Canine Renal Receptors for Parathyroid Hormone

DOWN-REGULATION IN VIVO BY EXOGENOUS PARATHYROID HORMONE

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ABSTRACT Chronic elevation of circulating parathyroid hormone (PTH) is associated with decreased target cell responsiveness to PTH. To study the subcellular mechanism of this phenomenon we evaluated PTH receptors and adenylate cyclase activity in renal cortical membranes prepared before and after infusion of bovine parathyroid gland extract (PTE) into thyroparathyroidectomized dogs. PTE infusion resulted in a 53% decrease in the number of high-affinity receptors ($P < 0.01$) associated with a 66% decrease in PTH-stimulated adenylate cyclase ($P < 0.01$) relative to paired base-line values. Both the equilibrium constant of dissociation (K_D) for PTH binding and the concentration of PTH that caused half-maximal stimulation of adenylate cyclase were in the range of 1 to 4 nM, and were unaffected by the PTE infusion. Responsiveness of the renal adenylate cyclase to sodium fluoride was 88% of base-line values. Infusion of the PTE vehicle alone did not affect PTH receptor number or blunt the adenylate cyclase response to PTH.

Pretreatment of the membranes made after PTE infusion with guanosine triphosphate (GTP), which is known to produce dissociation of receptor-bound PTH, failed to restore either receptor number or PTH-stimulated adenylate cyclase. This finding was not due to a lack of efficacy of the GTP pretreatment, because identical GTP pretreatment restored PTH binding to base-line values in membranes partially occupied by

incubation with PTH in vitro. Thus, simple residual occupancy of PTH receptors by the infused hormone did not appear to account for the observed receptor loss.

The results of this study suggest that target cell resistance to PTH in patients with hyperparathyroidism might occur, at least in part, due to down-regulation of PTH receptors by circulating hormone.

INTRODUCTION

Hyperparathyroid states in man are often associated with renal and skeletal resistance to the action of exogenous parathyroid hormone (PTH)[1] (1–5). The renal resistance is manifested by blunting of the phosphaturic and urinary cyclic (c)AMP responses to exogenous PTH, and appears to be related to the level of endogenous PTH (2). A similar state of PTH resistance has been induced by infusion of parathyroid extract (PTE) into normal humans (2). Although vitamin D deficiency may have additional effects, experimental evidence in calcium- and vitamin D-deficient rats supports the conclusion that elevated levels of PTH per se, and not alterations in extracellular phosphate or calcium concentrations, largely account for the loss of PTH responsiveness (desensitization) in the skeleton (6) and kidney (7). The subcellular mechanism(s) of this phenomenon has not been clearly defined. Forte et al. (8) and Carnes et al. (9) have demonstrated that the blunted phosphaturic response to PTH in rats with chronic secondary hyperparathyroidism is related to decreased activation of the renal cortical adenylate

[1] *Abbreviations used in this paper:* Gpp(NH)p, 5'-guanylimidodiphosphate; GTP, guanosine triphosphate; PTE, parathyroid extract; PTH, parathyroid hormone; TPTX, thyroparathyroidectomy.

cyclase. Activation by sodium fluoride (NaF) and other hormones was intact, suggesting a defect at the level of the PTH receptor, or of receptor-adenylate cyclase coupling (8, 9).

A variety of other peptide hormones, including insulin, glucagon, catecholamines, and gonadotropins are known to down-regulate their own receptors, and thereby attenuate target cell response (10, 11). Thus, down-regulation of PTH receptors is likely to account for the observed desensitization to PTH. However, loss of hormone receptors cannot be assumed to occur in all states of peptide hormone excess. Exposure of target cells to high levels of hormone in some cases results in altered binding affinity (12) or even an increase in receptor concentration (13). Furthermore, under some conditions, persistent occupancy of receptors by the desensitizing hormone mimics the appearance of receptor loss (10, 11). This has in fact been shown to occur in an in vitro model of PTH desensitization (14).

The hypothesis that increased levels of circulating PTH in vivo result in a decrease in PTH receptor number or affinity has not been tested directly. The recent report that the number of PTH receptors is decreased in chicks with chronic calcium or vitamin D deficiency does provide presumptive evidence that hyperparathyroidism results in a decrease in measured PTH receptors (15). However, the role of persistent occupancy has not been fully evaluated. We studied a canine model of PTH desensitization to evaluate the effects of increased levels of circulating PTH in vivo on renal PTH receptors and adenylate cyclase. Desensitization was produced by infusion of parathyroid extract (PTE) into thyroparathyroidectomized (TPTX) dogs. The PTH receptor in canine renal cortical plasma membranes has been characterized extensively (16–19) and, like that of human kidney (20), displays guanyl nucleotide-sensitive binding of PTH. This property was exploited to evaluate the role of simple residual occupancy in the down-regulation of PTH receptors.

METHODS

Protocol. The objective of the study was to evaluate the effects of increased circulating levels of PTH in vivo on renal PTH receptors and adenylate cyclase. Adult mongrel dogs were studied 1–2 wk after parathyroidectomy. We administered a crude preparation of bovine PTE by continuous intravenous infusion for 20 h ($n = 5$). Immediately before the infusion a left unilateral nephrectomy was performed for preparation of base-line renal cortical plasma membranes. At the end of the 20-h infusion period, the remaining kidney was removed for preparation of a second set of renal cortical plasma membranes (final). The paired renal membranes (base-line and final) from each individual animal were always assayed simultaneously. Thus, by expressing the effect of the infusion (final) as a percentage of the base-line values in the same animal, variability between both individual dogs and assays was decreased.

Controls ($n = 5$) were studied in an identical manner, but were infused with the PTE vehicle alone. By comparing the results of the PTE infusion to those of the vehicle infusion, we could differentiate the effects of the PTE per se from those of the procedure, including unilateral nephrectomy.

Animal preparation. Adult mongrel dogs weighing 15–25 kg underwent surgical TPTX 1–2 wk before study. TPTX was considered successful if the fasting plasma calcium fell by at least 2.0 mg/dl within 48 h after surgery. Animals were then maintained on levothyroxine 2.5 μg/kg per d; oral calcium carbonate (30–60 gm/d) and dihydrotachysterol (0–0.25 mg/d) to maintain the plasma calcium at ~8.0 mg/dl. At least 4 d before study, dihydrotachysterol was discontinued. This resulted in a fall of plasma calcium of 2.0±0.5 mg/dl by the time of study. Oral calcium supplements were continued, and intravenous calcium gluconate was given as needed to prevent tetany.

Infusion procedure. Surgery was performed under sodium pentobarbital anesthesia with mechanical ventilation. The left (base-line) kidney was removed via a flank incision. Prophylactic antibiotics (cephazolin [0.5 gm], and benzathine penicillin [1.2 million U]) were given as intramuscular injections. After recovery from anesthesia the awake animal was placed in a sling for the infusion. Bupivicaine was used for local anesthesia of the incision site and xylazine was given as needed for mild sedation. The experimental group was given an infusion of bovine PTE suspended at 2–5 U/ml in 5% dextrose, 0.2% NaCl, 1 mM acetic acid, 0.1% bovine serum albumin (BSA). The PTH preparations used are described under materials. Dogs 1–4 received a urea-TCA extract of bovine parathyroid gland. Dog 5 was given a Lilly bovine PTE. The PTE was continuously infused by a Sigma motor pump (Sigma Motor, Inc., Middleport, NY) into a forelimb vein at 2.0 ml/kg body wt per h for 20 h. The first two dogs received a bolus of PTH at the beginning of the infusion to ensure rapid attainment of steady-state levels of PTH. Measurement of PTH levels showed this to be unnecessary in the subsequent animals. The average total dose of PTE was 184±18 U/kg. At the end of the 20-h infusion period, the right (final) kidney was removed via a midline incision. Plasma samples for bioactive PTH levels and calcium were obtained every 2 h; plasma phosphate and creatinine every 6 h. Control animals were infused with the vehicle alone. Intravenous calcium gluconate was given to two control animals during the infusion to prevent tetany.

Renal cortical membrane preparation. Highly purified renal cortical plasma membranes were prepared from kidneys immediately after removal by a modification (21) of the method of Fitzpatrick et al. (22). Membranes were stored in aliquots at −80°C. No loss of receptor or adenylate cyclase activity was seen over a 1-yr period. Membrane protein was assayed by the method of Lowry et al. (23) using BSA as standard. Na^+-K^+ ATPase, a marker for renal basolateral membranes (24), was measured to exclude any systematic effect of the PTE infusion, or of unilateral nephrectomy, on the purity of the renal plasma membrane preparation. The specific activity of Na^+-K^+ ATPase was similar in final membranes of both groups: 436±53 nmol PO_4/min per mg protein PTE ($n = 4$); 470±49 control ($n = 5$). Enrichment of Na^+-K^+ ATPase activity over homogenate was also similar in final membranes of both groups: 7.4±0.7-fold PTE; 6.9±0.7-fold control.

Measurement of ^{125}I-*bPTH (1–34) binding.* Binding assays were done in a final volume of 0.1 ml containing 25 mM Tris/HCl (pH 7.5), 2.0 mM $MgCl_2$. 0.1% BSA, ~9,000 cpm (30–150 pg) receptor-purified ^{125}I-bPTH (1–34), 0–700 nM unlabeled bPTH (1–34), and 250 μg/ml of plasma mem-

branes. Incubations were carried out for 60 min at 30°C. Bound ^{125}I-bPTH (1–34) was separated by filtration through 0.2-μm cellulose acetate filters and analyzed as previously described (21). Binding in the presence of 700 nM bPTH (1–34) was considered nonspecific. Nonspecific and blank binding totaled <2.0% of the added ^{125}I-bPTH (1–34), and was routinely subtracted from all binding values. The number and equilibrium dissociation constant of high-affinity binding sites was determined by Scatchard analysis of the displacement binding curves between 0 and 7 nM unlabeled hormone. Putative low-affinity binding sites are not determined by this method.

In vitro PTH exposure. Membranes were incubated in 1-ml aliquots at 30°C for 60 min under the standard conditions used to assay PTH binding, with or without the addition of 24 nM bPTH (1–34). Membranes were then washed using the following procedure: the incubation was terminated by dilution with 4 vol of ice-cold incubation medium. Membranes were sedimented at 10,000 g for 10 min at 4°C. The pellet was resuspended in 5 ml of ice-cold 0.25 M sucrose/1.0 mM EDTA/5 mM Tris, pH 7.5 (SET buffer), and resedimented as above. The final pellet was resuspended at 1 mg/ml in SET buffer.

GTP pretreatment. Membranes were incubated for 5 min at 30°C under standard conditions used to assay binding, with or without 1.0 mM GTP. The washing procedure described above was repeated before measurement of total and nonspecific PTH binding.

Measurement of adenylate cyclase. Adenylate cyclase was measured by conversion of [α-^{32}P]ATP to [^{32}P]cAMP. Standard incubations were carried out in a final volume of 0.1 ml containing 50 mM Tris-Hepes, pH 7.5; 2.0 mM MgCl$_2$; 1.0 g/liter BSA; 0.1 mM [α-^{32}P]ATP (100–300 cpm/pmol); 1.0 mM cAMP; an ATP-regenerating system (30 μg creatinine phosphokinase; 10 mM creatine phosphate); with or without bPTH (1–34). NaF (10 mM) or 5'-guanylimidodiphosphate [Gpp(NH)p] (0.1 mM) were added where indicated. The assay was initiated by addition of 25 μg of membrane protein. Incubation at 30°C for 30 min was terminated by adding 0.1 ml, 50 mM Tris-HCl buffer containing 10 mM unlabeled ATP; 2.0% sodium dodecyl sulfate; and 1.0 mM [^{3}H]cAMP (~30,000 cpm). Assay tubes were placed immediately in a boiling water bath for 3 min. The double-column method of Salomon et al. (25) was used to isolate [^{32}P]cAMP. Each sample was corrected for recovery of [^{3}H]cAMP, which averaged 60%. All values given are the mean of triplicate determinations of 1–3 assays. Adenylate cyclase stimulation by PTH, NaF, and Gpp(NH)p is given as the increase over basal activity.

Biologically active PTH. Circulating levels of biologically active PTH were measured in a bioassay using guanyl nucleotide enhancement of canine renal cortical adenylate cyclase in vitro, as previously described by Nissenson et al. (26). The adenylate cyclase assay was performed as described here, with the addition of 100 μM Gpp(NH)p and 10 μl plasma. Hypoparathyroid canine plasma served as the vehicle for the PTH standards.

Measurement of Na$^+$-K$^+$ ATPase. Na$^+$-K$^+$ ATPase was measured as previously described (27), with the following modifications. Renal cortical homogenates and plasma membranes were incubated for 30 min on ice in 0.08% deoxycholate. Fractions were then incubated for 5 min at 37°C in 500-μl volume containing 75 mM Tris-HCl (pH 7.6), 100 mM NaCl, 20 mM KCl, 6 mM MgSO$_4$, and 3 mM ATP. Na$^+$-K$^+$ ATPase activity was calculated as that portion of total ATPase activity inhibited in the presence of 2.0 mM ouabain.

Statistics. Student's paired t test was used to test for statistical significance of the infusion procedure within groups (as a percentage of base line). The nonpaired t test was used for comparison between groups (28). All values given are the mean±SE.

Electrolytes. Plasma calcium was measured by atomic absorption spectrophotometry (29); phosphorus and creatinine by a colorimetric method (Technicon Autoanalyzer, Technicon Instruments Corp., Tarrytown, NY).

Materials. Partially purified bPTH (75 U/mg), was a generous gift of Dr. Claude Arnaud/University of California, San Francisco, CA. This material was derived by urea/HCl/cysteine extraction of pooled bovine glands carried through the TCA precipitation stage described by Rasmussen et al. (30). More than 95% of the biological activity (renal adenylate cyclase assay) in this preparation coeluted with purified bPTH (1–84) from Bio-Gel P-150 gel filtration columns (31). PTH extract (48 U/ml) was obtained from Eli Lilly & Co. (Indianapolis, IN). Highly purified synthetic bPTH (1–34) (6,000 U/mg) was obtained from Beckman Bioproducts, Palo Alto, CA. Receptor-purified, electrolytically labeled ^{125}I-bPTH (1–34) was prepared as previously described (21). Chromatoelectrophoresis demonstrated >95% radiohomogeneity. The specific activity of the radiolabeled PTH was 50–150 μCi/μg. [α-^{32}P]ATP was purchased from Amersham Corp., Arlington Heights, IL; [^{3}H]cAMP from New England Nuclear, Boston, MA; GTP and Gpp(NH)P from Boehringer Mannheim Biochemicals, New York; BSA, fraction V, fatty acid free, from Miles Laboratories, Elkhart, IN. All other reagents were purchased from standard laboratory suppliers.

RESULTS

Effects of PTE infusion on plasma bioactive PTH and electrolytes. Infusion of PTE for 20 h produced steady-state plasma levels of bioactive PTH ranging from 0.2 to 3.2 ngeq b-PTH (1–34)/ml (mean 2.2±0.7 ngeq/ml). A calcemic effect was seen in four animals in the PTE group, with an increase of plasma Ca from 6.6±0.8 mg/dl at base line, to a final value of 8.5±1.1 mg/dl. Plasma phosphate in the PTE group fell from 5.2±0.19 to 4.1±0.60 mg/dl. The control group did not differ significantly from the PTE group, in either base-line (7.8±0.3 mg/dl Ca; 5.6±0.7 mg/dl PO$_4$) or final plasma values (7.8±0.3 mg/dl Ca; 5.1±0.4 mg/dl PO$_4$).

Effects of PTE infusion on renal cortical adenylate cyclase in vitro. In vivo PTE infusion resulted in a marked loss of PTH-stimulated adenylate cyclase in renal cortical plasma membranes. Maximal PTH-stimulated adenylate cyclase activity in membranes made after PTE infusion (final) fell to one-third the activity seen in membranes made from base-line kidneys in the same animals (Fig. 1). This decrease in maximal PTH-stimulated activity occurred without a change in the concentration of PTH producing half-maximal adenylate cyclase activation (apparent K_A:4 nM). Basal adenylate cyclase activity was not affected by the PTE infusion (105±4% of base line).

NaF and Gpp(NH)p stimulation of adenylate cyclase activity were tested to determine whether the
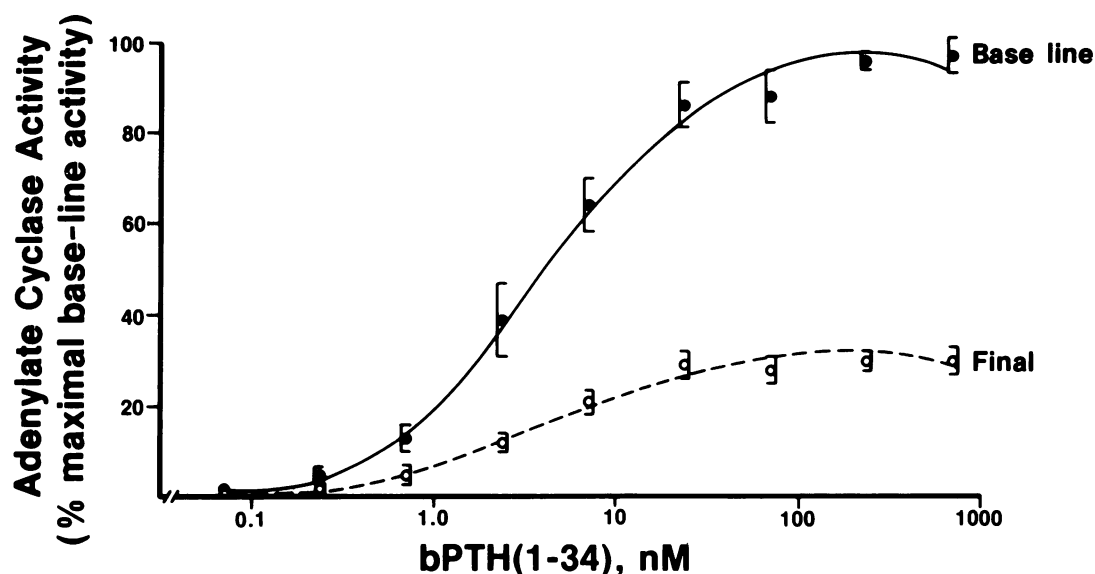
FIGURE 1 Effect of PTE infusion on PTH-stimulated adenylate cyclase activity of renal membranes in vitro. Renal cortical plasma membranes were prepared from one kidney before (base line) and the other kidney after (final) infusion of bovine PTE ($n = 5$) for 20 h. Results are expressed as a percentage of the maximum PTH-stimulated activity of the paired base-line membranes (627±72 pmol cAMP/30 min per mg protein). Basal adenylate cyclase activity has been subtracted from each point (104±7 pmol cAMP/30 min per mg protein base line vs. 109±6 final). Data represent mean±SE.

observed desensitization to PTH could be attributed to a postreceptor defect. Table I shows the adenylate cyclase activity of the base line and final membranes after PTE infusion compared with base-line and final membranes made after infusion of the vehicle alone (control, $n = 5$). The two groups did not differ signif-

TABLE I

Adenylate Cyclase Activity of Renal Cortical Plasma Membranes

	Basal		bPTH (1–34) 700 nM*		NaF, 10 mM		Gpp(NH)p 0.1 mM*	
	Base line	Final	Base line	Final	Base line	Final	Base line	Final
				(pmol cAMP/30 min/mg protein)				
PTE infusion								
Dog No. 1	78	90	423	131	3,130	2,930	1,580	1,990
2	110	116	814	277	8,780	7,060	1,540	2,460
3	121	126	607	212	7,160	6,900	3,300	5,130
4	99	108	762	284	8,940	7,110	3,410	4,940
5	114	104	531	168	ND‡	ND‡	3,180	2,710
Mean±SE	104±7	109±6	627±72	214±30§	7,000±1,352	6,000±1,024	2,600±426	3,480±660
Control infusion								
Dog. No. 6	97	150	533	766	4,200	6,350	1,270	2,650
7	200	214	1,207	638	11,360	8,960	5,920	5,480
8	97	92	973	685	9,830	7,420	1,570	1,470
9	125	172	519	685	12,620	9,850	6,710	8,330
10	131	142	826	589	10,650	8,130	6,480	7,200
Mean±SE	130±19	154±20	812±131	673±29	9,730±1,454	8,140±603	4,390±1,217	5,210±1,304

* Basal activity has been subtracted from all stimulated values.
‡ Not determined.
§ $P < 0.001$ compared with base-line PTE and final control.

icantly at base line. After the infusion, maximal PTH-stimulated adenylate cyclase activity was significantly lower in the PTE group than control (214±30 pmol cAMP/30 min per mg protein, final PTE vs. 673±29 final control, $P < 0.001$). In contrast, basal, NaF-, and Gpp(NH)p-stimulated activity did not differ significantly in final membranes of the two groups. Fig. 2 presents the adenylate cyclase activity of the final membranes as a percentage of the activity in the paired base-line membranes. The decrease of PTH-stimulated adenylate cyclase activity seen after PTE infusion (34±2% of base line) appeared to be a specific effect of the PTE, as no desensitization to PTH was seen in controls (94±18% of base line). In contrast, the effect of PTE infusion on basal, NaF-stimulated, and Gpp(NH)p-stimulated adenylate cyclase activity did not differ from control. NaF-stimulated adenylate cyclase activity was maintained at >88% of base-line activity after PTE infusion, while Gpp(NH)p-stimulated activity was even higher than base line, suggesting that the availability of catalytic cyclase was not limiting PTH responsiveness. These results support the conclusion that PTE infusion induced a state of refractoriness of the renal adenylate cyclase to subsequent PTH stimulation, which is likely to involve a lesion at the level of the PTH receptor.

Studies of ^{125}I-bPTH (1-34) binding. To test directly the hypothesis that the observed desensitization to PTH involves a decrease in the number of PTH receptors, we evaluated binding of ^{125}I-bPTH (1-34) to the renal cortical plasma membranes in vitro. A representative pair of binding curves for one PTE-infused dog is shown in Fig. 3 A. Maximum binding fell in the final membranes of all five PTE-infused dogs, to 61±3% of base-line values, but remained at 99±6% of base-line values after infusion of the vehicle alone (Table II). Scatchard transformation of the binding curves (Table II) showed that a decrease in the number of PTH receptors from 1.89 pmol/mg protein at base line to 0.88 pmol/mg protein after PTE infusion ($P < 0.001$) accounted for the decreased binding seen in the final membranes of the PTE group. No significant change in receptor number occurred in controls. The equilibrium constant of dissociation (K_D) was not altered in either group (Table II).

Fig. 3 B shows the number of PTH receptors in the final membranes of both groups, expressed as a percentage of the paired base-line values. The number of receptors fell to 47% of base-line values after PTE infusion, while no significant change occurred in the controls ($P < 0.01$). The magnitude of the decrease in the number of PTH receptors after PTE infusion is
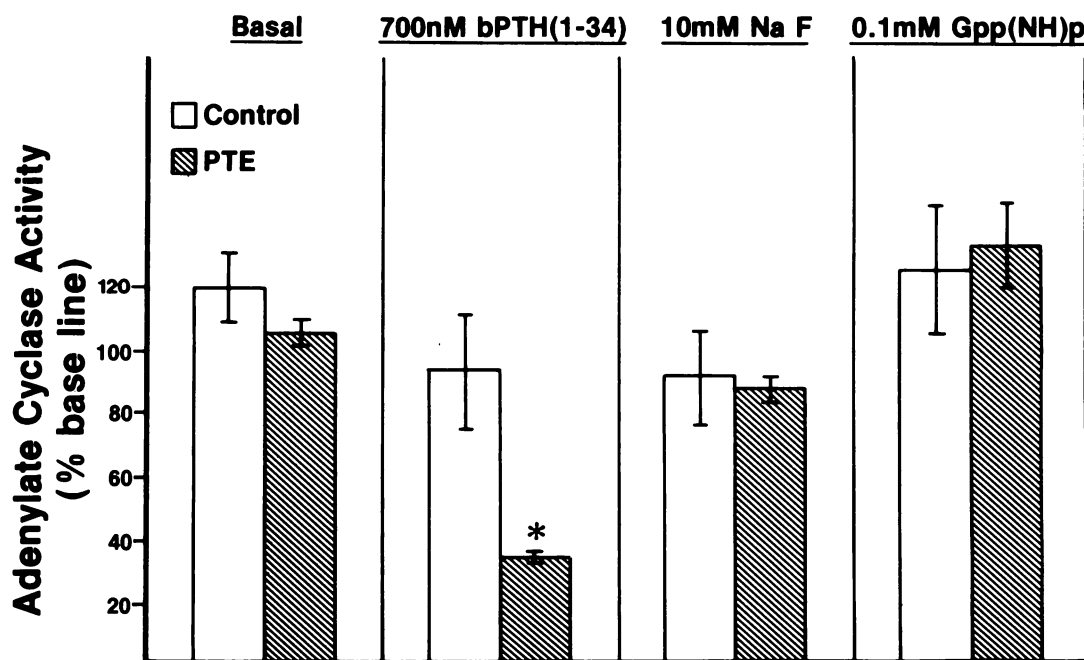

FIGURE 2 Adenylate cyclase activity of renal cortical plasma membranes prepared after PTE ($n = 5$) or control ($n = 5$) infusions. Membranes were assayed in triplicate for basal adenylate cyclase activity and maximal activity stimulated by 700 nM bPTH (1-34), 10 mM NaF, and 0.1 mM Gpp(NH)p. Results are expressed as a percentage of the activity seen in paired membranes made from the opposite kidney of the same animal before infusion (percent base line). Data represent mean±SE. *$P < 0.02$ compared with control.
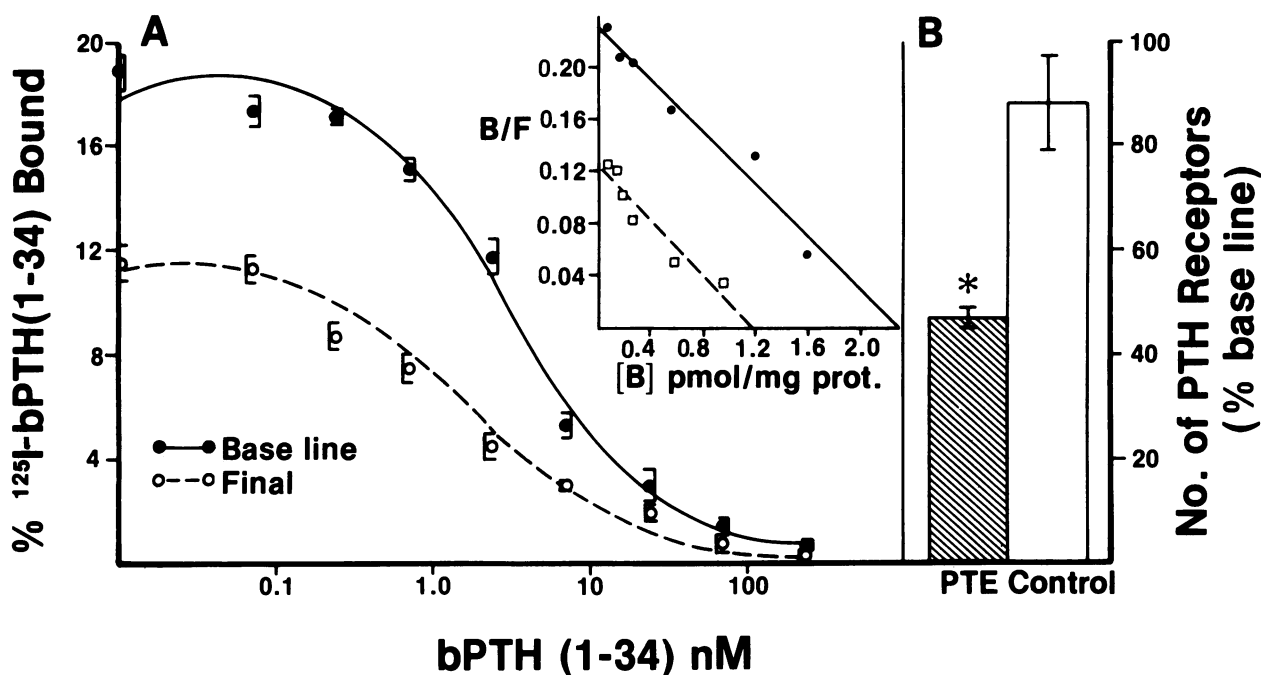
FIGURE 3 (A) Competitive binding of ^{125}I-bPTH (1-34) to renal membranes before (base-line) and after (final) infusion of bovine PTE for 20 h. Each point is the mean±SE of quadruplicate determinations. Percent bound refers to the percent of added ^{125}I-bPTH (1-34) specifically bound. Scatchard transformation of this binding curve is shown in the inset. The data shown are representative of results in five PTE-infused animals. (B) Effect of PTE infusion on the number of PTH receptors in renal cortical plasma membranes. PTH receptor number was derived by Scatchard analysis of ^{125}I-bPTH (1-34) competitive binding between 0 and 7 nM unlabeled hormone as shown in A. Receptor number is expressed as a percentage of the number of PTH receptors in paired base-line membranes in PTE-infused (n = 5) and control (n = 5) animals. Base-line receptor number was 1.89±0.35 pmol/mg protein, PTE; 1.58±0.49, control. Data represent mean±SE. *P < 0.01 compared to preinfusion values and to control.

sufficient to account for at least 80% of the previously demonstrated decrease in PTH-stimulated adenylate cyclase activity (53% decrease in receptor number; 66% decrease in PTH-stimulated adenylate cyclase activity).

Effect of guanyl nucleotides on desensitized membranes. To determine whether persistent occupancy of receptors by infused PTE could account for the loss of hormone binding and adenylate cyclase stimulation, we exploited the ability of guanyl nucleotides to induce dissociation of PTH bound to canine renal receptors (17-19). We first established that pretreatment of membranes with GTP reversed the persistent occupancy of receptors that occurred when membranes were exposed to PTH in vitro. Base-line membranes were incubated with a concentration of PTH chosen to reproduce the same magnitude of binding loss seen after PTE infusion. GTP pretreatment completely restored subsequent ^{125}I-bPTH (1-34) binding in these membranes (Fig. 4). In contrast, GTP pretreatment did not alter binding in final membranes prepared after exposure to PTE in vivo.

To ensure that the failure of guanyl nucleotide treatment to restore PTH receptors was not due to rapid metabolism of GTP by the membranes, we measured ^{125}I-bPTH (1-34) binding to desensitized membranes in the presence of the hydrolysis-resistant GTP analogue Gpp(NH)p (Table III). There was a 60% decrease in PTH binding to all membranes in the presence of Gpp(NH)p, consistent with the reported effect of Gpp(NH)p to decrease binding affinity (16). Because PTH binding fell proportionately in both base-line and final membranes, binding in the desensitized membranes remained at only 65% of base line. Thus, although Gpp(NH)p clearly affected binding, it failed to equalize the PTH binding of desensitized membranes to that of base line.

In theory, guanyl nucleotide treatment of the final PTE membranes could release persistently bound PTH, thus restoring receptor number, but fail to restore maximal ^{125}I-bPTH (1-34) binding because of a change in binding affinity of the reexposed receptors. To evaluate this possibility, receptor number and affinity were remeasured after GTP pretreatment in

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TABLE II
Binding of ^{125}I-bPTH (1–34) to Desensitized Canine Renal Membranes

	^{125}I-bPTH (1–34) maximum bound		No. of PTH receptors*		Binding affinity (K_D)*	
	Base line	Final	Base line	Final	Base line	Final
	%		pmol/mg protein		nM	
PTE infusion						
Dog. No. 1	11.3	7.1	0.84	0.44	1.6	1.4
2	17.8	12.8	1.25	0.55	1.2	0.9
3	18.9	11.7	2.28	1.14	2.2	1.9
4	14.2	8.3	2.69	1.20	3.8	3.5
5	9.7	5.0	2.27	1.05	3.4	3.9
Mean±SE	14.4±1.8	9.0±1.4‡	1.89±0.35	0.88±0.16‡	2.4±0.5	2.3±0.6
Control infusion						
Dog. No. 6	6.5	6.8	0.98	1.11	1.7	2.1
7	14.8	12.3	0.55	0.35	2.4	0.7
8	9.9	9.3	0.84	0.62	1.8	1.5
9	13.7	13.0	2.61	2.76	3.7	4.6
10	10.2	12.2	2.94	2.45	3.2	4.2
Mean±SE	11.0±1.5	10.7±1.2	1.58±0.49	1.46±0.49	2.6±0.4	2.7±0.8

* Determined by Scatchard analysis of displacement binding curves.
‡ $P < 0.001$ compared with base-line values.

both base-line and final membranes of the PTE-infused group ($n = 4$). The absolute number of receptors determined after GTP pretreatment (1.76±0.43 pmol/mg protein base-line [+]GTP; 0.83±0.20 final [+]GTP) was virtually identical to the values previously obtained without GTP pretreatment (1.89±0.39 pmol/mg protein base line; 0.81±0.18 final PTE). Binding affinity decreased slightly in all GTP pretreated membranes ($K_D = 4$ nM).

GTP pretreatment also failed to restore PTH-stimulated adenylate cyclase activity to base-line values in the desensitized membranes (Fig. 5). Absolute PTH-stimulated activity was increased in all membranes after GTP pretreatment, presumably due to a residual effect of tightly bound GTP on the membranes. However, activity in both the base-line and final membranes was increased proportionately (2,845±603 pmol cAMP/30 min per mg protein, base line; 1,436±456, final). Basal adenylate cyclase activity in desensitized membranes was not affected by GTP pretreatment, either in absolute terms (119±19 pmol cAMP/30 min per mg protein [+] GTP; 110±7 [−] GTP) or relative to base-line values (Fig. 5).

In summary, although GTP pretreatment restored the loss of binding seen after exposure of isolated membranes to PTH in vitro (Fig. 4), GTP pretreatment reversed neither the receptor loss nor the adenylate cyclase desensitization induced by PTE exposure in vivo (Fig. 5).

DISCUSSION

The results of this study demonstrate that maintenance of circulating, biologically active PTH at hyperparathyroid levels for 20 h induces a state of renal refractoriness to the hormone that can largely be attributed to a loss of available PTH receptors on renal cortical plasma membranes. Thus, administration of PTE to thyroparathyroidectomized dogs resulted in a 53% loss of renal PTH receptors, which was associated with a 66% decrease in maximal PTH-stimulated adenylate cyclase activity. We suggest that down-regulation of PTH receptors by circulating hormone may play a primary role in the PTH resistance associated with hyperparathyroidism in man.

This hypothesis is consistent with previous data from animal models of secondary hyperparathyroidism. Loreau et al. (32) reported decreased binding of human [^{3}H]PTH (1–34) to renal membranes from rats fed a calcium- and vitamin D-deficient diet. This could not be attributed to hypocalcemia per se because parathyroidectomy of rats fed a normal diet resulted in increased renal PTH receptor binding despite marked hypocalcemia. Similarly, Forte et al. (10) have recently reported decreased binding of an ^{125}I-labeled PTH analogue, norleucyl[8]-norleucyl[18]-tyrosinyl[34] [Nle[8], Nle[18], Tyr[34]] bPTH (1–34) amide, to renal membranes from chicks maintained on either a calcium- or vitamin-D deficient diet. Neither of these studies, however, di-
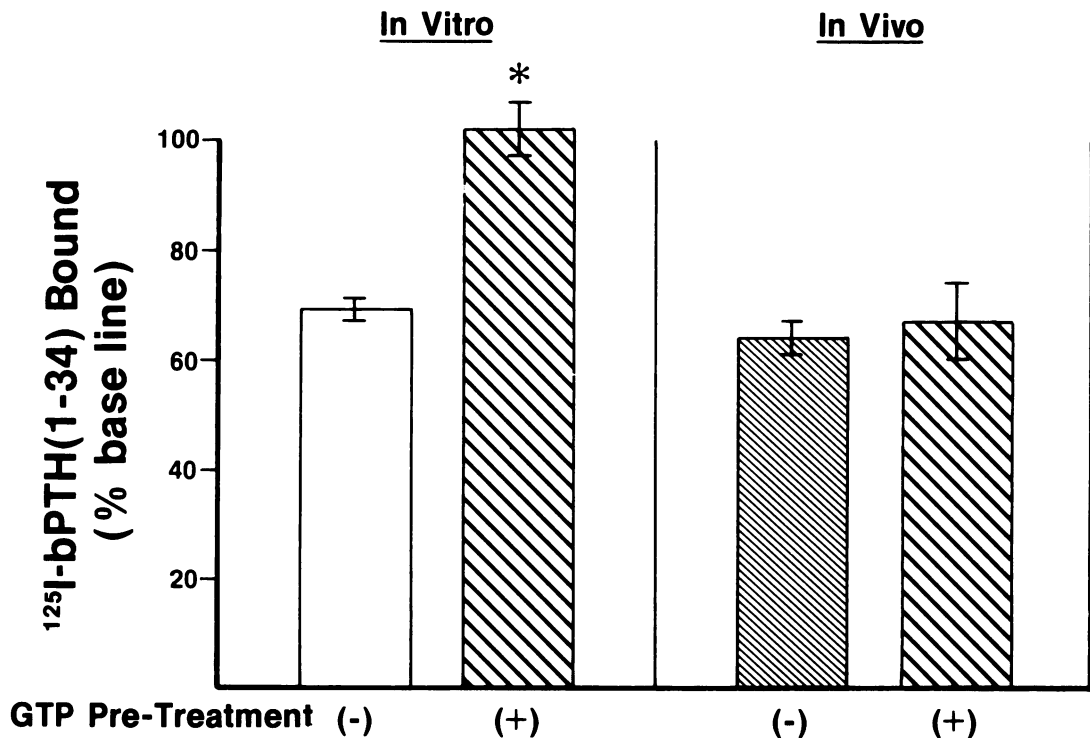
In Vitro   **In Vivo**

FIGURE 4 Effect of GTP pretreatment on PTH binding after in vitro vs. in vivo PTH exposure. Base-line membranes of the PTE group (n = 4) were pretreated with PTH in vitro (□) to achieve the same degree of binding loss seen in the final membranes (in vivo PTH exposure ▨). All membrane pairs were then treated with GTP (▧), followed by extensive washing. Binding is expressed relative to that seen in the paired base-line membranes that had not been exposed to PTH (range 7 to 15% specific binding at base line). Data represent mean±SE. °P < 0.01 vs. in vitro (−) GTP treatment and in vivo (+) GTP treatment.

rectly establishes that circulating PTH plays a causal role in regulating receptor activity in these models of secondary hyperparathyroidism. The decreased PTH receptor activity seen in the present study following infusion of PTE clearly implicates PTH as the causal factor in this phenomenon. This is presumably due to a direct effect of infused PTH to down-regulate its

TABLE III

Effect of Gpp(NH)p on PTH Binding to Desensitized Renal Cortical Plasma Membranes

| | [125]I bPTH (1–34) Specific Binding | |
| | Base line | Final |
		Percent base line
(−)Gpp(NH)p	16.8±1.8	11.2±1.7 66±5
(+)Gpp(NH)p°	7.1±1.7	4.0±1.4 64±4‡

Data represent mean±SE.
° Incubated in the presence of 0.1 mM Gpp(NH)p.
‡ NS from (−)Gpp(NH)p.

renal receptors, although we cannot rule out a modulating role of indirect factors (such as vitamin D metabolites) potentially altered by circulating PTH.

The molecular mechanism of PTH-induced down-regulation of its own receptor is uncertain. However, this phenomenon almost certainly did not occur as a result of simple residual occupancy of PTH receptors by infused hormone. Thus, pretreatment of renal membranes from PTE-infused animals with GTP, which is known to produce dissociation of receptor-bound PTH (17–19), failed to restore available PTH receptors to preinfusion levels. Our findings differ from those of Tamayo et al. (14) who recently reported that down-regulation and desensitization of PTH receptors following perfusion of isolated dog kidneys with bPTH (1–34) in vitro were attributable to persistent occupancy of receptors by infused hormone. In those studies, pretreatment of desensitized membranes with GTP restored PTH receptor binding and PTH stimulation of adenylate cyclase to control values. The basis for the difference between these results and our own, although undefined, is not a lack of efficacy of GTP treatment under our experimental conditions because
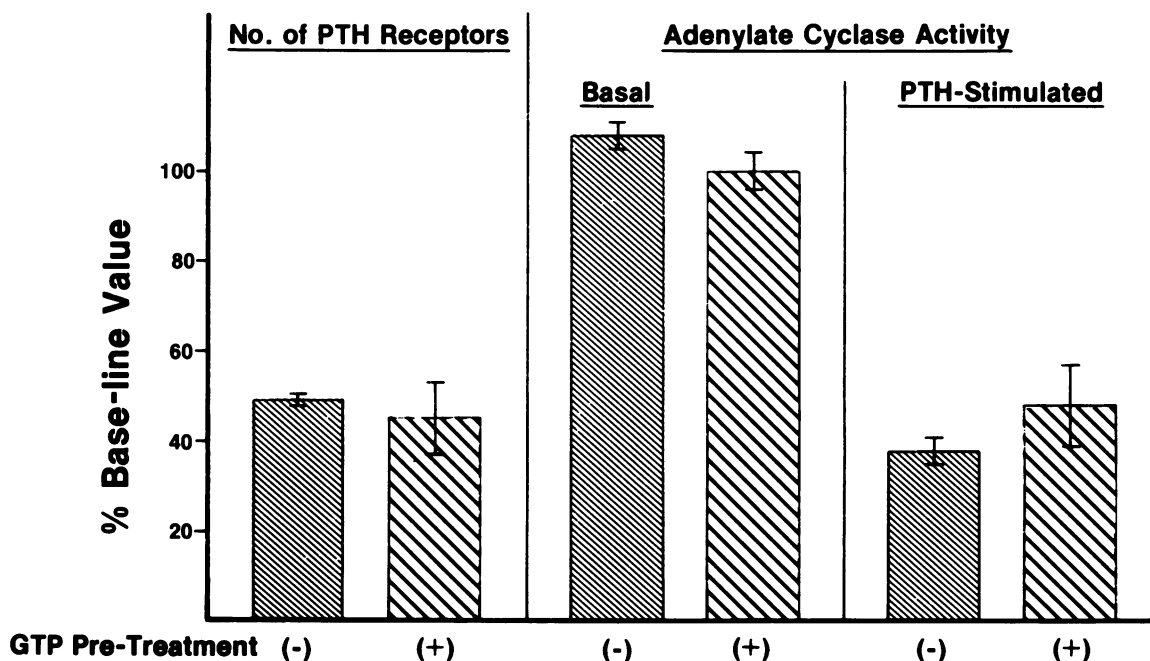
FIGURE 5 Effect of GTP pretreatment on the number of PTH receptors and on PTH-stimulated adenylate cyclase activity in desensitized membranes. Both base-line and final membranes of the PTE group ($n = 4$ pairs) were pretreated with GTP, followed by extensive washing. The number of PTH receptors was determined by Scatchard analysis of ^{125}I-bPTH (1–34) competitive-binding curves. PTH-stimulated adenylate cyclase activity was measured in the presence of 700 nM bPTH (1–34). Values for final membranes are presented as a percentage of the paired base-line values. Data represent mean±SE.

(a) identical GTP pretreatment restored to base-line values PTH receptors partially occupied by incubation with PTH in vitro; and (b) ^{125}I-bPTH (1–34) binding to membranes from PTE-infused animals remained depressed (relative to base-line membranes), even in the presence of the stable GTP analogue Gpp(NH)p. It is conceivable that the residual occupancy of PTH receptors observed by Tamayo et al. (14) was due to in vitro perfusion of PTH at a relatively low temperature (18°–20°C), which might have decreased the rate of dissociation of perfused PTH from renal receptors and/or blocked metabolic events subsequent to PTH binding (e.g., receptor internalization) (33).

In this study, the renal PTH receptors that remained after the 20-h infusion of PTE appeared to be functionally normal. These high-affinity receptors continued to be of a single class (i.e., linear Scatchard plot) with an unaltered affinity for PTH. In addition, they were able to initiate PTH-dependent activation of adenylate cyclase, albeit at a level reduced proportionally with the loss of total receptors.

Components of the adenylate cyclase system beyond the PTH receptor appear to be essentially intact in this model of desensitization. Enzyme activation by NaF and Gpp(NH)p (which activate adenylate cyclase via

postreceptor mechanisms) were not different than that seen in controls (34). The slight decrease in NaF-stimulated adenylate cyclase activity and the increase in Gpp(NH)p-stimulated adenylate cyclase activity that occurred after PTE infusion appeared after the vehicle infusion as well, suggesting that these changes might be related to the compensatory renal hypertrophy known to occur after unilateral nephrectomy (35, 36). We cannot exclude the possibility that the small (12%) decrease in NaF-stimulated adenylate cyclase activity seen after PTE infusion plays a role in the observed desensitization, but its contribution appears minor.

The results of the present study raise the possibility that target cell resistance to PTH in patients with hyperparathyroidism might occur, at least in part, due to down-regulation of PTH receptors by circulating hormone. Important in this regard is our observation that down-regulation occurred at circulating bioactive PTH levels (0.2–3.2 ngeq bPTH [1–34]/ml), which although higher than generally seen in primary hyperparathyroidism, are similar to those seen in some patients with hyperparathyroidism secondary to chronic renal failure (26). However, the duration of high circulating levels of PTH in the present study was only 20 h. Further work is needed to determine

whether loss of PTH receptors and/or additional loci of desensitization might appear with chronically elevated PTH levels. Nevertheless, down-regulation of renal PTH receptors in response to elevations in circulating PTH, with resultant desensitization of PTH-stimulated adenylate cyclase, may constitute an important etiologic factor in the PTH resistance associated with hyperparathyroidism.

ACKNOWLEDGMENTS

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