Effect of Synthetic 1-34 Fragment of Human Parathyroid Hormone on Plasma Adenosine 3', 5'-Monophosphate (cAMP) Concentrations and the Diagnostic Criteria Based on the Plasma cAMP Response in Ellsworth-Howard Test*

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Abstract

The effect of synthetic 1-34 fragment of human parathyroid hormone (hPTH(1-34)) on plasma adenosine 3', 5'-monophosphate (cAMP) in human subjects and the diagnostic criteria for the plasma cAMP response in an Ellsworth-Howard test were studied. 20 or 30 μ g hPTH(1-34) and 200 USP Parathormone (Eli Lilly & Co.), infused intravenously over 5 min, produced very similar patterns of response in plasma cAMP, peak values being observed within 5 or 10 min after the end of the infusion. The maximum levels of plasma cAMP were over 111.5 pmol/ml in all of the normal subjects (n=5) and patients with idiopathic hypoparathyroidism (n=22), including those of children, but the plasma cAMP did not rise above 65.0 pmol/ml in pseudo-hypoparathyroidism (n=7). There existed a significant correlation between the maximum plasma cAMP concentrations and increases in urinary cAMP excretion after infusions of both hPTH(1-34) and Parathormone. These results suggest that hPTH(1-34) has effects essentially identical to those of native PTH on plasma cAMP. We would like to propose a new diagnostic criterion in the Ellsworth-Howard test: a peak value of plasma cAMP over 100 pmol/ml after 30 μ g hPTH(1-34) infusion is regarded as a normal response.

The Ellsworth-Howard test (Ellsworth and Howard, 1934) is essential for the diagnosis of pseudohypoparathyroidism. Bovine parathyroid extract (Parathormone; Eli Lilly & Co., Indianapolis, IN), once used also in Japan, is now not available, so this test has come to be performed with synthetic 1-34 fragment of human parathyroid

hormone (hPTH(1-34)). Neer *et al.* (1977) have already demonstrated that hPTH(1-34) had sufficient effects and could be used for the Ellsworth-Howard test.

Although this test has been based on the responsiveness of phosphate (Ellsworth and Howard, 1934; Bronsky *et al.*, 1958) and adenosine 3', 5'-monophosphate (cAMP) in urine (Chase *et al.*, 1969a), it has been shown that plasma cAMP is also useful as a response index in this test (Tomlinson *et al.*, 1976a). We had also previously described similar data for plasma cAMP fol-

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lowing infusion of Parathormone (Sohn *et al.*, 1980). Tomlinson *et al.* (1976b) studied the effect of hPTH(1-34) on plasma cAMP concentrations, but the diagnostic criteria based on the plasma cAMP response in Ellsworth-Howard test have not yet been established.

We have now studied responsiveness of plasma cAMP to hPTH(1-34) in normal subjects, patients with idiopathic hypoparathyroidism and patients with pseudohypoparathyroidism. On the basis of these results, we should like to propose a new diagnostic criterion in Ellsworth-Howard test.

Materials and Methods

Testing was carried out with the informed consent of all patients and control subjects. Five control subjects, sixteen idiopathic hypoparathyroid patients, and five pseudohypoparathyroid type I patients were studied. The main clinical and laboratory data on these hypoparathyroid patients are summarized in Table 1. No patients had serum levels of magnesium lower than 1.9 mg/dl and GFR less than 41 ml/min. The ages of control subjects ranged from 19 to 21 years. In addition, the previous data about the effect of 200 USP of Parathormone on plasma cAMP in seven idiopathic hypoparathyroid patients (3 males, 4 females, aged from 27–70 years) and four pseudohypoparathyroid type I patients (2 males, 2 females, aged from 23–38 years) were also evaluated. Pseudohypoparathyroidism type I was defined by chronic hypocalcemia, hyperphosphatemia, and elevated levels of immunoreactive PTH, plus the lack of both urinary cAMP and phosphate responses to injected PTH. Three of seven pseudohypoparathyroid type I patients had the characteristic Albright's osteodystrophy.

The Ellsworth-Howard test was performed with hPTH(1-34). The doses were 20 μ g in three idiopathic hypoparathyroid and two pseudohypoparathyroid patients, and 30 μ g in the remainder, including control subjects. The changes in plasma cAMP in response to hPTH(1-34) administration were examined. In only three patients, one with idiopathic hypoparathyroidism and two with pseudohypoparathyroidism, the

effects of these two hormones were compared. The time interval between two tests was over three months in each case.

hPTH(1-34) was synthesized by the liquid-phase techniques and had an assayed potency of 3,300 International Units/mg (*in vitro* rat renal cortical adenylate cyclase assay using bovine PTH (67/342, WHO) as a standard substance, Toyo Jyozo Co., Japan) (Igarashi *et al.*, 1982).

The Ellsworth-Howard test was performed according to the protocol as described previously (Aida *et al.*, 1975). The hPTH(1-34) was administered at 1600 h in cases 1, 3 and 8, and at 1300 h in the remainder. Two child patients were not given aluminum hydroxide prior to the test. hPTH(1-34), dissolved in 5 ml of normal saline, was injected via an antecubital vein over 5 min similar to Parathormone (2 ml) and blood samples were obtained via an indwelling canula in the contralateral antecubital vein immediately before, and 0, 5, 10, 20, 30, 60 and 120 min after the end of the infusion. For the measurement of plasma cAMP, whole blood was immediately mixed with a 1–2 % volume of 0.5 M EDTA-4Na (pH 7.4) in a cooled tube, and centrifuged at 3,000 rpm, at 4°C for 10 min and the plasma was stored at –20°C.

Plasma cAMP was measured by RIA with a YAMASA RIA kit (Yamasa Shoyu Co., Japan) according to the method reported previously (Honma *et al.*, 1977). In this method, the plasma is directly assayed for cAMP by succinylation. When the plasma contained high levels of cAMP, it was diluted after succinylation, so the amount of the succinic reagent was kept constant throughout the RIA. The intra- and interassay coefficients of variation were 12.8 % and 9.2 %, respectively. With this method, the mean normal levels of plasma cAMP (0800–0900 h) were 13.6 ± 3.62 pmol/ml [mean $\pm$ SD; range, 6.4–20.8 pmol/ml; n=43].

Serum PTH was measured by RIA with an antibody recognizing the C-terminal of PTH (Eiken ICL, Japan). The minimum detectable level was 0.1 ng/ml, and the upper limit of the normal range was 0.4 ng/ml. Calcium (Ca) was measured by the EGTA titration method employing a Corning autoanalyzer; phosphate (P) by the Fiske and Subbarow method (Fiske and Subbarow, 1925); and creatinine by the Brod and Sirota method (Brod and Sirota, 1948), respectively.

Table 1. Clinical and laboratory data

Subject No.	Age, sex (yr)	Body weight (kg)	Serum Ca (mg/dl)	Serum P (mg/dl)	Serum iPTH (ng/ml)	NcAMP (n mol/dl GF)	Responses to hPTH(1-34) ^a			Treatment
							Dose (μg)	Urine cAMP (μmol/h)	Urine P (mg/2h)	
Idiopathic hypoparathyroidism										
1	50, M	70.0	8.3	4.2	ND	0.66	20	7.54	107.14	97.6 1, 25-(OH) ₂ D ₃ 2.0 μg
2	39, M	65.0	6.3	4.2	ND	0.76	20	8.15	137.75	48.5 (—)
			7.9	3.9	ND	0.42	20	7.51	138.72	76.7 1, 25-(OH) ₂ D ₃ 2.0 μg
3	9, F	25.0	6.7	5.5	ND		19	5.96	138.85	56.7 (—)
4	2mo, M	5.5	5.8	8.1	ND		8.1	0.91		3.6 (—)
5	29, M	50.0	5.5	6.1	ND	0.14	30	7.20	151.97	73.4 (—)
6	49, M	57.0	4.7	5.1	0.15	0.14	30	8.86	108.45	86.8 (—)
7	31, M	53.5	5.8	5.4	0.25	0.74	30	4.33	107.79	54.7 (—)
8	23, M	63.0	7.4	3.3	ND	0.49	30	8.73	115.11	20.6 (—)
9	39, M	45.0	6.9	4.9	0.11	0.64	30	9.06	174.62	65.0 (—)
10 ^b	30, F	44.5	6.9	3.7	0.18	3.16	30	14.01	319.01	35.2 (—)
11	33, M	63.0	9.0	5.5	ND	0.29	30	14.38	269.20	106.4 Vitamin D ₂ 10 mg
12	42, M	66.0	9.3	3.5	0.18	0.30	30	7.86	115.81	1α-OHD ₃ 4.0 μg
13	28, M	65.0	8.5	3.0	ND	0.22	30	8.79	123.43	1α-OHD ₃ 2.0 μg
14	28, M	44.0	8.4	3.5	ND	0.08	30	9.00	171.61	1α-OHD ₃ 2.0 μg
15	34, M	61.0	8.8	3.4	ND	0.01	30	11.05	147.54	63.2 1α-OHD ₃ 4.0 μg
16	66, F	46.0	10.2	4.4	ND	0.47	30	5.15	270.78	53.7 1, 25-(OH) ₂ D ₃ 1.0 μg
Pseudohypoparathyroidism										
17 ^c	40, F	54.0	7.7	4.9	0.45	1.04	20	0.98	18.38	12.6 (—)
			9.3	4.4	ND	1.04	20	0.80	15.63	18.1 1, 25-(OH) ₂ D ₃ 1.5 μg
18	26, M	45.5	8.6	4.3	0.30	0.06	30	0.31	5.76	25.4 (—)
			9.1	4.3	ND	0.25	30	0.20	5.31	26.5 1, 25-(OH) ₂ D ₃ 1.0 μg
19	16, M	78.0	9.1	4.3	0.34	0.99	30	0.10	3.29	49.1 ^e 1, 25-(OH) ₂ D ₃ 0.5 μg
20	48, M	80.5	8.4	4.2	0.51	1.13	30	0.12	3.29	29.9 1α-OHD ₃ 2.0 μg
21	26, M	58.5	9.7	4.2	0.44	0.30	30	0.18	4.07	33.5 1α-OHD ₃ 2.0 μg

^a Following a suggestion by the Ministry of Health and Welfare Disorders of Hormone Receptors Research Committee, Japan, the diagnostic criteria for this test performed with 200 USP of Parathormone (now, 30 μ g hPTH(1-34)) in adults are as follows; (1) on urinary phosphate, an increment more than 35 mg in 2 h after hormone infusion, and (2) on urinary cAMP, increments more than 1.0 μ mol and 10-fold in 1 h after hormone infusion as compared with the basal value, are regarded as normal responses (Yamamoto *et al.*, 1977). ^b Idiopathic hypoparathyroidism associated with hyperthyroidism. ^c Normocalcemic pseudohypoparathyroidism. ^d Maximum increase within 1 h after hormone infusion. ^e This value indicates a normal response, but before vitamin D treatment, the increase in urinary phosphate was defective; 28.1 mg in 2 h after infusion of 200 USP of Parathormone. In cases 3 and 4, basal excretions in total urinary cAMP were 2.88 nmol/dl GF and 3.87 nmol/mg creatinine, while the doses of hPTH(1-34) were 20 μ g and 30 μ g per 1 m² body surface area, respectively. Normal ranges: serum calcium, 8.4–10.2 mg/dl; serum phosphate, 2.8–4.4 mg/dl; nephrogenous cAMP (NcAMP), 0.58–2.82 nmol/dl GF.

Results

Basal values of plasma cAMP were not significantly different between patients with idiopathic hypoparathyroidism; 12.6 ± 2.82 pmol/ml ($n=14$) and pseudohypoparathyroidism; 9.8 ± 3.01 pmol/ml ($n=5$) except for two children with hypoparathyroidism showing a tendency toward high values ($p > 0.05$). The pattern of changes in plasma cAMP concentrations after a 5 min infusion of hPTH(1-34) was very similar to that following Parathormone (Fig. 1).

Plasma cAMP increased to over 100 pmol/ml within 5 or 10 min after the end of hPTH(1-34) infusion in all patients with idiopathic hypoparathyroidism and normal

subjects. The mean values for maximum plasma cAMP concentrations were 170.0 ± 43.63 pmol/ml (6.0–12.9-fold increase over the basal values) with $20 \mu\text{g}$ ($n=4$), 211.0 ± 71.42 pmol/ml (6.6–31.9-fold increase) with $30 \mu\text{g}$ ($n=30$) in patients with idiopathic hypoparathyroidism, and 204.8 ± 59.49 pmol/ml (9.8–22.4-fold increase) with $30 \mu\text{g}$ ($n=5$) in normal subjects. Increases in plasma cAMP to over 100 pmol/ml were observed at both 5 min and 10 min after the end of hPTH(1-34) infusion except in only two patients with idiopathic hypoparathyroidism who received $20 \mu\text{g}$ (case 1), and $30 \mu\text{g}$ (case 8). Then the cAMP decreased in all cases, as shown in Fig. 1. By 20 min, plasma cAMP had declined to less than 100 pmol/ml in 13 patients with idiopathic hypoparathyroidism and in 2 normal subjects.

Fig. 1. Changes in plasma cAMP concentrations in an Ellsworth-Howard test performed with hPTH(1-34). The arrows indicate intravenous hormone administration over 5 min. Closed symbols (■, ▲) represent the results obtained before treatment and open symbols (□, △), during maintenance treatment with vitamin D₂ or active vitamin D metabolites. Data are expressed as the mean $\pm$ SEM except for two patients with pseudohypoparathyroidism.

Fig. 2. Maximum plasma cAMP concentration after administration of 20–30 μ g hPTH(1-34) or 200 USP Parathormone. Dashed line indicates both tests were performed in the same patients. Closed and open symbols indicate the results examined before and during treatment as in Fig. 1.

In 5 patients with pseudohypoparathyroidism plasma cAMP did not rise above 44.4 pmol/ml (1.3–2.9-fold increase) after infusion of 20 or 30 μ g hPTH(1-34), and these low responses were not improved during treatment.

Fig. 2 shows maximum plasma cAMP concentrations after hPTH(1-34) and Parathormone infusions in all patients including those of children. In either test of the two hormones, there was no overlap between idiopathic hypoparathyroidism or normal subjects and pseudohypoparathyroidism; the increase in plasma cAMP was more than

Fig. 3. Relationship between increase in urinary cAMP and maximum plasma cAMP concentration in Ellsworth-Howard test. Since increases in urinary cAMP, expressed as an absolute term (μ mol/h) in children, are little, two children with hypoparathyroidism (case 3, 4) were excluded. One patient with anti-convulsant induced osteomalacia (marked by a) and one with primary hyperparathyroidism (marked by b) were included. The symbols correspond to those in Fig. 2.

111.5 pmol/ml in the former, but less than 65.0 pmol/ml in the latter.

When the effects of 20 μ g hPTH(1-34) were compared with those of 200 USP Parathormone, the peak value for plasma cAMP after hPTH(1-34) infusion was 70.7% of that after Parathormone infusion in idiopathic hypoparathyroidism (case 1), and also the former produced a peak corresponding to 67.7% of that of the latter in pseudo-

hypoparathyroidism (case 8, Fig. 2). On the other hand, the mean maximum levels of plasma cAMP after infusion of 200 USP Parathormone in idiopathic hypoparathyroidism ($n=8$) were 194.3 ± 61.75 pmol/ml (6.0–21.0-fold increase), which were nearly identical to those after 30 μ g hPTH(1-34).

As shown in Fig. 3, after infusions of hPTH(1-34) or Parathormone there existed a significant correlation between maximum levels of plasma cAMP and increases in urinary cAMP excretion (hPTH(1-34): $r=0.861$, $p<0.001$ v.s. Parathormone: $r=0.823$, $p<0.001$).

Discussion

Neer *et al.* (1977) demonstrated that hPTH(1-34) and native PTH(1-84), when given in large doses, produced essentially identical effects as estimated by urinary cAMP and phosphate in humans. In the present study, the increment in plasma cAMP after hPTH(1-34) infusion was marked in normal controls and idiopathic hypoparathyroidism, while in pseudohypoparathyroidism, its increment was clearly low. Furthermore, the hPTH(1-34) infusion produced a parallel increase in the levels of plasma and urinary cAMP. These results are quite similar to those examined with Parathormone (Tomlinson *et al.*, 1976a; Sohn *et al.*, 1980), and suggest that hPTH(1-34) and native PTH are essentially equipotent in their effect on plasma cAMP.

Considering that PTH stimulates adenylate cyclase activity in certain tissues such as bone (Chase *et al.*, 1969b) and liver (Canterbury *et al.*, 1974), one cannot deny that cAMP generated from these extrarenal organ(s) might be released into plasma after PTH administration. On the other hand, there has been some evidence which suggests that the increase in plasma cAMP after the infusion of PTH originates from the kidney (Kaminsky *et al.*, 1970; Tomlinson *et al.*,

1974). As shown in Fig. 3, the positive correlation between plasma and urinary cAMP in the Ellsworth-Howard test may be compatible with the idea that the PTH-induced increase in plasma cAMP is of renal origin as is the case in urinary cAMP.

Generally, in pseudohypoparathyroidism, plasma cAMP response to injected PTH has been suggested to be defective to the same degree as urinary cAMP response (Tomlinson *et al.*, 1976a; Sohn *et al.*, 1980). In addition we demonstrated that plasma and urinary cAMP responses were quite low also in a patient with subperiosteal bone resorption (case 19) and in cases with normocalcemia during vitamin D treatment. In case 17 the response in urinary cAMP showed borderline values suggesting an incomplete refractoriness. The observations that the increases in plasma cAMP after hPTH(1-34) infusion were also defective in this patient appeared to confirm the diagnosis of pseudohypoparathyroidism. However, some cases of pseudohypoparathyroidism were reported to have normal urinary cAMP response to PTH (type II (Drezner *et al.*, 1973)) or to restore the normal response to PTH in bone (Drezner *et al.*, 1976) and kidney (Rodríguez *et al.*, 1974) following vitamin D treatment. Further study is required to elucidate how much the plasma cAMP would increase after PTH administration in these patients.

There remain a few problems concerning the evaluation of the Ellsworth-Howard test results with respect to responsiveness of urinary cAMP and phosphate (Aida *et al.*, 1975). In contrast to the traditional method based on urine collection which may be time consuming and incorrect, the method based on the changes in plasma cAMP can evaluate the results correctly within a short time and has been considered to be clinically useful (Tomlinson *et al.*, 1976a; Sohn *et al.*, 1980; Lewin *et al.*, 1978; Mallet *et al.*, 1979). Now, evaluation of the Ellsworth-Howard test on the basis of plasma cAMP must be standardized. In the present study,

we tried to evaluate the Ellsworth-Howard test with its peak value. As shown in Fig. 2, a value of 100 pmol/ml was regarded as an indication of a normal response in plasma cAMP and this value discriminates quite clearly pseudohypoparathyroidism type I from hypoparathyroidism and normal controls. Although different centers use different infusion protocols, the multicenter assessments in Japan (Ishida *et al.*, 1982) including the author's data support the validity of this criterion. The peak values for plasma cAMP after an intravenous infusion (15 min) or a single bolus injection of 200 USP of Parathormone in 24 idiopathic hypoparathyroid patients and 15 pseudohypoparathyroid patients reported by Tomlinson *et al.* (1976) and Lewin *et al.* (1978) are in good agreement with our results mentioned above. In addition, it is also possible to apply this criterion to children with hypoparathyroidism as reported by Mallet *et al.* (1979).

The time of drawing blood samples is of prime importance in this test. Because plasma cAMP rapidly decreases after 10 min, especially after 20 min from the end of the hormone infusion for 5 min, it is desirable to take blood samples for plasma cAMP measurements at least 3 times: before, and 5 and 10 min after the intravenous hormone administration (Tomlinson *et al.*, 1976a).

In future, the Ellsworth-Howard test should be performed with hPTH(1-34) in Japan. But instead of Parathormone, purified bovine PTH(1-84) is also used in other countries. We expect that the criterion proposed above will be further confirmed by other laboratories using these two hormones.

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