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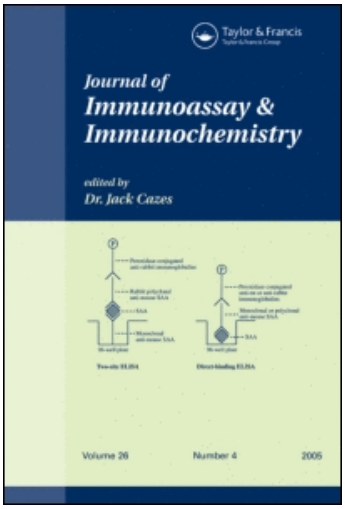

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Bettina Niepel[a]; Heinfried Radeke[a]; Michael J. Atkinson[a]; Harald Jueppner[a]; Rolf-Dieter Hesch[a]
[a] Abteilung Klinische Endokrinologie, Medizinische Hochschule, Hannover, West Germany

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A HOMOLOGOUS BIOLOGICAL PROBE FOR PARATHYROID HORMONE IN HUMAN SERUM

Bettina Niepel, Heinfried Radeke, Michael J.Atkinson,
Harald Jueppner and Rolf-Dieter Hesch
Abteilung Klinische Endokrinologie, Medizinische Hochschule,
D-3000 Hannover 61, West Germany.

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ABSTRACT

A method of measuring the biological activity of parathyroid hormone (PTH) in human serum that depends on the activation of its natural target enzyme, human renal cortical adenylate cyclase, is described. Optimal sensitivity ranging in different assays from 14 to 20 pg 1-34 hPTH/ml was achieved in the presence of the GTP-analogue GppNHp (10 µmol/L), 5 mmol/L $MgCl_2$ and 1.25 mmol/L EGTA. Basal and stimulated cAMP production was reproducible within assays (c.v. below 7%,S.E.M.,n=3) and between assays (c.v. 5 to 14%, S.E.M.,n=4). The recovery of 1-34 hPTH added to individual test sera averaged 94%.

The specificity of the method was established as follows: 1.) Other tested hormones, at 100 ng/ml, were ineffective; 2.) In the majority of peripheral sera from patients with hyperfunctioning parathyroid glands elevated bio-activity was detected; 3.) The circulating bio-activity fell rapidly after removal of parathyroid adenomata; 4.) Treatment with antisera for hPTH reduced the bio-activity; 5.) A PTH-antagonist inhibited the bio-activity.
(KEY WORDS: Parathyroid hormone, bioassay, adenylate cyclase)

INTRODUCTION

Parathyroid hormone (PTH) circulates as a family of peptides with different immunoreactivity and biological activity (bio-activity). Because this pattern of parathyroid peptides is

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incompletely understood there is a longstanding need for a direct measurement of bio-active PTH (bio-PTH) in human serum. Sufficient sensitivity for this purpose was first achieved by a cytochemical bioassay (1); however, this assay is difficult to perform and has limited sample capacity.

The classical in vitro bioassay using the PTH-sensitive adenylate cyclase from kidney cortex has until recently had a lower limit of detection of about 10 ng/ml. This lack of sensitivity has been overcome by the addition to the assay incubation mixture of the non-hydrolysable GTP-analogue 5'-guanylyl-imido-diphosphate (GppNHp) (2).

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The use of canine kidney plasma membranes made the assay heterologous with respect to the species origin of measured hormone and target enzyme. Species differences exist in the amino acid sequences of PTH from animals and humans, and there is evidence for differences in the PTH-receptor-cyclase systems of different species (3). We therefore prepared the PTH-sensitive adenylate cyclase from human kidney cortex, examined the effects of GTP (known to act as cofactor) and other nucleotides on the sensitivity of this enzyme system and adapted it to the measurement of bio-activity in serum. Development and characteristics of this assay, published in preliminary form (4), are described here.

MATERIALS AND METHODS

GTP, ATP, creatine phosphate, creatine kinase (from rabbit muscle; specific activity per mg lyophilisate at 37°C: 800 U) and

cAMP were bought from Boehringer, Mannheim, FRG. GDP, GppNHp and inosine triphosphate (ITP) were from Sigma, St.Louis, MA, USA. DASP anti-rabbit for the cAMP-radioimmunoassay was from Organon Technica, Holland. Human and bovine serum albumin were from Behringwerke, Marburg, FRG. Percoll^{R}(densitiy 1.1 g/ml) was from Pharmacia, Uppsala, Sweden.Partially purified 1-84 hPTH from adenoma tissue culture medium ($P_{2/7}$;ref.5) was a gift from Drs. G.Dorn and R.Montz, Hamburg, FRG. Synthetic 1-34 hPTH was from Paesel, Frankfurt, FRG. This peptide was used as a standard against which all serum measurements were performed. The total content of the vial (500 µg according to manufacturer) was dissolved in 500 µl 0.01 mol/L acetic acid and stored in 10µl-aliquots at -70°C. No potency estimation of this 1-34 hPTH was given by the manufacturer; calibrated against the ampouled hPTH-standard (code 79/500, kindly donated by Dr.J.Zanelli of NIBSC, London, United Kingdom) in our experimental system in the presence of 10^{-5} mol/L GppNHp (data not shown) the potency was about 15000 I.U./mg. The inhibitor (8,18-Nle,34-Tyr)3-34 bPTH-amide (NTA) was from Peninsula Lab., CA, USA. Human calcitonin was a gift from Dr.W.Rittel, Ciba-Geigy, Basle, Switzerland. Antiserum Goat 10, directed against the N-terminal region of PTH (6) was generously provided by Dr.C.Desplan and Professor M.S.Moukhtar, Paris. Ethylene glycol-bis(2-aminoethylether-N,N')-tetraacetic acid (EGTA) was from Serva, Heidelberg, FRG. All other reagents were from Merck, Darmstadt, FRG.

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Preparation of Particulate Enzyme

Human kidneys were obtained during surgery for malignant tumours. Cortex free of tumour was dissected off, rinsed in buffer (50 mmol/L Tris/HCl, pH 7.4, 1 mmol/L EDTA, 250 mmol/L sucrose) and stored for up to three months at -70°C. The preparation was carried out according to Mohr and Hesch (7) using pooled tissue. In brief, the homogenised material was subjected to differential centrifugation essentially as described (8) followed by isopycnic centrifugation on a self-generating Percoll^{R}-gradient. By this method we consistently obtained an enrichment of the plasma membrane marker Na/K-ATPase of 300 to 400% compared to the homogenate. The specific activity of the PTH-sensitive adenylate cyclase increased to 200%. The protein yield was 0.3% of kidney cortex fresh weight.

Aliquots of the membrane preparation were stored at -70°C where they remain stable for over three months. Freeze-drying of the membranes did not reduce their responsiveness to PTH. Immediately before use, the membrane preparation was thawed at room temperature, the membranes were harvested at 4000g x 1 min and resuspended in "cyclase buffer" (50 mmol/L Tris/HCl, pH 7.4).

Adenylate Cyclase Assay

The final reaction volume was 200 µl containing, in cyclase buffer, 3-isobutyl-1-methylxanthine (1 mmol/L), ATP (1.8 mmol/L), an ATP-regenerating system (creatine phosphate, 8 mmol/L, and 100 µg creatine kinase) and 25 µg membrane protein; $MgCl_2$ was

3 mmol/L. For cyclase assays of serum samples, $MgCl_2$ was increased to 5 mmol/L and 1.25 mmol/L EGTA was added (see below). NaF, nucleotides and $CaCl_2$ were diluted in cyclase buffer. All hormone dilutions were made in the presence of protein to reduce adsorptive loss: We used cyclase buffer with 2% human serum albumin or human serum from a healthy donor depleted of N-terminal immunoreactive PTH ("hyposerum") by affinity chromatography using antiserum Goat 10.

Serum was used because EDTA and heparin interfered in the assay. Loss of bio-activity in serum upon storage at -20°C and subsequent thawing was between 0 and 76% ($\bar{x}$=53%, n=7), loss of activity of the synthetic standard diluted in "hyposerum" averaged 39% (n=6). Therefore, all serum samples were tested against fresh dilutions of the standard within three hours after blood centrifugation. For assessment of recovery of PTH-bioactivity in individual sera, a mixture of equal amounts of the patient's serum and of a PTH standard (100 pg/ml) was assayed. The actual bioactivity of this mixture was read from the standard curve and compared with the ideal value as calculated from bioactivities of the standard and the serum assayed seperately. The results were expressed as % of this calculated value.

The incubation was started by the addition of membranes to the otherwise complete incubation mixtures and transferral of the tubes from 4°C to 30°C and was stopped after 30 min by protein denaturation at 85°C (5 min). A blank value was obtained by boiling the incubation mixture without prior incubation at 30°C.

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1 ml 0.1 mol/L Na-acetate, pH 6.2, was mixed with the content of each tube. cAMP was measured in an aliquot of the supernatant (4000g x 5 min) after acetylation in a specific radioimmunoassay developed by Scholz et al.(manuscr. in prep.) according to the method of Steiner et al.(9). The recovery for cAMP from the incubation mixture was 85 to 95%. The interassay c.v. of the RIA did not exceed 5% (S.E.M.,n=13) for any point on the standard curve. Other nucleotides did not cross-react in the concentrations in which they occurred in the enzyme assays. cAMP production was proportional to added membrane protein from 4 to 50 µg per tube. The production rate was linear with time between the 5th and 30th minute of incubation. Adenylate cyclase activity was expressed as pmol cAMP produced per mg membrane protein per min incubation time.

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Chromatography of Nucleotides

Nucleotides as purchased or after incubation were analysed by high performance liquid chromatography (HPLC) on Nucleosil 10 SB with a Model 440 UV detector at 254 nm (equipment from Waters Assoc.). The buffer was 50 mmol/L KH_2PO_4, 200 mmol/L KCl, pH 6.9. Complete separation of the mono-, di- and triphosphates of adenosine and guanosine was achieved. Relative amounts of nucleotides were estimated by computing peak areas.

Other Methods

Mid-regional immunoreactive PTH (44-68 PTH) was determined as described (10). Protein was measured using bovine serum albumin as a standard (11).

RESULTS

Nucleotide Effects

In our first set of experiments we examined the effects of nucleotides on the enzyme system under study. Dose-response relationships in basal, PTH-stimulated and NaF-stimulated systems for the naturally occurring cofactor GTP, the structurally related ITP and the non-hydrolysable analogue GppNHp are shown in Fig.1. Dose-response curves for the hPTH preparation $P_{2/7}$ with and without nucleotides are shown in Fig.2 and the effects of competing nucleotides are shown in Fig.3. The nucleotides did not have any significant effect on the NaF dependent cAMP production but they stimulated both the basal and PTH-dependent cAMP production (Fig.1 a - c). The most effective nucleotide was GppNHp. At 10^{-5} mol/L, when incubated with saturating hormone concentrations, it yielded the highest cAMP production rate seen in this system (Fig.2) greatly exceeding that produced by saturating (10 mmol/L) NaF (Fig.1 c). In addition to potentiating both the basal and PTH-stimulated cyclase activity, the nucleotides decreased the minimal effective PTH-dose in this system (Fig.2). At saturating concentrations, GppNHp (10^{-5}mol/L) and GTP (10^{-6}mol/L) decreased the minimal effective PTH level to about 1/50 and 1/2, respectively, of that observed in the absence of exogenous guanyl nucleotides. As the maximal effect of ITP barely exceeded the GTP-effect, this nucleotide was not tested at different PTH-concentrations. Additive stimulation was found with combinations of GTP and ITP at subsaturating concentrations, and at saturating

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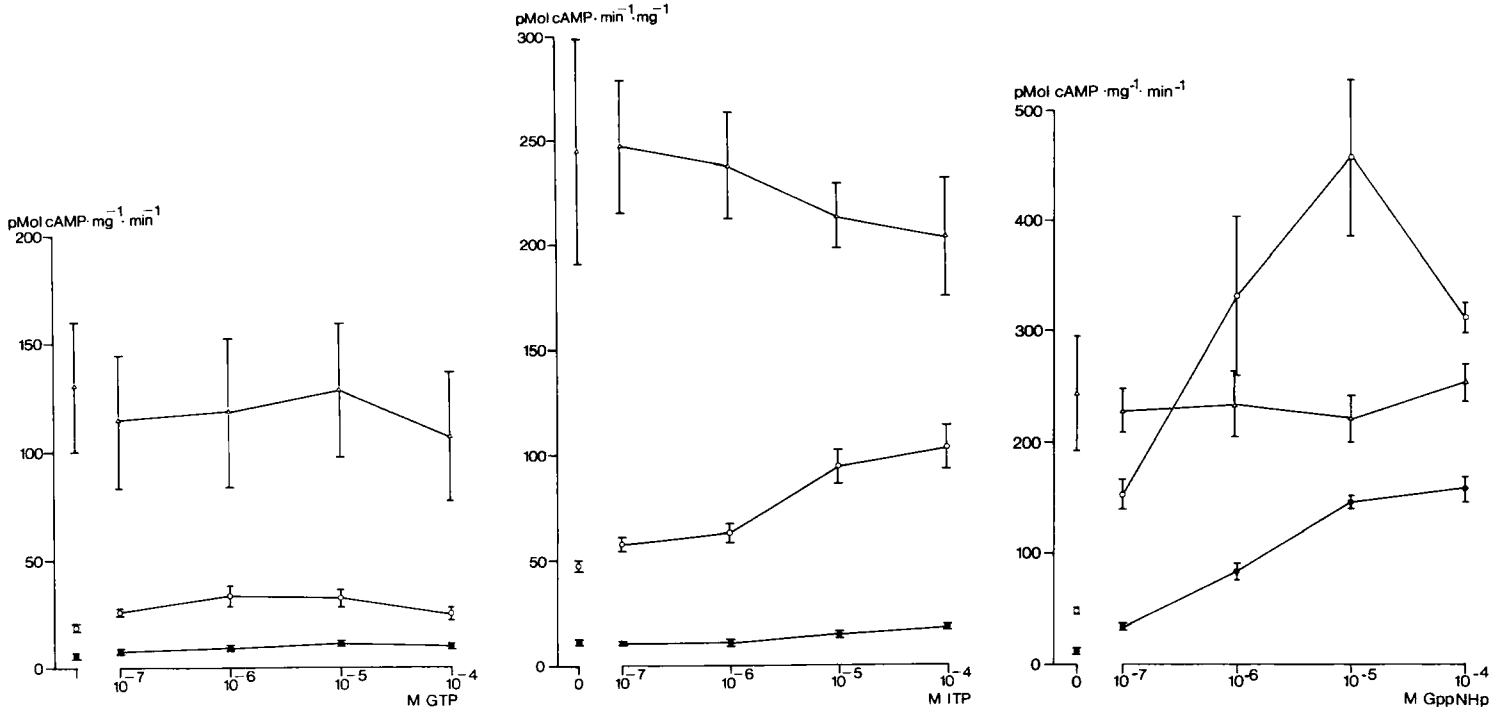

FIGURE 1 Effects of nucleotides on basal (closed circles), PTH-stimulated (10 µl $P_{2/7}$/ml, open circles) or NaF-stimulated (10 mmol/L, triangles) cAMP production.
a) Effect of GTP b) Effect of ITP c) Effect of GppNHp
$\bar{x} \pm$ S.E.M. (n=3 to 6 separate experiments)

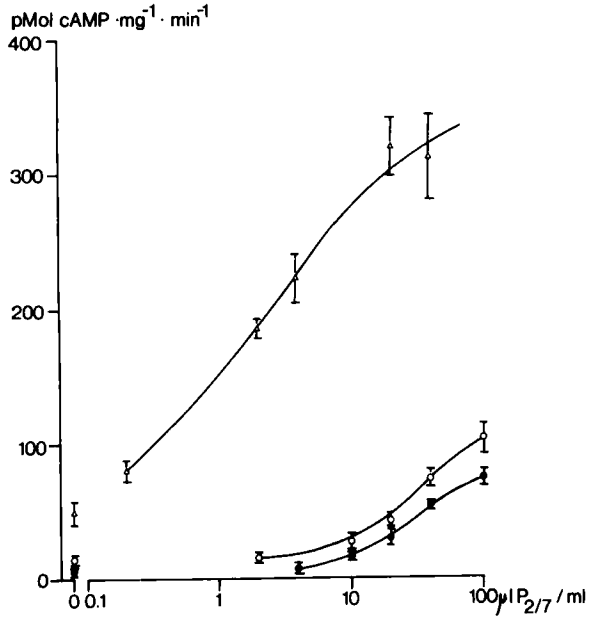

FIGURE 2 PTH-activation curves of cAMP production in the absence of exogenous nucleotides (closed circles) or with GTP (1 µmol/L, open circles) and with GppNHp (10 µmol/L, triangles). Effects of 1o µmol/L GTP were not considerably higher (not shown) than with 1 µmol/L GTP. $\bar{x} \pm$ S.E.M.(n=3)

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concentrations of either nucleotide the other was ineffective (not shown). In contrast, ITP and GTP inhibited the effect of saturating GppNHp (Fig.3).

In all experiments GDP behaved like GTP (not shown). To clarify this observation we performed HPLC analysis of the nucleotides before and after incubation. The purchased GTP preparation contained 75 % GTP, 22 % GDP and 3 % GMP (assayed several weeks after purchase). The GDP preparation was 95 % pure, the impurities coeluting with GTP and GMP. After 2 min incubation in the adenylate cyclase assay in the presence of the ATP

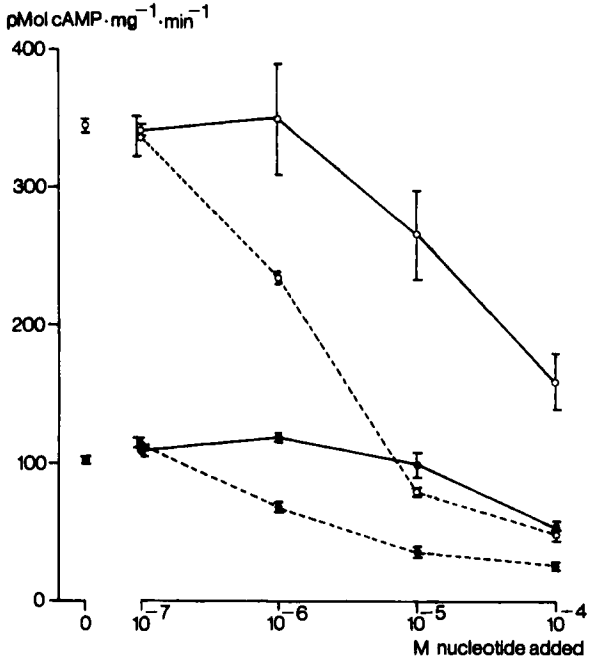

FIGURE 3 Effects of competing nucleotides on basal (closed circles) or PTH-stimulated (5 µl $P_{2/7}$/ml,open circles), GppNHp-augmented (10 µmol/L) cAMP production. Solid line: Inhibition by ITP. Broken line: Inhibition by GTP. $\bar{x} \pm$ S.E.M. (n=3)

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regenerating system, only GTP was detectable, no matter whether GDP or GTP (10^{-4}mol/L) had been added. The same short incubation with membranes alone also converted most of the added GDP to GTP.

In addition, HPLC analysis of the ATP preparation revealed a purity of more than 98 %. Upon incubation, ADP and AMP only became detectable when the ATP regenerating system was omitted and amounted together to 14 %.

Adaptation of the Assay to the Presence of Serum

We included 10^{-5}mol/L GppNHp in the incubation mixture in all subsequent experiments (except in Fig.4). The addition of 25 %

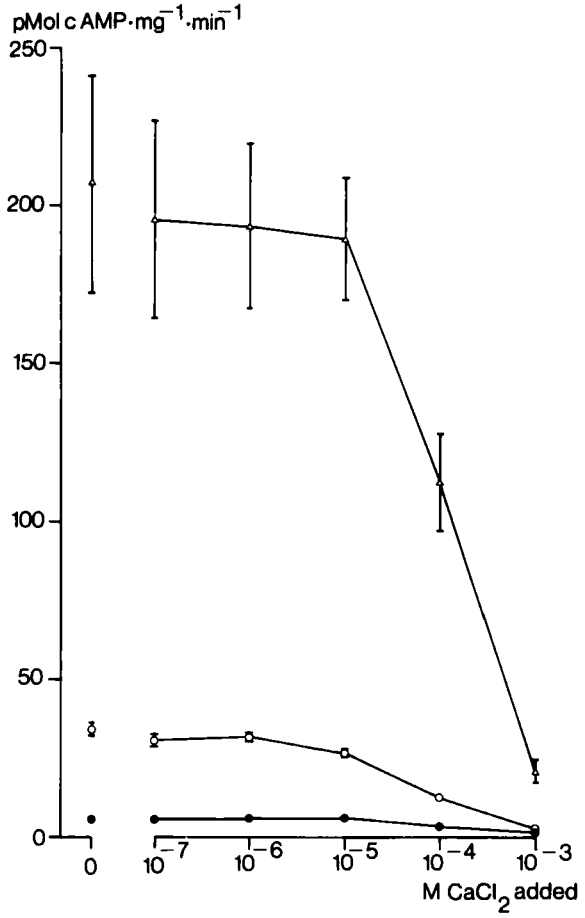

FIGURE 4 Effects of added $CaCl_2$ on basal (closed circles), PTH-stimulated (10 µl $P_{2/7}$/ml, open circles) or NaF-stimulated (10 mmol/L, triangles) cAMP production in membranes from human renal cortex. $\bar{x} \pm$ S.E.M.(n=3 to 6 separate experiments)

human serum to the assay decreased the cAMP production to nearly zero (Table 1,first line). We suspected the Ca-ions from the serum to be responsible, at least in part, for this inhibition, because in a preliminary experiment the inhibitory effect of Ca on the enzyme under study had been shown (Fig.4). We therefore attempted

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to selectively reduce the Ca-concentration in the assay mixture by adding the chelator EGTA. As discussed in (12) the chelator binds Mg^{2+} with low affinity. The optimal reversal of the inhibition by serum was obtained with 1.25 mmol/L EGTA and a simultaneous increase of added Mg^{2+} from 3 to 5 mmol/L (Table 1, fourth line).

Fig.5 depicts the dose-response curves both in the absence and presence of serum and shows that, in spite of similar basal cAMP production and sensitivity under both assay conditions, the maximal enzyme activity was depressed by about 45% when serum conditions were chosen.

The intraassay c.v. of the cAMP production rate was from 1.9 to 6.1% S.E.M.(n=3), the interassay c.v. ranged from 5 to 14% over the entire standard curve (S.E.M.,four consecutive assays). Table 2 shows the results from an assay with a lower limit of detection (response 2 S.D. above control) of 14 pg/ml, the optimal sensitivity which could be achieved in the presence of serum. Activation curves with dilutions of sera from two patients with hyperparathyroidism were superimposable on the standard curve (in "hyposerum")(Fig.6).

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Interference of Other Hormones

In the following experiments, the incubation mixture contained, in addition to the substances listed in the Methods section, 1.25 mmol/L EGTA, 5 mmol/L $MgCl_2$, 10^{-5} mol/L GppNHp as well as 25% human serum.

TABLE 1

Evaluation of Different Incubation Media for the Measurement of Bio-PTH in Serum

Additions				cAMP production (pMol $mg^{-1} min^{-1}$)		
$MgCl_2$ (mM)	EGTA (mM)	GppNHp (µM)	$CaCl_2$ (mM)	basal	100pg/ml 1-34hPTH	Stimulation (%)
3	0	10	0	~0	~0	~0
3	1.25	10	0	21	38	131
3	2.5	10	0	19	27	142
5	1.25	10	0	51	106	203
10	2.5	10	0	113	180	159
3	2.5	10	0.25	11	21	191
10	2.5	100	0	120	204	170
For comparison: no serum present						
3	0	10	0	56	130	232

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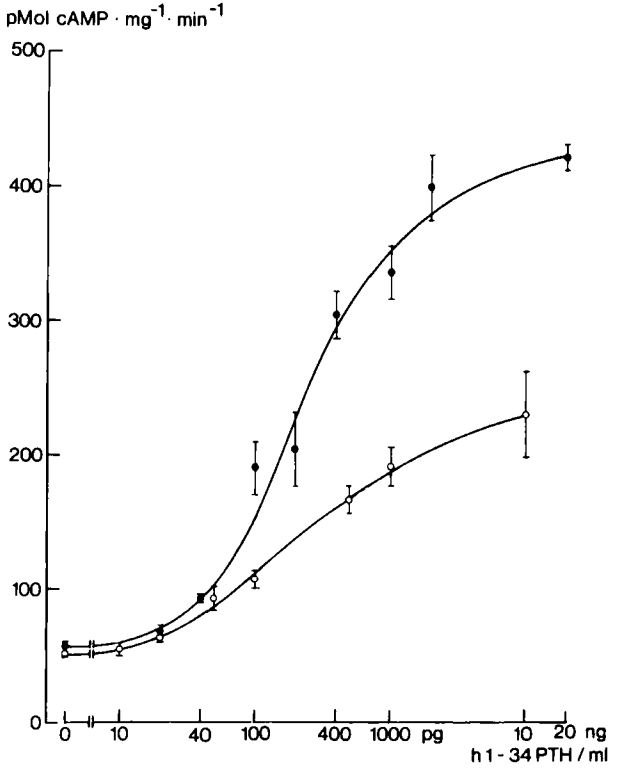

FIGURE 5 Stimulation of human renal adenylate cyclase by 1-34 hPTH in the presence (open circles; 1.25 mmol/L EGTA, 5 mmol /L $MgCl_2$) or absence (closed circles; 3 mmol/L $MgCl_2$) of 25% human serum. 1o µmol/L GppNHp was included in the assay. $\bar{x} \pm$ S.E.M. (n=5 separate experiments)

TABLE 2

Stimulation of Human Renal Adenylate Cyclase by Low Doses of 1-34 hPTH in the Presence of 25% Serum and 1.25 mmol/L EGTA, 5 mmol/L $MgCl_2$ and 10 µmol/L GppNHp.

1-34 hPTH (pg/ml)	cAMP production (pmol mg^{-1} min^{-1}) $\bar{x} \pm$ S.D., n=3	difference to control (Student's t-test)
0	63.9 ± 2.2	-
10	67.5 ± 2.0	n.s.
20	70.0 ± 2.2	$p < 0.05$
50	75.5 ± 3.9	$p < 0.02$

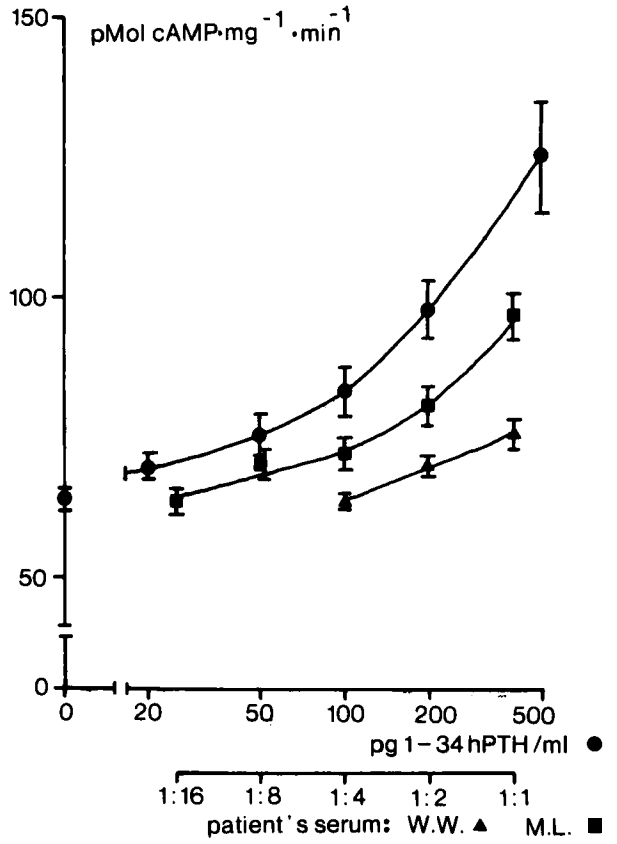

FIGURE 6 Activation curves produced by 1-34 hPTH diluted in serum depleted of N-regional immunoreactive PTH and by dilutions of sera from two patients. $\bar{x} \pm$ S.E.M. (n=3)

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TABLE 3

Effects of Various Hormones Incubated Alone or Together With 1-34 hPTH on Human Renal Adenylate Cyclase

Addition(s)	Concentration (µg/ml)	cAMP production (pMol mg^{-1} min^{-1})
none		47.5
physiol.saline		68.5
2% ascorbic acid		76.7
human calcitonin	0.1	46.8
	1	54.3
	10	57.3
Suprarenin® =	1	46.3
epinephrine	10	56.8
	100	71.2
Minirin® =	0.1	63.7
8-Arg-vasopressin	1	73.9
	10	80.8
	100	119
Glucagon-Novo®	0.1	48.6
	1	95.8
	10	91.7
	100	95.8
Synacthen® =	2.5	56.2
ACTH 1-39	25	59.7
	250	64.6
1-34 hPTH (200 pg/ml)		125
dito,+ calcitonin	1	134
dito,+ Suprarenin®	1	137
dito,+ Minirin®	1	148
dito,+ Glucagon-Novo®	1	140
dito,+ Synacthen®	2.5	157

We investigated the effects of other hormones in this system. Table 3 shows that none of the tested hormones had any considerable effect on the enzyme at 100 ng/ml. At higher concentrations, glucagon and 8-Arg-vasopressin stimulated the cAMP production to values above those obtained with buffer or vehicle, but maximal stimulation with these hormones was far below that elicited by maximal PTH-doses (Fig.5).

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Measurement of Bio-active PTH in Human Serum

Table 4 shows arbitrary serum levels of bio-PTH calibrated against the biological activity of the synthetic 1-34 hPTH standard. Healthy persons appeared to have levels of bio-PTH near the detection limit of the assay (here: 14 pg/ml) and not higher than 20 pg/ml. An a-parathyroid patient had undetectable bio-PTH. Parathyroid carcinoma and adenomata were always reflected by elevated bio-PTH levels in the serum of patients with these abnormalities. The assay did, however, not detect any elevation of bio-PTH in two out of three patients with hyperplastic parathyroid glands. Measurable bio-PTH was not elevated in 4 out of 10 patients with renal insufficiency who had clearly elevated mid-regional immunoreactive PTH (not shown). The fast disappearance of bio-PTH after removal of an adenoma is depicted in Fig.7.

The recovery of exogenous PTH, added to the individual serum samples, varied from 43 to 174% with a mean of 94% close to the ideal value (100%) (Table 4).

Reduction of Bio-activity in Human Serum

Biological activity detected in human serum by this assay, i.e. presumptive bio-PTH, was inhibited when the serum was preincubated with antisera for hPTH (Table 5 a). The N-terminal specific antiserum Goat 10, preincubated for 1/2 h at 4°C with the serum sample in a dilution of 1: 10 000, inhibited the bioactivity in two sera by about 70%. Another antiserum, Giselle

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TABLE 4

Endogenous Biologically Effective PTH in Peripheral Sera from Healthy Adults and Patients

Total n = 22 Range of bio-PTH: < 14 - 295 pg/ml
Mean recovery: 94 % Range of recovery: 43 - 174 %

Group I Healthy volunteers

No.	bio-PTH (pg/ml)	recovery (%)
1	< 14	146
2	< 14	80
3	< 14	55
4	< 14	51
5	15	96
6	16	74
7	17	88
8	17	43
9	19	72
10	19	72
11	19	140

Normal range of iPTH: 100 - 300 pg/ml

n = 11 range: < 14 - 19 mean: 84
range: 43 - 146

Group II Patient without parathyroid glands

bio-PTH: < 14 pg/ml recovery: 100 % iPTH: 55 pg/ml

Group III Patients prior to parathyroid surgery

No.	creatinine clearance (ml/min)	iPTH (pg/ml)	bio-PTH (pg/ml)	recovery (%)	parathyroid histology
1	84	500	< 14	n.d.	hyperplasia
2	42	430	15	174	hyperplasia
3	80	665	29	100	hyperplasia
4	55	470	77	113	adenoma
5	85	1300	85	n.d.	adenoma
6	65	>2500	95	100	adenoma
7	14	>2500	125	n.d.	adenoma
8	< 3	>2500	295	87	adenoma
9	48	499	21	95	carcinoma
10	73	1590	26	98	carcinoma

n = 10 range: < 14 - 295 range: 87 - 174 mean: 109

n.d.= not determined iPTH: values expressed as equivalents of the synthetic standard (44-68 hPTH) used.

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TABLE 5

Effects of Preincubation With Antisera to hPTH (a), and of Coincubation With (8,18-Nle,34-Tyr)3-34 bPTH-amide (NTA) (b), on the Stimulation of Human Renal Adenylate Cyclase by Human Serum or Synthetic 1-34 hPTH

a)

	Bio-activity (pg/ml)	Inhibition (%)
Patient I	195	-
Patient I, serum preincubated with antiserum Goat 10 (1:10 000)	53	73
Patient II	88	-
Patient II, serum preincubated with antiserum Goat 10 (1:10 000)	26	68
Patient II, serum preincubated with antiserum Giselle III (1:1000)	< 20	~ 100

b)

Agonist	NTA (µg/ml)	Bio-activity (pg/ml)	Molar ratio (Agonist:NTA)	Inhibition (%)
Serum Patient III	-	45	-	-
- " -	0.001	45	1:20	0
- " -	0.01	41	1:200	9
- " -	0.1	35	1:2000	22
- " -	1	< 20	1:20 000	~ 100
Serum Patient IV	-	170	-	-
- " -	1	80	1:5000	53
250 pg 1-34 hPTH	5	42	1:20 000	83
500 /ml	1	100	1:2000	80
-	1	< 20	-	-
-	10	66	-	-

III, shown to crossreact to some extent with 1-34 hPTH but having high affinity towards the 44-68 peptide (12), added at a 1 : 1 000 dilution, inhibited by about 100%.

Activation of the cyclase by 1-34 hPTH or serum was also inhibited, in a dose-dependent manner, by NTA (Table 5 b).

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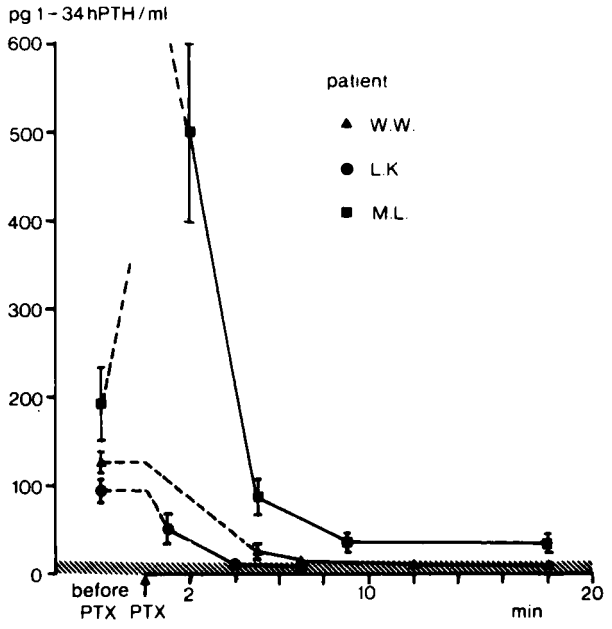

FIGURE 7 Serum bio-PTH levels before and after parathyroid adenoma removal (PTX). Blood was drawn from a central catheter (W.W. and M.L.) or from the forearm (L.K.). $\bar{x} \pm$ S.E.M.(n=3). Shaded area: values not significantly different from control.

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DISCUSSION

The most important observation, from a practical point of view, was that the sensitivity of our human adenylate cyclase preparation for PTH was efficiently increased towards presumptive serum levels of bio-active PTH (14) by GppNHp. It was thereby established that in this respect the human enzyme behaved similarly to the analogous enzymes from chick (15,16) and dog (2,17) and adenylate cyclases responsive to several other hormones (18).

The modest ability of GTP to stimulate the adenylate cyclase and to sensitise it towards the hormonal stimulus, and the

competitive inhibition of the GppNHp effect by GTP shown in our system, have also been described for PTH-responsive adenylate cyclases from other species (15,19). This is consistent with the hypothesis that the natural nucleotides experience dephosphorylation at their site of action with loss of activation of the cyclase. GppNHp, acting at the same site as GTP and ITP, is not hydrolysed by adenylate cyclase and is thought to lock the catalytic subunit of the adenylate cyclase into a persistently activated state (20). The reason for the greater maximal effect of ITP as compared to that of GTP, for which higher nucleotide concentrations are required, already described by one group (21), might also be connected with a weaker binding and a slower dephosphorylation of the inosine nucleotide.

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Recent studies (22) have attributed a stimulatory role to GDP in some adenylate cyclase preparations. In our system, rapid conversion of the added GDP to GTP, as revealed by HPLC, explains the equal effectiveness of GDP and GTP added to the enzyme preparation. It could also be shown by HPLC that the ATP regenerating system was able to maintain both the adenosine and guanosine nucleoside in the triphosphorylated state, which is a necessary prerequisite for a constant cAMP production rate throughout the incubation period.

The observed inhibition by Ca-ions in the range of 10^{-4} to 10^{-3} mol/l is a feature common to many adenylate cyclases including the PTH-responsive enzymes from rat (23,24) and dog (25). In the micromolar range Ca^{2+} is known to stimulate some adenylate

cyclase preparations (reviewed in 26), but we could not demonstrate this effect. As already pointed out in the Results section, much of the inhibitory effect of serum was probably due to Ca-ions as it could be substantially reduced by the inclusion of the Ca-chelator EGTA.

The modified assay showed a dose response relationship from about 20 to 2000 pg/ml for 1-34 hPTH, but 5000fold, i.e. supraphysiological concentrations of other hormones were necessary to show an effect. This degree of specificity is comparable to that reported for the canine system (2), although in the latter no stimulation by vasopressin was found. This may be due to a species difference in the distribution of hormone receptors along the kidney tubule (27). The cortical parts of the human kidney tubule have been described to contain mainly PTH-receptors and few for calcitonin and vasopressin (28).

As was to be expected of a bio-assay for PTH it detected elevated circulating bio-activity in the majority of patients with surgically proven hyperparathyroidism as compared to normals who had concentrations from undetectable to about 20 pg/ml; yet it would appear that the sensitivity (and specificity; see below) of the assay limit the accurate determination of bio-PTH in normals and the complete separation of the normal from the hyperparathyroid group. However, compared to the study using canine adenylate cyclase which only included sera from the vein draining the adenomatous parathyroid gland(s), this report is an advance as it shows elevation of bio-active PTH in peripheral serum samples from hyperparathyroid patients.

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In a group of patients with renal failure Nissenson et al.(2) did not observe any correlation between bio-PTH and C-regional immunoreactive PTH. This finding was confirmed using our bio-assay and a RIA with mid-regional specificity which detects elevated immunoreactivity in the plasma of all patients with impaired kidney function (10).

The bio-active PTH-peptides were shown to disappear rapidly from the circulation after the removal of the hyperfunctioning glands. The initial rise of bio-PTH in the serum of one patient probably reflects PTH release from the adenoma upon mechanical manipulation before its venous drainage was interrupted.

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Inhibition of bio-activity with antisera to PTH and the PTH antagonist NTA was demonstrated further indicating that the observed bio-activity was due to PTH. The high molar excess of NTA required to inhibit the stimulation arising from serum or the synthetic standard - whereas in the original report on this inhibitor (29) complete inhibition was observed with a molar ratio of agonist over antagonist of 1:100 - may possibly be due to the presence of GppNHp as suggested in early studies (30).

While in previous publications (1,2) no data on the recovery of added exogenous PTH to individual serum samples were given, the present work attempted to assess this question showing a highly variable recovery rate under the chosen assay conditions. Since unfractionated serum was assayed, the method is expected to reflect the combined action of all serum constituents, stimulatory or inhibitory, on the adenylate cyclase in vitro. Inasmuch as the

same integration of stimulatory and inhibitory effectors takes place at the renal receptor in vivo, it is a desirable characteristic of the assay, because it measures the effective concentration of PTH-bio-activity rather than the absolute concentration of a single peptide the action of which could be modulated by other factors present in a particular serum (31,32, 33). In contrast, serum factors may occur which influence the cAMP production only in vitro. If these are not present to the same degree in both the test serum and in the serum used to dilute the standards an accurate quantification is not possible.

Another concern relates to the nature of the target enzyme in this assay. Recent experimental data suggest that the renal adenylate cyclase-linked receptor differs from that in bone (34,35). There is also growing evidence that, at least in bone, PTH may exert clinically relevant effects which are not mediated via cAMP and which differ with respect to their requirement of the amino-acid-sequence of the PTH peptides which are effective in this respect (36). Therefore a good correlation may be expected between bio-PTH levels estimated with this assay and clinical parameters associated with the phosphaturic and calcium-retaining actions of PTH assumed to operate through renal cortical adenylate cyclase (37,38), whereas a correlation with the metabolic state of the bones may not occur.

In conclusion, we have developed a bioassay suitable for the specific determination of biologically effective PTH in serum. It is less sensitive but more practicable than the cytochemical

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bioassay and may be of great help in the investigation of PTH status in man.

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