Stimulation of 1,25-Dihydroxyvitamin D Production by Parathyroid Hormone and Dibutyryl 3',5'-Cyclic AMP in Normal Subjects, Hypoparathyroidism and Pseudohypoparathyroidism

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UNAKAMI, H., FURUKAWA, Y., SOHN, H.E., YUMITA, S., MIURA, R., HANEW, K. and YOSHINAGA, K. *Stimulation of 1,25-Dihydroxyvitamin D Production by Parathyroid Hormone and Dibutyryl 3',5'-Cyclic AMP in Normal Subjects, Hypoparathyroidism and Pseudohypoparathyroidism*. Tohoku J. exp. Med., 1982, **138** (2), 167-175. Parathyroid extract (PTE) or synthetic 1-34 human parathyroid hormone (1-34 hPTH) was injected intravenously as a bolus in 4 normal subjects, 4 patients with PTH deficient hypoparathyroidism (HP) and 3 patients with pseudohypoparathyroidism (PHP). In normal subjects and HP, plasma 1,25(OH)₂D was markedly increased at 6 hr and reached the peak at 12 or 14 hr after administration of 200 units of PTE or 20 to 30 µg of 1-34hPTH. On the other hand, 500 units of PTE or 20 µg of 1-34hPTH failed to increase plasma 1,25(OH)₂D in PHP. However, 2.5 mg/kg of dibutyryl cAMP remarkably increased plasma 1,25(OH)₂D in a patient with PHP. Maximal increments of plasma 1,25(OH)₂D in 3 patients with HP (21.7±5.6 pg/ml, mean±s.d.) were nearly as high as in normal subjects (20.6±7.0 pg/ml), whereas those in 3 patients with PHP (2.3±2.3 pg/ml) were distinctly lower than in normal subjects or HP. It is suggested that 1,25(OH)₂D production by PTH is intact in HP, but is impaired in PHP mainly due to a defect in the activation of adenylate cyclase system. — — — parathyroid extract; synthetic 1-34 human parathyroid hormone; dibutyryl 3',5'-AMP; 1,25-dihydroxyvitamin D; adenylate cyclase system

It is generally accepted that the conversion of 25-hydroxyvitamin D₃ to 1,25-dihydroxyvitamin D₃ in the kidney is enhanced by parathyroid hormone (PTH) (Garabedian et al. 1972; Fraser and Kodicek 1973). In addition, Rasmussen et al. (1972), Larkins et al. (1974) and Horiuchi et al. (1977) reported that 3',5'-cyclic AMP(cAMP) or dibutyryl 3',5'-cyclic AMP(DBcAMP) reproduced the stimulating effect of PTH on 1,25-dihydroxyvitamin D₃ production, suggesting that PTH action is mediated by cAMP.

Therefore, it is plausible that serum concentration of 1,25-dihydroxyvitamin D₃ is low due to the lack of PTH in patients with idiopathic or postoperative hypoparathyroidism (HP) (Haussler et al. 1976; Lund et al. 1980; Lambert et al.

1980; Mason et al. 1980), or due to the unresponsiveness of the kidney tissue to the action of PTH in pseudohypoparathyroidism (PHP) (Drezner et al. 1976; Sinha et al. 1977; Lawoyin et al. 1979; Lambert et al. 1980).

In this study, we examined the responsiveness of vitamin D metabolites to PTH administration in normal subjects, HP and PHP. Additionally, we studied the effect of DBcAMP on the production of vitamin D metabolites in order to demonstrate the impaired cAMP system in a patient with PHP.

MATERIALS AND METHODS

Four normal subjects (all were males, aged 24 years), 4 patients with HP (2 males and 2 females, aged 39–70 years) and 3 patients with PHP (2 males and 1 female, aged 13–39 years) were studied. Diagnosis of HP or PHP were made according to the amounts of increments in urinary excretion of P (Δ UP) and cAMP (Δ cAMP) after the intravenous injection of 200 units of PTE (Parathormone, Lilly Co., Ltd.) or 20 μ g of 1-34hPTH (synthetic human parathyroid hormone, Toyo Jozo Co., Ltd.) (HP: Δ UP > 35 mg/2 hr, Δ UcAMP > 1.0 μ mole/hr. PHP: Δ UP < 35 mg/2 hr, Δ UcAMP < 1.0 μ mole/hr). By these criteria Cases 5, 6, 7 and 8 were diagnosed as HP, while Cases 9, 10 and 11 were as PHP (Table 1).

TABLE 1. *Summary of the experimental subjects*

Subject No.	Diagnosis	Sex/Age	Serum		Plasma			
			Ca (mg/100 ml)	P (mg/100 ml)	iPTH* (ng/ml)	25OHD (ng/ml)	24,25 (OH) ₂ D (ng/ml)	1,25 (OH) ₂ D (pg/ml)
1	Normal	M/24	9.4	3.2	<0.3	10.9	1.47	46.6
2	Normal	M/24	9.3	2.7	<0.3	8.7	2.13	51.5
3	Normal	M/24	9.7	3.0	<0.3	11.1	2.42	29.9
4	Normal	M/24	9.4	3.2	<0.3	20.0	2.24	25.3
5	HP	F/70	5.8	5.9	<0.3	17.2	1.45	<5.0
6	HP	F/45	5.9	5.7	<0.3	17.2	1.57	<5.0
7	HP	M/50	4.9	6.6	<0.3	8.0	1.95	21.2
8	HP	M/39	6.2	4.2	<0.3	9.6	2.77	33.7
9	PHP	M/24	7.7	3.9	0.60	6.8	1.15	10.3
10	PHP	M/13	7.3	5.4	0.72	10.9	1.17	41.6
11	PHP	F/39	7.7	4.9	0.46	8.8	1.42	32.3

* Normal range was less than 0.4 ng/ml.

After an overnight fast, 200 units of PTE were intravenously injected over 10 min to 3 patients with HP (Cases 5, 6, 7) and 500 units of PTE were intravenously infused with 250 ml of normal saline for 1 hr to 2 patients with PHP (Cases 9, 10) to evaluate the responsiveness of vitamin D metabolites to PTH. Similarly, 30 μ g of 1-34 hPTH were injected to 4 normal subjects, and 20 μ g of 1-34hPTH to a patient with HP (Case 8) and a patient with PHP (Case 11), respectively. In addition, 2.5 mg/kg of DBcAMP was intravenously infused with 250 ml of normal saline for 1 hr to a patient with PHP (Case 9). Blood was drawn before and 3, 6, 12 (14), and 24 hr after the administration of PTH or DBcAMP for the determination of serum calcium (Ca), phosphate (P) and plasma vitamin D metabolites. Furthermore, hourly urine in each subject was collected for 3 hr before and after the administration of PTH or DBcAMP to quantitate urinary P and cAMP.

Serum Ca was measured by EGTA titrimetry, and serum or urinary P were by Fiske and Subbarow's method (1925). Plasma immunoreactive PTH (iPTH) was quantitated by radioimmunoassay utilizing a PTH assay kit (Eiken Co., Ltd.). Urinary cAMP was

measured by radioimmunoassay using a cAMP assay kit (Yamasa Shoyu Co., Ltd.) (Honma et al. 1977).

Plasma vitamin D metabolites (25-hydroxyvitamin D [25OHD], 24,25-dihydroxyvitamin D [24,25(OH)₂D] and 1,25-dihydroxyvitamin D [1,25(OH)₂D] were quantitated as follows: 3 to 5 ml of blood plasma were extracted with methylene chloride after addition of 6000 dpm of [23,24(n)-³H]-25OHD₃ (70–110 Ci/mmol), 6000 dpm of [23,24(n)-³H]-24R,25(OH)₂D₃ (50–80 Ci/mmol) and 6000 dpm of [23,24(n)-³H]-1 α ,25(OH)₂D₃ (70–110 Ci/mmol) (Amersham) for evaluating the recovery rates. The extracts were applied to the Sephadex LH-20 column (0.7×14 cm) with a mixed solvent of chloroform and hexane (60:40, v/v) as described by Kano et al. (1979). 25OHD fraction (4–10 ml), 24,25(OH)₂D fraction (10–20 ml) and 1,25(OH)₂D fraction (20–40 ml) were separately collected. Then, both 24,25(OH)₂D and 1,25(OH)₂D fractions were applied to high pressure liquid chromatography (Yanagimoto, L-2000. μ porasil silicic acid column, ϕ 4×250 mm) with a mixed solvent of 3% methanol in methylene chloride at flow rate of 1.0 ml/min for further purification. The overall recovery of each metabolite was; 25OHD 35.4±6.1%, 24,25(OH)₂D 59.6±11.6%, 1,25(OH)₂D 57.3±9.9% (mean±s.d.). The assays for 25OHD and 24,25(OH)₂D were carried out by the method of Belsey et al. (1971) and Talor et al. (1976), respectively. 1,25(OH)₂D was quantitated by radioreceptor assay using the intestinal cytosol receptor from vitamin D deficient chickens according to the method of Eisman et al. (1976). The within- and between-assay coefficients of variation for the determination of the metabolites were; 25OHD 9.9% and 17.7%, 24,25(OH)₂D 10.4% and 18.1%, 1,25(OH)₂D 13.1% and 18.4%, respectively. Normal ranges of the metabolites in adult volunteers were; 25OHD 20.5±5.4 ng/ml ($n=6$, from April to September) or 16.7±5.4 ng/ml ($n=13$, from October to March), 24,25(OH)₂D 1.73±0.62 ng/ml ($n=17$), 1,25(OH)₂D 37.2±13.8 pg/ml ($n=21$) (mean±s.d.).

Student's *t* test was used for statistical analyses, and the variance of the mean was expressed as s.d.

RESULTS

In 4 normal subjects, the basal values of serum Ca and P were 9.5±0.2 mg/100 ml and 3.0±0.2 mg/100 ml, and those of plasma vitamin D metabolites were; 25OHD 12.7±5.0 ng/ml, 24,25(OH)₂D 2.07±0.41 ng/ml, 1,25(OH)₂D 38.3±12.7 pg/ml. 4 patients with HP showed a low basal serum Ca (5.7±0.6 mg/100 ml) and a high basal serum P (5.6±1.0 mg/100 ml). The basal value of plasma 25OHD was 13.0±4.9 ng/ml and that of plasma 24,25(OH)₂D was 1.94±0.60 ng/ml. In 2 of these patients, plasma 1,25(OH)₂D were less than 5.0 pg/ml, but were within normal range in the remaining 2 patients (Cases 7, 8). In 3 patients with PHP, the basal values of serum Ca, serum P, plasma 25OHD and plasma 24,25(OH)₂D were 7.6±0.2 mg/100 ml, 4.7±0.8 mg/100 ml, 8.8±2.1 ng/ml and 1.25±0.15 ng/ml, respectively. The basal value of plasma 1,25(OH)₂D was lower than normal in a patient, but within normal range in the other 2 patients (Cases 10, 11) (Table 1).

After the administration of 200 units of PTE, plasma 1,25(OH)₂D was markedly increased at 6 hr, reached its peak at 14 hr and remained plateau or slightly decreased at 24 hr in 3 patients with HP (Cases 5, 6, 7). In contrast, 500 units of PTE failed to increase plasma 1,25(OH)₂D in 2 patients with PHP (Cases 9, 10) (Fig. 1). Plasma 24,25(OH)₂D was distinctly decreased in patient with HP (Case 6), but showed no significant changes in another patient with HP and 2 patients with PHP. After the administration of 1-34 hPTH, plasma 1,25(OH)₂D was increased, as was seen by PTE, in 4 normal subjects and a patient with HP

Fig. 1. Effect of PTE on plasma 1,25(OH)₂D production in patients with HP (○—○ Cases 5, 6, 7) and PHP (●—● Cases 9, 10). Marked increments in plasma 1,25(OH)₂D were seen only in HP.

Fig. 2. Effect of 1-34hPTH on plasma 1,25(OH)₂D production in normal subjects (■—■ $n=4$, mean $\pm$ S.D.), a patient with HP (○—○ Case 8) and a patient with PHP (●—● Case 11). Increments in plasma 1,25(OH)₂D were seen in normal subjects and HP, but not in PHP.

(Case 8), but no increment was seen in a patient with PHP (Case 11) (Fig. 2). Plasma 24,25(OH)₂D was decreased in a patient with HP, whereas no significant changes were seen in 4 normal subjects or a patient with PHP.

There were no significant changes in serum Ca, P or plasma 25OHD in any subjects with PTE or 1-34hPTH.

TABLE 2. Changes in urinary P, urinary cAMP and plasma 1,25(OH)₂D by PTH administration

Subject No.	Administered PTH	ΔUrinary P (mg/2 hr)	ΔUrinary cAMP (μmoles/hr)	Maximal Δplasma 1,25(OH) ₂ D (pg/ml)
1	1-34hPTH 30 μg	—	14.09	25.9
2	1-34hPTH 30 μg	—	12.26	25.7
3	1-34hPTH 30 μg	—	8.22	11.0
4	1-34hPTH 30 μg	—	14.26	19.6
5	Parathormone 200U	101.7	7.99	56.2
6	Parathormone 200U	44.3	9.08	27.7
7	Parathormone 200U	156.2	15.29	20.8
8	1-34hPTH 20 μg	48.5	8.15	16.6
9	Parathormone 500U	30.5*	0.21	2.3
10	Parathormone 500U	74.9†	0.24	0.2
11	1-34hPTH 20 μg	12.6	0.99	4.6

* 12.2/200 units of Parathormone.

† 30.0/200 units of Parathormone.

Fig. 3. Effects of PTE (upper) and DBcAMP (lower) on plasma vitamin D metabolites production in a patient with PHP (Case 9). DBcAMP caused a marked increment of plasma 1,25(OH)₂D (●—●) and a reciprocal decrement of plasma 24,25(OH)₂D (○—○), though no responses were seen by PTE administration.

By PTH administration, the urinary excretion of P increased to more than 35 mg/2 hr in all patients with HP, but less than 35 mg/2 hr (200 units of PTE or 20 μ g of 1-34hPTH) in all patients with PHP. While, increments in the urinary excretion of cAMP in normal subjects and HP were more than 1.0 μ mole/hr, and less than 1.0 μ mole/hr in all patients with PHP. The maximal increment of plasma 1,25(OH)₂D in 3 patients with HP (Cases 6, 7, 8,) was 21.7 ± 5.6 pg/ml, which was as high as in normal subjects (20.6 ± 7.0 pg/ml), although a patient with HP (Case 5) showed an extremely high value (52.6 pg/ml). In 3 patients with PHP, the maximal increment of 1,25(OH)₂D was 2.3 ± 2.3 pg/ml, and it was significantly lower than in normal subjects ($p < 0.01$) or in HP ($p < 0.05$) (Table 2). But there were no significant correlations between the maximal increments of plasma 1,25-(OH)₂D and the increments of urinary excretion of P or those of cAMP in any subjects.

In a patient with PHP (Case 9) who failed to demonstrate any change in plasma vitamin D metabolites to 500 units of PTE, 2.5 mg/kg of DBcAMP brought about marked increment of plasma 1,25 (OH)₂D and reciprocal decrement of plasma 24,25(OH)₂D (Fig. 3). Besides, urinary excretion of P was markedly increased (4 UP=113.3 mg/2 hr).

DISCUSSION

As has been reported by other workers (Haussler et al. 1976; Lund et al. 1980; Lambert et al. 1980), plasma 1,25(OH)₂D in HP seems to be low due to the lack of PTH. However, as the 1 α -hydroxylation system is thought to be intact in this disease, exogenous PTH may be able to stimulate 1 α -hydroxylase which subsequently increases plasma 1,25(OH)₂D. Mason et al. (1980) reported that a single injection of PTE (400 units) markedly increased the plasma 1,25(OH)₂D level and reached the peak value at 14 hr after the injection in surgical HP. Eisman et al. (1979) also reported that twice injections of 2.5 U/kg of PTE (up to 200 U) caused an increment of serum 1,25(OH)₂D (peak 8–40 hr) in healthy volunteers. In our study, a single injection of 200 units of PTE or 20 to 30 μ g of 1-34 hPTH clearly increased plasma 1,25(OH)₂D in either patients of HP or normal subjects. Peak values of plasma 1,25(OH)₂D after injection were obtained at 12 or 14 hr in HP and at 24 hr in normal subjects, the peak time being nearly the same as those reported previously. Additionally, peak values of plasma 1,25(OH)₂D might be similar to those of other reports if injection doses were considered. Some workers proposed the inhibitory effect of PTH on 24-hydroxylase activity from animal or in vitro experiments (Tanaka et al. 1975; Juan and DeLuca 1977), but few workers reported the decrement of plasma 24,25(OH)₂D after PTH administration in man. In our patients with HP and normal subjects, changes in plasma 24,25(OH)₂D after injection of PTE or 1-34 hPTH were varying, i.e. plasma 24,25(OH)₂D was distinctly decreased only in 2 patients with HP, there being no changes in others. The reason of this difference in responsiveness to PTH is still unknown.

In PHP, as many authors reported, plasma 1,25(OH)₂D seems to be low

depending upon the unresponsiveness to PTH in the kidney tissue (Drezner et al. 1976; Sinha et al. 1977; Metz et al. 1977). Furthermore, other authors reported that stimulating effect of PTH on 1,25(OH)₂D production was not seen in this disease (Lambert et al. 1980; Mason et al. 1980) unless PTH was repeatedly administered (Lawoyin et al. 1979). In our study, neither increments of plasma 1,25(OH)₂D nor decrements of plasma 24,25(OH)₂D were seen in 2 patients injected with PTE or in a patient injected with 1-34hPTH. Even large doses of PTE (500 units) failed to cause any change in vitamin D metabolites in PHP. This lack of changes in plasma vitamin D metabolites was related to the lack of changes in cAMP and P excretion.

As to the maximal increments of plasma 1,25(OH)₂D, there was a significant difference between normal subjects and PHP ($P < 0.01$) or between HP and PHP ($p < 0.05$). Moreover no overlaps were seen in the values of maximal increment of plasma 1,25(OH)₂D between normal subjects and PHP or between HP and PHP, though injection doses of PHP were varying. Therefore, it seemed possible to differentiate HP and PHP from each other by evaluating the maximal increment of plasma 1,25(OH)₂D using the method of a single PTH administration, even though there were overlaps in their basal values of plasma 1,25(OH)₂D.

In our study, there were no significant correlations between the maximal increments of plasma 1,25(OH)₂D and the increments of urinary excretion of P or those of cAMP in any subjects. But, the responses of 1,25(OH)₂D production to PTH might be correlated to the increments of urinary excretion of P or cAMP if more subjects were studied, as they showed differences between normal subjects and PHP, or between HP and PHP.

The impairment of the adenylate cyclase system in PHP is well known (Chase et al. 1969; Bell et al. 1972). Drezner et al. (1973) classified PHP into 2 types; impairment of an increase in intracellular cAMP (Type I), an inability of intracellular cAMP to initiate the chain of metabolic events (Type II). In our study, 2.5 mg/kg of DBcAMP remarkably increased plasma 1,25(OH)₂D in a patient with PHP of Type I (Case 9) with a reciprocal decrease in plasma 24,25-(OH)₂D. Relating to these findings, some workers demonstrated that cAMP or DBcAMP reproduced the stimulating effect of PTH on 1,25(OH)₂D production by in vitro or in vivo animal experiments (Rasmussen et al. 1972; Larkins et al. 1974; Horiuchi et al. 1977). Taken together, it is suggested that DBcAMP stimulates 1 α -hydroxylase activity and inhibits 24-hydroxylase activity in Type I PHP, and that the mechanism of production or inhibition of vitamin D metabolites after cAMP producing system is not impaired.

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