Effects of Dietary-induced Hyperparathyroidism on the Parathyroid Hormone-Receptor-Adenylate Cyclase System of Canine Kidney

EVIDENCE FOR POSTRECEPTOR MECHANISM OF DESENSITIZATION

JUAN TAMAYO, EZEQUIEL BELLORIN-FONT, and KEVIN J. MARTIN, *Renal Division, Department of Internal Medicine, Washington University School of Medicine, St. Louis, Missouri 63110*

ABSTRACT The present studies were designed to examine the consequences of chronic mild elevations of endogenous parathyroid hormone (PTH) in vivo on the PTH receptor-adenylate cyclase system of canine kidney cortex. Hyperparathyroidism was induced in normal dogs by feeding a diet low in calcium, high in phosphorus to the animals for a period of 6–9 wk. This maneuver resulted in a two to threefold increase in the plasma levels of carboxy-terminal immunoreactive PTH. This degree of hyperparathyroidism is similar to that seen in patients with hyperparathyroidism and normal renal function. After 6–9 wk on the diet the animals were killed and basolateral renal cortical membranes prepared for the study of the PTH receptor-adenylate cyclase system in vitro. The dietary hyperparathyroidism resulted in desensitization of the PTH-responsive adenylate cyclase (V_{max} 3,648±654 pmol cyclic (c)AMP/mg protein per 30 min in hyperparathyroid animals vs. 5,303±348 in normal controls). The K_{act} (concentration of PTH required for half-maximal enzyme activation) was unchanged. However, PTH receptor binding (^{125}I-norleucyl^{8}-norleucyl^{18}-tyrosinyl^{34}, ^{125}I[Nle^{8}, Nle^{18}, Tyr^{34}] bPTH (1–34) NH_2 as radioligand) was not different in the two groups of animals. Thus, dietary hyperparathyroidism resulted in an uncoupling of the PTH receptor-adenylate cyclase system. This defect was not corrected by guanyl nucleotides in vitro, and the effects of guanyl nucleotides on PTH binding and enzyme activation appeared normal. NaF-stimulated enzyme activity was reduced in the hyperparathyroid animals (8,285±607 pmol cAMP/mg protein per 30 min vs. 10,851±247 in controls). These data indicate that desensitization of the PTH-responsive adenylate cyclase system of canine kidney as a result of mild chronic elevations of endogenous PTH is due to a postreceptor defect, demonstrable by NaF activation, not corrected by guanyl nucleotides, leading to abnormal PTH-receptor adenylate cyclase coupling.

INTRODUCTION

Constant exposure of the parathyroid hormone (PTH)[1] receptor-adenylate cyclase system of the renal cortex to high levels of PTH results in a decreased response to further stimulation of the enzyme with the same hormone. This general phenomenon, known as desensitization, has been demonstrated both in vivo and in vitro not only for PTH but also for a variety of other hormone- and neurotransmitter-sensitive adenylate cyclase systems (1–9). This phenomenon may be an important mechanism for the regulation of the effects of these hormones and/or neurotransmitters (10). The precise alterations that can lead to desensitization

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[1] *Abbreviations used in this paper:* bPTH, bovine PTH; C, catalytic subunit; G/F, nucleotide regulatory protein; Gpp(NH)p, 5′-guanylyl imidodiphosphate; GTP, guanosine triphosphate; iPTH, immunoreactive PTH; K_{act}, concentration of PTH required for half-maximal enzyme activation; ^{125}I[Nle^{8}, Nle^{18}, Tyr^{34}], ^{125}I-norleucyl^{8}-norleucyl^{18}-tyrosinyl^{34}; PTH, parathyroid hormone; syn, synthetic.

within the hormone-receptor-adenylate cyclase system are ill defined and vary according to the tissue studied and the experimental conditions (10, 11). Adenylate cyclase activation is dependent on the interaction of at least three classes of components: the hormone-receptor complex, the nucleotide regulatory protein (G/F), and the catalytic subunit (C). Accordingly, the desensitization process can potentially involve different regulatory steps in the adenylate cyclase complex.

Previous studies have examined the mechanisms of desensitization of the PTH-responsive adenylate cyclase system of kidney cortex in different experimental models in vivo. Mahoney and Nissenson (12) used the constant infusion of bovine parathyroid extract to parathyroidectomized dogs for a period of 20 h and demonstrated that desensitization of PTH-responsive adenylate cyclase in vitro was due, at least in part, to apparent receptor loss. Similar findings were obtained by Forte et al. (13) in renal cortical membranes from chickens rendered hyperparathyroid for up to 3 wk by dietary calcium and/or vitamin D restriction. While high concentrations of PTH were achieved in plasma in the studies in dogs, no direct measurements of plasma PTH were obtained in the studies in chickens. However, it is likely that the degree of hyperparathyroidism was severe due to persistent marked hypocalcemia. The present studies were designed to examine the PTH-receptor-adenylate cyclase system of canine kidney cortex during a mild chronic hyperparathyroid state, similar in degree to that seen in human parathyroid disease states.

METHODS

Studies were performed in 14 adult normal mongrel dogs. The experimental group (seven dogs) received a low calcium, normal phosphorus diet (0.01% g/dry wt of Ca, 0.8–1% g/dry wt of Pi) supplemented with 6 g of inorganic phosphorus for a period of 6–9 wk. The control group (seven dogs) received normal dog chow (0.8–1% g/dry wt of Ca, 0.8–1% g/dry wt of Pi). Blood for determination of the levels of immunoreactive (i)PTH, total and ionized calcium, phosphorus, magnesium, blood urea nitrogen, and creatinine were obtained weekly in the experimental animals throughout the study. After a period of 6–9 wk, under general anesthesia (pentobarbital 30 mg/kg of body wt i.v.) and heparin (5,000 IU, i.v.), the kidneys were removed, perfused with ice-cold saline to remove residual blood and basolateral membranes were prepared from the cortex by differential centrifugation and sucrose gradient ultracentrifugation as previously described (14, 15). The enrichment in Na-K-ATPase was identical in membranes from both groups of animals. All values shown are mean±SEM. Statistical analysis was performed by Student's t test for paired or unpaired data as appropriate.

Adenylate cyclase assay. Adenylate cyclase activity was measured in this preparation by quantitating the formation of cyclic (c)AMP from ATP according to the method of Steiner et al. (16) with the following modifications. Incubations were carried out at 25°C for 30 min in a mixture containing 50 mM Tris HCl, 25 mM KCl, 1 mM 3-isobutyl-1-methylxanthine, 0.2 mM EDTA, pH 7.5, 5% hypoparathyroid dog serum, 0.2 mM ATP, 25 mM phosphocreatinine, 1 mg/ml creatinine phosphokinase, 5 mM $MgCl_2$ and 20–50 μg membrane protein in a total volume of 100 μl. When necessary, divalent cations, PTH, guanosine triphosphate (GTP), or 5′-guanylyl imidodiphosphate (Gpp[NH]p) were added at the indicated concentrations. The reaction was initiated by addition of the membranes and terminated by boiling the samples for 3 min. The samples were diluted 1:10 in 50 mM sodium acetate, pH 6.2 and the cyclic (c)AMP was measured by radioimmunoassay by the method of Steiner et al. (17), using the antibody BR-1 produced in our laboratory (18).

Receptor binding assay. Receptor binding was measured by incubating the basolateral membranes (30–50 μg protein) with 30,000–50,000 cpm of the agonist radioligand ^{125}I[Nle^{8}, Nle^{18}, Tyr^{34}] bovine bPTH (1–34) NH_2 (sp act 280–320 μCi/μg) in 50 mM Tris HCl, 25 mM KCl, and 5% hypoparathyroid dog serum as described (18). Synthetic (syn) bPTH (1–34) was added at the indicated concentrations. The binding of the radioligand to kidney membranes is specific and saturable and correlates with biologic activity in the adenylate cyclase assay (14, 19, 20). The incubations were performed at 16°C for 60 min. Free radioligand was separated from membrane-bound radioligand by the microcentrifuge method. Radioactivity in the tips of the microcentrifuge tubes containing membrane-bound hormone was counted in a gamma counter (model 4600, Micromedic Systems Div., Rohm & Haas Co., Horsham, PA). Under the experimental conditions used, nonspecific binding (radioactivity remaining in the tips of the microfuge tubes from incubations containing membranes and 10^{-5} M syn bPTH (1–34) was 5.9±0.6% of the total radioactivity added. The specific binding calculated by subtracting the nonspecific binding from the total binding was 17.8±1% of the total radioactivity added in the absence of exogenous guanyl nucleotides.

To evaluate PTH receptor binding in membranes from control and hyperparathyroid kidneys, inhibition of binding of ^{125}I[Nle^{8}, Nle^{18}, Tyr^{34}] bPTH (1–34) NH_2 was measured in the presence of increasing concentrations of syn bPTH (1–34). PTH bound was calculated by multiplying the fraction of the radioligand bound by the quantity of syn bPTH (1–34) added. However, since displacement of the radioligand by [Nle^{8}, Nle^{18}, Tyr^{34}] bPTH (1–34) NH_2 and syn bPTH (1–34) give parallel curves with slightly different affinities (14, 19), these calculations will overestimate the amount of PTH bound by a factor equal to the difference in affinities for the two peptides (approximately twofold) (14). Accordingly, the amount of PTH bound is expressed as femtomoles equivalents of [Nle^{8}, Nle^{18}, Tyr^{34}] bPTH (1–34) NH_2. Thus, 1 fmol equivalent of the analogue is the amount of syn bPTH (1–34) required to produce an equivalent inhibition of radioligand binding as 1 fmol of [Nle^{8}, Nle^{18}, Tyr^{34}] bPTH (1–34) NH_2. The amount of PTH bound as femtomoles equivalents of [Nle^{8}, Nle^{18}, Tyr^{34}] bPTH (1–34) NH_2 is the fraction of syn bPTH (1–34) added that results in the same fractional binding of the radioligand.

Determination of iPTH. Immunoreactive PTH was measured in serum by radioimmunoassay using the antibody CH-9 that has antigenic determinants for the middle and carboxy-terminal regions of the PTH molecule as previously described (21). Hyperparathyroid dog serum was used as assay standard. Plasma samples were extracted and concentrated following the procedure of Bennett et al. (22) with the following modifications: briefly C_{18} ODS-Silica cartridges (Sep-Pak, Waters Associates, Millipore Corp., Milford, MA) were equilibrated with 5 ml of a solution containing 0.1%

trifluoroacetic acid (vol/vol) and 80% (vol/vol) acetonitrile and washed with 5 ml of 0.1% (vol/vol) trifluoroacetic solution. Then 2 ml of plasma was applied followed by 20 ml of 0.1% trifluoroacetic acid solution. Finally, the cartridges were eluted with 3 ml of the solution containing 80% acetonitrile and 0.1% trifluoroacetic acid. This eluate was collected lyophylized and reconstituted with 1 ml of PTH assay buffer and assayed for iPTH. Preliminary studies with plasma containing high levels of canine PTH showed good agreement between the results obtained with extracted and unextracted plasma.

RESULTS

As a result of the low calcium, high phosphorus diet, iPTH values rose from control values of 46±12 μleq/ml to 80±17 μleq/ml after 4–5 wk, to 107±19 μleq/ml at the completion of the study as shown in Fig. 1. The levels of iPTH in the control period were not different from those of normal control dogs (45±11 μleq/ml, range 17–81 μleq/ml). The values for ionized calcium, phosphorus, and creatinine in the dogs fed low Ca diet are shown in Table I. There was an increase in ionized calcium after 4–5 wk of the diet, which was not maintained at 8–9 wk. Plasma phosphorus decreased throughout the period of the experimental diet, consistent with the elevation in plasma iPTH. Plasma creatinine values did not change significantly. The dogs were healthy at the completion of the diet as judged for their weights and physical activity.

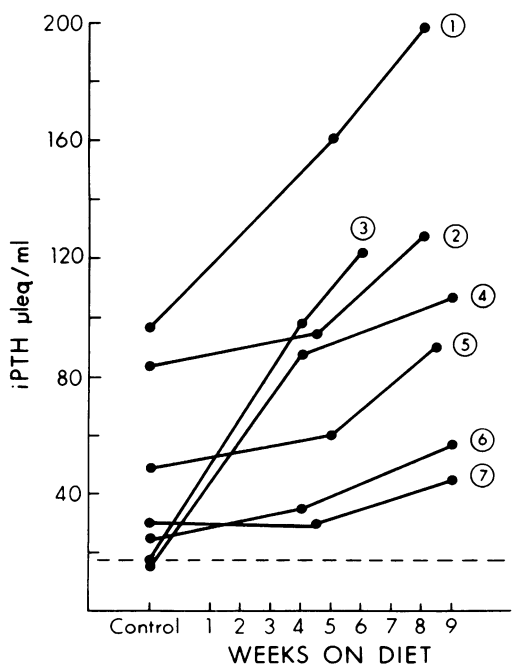

FIGURE 1 Changes in serum iPTH in seven dogs before, during, and at the completion of treatment with a low calcium, high phosphorus diet. The dashed line represents the detection limit of the assay. The numbers in circles represent individual animals. The mean values are 46±12 μleq/ml in the control period, 80±17 μleq/ml after 4–5 wk ($P < 0.05$), and 107±19 μleq/ml at the end of the study period ($P < 0.01$).

Adenylate cyclase activity. The data of basal and syn bPTH (1–34)-stimulated adenylate cyclase activity in renal cortical membranes from experimental and control animals are shown in Table II. Basal enzyme activity was slightly but not significantly reduced in the membranes of the hyperparathyroid dogs (352±42 pmol cAMP/mg protein per 30 min vs. 506±68 in control animals, NS). PTH-stimulated enzyme activity was significantly reduced in the experimental animals. As shown in Fig. 2, net PTH-stimulated enzyme activity was significantly decreased ($P < 0.05$) in the membranes of the hyperparathyroid animals (V_{max} 3,648±654 pmol cAMP/mg protein 30 min vs. 5,303±348 in control). The concentration of PTH required for half-maximal enzyme stimulation (K_{act}) was not changed. The correlation between maximal PTH-stimulated adenylate cyclase activity and the final plasma level of iPTH is shown in Fig. 3.

Basal and glucagon (10^{-6} M)-stimulated adenylate cyclase activities in renal cortical homogenates from these two groups of animals were not different (basal 150±19 vs. 160±20, glucagon stimulated 354±34 vs. 355±25 pmol/mg protein per 30 min) in control plus experimental animals, respectively.

PTH-receptor binding. In sharp contrast to the decrease in adenylate cyclase activity there was no difference in the amount of PTH bound per microgram of membrane protein in both groups (Fig. 4). B_{max} 2.76±0.2 fmol equivalent bound/μg protein in hyperparathyroid animals and 2.85±0.2 in controls (Table III). The concentration of syn bPTH (1–34) required to demonstrate half-maximal binding of radioligand was identical in both groups.

Effects of exogenous guanyl nucleotides on adenylate cyclase activity. Based on the known effects of guanyl nucleotides in the regulation of the hormone-receptor-adenylate cyclase interactions, additional experiments were performed in the presence of the naturally occurring nucleotide GTP or its nonhydrolyzable analogue Gpp(NH)p.

As shown in Fig. 5, PTH-stimulated adenylate cyclase activity in the presence of Gpp(NH)p (10^{-4} M) remained markedly reduced in the membranes from the hyperparathyroid animals. Maximal enzyme activity was 6,453±896 pmol cAMP/mg protein per 30 min in the hyperparathyroid group vs. 9,080±577 pmol cAMP/mg protein per 30 min in controls ($P < 0.05$). The concentration of PTH required for half-maximal enzyme activation was similar in both groups. Similar results were obtained with GTP (10^{-3}

TABLE I

Plasma Ionized Calcium, Phosphorus, and Creatinine Before and During Administration of Low Calcium, High Phoshorus Diet

Animal no.	Normal diet	Low calcium, high phosphorus diet		
		2 wk	4–5 wk	8–9 wk
Ionized calcium (*mg/dl*)				
1	4.88	5.16	5.44	5.60
2	4.82	4.94	5.08	4.58
3	5.14	5.14	5.14	4.56
4	5.08	5.18	5.20	4.90
5	4.06	4.96	5.06	5.16
6	5.14	5.22	5.58	5.26
7	4.36	5.06	5.04	4.92
Mean±SEM	4.78±0.16	5.09±0.05	5.22±0.07	4.99±0.14
		NS	$p < 0.02$	NS
Phosphorus (*mg/dl*)				
1	4.81	4.16	4.82	3.66
2	4.10	2.96	3.03	2.71
3	4.35	3.80	3.81	2.87
4	4.09	3.56	3.68	3.55
5	4.06	2.81	2.72	2.65
6	6.69	5.05	5.17	5.00
7	3.81	3.70	2.87	2.86
Mean±SEM	4.56±0.37	3.72±0.28	3.72±0.36	3.32±0.32
		$p < 0.01$	$p < 0.01$	$p < 0.001$
Creatinine (*mg/dl*)				
1	1.00	0.72	0.59	0.77
2	0.61	0.55	0.58	0.50
3	0.73	0.68	0.57	0.58
4	0.65	0.64	0.75	0.61
5	0.52	0.60	0.59	0.62
6	0.80	0.65	0.68	0.71
7	0.77	0.78	0.75	0.71
Mean±SEM	0.73±0.06	0.66±0.03	0.64±0.03	0.64±0.03
		NS	NS	NS

M). It is important to note that the ratio of PTH-stimulated enzyme activity in the presence of Gpp(NH)p was identical in both groups to that in the absence of the guanyl nucleotide. Furthermore, adenylate cyclase activity in response to Gpp(NH)p in the absence of PTH was similar in both groups (Fig. 6).

Effects of guanyl nucleotides on PTH receptor binding. Fig. 7 illustrates the influence of increasing concentrations of Gpp(NH)p on the equilibrium binding of ^{125}I[Nle^{8}, Nle^{18}, Tyr^{34}] bPTH (1–34) NH_2 to membranes of both groups of animals. It is clear that under these circumstances the guanyl nucleotide decreased equilibrium binding to the same extent in both sets of membranes.

Effects of NaF on adenylate cyclase activity. In view of the results obtained with guanylnucleotides, it was of interest to examine the NaF-stimulated enzyme activities as an additional probe for the interaction of G/F and the catalytic moiety of the enzyme. Fig. 8 shows the adenylate cyclase activity in the presence of 10 mM NaF. It is clear that the membranes from the hyperparathyroid dogs have a decreased response to NaF compared with control animals (8,285±607 pmol cAMP/mg protein per 30 min vs. 10,851±247 in control, $P < 0.01$).

Effects of manganese on adenylate cyclase activity. Since basal adenylate cyclase activity was slightly lower (albeit not significantly) in the membranes from

TABLE II

Basal and Syn bPTH (1–34)-stimulated Adenylate Cyclase Activity in Renal Cortical Membranes from Control and Experimental Animals

	Experimental animals						
		Syn bPTH 1-34					
Animal no.	Basal	0.1	1	2	5	10	100
				nM			
Experimental animals							
1	197	285	731	900	1,256	1,220	1,639
2	440	622	1,531	1,975	2,217	2,474	2,378
3	529	688	1,979	3,120	3,982	4,322	5,336
4	380	545	1,657	2,351	3,143	4,045	3,954
5	273	441	1,472	1,584	2,069	2,127	2,865
6	280	753	3,156	3,634	4,657	5,598	5,619
7	369	657	2,928	4,112	5,680	6,116	6,216
Mean±SEM	352±42	570±61	1,922±323	2,525±435	3,286±594	3,700±691	3,999±669
Control animals							
1	633	1,038	2,334	2,612	3,223	3,812	5,052
2	548	896	2,697	3,496	4,107	4,411	4,452
3	632	1,009	3,626	5,473	7,524	7,327	7,212
4	430	706	2,890	3,559	6,152	5,904	6,153
5	740	841	2,370	3,296	4,708	5,694	6,533
6	249	577	3,065	3,745	6,221	6,268	5,994
7	316	351	2,953	3,340	5,851	5,441	5,003
Mean±SEM	506±68	774±93	2,847±168	3,645±333	5,398±553	5,551±440	5,771±368
	NS	NS	$p < 0.05$	NS	$p < 0.05$	$p < 0.05$	$p < 0.05$

All values shown are picomoles cyclic AMP per milligram protein per 30 min.

hyperparathyroid animals compared with normal animals, the last series of experiments were designed to analyze enzyme activity in the presence of high concentrations of Mn (20 mM) and in the absence of magnesium. Under these conditions, adenylate cyclase activity is devoid of the influence of hormone or guanine nucleotides (15, 23), which may have influenced enzyme activity in vivo. It was found that adenylate cyclase activity in the presence of 20 mM Mn^{++} was identical in both groups of membranes (1,631±142 pmol cAMP/mg protein per 30 min in hyperparathyroid vs. 1,744±133, NS, in control animals).

DISCUSSION

The administration of a low calcium, high phosphorus diet to adult normal dogs resulted in the development of secondary hyperparathyroidism (24, 25), as reflected in a rise in plasma iPTH and a decline in plasma phosphorus. The two- to threefold elevation of plasma carboxy-terminal iPTH is similar in degree to that seen in patients with hyperparathyroidism and normal renal function. Thus, this experimental maneuver provides the basis for the study of the consequences of

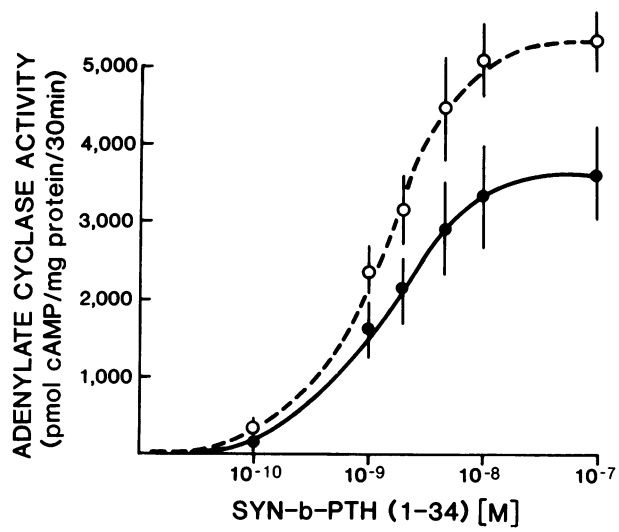

FIGURE 2 Adenylate cyclase activity in response to increasing concentrations of syn bPTH (1–34) in basolateral renal cortical membranes from normal (○) and hyperparathyroid (●) dogs. The data are shown as mean±SEM for results of seven dogs in each group. Basal enzyme activities have been subtracted from each point (506±68 pmol cAMP/mg protein per 30 min in control and 352±42 in hyperparathyroid animals, NS).

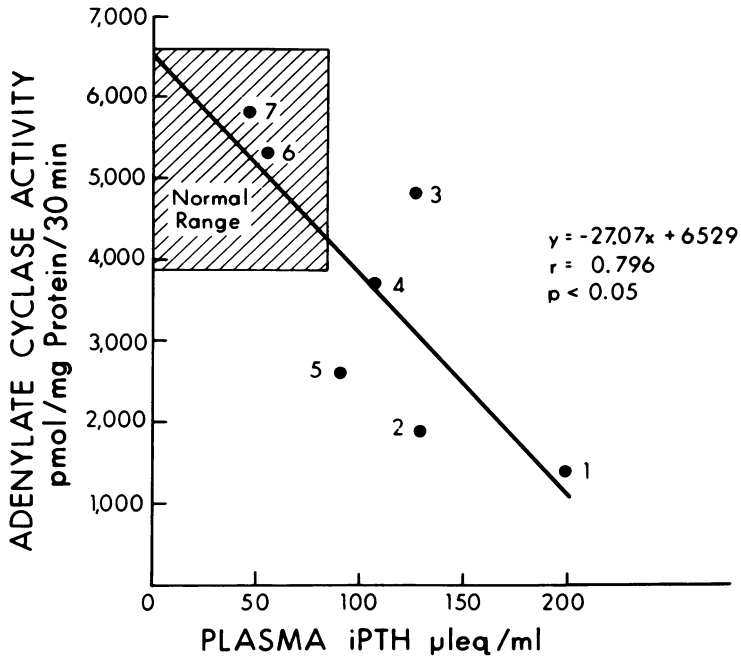

FIGURE 3 Correlation between maximal syn bPTH (1–34) stimulated-adenylate cyclase activity and plasma iPTH in dogs fed a low calcium, high phosphorus diet. The numbers identify the individual animals. The range of values for iPTH and adenylate cyclase activity in the control animals is shown in the hatched area.

chronic exposure to mildly elevated concentration of endogenous PTH in vivo on the PTH-receptor-adenylate cyclase system of canine kidney.

Dietary-induced hyperparathyroidism resulted in desensitization of the PTH-sensitive adenylate cyclase system of kidney cortex, as reflected in a diminished response to in vitro stimulation of the enzyme by syn bPTH (1–34) when compared with membranes of control animals. The affinity of the enzyme for PTH was not changed. This decrease in PTH-stimulated enzyme activity correlated with the increased plasma levels of iPTH achieved by the experimental diet (Fig. 3). Animals 6 and 7, with the lowest final levels of plasma iPTH, showed no reduction in PTH-stimulated adenylate cyclase activity while animal 1, with the highest level of plasma iPTH, showed the greatest reduction in enzyme activity. The correlation of final plasma iPTH values with in vitro PTH-stimulated adenylate cyclase activities is not apparent, however, if the fold rise in plasma iPTH is considered. Thus, animals 3 and 4, with the highest fold rise in iPTH had PTH-stimulated adenylate cyclase activities in vitro within or slightly below the range found in the control animals. Further studies will be required to clarify the relationship of in vitro PTH-stimulated adenylate cyclase activity to circulating levels of biologically active PTH.

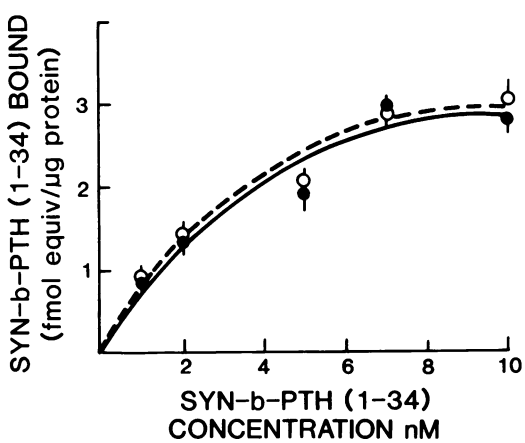

FIGURE 4 Specific binding of syn bPTH (1–34) expressed as femtomoles equivalents per microgram protein to membranes from control (○) and hyperparathyroid (●) dogs in the presence of increasing concentrations of syn bPTH (1–34).

Since the degree of desensitization of PTH-stimulated adenylate cyclase activity was greatest in animals 1 and 2, it is necessary to consider whether these two animals were anomalous in some way at the start of the study. However, they were normal healthy-looking

TABLE III

PTH Receptor Binding in Renal Cortical Membranes from Normal Animals and Animals with Dietary-induced Hyperparathyroidism

Normal animals			Experimental animals		
Animal no.	K_D	B_{max}	Animal no.	K_D	B_{max}
	nM	*fmol equiv/ μg protein*		*nM*	*fmol equiv/ μg protein*
1	1.8	2.58	1	2.4	2.46
2	2.0	3.36	2	1.8	2.41
3	1.8	2.87	3	1.9	2.44
4	2.2	3.43	4	1.8	2.79
5	2.1	2.24	5	2.0	2.65
6	1.9	2.60	6	2.1	3.37
7	1.7	2.87	7	2.0	3.24
Mean±SEM	1.93±0.07	2.85±0.16		2.0±0.08 NS	2.76±0.15 NS

animals with normal serum chemistry profiles, normal iPTH values, and without any abnormal findings at laparotomy. Furthermore, in renal cortical membranes prepared in our laboratory from >50 normal dogs, adenylate cyclase activity was never close to that seen in these animals. Thus, one has to attribute the findings in these animals as due to an effect of the dietary-induced hyperparathyroidism.

The desensitization of the adenylate cyclase was not accompanied by down-regulation of PTH receptors, which showed normal binding capacity and affinity in each animal. These observations indicate that the de-

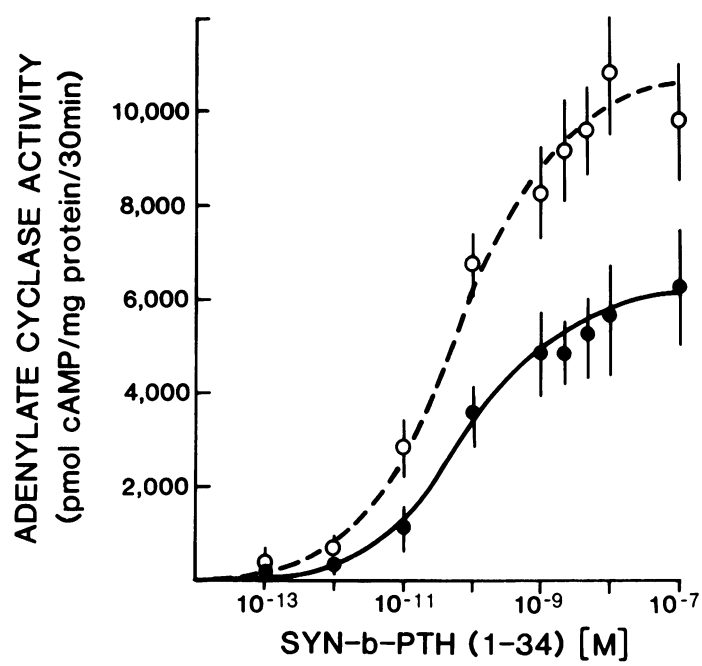

FIGURE 5 Syn bPTH (1-34)-stimulated adenylate cyclase activity in the presence of Gpp(NH)p 10^{-4} M in membranes from control (○) and hyperparathyroid animals (●). Basal enzyme activities in the absence of hormone have been subtracted from each point (1,556±121 pmol cAMP/mg protein per 30 min in hyperparathyroid dogs and 1,928±160 in control, NS).

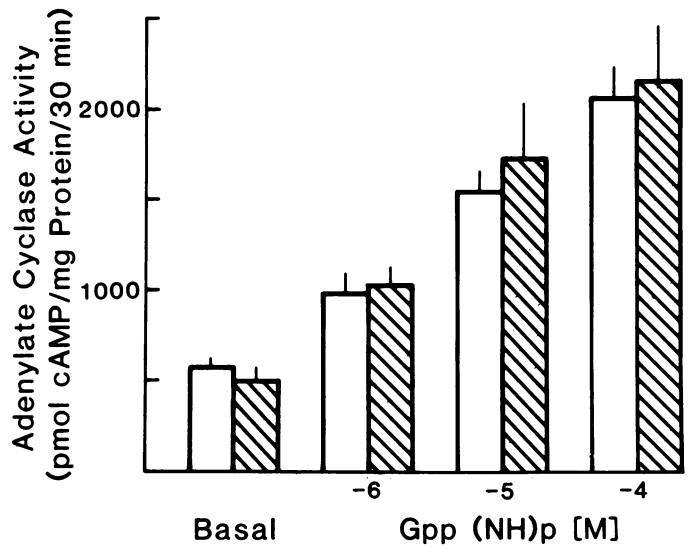

FIGURE 6 Adenylate cyclase activity in the presence of increasing concentrations of Gpp(NH)p and in the absence of PTH in basolateral cortical membranes from control (□) and hyperparathyroid animals (▨).

sensitization to PTH produced during dietary hyperparathyroidism is due to an uncoupling lesion of the PTH-receptor-adenylate cyclase complex.

Since guanine nucleotides play a major role in the regulation of receptor-cyclase coupling (14, 26), experiments were performed to examine the effects of GTP and GppNHp (a nonhydrolyzable analogue of GTP) on PTH-stimulated enzyme activity. Although in the presence of guanine nucleotides PTH-stimulated enzyme activity increased in both membranes from

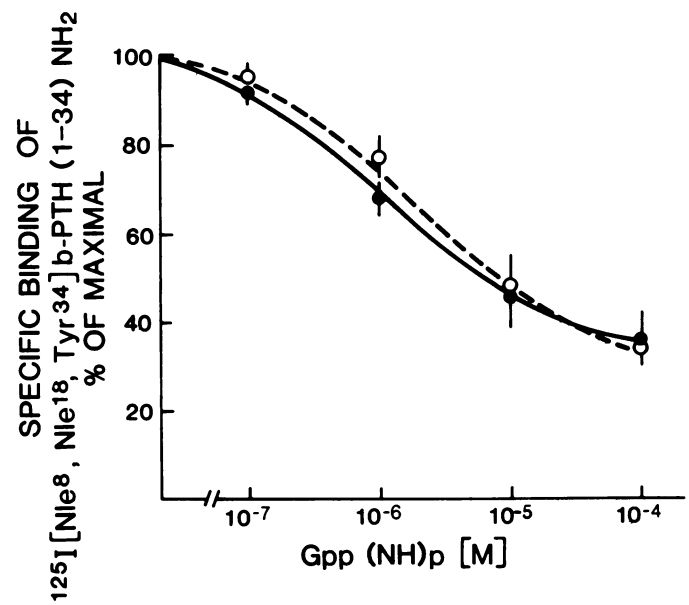

FIGURE 7 Equilibrium binding of ^{125}I[Nle^{8}, Nle^{18}, Tyr^{34}] bPTH (1-34) NH_2 in the presence of increasing concentrations of Gpp(NH)p in membranes from control (○) and hyperparathyroid (●) dogs.

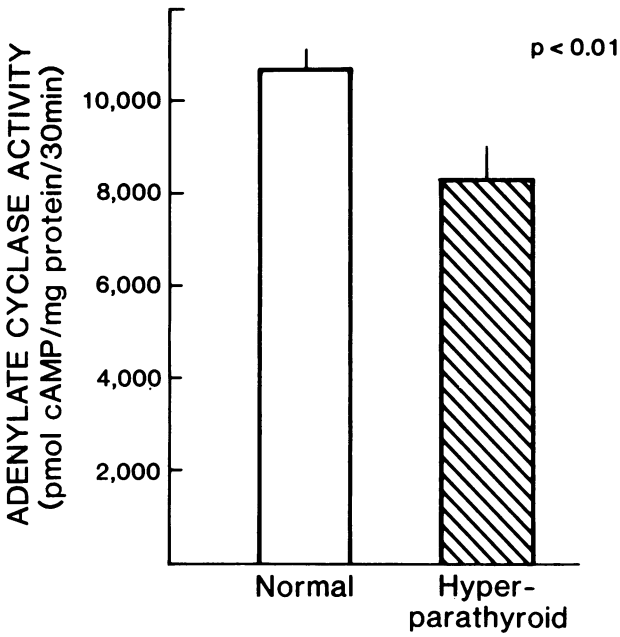

FIGURE 8 Adenylate cyclase activity in the presence of NaF 10 mM in basolateral cortical membranes from control and hyperparathyroid dogs. Data shown represent the mean±SEM for results obtained in seven dogs in each group. Basal enzyme activities were 352±42 pmol/mg protein per 30 min in control and 506±68 in hyperparathyroid dogs.

control and hyperparathyroid animals, the relative difference between the two groups was preserved. Moreover, GppNHp-stimulated enzyme activity in the absence of PTH was not different between hyperparathyroid and normal animals. These findings suggest that desensitization was not due to abnormal regulation of the system by guanine nucleotides. Furthermore, the effects of guanine nucleotides in decreasing the equilibrium binding of the radioligand ^{125}I[Nle^{8}, Nle^{18}, Tyr^{34}] bPTH (1–34) NH_2 was comparable in both groups of membranes.

Accordingly, further experiments were performed using NaF as an additional probe for the interaction of G/F and the catalytic subunit (C). Enzyme activity in the presence of NaF was significantly reduced in the membranes from the hyperparathyroid animals. The magnitude of this reduction was similar in degree to the reduction in PTH-stimulated enzyme activity in the presence or absence of guanine nucleotides. Thus, as assessed with NaF, the interaction of G/F with C appears to be impaired in the membranes from the hyperparathyroid animals. The explanation for the different results obtained with nonhormonal stimulators of adenylate cyclase (GppNHp and NaF) is not clear. It is possible that this difference is related to the markedly greater enzyme activity attained with NaF compared with Gpp(NH)p. Alternatively, since there is evidence that the effects of NaF and GppNHp on the regulatory protein have some differences, for example, heat stability, trypsin sensitivity, and other indirect criteria (27, 28) (hence the designation G/F), it is possible that the nature of the defective G/F to C interaction in the membranes from the hyperparathyroid animals is more closely related to the site (or nature) of the action of NaF than to Gpp(NH)p. Since there is increasing evidence for the existence of other poorly characterized factors (cytoskeletal or intrinsic to the membrane) that can play a role in the regulation of the interactions between the components of adenylate cyclase system (29–33), it is possible that such factors may play a role in the mechanisms of the altered receptor-cyclase coupling of the present studies.

The results of the present studies differ from previous investigations by our laboratory (18) and others (12, 13) on PTH desensitization in vitro or in vivo. Thus, we have shown that in the isolated perfused dog kidney, short-term constant exposure to syn bPTH (1–34) results in desensitization, which can be attributed to persistent receptor occupancy (18). Studies of short-term infusions of bovine parathyroid gland extract in the dog in vivo by Mahoney and Nissenson (12) have suggested that desensitization in this model is due to receptor loss. Similar results were obtained by Forte et al. (13) in calcium- or vitamin D-deprived chicks. Therefore, it is evident that different results may be obtained according to the experimental circumstances. The refractoriness to agonist-induced adenylate cyclase activation in many tissues with several receptor types in both intact cells and cell-free systems have also revealed that several mechanisms within the multicomponent adenylate cyclase system (11) may be invoked to explain this phenomenon.

It is important to emphasize that the present studies were performed in an experimental model with mild elevations of endogenous PTH similar to those seen in clinical parathyroid dysfunction. This situation may not be strictly analogous to results obtained during constant exposure of high levels of exogenous PTH (or PTH fragments) from a different species. For example, since there is evidence that PTH secretion in the dog is episodic (34–36), it is possible that such episodic exposure to PTH may influence the PTH receptor-adenylate cyclase system in a manner different from constant exposure to the hormone. Further studies will be required to evaluate this possibility.

In summary, the induction of dietary hyperparathyroidism in adult mongrel dogs results in a variable degree of desensitization of the PTH-responsive adenylate cyclase system of kidney cortex to further stimulation with syn bPTH (1–34) without any alteration

in PTH receptor binding. This defect, demonstrable by NaF activation, not corrected by guanyl nucleotides, appears to be due to a postreceptor event leading to abnormal receptor-cyclase coupling. The precise nature of the factor(s) other than guanyl nucleotides that may regulate receptor-adenylate cyclase interactions will require further investigation.

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