen-free 30-ml glass vial. This vial was kept in the refrigerator for the rest of that week and used as the master solution from which the infused solutions were prepared. Maintenance of sterility was documented by negative cultures of these solutions at the end of each week; also, a limulus lysate assay of this preparation for pyrogens was negative. The bioactivity of these solutions, based on the guanyl nucleotide-amplified activation of canine renal cortical plasma membrane adenyl cyclase (17), was stable during refrigeration for 4 d.

Preparation of infused solutions. A single syringe pump (model 975, Harvard Apparatus, Co., Inc., S. Natick, MA) was used for all studies and was calibrated to deliver 48 ml in 15 min. For each infusion, a sterile 50-ml glass syringe was inverted and the plunger withdrawn far enough to accommodate 55 ml of infusion solution. 2.2 ml of 25% HSA (Buminate 25%, Baxter Travenol Laboratories, Deerfield, IL) was sterilely withdrawn from a refrigerated vial and injected through the tip of the 50-ml glass syringe into the barrel. Sterile water for injection USP, in an amount calculated to yield an ultimate infusion solution volume of 55 ml, was added to the 50-ml syringe as above. This syringe was then sterilely capped and gently inverted several times to insure uniform distribution of the albumin. Finally, the calculated volume of bPTH 1–34 stock solution (20 μg/ml) was sterilely aspirated from the master vial and added to the 50-ml glass syringe. The syringe was capped and mixed as above, and then connected to a 76-cm, small diameter, IV extension tubing that was attached directly to the patient's intravenous catheter for the infusion.

Infusion protocols. Each subject completed two separate infusion protocols, designated "separate day" and "single day" administration, which are depicted in Fig. 1. All studies were completed within a single 8-wk interval (fall, 1981). The timing of urine collection, blood sampling, and bPTH 1–34 infusion was as indicated. 90 min before the first infusion, we placed a catheter in each forearm, one for bPTH 1-34 infusion and one for blood sampling. The volunteers voided 60 min before the infusion that began each day's study, and remained seated in a lounge chair except to urinate. Each subject underwent the separate day administration protocol first; on 5 separate days, they received in sequence 10, 30, 75, 150, and 300 U of bPTH 1-34/70 kg. Although Tomlinson et al. showed that the urinary cAMP response to a 200-U bolus of bPTH 1-84 after an infusion of 1,000 U bPTH over 2 h is fully recovered within 24 h (9), we wanted to minimize the possibility of dose-dose interactions. Therefore, in our studies, the doses were given in ascending order; each dose in the separate day protocol was administered no less than 3 d after the preceding one, and at least 5 d were allowed to elapse after the 300 U/70 kg separate day infusion before the single day infusions were administered. In both protocols, the PTH infusions were performed during the first 15 min of a 30-min urine collection period, which was followed by another 30-min collection interval. In the single day protocol, the subsequent 15-min period served as a base line for the next infusion. Immediately before the first exposure to the hormone, hypersensitivity to this preparation was excluded by the instillation of two drops of a solution containing 3 U/ml of bPTH 1-34 in 0.9% NaCl into the lower conjunctival sac of one eye. There were no adverse effects of any of the studies.

Collection and preparation of samples. An aliquot of each urine sample was frozen at −20°C until analyzed. Serum specimens obtained at each blood sampling point were also kept frozen at −20°C. Plasma samples for phosphorus and cAMP determinations were collected in iced 3-ml glass tubes containing heparin and theophylline. These were prepared using the method of Feinglos et al. (18) with a minor modification. The relative insolubility of theophylline in water required excessive heating of the solution, which resulted in uncertain volume loss and precipitation of theophylline in the transferring pipette tips. Therefore, 3.6 g of theophylline and 6.7 ml of Na heparin (10,000 U/ml) were dissolved in 293 ml of distilled water. 300 μl of this solution was then pipetted into each tube and lyophilized to dryness. This more dilute solution was found to dissolve the theophylline at a much lower temperature and to allow transfer without precipitation.

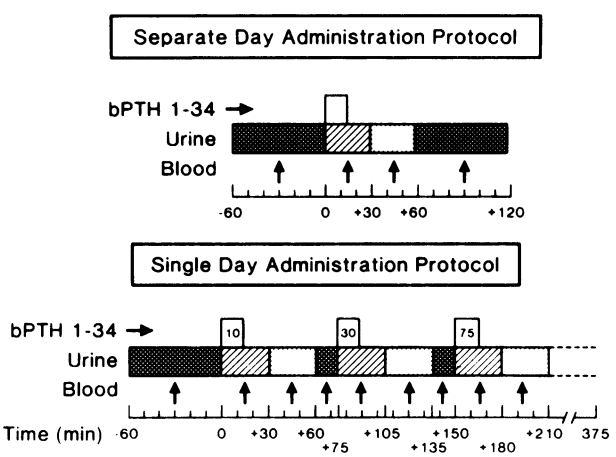

FIGURE 1 Diagrams of bPTH 1–34 infusion schemes. Times of drug administration are indicated by the open boxes, urine collection intervals by the shaded bars, and blood collection times by the vertical arrows. For the single day protocol, the crosshatched areas signify control urine collections for the subsequent PTH dose. For the single day administration protocol, the 150 and 300 U doses of bPTH 1–34 are omitted for clarity.

Plasma specimens were prepared for cAMP analysis using a modification of the method of Wray et al. (19, 20). 500 μl of each plasma sample was added suddenly to 750 μl of preheated 0.05 M Na acetate assay buffer at pH 4.9 and was incubated at 90°C for 10 min. The tubes were then centrifuged at 3,000 rpm for 15 min and the cAMP-containing supernatant (which was now at the assay buffer pH of 6.2) was removed and frozen at −20°C until analyzed.

Analyses. All samples from a given infusion were analyzed in the same assay. Serum and urinary creatinine were determined using standard autoanalyzer methods. Serum and urinary calcium were measured by atomic absorption spectrophotometry (model 2380, Perkin-Elmer Corp., Norwalk, CT). Plasma and urinary phosphorus were determined in duplicate by a colorimetric procedure (21) on a microsample spectrophotometer (model 300-N, Gilford Instrument Laboratories, Oberlin, OH). Phosphate excretion was calculated as the ratio of phosphate clearance to creatinine clearance or as percent fractional excretion of phosphate (FEP) (22).

Urinary and plasma cAMP were determined in triplicate by radioimmunoassay using a rabbit antiserum at a final dilution of 1:400,000 (23). The intraassay coefficient of variation was 9.9% (21 assays). NcAMP excretion was expressed as a function of glomerular filtration rate (nmol/100 ml glomerular filtrate) as described by Broadus et al. (24). To assure that any apparent desensitization of the NcAMP response was not caused by induction of endogenous antibodies to bPTH 1-34, we assayed paired specimens from each subject for binding of ^{125}I-bPTH 1-34 in the absence of added antiserum (25). Samples were taken prior to the first exposure to the hormone and compared with samples taken on the morning of the last study. The mean percent binding was 7.6±0.1 (mean±SE) for the base-line specimens and 7.7±0.2 for the postexposure specimens (NS). These values were also not significantly different from nonspecific binding in other control plasmas.

Statistical analyses. The results are tabulated and depicted as mean±SE. Data were analyzed by calculation of linear regressions of responses vs. dose or log dose, and pair comparisons were made by *t* test, accepting significant differences at the 99% level.

RESULTS

NcAMP. For each dose in both the separate day and single day protocols, the NcAMP response was prompt and brief (Table I). Peak NcAMP values were always found in the 0–30-min period encompassing the bPTH 1-34 infusion, and base-line NcAMP values were indistinguishable for all doses. NcAMP increased linearly with increasing doses of bPTH 1-34 in both protocols (separate day, $r = 0.94$; single day, $r = 0.88$;

TABLE I
Responses to bPTH 1–34 Infusions

bPTH 1-34 dose	Urine collection period	NcAMP (nmol/100 ml glomerular filtrate)		FEP (%)		C_{Ca}/C_{Cr} $\times 10^{-3}$	
		Sep. d.*	Single d.*	Sep. d.	Single d.	Sep. d.	Single d.
U/70 kg	*min*						
	C	0.1 (0.1)‡	1.2 (0.5)	0.13 (0.02)	0.17 (0.03)	6.1 (1.4)	5.6 (0.6)
10	0–30	1.2 (0.5)	2.4 (0.5)	0.16 (0.02)	0.21 (0.02)	4.1 (1.0)	4.9 (0.4)
	30–60	0.5 (0.3)	0.9 (0.4)	0.18 (0.02)	0.21 (0.02)	2.4 (0.6)	2.2 (0.3)
	C§	1.7 (0.6)	0.9 (0.5)	0.15 (0.03)	0.19 (0.03)	8.0 (2.0)	2.4 (0.5)
30	0–30	4.4 (1.7)	8.1 (1.2)	0.22 (0.03)	0.29 (0.03)	8.0 (2.0)	3.9 (0.5)
	30–60	1.1 (0.6)	0.8 (0.3)	0.21 (0.02)	0.23 (0.02)	2.8 (0.8)	1.6 (0.3)
	C§	1.9 (0.5)	1.4 (0.4)	0.14 (0.02)	0.23 (0.02)	5.4 (1.5)	1.9 (0.4)
75	0–30	34.3 (9.1)	19.2 (3.8)	0.26 (0.04)	0.30 (0.04)	9.0 (2.3)	5.3 (0.8)
	30–60	3.0 (0.9)	1.6 (0.5)	0.22 (0.03)	0.31 (0.04)	3.5 (0.8)	2.0 (0.4)
	C§	1.7 (0.5)	0.7 (0.3)	0.13 (0.02)	0.22 (0.02)	6.5 (1.2)	1.5 (0.4)
150	0–30	91.3 (13.6)	57.2 (10.0)	0.29 (0.03)	0.34 (0.03)	11.0 (1.3)	7.5 (1.0)
	30–60	5.4 (1.5)	4.3 (1.5)	0.26 (0.03)	0.36 (0.04)	3.4 (0.8)	2.0 (0.3)
	C§	0.9 (0.2)	1.1 (0.5)	0.13 (0.01)	0.20 (0.02)	11.0 (2.8)	1.5 (0.4)
300	0–30	237 (22.4)	85.3 (9.6)	0.27 (0.01)	0.34 (0.04)	17.0 (4.7)	9.5 (1.6)
	30–60	10.7 (2.8)	12.2 (4.4)	0.25 (0.01)	0.34 (0.04)	4.5 (1.1)	2.4 (0.6)

* "Sep. d." refers to effects of single-dose PTH infusions on separate days; "Single d." refers to infusion of all five doses on 1 d.
‡ All data shown as mean±SE.
§ For the single day studies, this control value is the 60–75-min urine collection period after the preceding bPTH 1-34 dose.

$P < 0.001$ for both) (Fig. 2). However, the peak values obtained from the larger doses in the single day study were significantly less than the values obtained when the same doses were given on separate days. The mean slope of the individual regressions of NcAMP on bPTH 1-34 doses for separate day administration (0.83±0.08) was nearly three times the slope of response for doses given on 1 d (0.30±0.03, $P < 0.001$). After a cumulative dose of only 40 U (10 + 30 U) of bPTH 1–34/kg, the NcAMP response to 75 U/70 kg for the single day study was reduced 44% from the effect of the same dose when given alone, and the response to 300 U/70 kg given as the last of the sequential infusions was reduced 64% ($P < 0.001$) (Fig. 2).

Serum calcium and plasma phosphorus. There was no significant change in serum calcium or plasma phosphorus during any of the separate day studies. During the 7¼-h single day protocol, serum calcium increased slightly but progressively: basal, 9.1±0.1 mg/dl; last sample, 9.4±0.1 mg/dl ($P < 0.01$). During this same period, plasma inorganic phosphorus concentrations decreased slightly but not significantly: basal, 3.0±0.3 mg/dl; last sample, 2.6±0.2 mg/dl ($0.1 > P > 0.05$). For both ions, changes during each dose interval were inconsequential.

Phosphaturia. The effect of bPTH 1-34 on phosphate excretion, expressed as percent FEP (22) (Table I), occurred acutely with both separate and single day

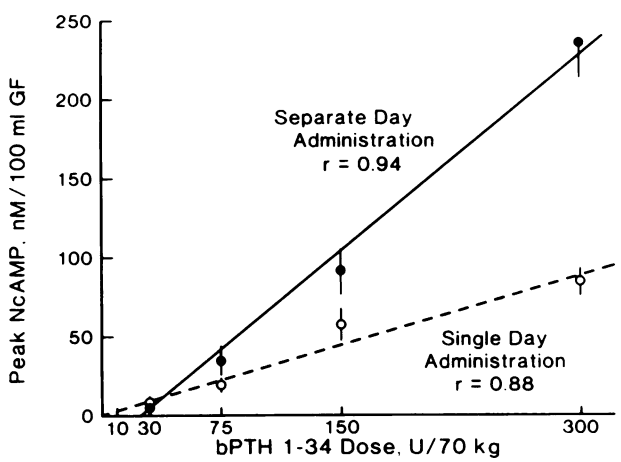

FIGURE 2 Peak NcAMP values after bPTH 1–34 infusions are plotted as a function of dose (U/70 kg body wt.). The five doses administered sequentially on one day (○) engender significantly less NcAMP excretion than when the individual doses are given on separate days (●). Here and in later figures, data are mean±SE. For both regressions, $P < 0.001$.

administration. FEP increased linearly with the log of bPTH 1-34 dose (Fig. 3). In contrast to the effect on NcAMP excretion, single day administration did not blunt the phosphaturic response to bPTH 1-34; the mean slopes of FEP vs. log dose bPTH 1-34 for separate day and single day administration were, respectively, 0.088±0.013 and 0.084±0.001 ($P > 0.10$).

Calcium excretion. The base-line ratio of calcium clearance to creatinine clearance (C_{Ca}/C_{Cr}) gradually fell between the 10 U and 300 U/70 kg doses during the single day protocol. Despite the differing base-line values, we observed an initial increase of calcium excretion in both protocols after all except the 10 U/70 kg dose (Table I). This effect is clearly shown in Fig. 4, which relates the log dose of bPTH 1-34 to the change from base line in C_{Ca}/C_{Cr} that occurs during the first 30-min urine collection period (which included the PTH infusion). Interestingly, there was no difference between the two protocols in the amplitude of this calciuric response.

DISCUSSION

The capability of target cells to rapidly diminish their biologic responses to prolonged or repetitive hormonal stimulation has been referred to by various terms, including "tachyphylaxis," "resistance," "down-regulation," and "homologous desensitization." This effect has been extensively studied in target cells for insulin, growth hormone, catecholamines, glucagon, angiotensin II, thyrotrophin-releasing hormone, and gonadotropins, and has been correlated with apparent loss of receptor sites in many cases (12). In addition, insulin receptors have been postulated to exhibit "negative cooperativity," whereby exposure of target cells to increased insulin concentrations results in diminished

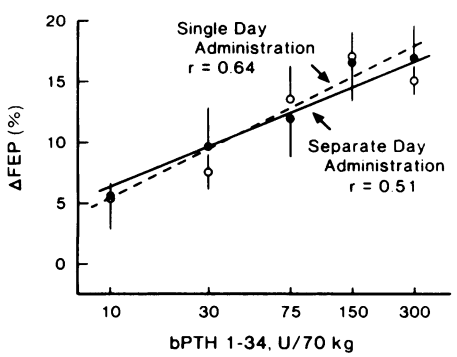

FIGURE 3 Maximal changes (Δ) in fractional excretion of phosphate (FEP, percent) following infusion of bPTH 1-34 plotted as a function of dose (U/70 kg, note log scale). There are no significant differences between slopes of the two curves (— ● —, doses on separate days; --- O ---, doses sequentially on one day); for both regressions, $P < 0.001$.

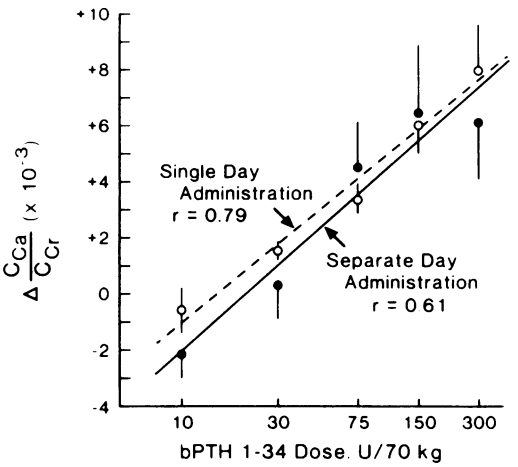

FIGURE 4 Change (Δ) in calcium clearance as a function of creatinine clearance (C_{Ca}/C_{Cr}) during the first 30 min from the start of bPTH 1-34 infusions plotted as a function of dose (U/70 kg, note log scale). — ● —, doses given on separate days; --- O ---, doses given sequentially on a single day. There are no significant differences between the early hypercalciuric responses in the two protocols; for both regressions, $P < 0.001$

affinity of the remaining, unoccupied receptor sites and an acute decrease in target cell sensitivity to the hormone (26). Another possible mechanism for hormone-induced tachyphylaxis is prolonged occupancy of the receptor site by undissociated ligand, which results in a functional receptor loss (27). In a canine renal system, such receptor loss may be reversed by addition of guanosine triphosphate (GTP) (28).

The phenomenon of homologous desensitization induced by exogenous PTH has received relatively little attention, and the mechanism(s) of this effect are uncertain. Most investigations of this subject have emphasized the cAMP response to PTH administration rather than effects on phosphate or calcium transport. Chao and Forte (3) noted a time- and dose-dependent, PTH-mediated homologous tachyphylaxis in cultured rat kidney cells, despite the presence of normal cAMP responsiveness to cholera toxin. They interpreted this as indicating that the catalytic and coupling units of the adenylate cyclase enzyme system were functioning normally, and then suggested that the locus for down-regulation was at the receptor sites. Nichols and colleagues (2) had previously found that the infusion of very large doses of PTH (175 U/kg) for 8 h in rats caused a diminution both in the cAMP response of renal cortical slices to PTH and in the PTH-dependent adenylate cyclase activity of renal cortical cell membranes. A concurrent decrease in responsiveness of the enzyme to calcitonin and fluoride, but not to vasopressin, was noted in this study; this suggests that acute

infusion of PTH inhibited the catalytic activity of adenyl cyclase as well as receptor-mediated activation of the enzyme by subsequent PTH challenge. Tamayo et al. (28) examined the mechanism of PTH-induced homologous desensitization in purified basolateral cortical membranes from isolated perfused canine kidneys. They found that bPTH 1–34 induced a decrease in PTH binding and in PTH-stimulated adenylate cyclase activity that could be normalized by preincubation of the membranes with GTP (which dissociates bound PTH). This suggests that tachyphylaxis was caused by persistent receptor occupancy in their in vitro model (28).

The characteristics of PTH receptors and their functional relationship to adenyl cyclase are currently under active investigation (16, 29). Two earlier studies in parathyroidectomized rats, which were published before the concept of homologous down-regulation appeared, presented data which suggest that tachyphylaxis occurs in phosphaturic (1, 4) and urinary cAMP (1) responses to prolonged or repetitive exogenous PTH stimulation. However, in the intact rat, even large doses of PTH given as prolonged infusions or as serial bolus injections failed to elicit refractoriness in vivo, despite the concurrent in vitro evidence of this phenomenon (2). Studies in chicks (29) and rats (30) that were made secondarily hyperparathyroid by dietary deficiency of vitamin D or calcium have shown evidence of tachyphylaxis both in vivo and in vitro; however, the possible influence of concomitant hypocalcemia on these results was not evaluated. The applicability of these findings to human studies is uncertain because there are significant interspecies differences in the effect of PTH on cAMP production and excretion (13, 20, 31) as well as intraspecies variability in the responses to different PTH preparations (13).

Most studies of the plasma or urinary cAMP responses to PTH administration in humans (5–10, 32, 33) have been performed in patients with primary or secondary hyperparathyroidism (HPT), which is characterized by states of chronic endogenous PTH excess. The majority of these reports (5, 6, 9, 10, 31–33) used modes of expression for cAMP excretion that fail to compensate for differences in body mass and/or renal function. A spectrum of results has been noted. Some investigators (6–8) reported a clearly diminished cAMP response in HPT relative to controls; others (5, 9, 32) observed a decrease in some, but not all, patients, and still others (10, 33) found no significant differences between HPT and normals. These discrepancies cannot be reconciled from the published data because of the variability in PTH preparations, doses, and modes of administration; the differences in base-line serum calcium levels; and the inequatable modes of expression for cAMP excretion. However, we conclude that the urinary cAMP response to PTH is blunted in at least some patients with HPT and that this phenomenon tends to occur in patients with the highest endogenous PTH concentrations (9). To our knowledge, the only studies of homologous desensitization of the renal and plasma responses to exogenous PTH in normal man have been done by Tomlinson et al. (9) and are described earlier in this report. The physiological importance of these experiments is unclear because of small patient numbers and extremely large PTH doses.

We wished to investigate the effects of dose and time on the development of homologous down-regulation of renal responses to PTH in normal humans. To learn whether these responses differed in the degree of tachyphylaxis engendered, we evaluated the effects of acute and repetitive PTH administration on the urinary excretion of phosphate and calcium as well as cAMP. NcAMP response vs. bPTH 1-34 dose was linear in both protocols between 10 and 300 U/kg per 15 min, despite a 64% reduction in the slope of this regression for the single day study. Although our experimental design does not provide insight into the mechanism(s) of this phenomenon, our results clearly document that desensitization of the NcAMP response to infused bPTH 1-34 in humans occurs rapidly and follows brief exposure to doses of PTH much smaller than those customarily used to test PTH action on the kidney.

The tachyphylaxis for NcAMP excretion was not accompanied by clearcut decreases in the phosphaturic responses to the same bPTH 1-34 doses. Phosphate excretion was linearly related to the logarithm of the PTH dose; it demonstrated a progressively smaller increment in phosphaturic response to a given increment in bPTH 1-34 dose. This is in contrast to the linear relationship that we found for bPTH 1-34 dose vs. NcAMP response in both protocols and suggests differential sensitivities of renal responses to PTH. In this regard, Besarab et al. (34), using an isolated rat kidney preparation, reported in abstract form that an increase in urinary cAMP was induced by a dose of bPTH 1-34 that was only one-third of that necessary to provoke a detectable increase in phosphaturia. The absence of tachyphylaxis for the early hypercalciuric effect of PTH provides further evidence that the urinary cAMP response to PTH is not always directly proportional to other biologic effects of PTH (35). We must point out that the abbreviated urine collection intervals required for the single day multidose study prevented us from obtaining complete curves of the effect of bPTH 1-34 on phosphate excretion. Perhaps this obscured some PTH-mediated refractoriness of the phosphaturic response. Similarly, for the separate day studies, calculation of changes in FEP as area under the curve might yield a more complete picture of the re-

sponses. We did not calculate this because there is no way to compare such data with those from the single day studies.

Resistance to PTH has been provoked in rats by hypercalcemia (36), hypermagnesemia (37), and hypokalemia (38), and in both rats and chicks by hypocalcemia associated with either vitamin D deficiency or a calcium-deficient diet (27, 39). Humans with chronic renal failure (5, 9), vitamin D deficiency (32), and some patients with primary hyperparathyroidism (6–9) are also refractory to stimulation by exogenous PTH. However, our patients maintained normal blood levels of creatinine, potassium, magnesium, and calcium throughout their study periods and had normal endogenous PTH levels on the morning of the single day protocol. In addition, there was no evidence for the induction of endogenous antibodies to bPTH 1-34. The decrease of urinary cAMP responses seen in our subjects with closely spaced, small doses of PTH is consistent with the general mechanism of homologous down-regulation of peptide hormone receptors, which has been elucidated for other hormones (12). Alternative explanations might include an effect of repetitive PTH administration on cAMP metabolism, such as an increase in phosphodiesterase activity. The recent work of Marcus and Grant (40) provides some experimental support for such a hypothesis. Repeated infusions of bPTH 1-34 may have suppressed endogenous PTH levels, but one cannot see how this would decrease renal response to the exogenous hormone.

The results of these experiments have several important practical implications. Because of the dissociation of urinary cAMP, phosphaturic, and calciuric responses to bPTH 1-34, studies, if they are to have physiologic relevance, must assess all of these variables instead of relying only upon changes in cAMP excretion. The transient hypercalciuria elicited by the larger PTH doses may help to explain discrepancies in the reported actions of PTH on calcium excretion[2]; it emphasizes the need to collect frequent urine specimens during in vivo studies of this effect. Finally, to circumvent the complicating effects of homologous desensitization, investigations of renal responses to PTH in vivo should avoid prolonged or closely spaced exposures to the hormone.

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[2] We have examined the mechanism of this early hypercalciuric response and are preparing a separate manuscript.

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