1,25-Dihydroxyvitamin D Production after Stimulation with Synthetic Human Parathyroid Hormone (1-34) in Hypoparathyroid and Renal Tubular Disorders

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Abstract

1,25-dihydroxyvitamin D production in response to two successive infusions of synthetic active 1-34 fragment of human PTH [hPTH-(1-34)] was evaluated in order to develop an understanding of the vitamin D metabolism and the rationale of vitamin D therapy in calcium disorders. Five normal controls, six hypoparathyroid patients, two patients with hypophosphatemic vitamin-D-resistant rickets, one patient with Lowe's synd. and one patient with primary Fanconi's synd. were investigated, and the following results were obtained.

All normal controls showed a significant increase in serum $1,25(\text{OH})_2\text{D}$ [43 ± 3.8 ($m \pm \text{SEM}$, $n=5$, basal), 53 ± 4.3 (three hours after the first PTH infusion), 65 ± 7.7 (six hours) and 66 ± 4.4 (nine hours) pg/ml]. All patients with PTH-deficient hypoparathyroidism showed a significant increase in serum $1,25(\text{OH})_2\text{D}$, and serum $1,25(\text{OH})_2\text{D}$ values were within the normal range after hPTH-(1-34) stimulation. Serum $1,25(\text{OH})_2\text{D}$ remained low after hPTH-(1-34) infusions in a patient with pseudohypoparathyroidism type I who showed a significant increase in this value after infusion of dibutyryl cyclic AMP. On the other hand, a patient with normocalcemic pseudohypoparathyroidism type I had a high basal $1,25(\text{OH})_2\text{D}$ value, which increased further after hPTH-(1-34) infusions. An almost normal increase in serum $1,25(\text{OH})_2\text{D}$ was observed in two patients with hypophosphatemic vitamin-D-resistant rickets, one with Lowe's syndrome and the other with primary Fanconi's syndrome.

We conclude that these results are important in obtaining an understanding of calcium and vitamin D metabolism in these disorders and that this PTH stimulation test is a useful method to use in evaluating renal responsiveness in $1,25(\text{OH})_2\text{D}$ production to PTH in various calcium disorders.

1,25-dihydroxyvitamin D [$1,25(\text{OH})_2\text{D}$], the most active vitamin D metabolite, is synthesized in the proximal renal tubule from 25-hydroxy-vitamin D (25-OHD), and it in turn promotes intestinal calcium (Ca) absorption and mobilization of Ca from

bones. The renal 25-OHD-1 α -hydroxylase activity is considered to be stimulated by parathyroid hormone (PTH) (Rasmussen *et al.*, 1972; Garabedian *et al.*, 1972) via low serum Ca^{2+} stimulation of PTH secretion and also by low extracellular Ca^{2+} (Boyle *et al.*, 1971; Fukase *et al.*, 1982). Other factors, such as serum phosphate (P) (Ta-

naka *et al.*, 1973; Baxter and DeLuca, 1976; Lobaugh and Drezner, 1983), blood pH (Kawashima *et al.*, 1982), sex steroids (Spanos and Haussler, 1976), glucocorticoids (Chesney *et al.*, 1978; Hahn *et al.*, 1980), growth hormone (Spanos and Haussler, 1978) and 1,25(OH)₂D itself (Larkins *et al.*, 1974; Spanos, 1983) modulate serum 1,25(OH)₂D levels either directly or indirectly. Among these factors, PTH is considered to be the most potent stimulator of 1 α -hydroxylase activity, and deficient actions of PTH, either due to insufficient PTH secretion or peripheral PTH resistance, result in low serum 1,25(OH)₂D and low serum Ca (Markowitz *et al.*, 1982; Unakami *et al.*, 1982). Therefore, the PTH stimulation test is thought to be a useful method in evaluating 1,25(OH)₂D secretion in various disorders, which is important in obtaining an understanding the rationale of vitamin D therapy.

Thus, we investigated the response of serum 1,25(OH)₂D after stimulation with synthetic active 1-34 fragment of human PTH in hypoparathyroid and renal tubular disorders.

Materials and Methods

1) Subjects

Five normal controls (5-32 years old, serum Ca 9.3 ± 0.4 mg/dl and serum P 3.8 ± 0.9 mg/dl, $m \pm SD$), four patients with PTH-deficient hypoparathyroidism (HP) (aged 3, 13, 15, and 38 years, respectively), two patients with pseudohypoparathyroidism Type I (PHP-I) (2 and 34 years), two patients with hypophosphatemic vitamin-D-resistant rickets (XLH), one patient with Lowe's syndrome and one patient with primary Fanconi's syndrome were investigated (for detailed data see Table 1). All had normal creatinine (Cre) clearance, and either had no previous experience of vitamin D therapy or had discontinued therapy for more than four days prior to this study (Table 1). One patient with PHP-I (case 6) was previously reported by Nakajima *et al.*, (1966).

Table 1. Serum Ca, P, iPTH and 25-OHD values before the first hPTH-(1-34) infusion.

Case No.	Age	Serum values			
		Ca mg/dl	P mg/dl	iPTH ng/ml	25-OHD ng/ml
1 HP a	3 y	6.7	6.5	<0.2	21
2 HP b	13 y	5.4	6.2	0.3	16
3 HP a	15 y	6.0	6.3	nd	49
4 HP b	38 y	7.7	3.3	nd	22
5a PHP b	2 y	6.6	6.5	0.67	30
5b PHP dbc	2 y	5.8	7.1	nd	26
5c PHP a	2 y	8.9	5.3	nd	26
6 PHP a	34 y	8.5	3.3	1.0	34
7 XLH	21 m	8.7	2.2	0.42	18
8 XLH a	10 y	9.0	2.1	0.35	25
9 Lowe	4 m	9.3	4.7	0.2	20
10 Fanconi a	16 y	8.5	1.9	0.4	46
Normal values		9.5 ± 1.0	2.5-4.5	<0.55	14-50
		Adults			

Abbreviations: No.=number; y=years; m=months; nd=not done; HP=PTH-deficient hypoparathyroidism; PHP=Pseudohypoparathyroidism type I; XLH=X linked hypophosphatemic vitamin-D-resistant rickets.

b: In these patients hPTH-(1-34) was infused at 1000 h and 1900 h.

dbc: Dibutyryl cAMP infusion test.

a: In these patients the PTH stimulation was performed after cessation of treatment. Case No., previous treatment, and duration of cessation of treatment are shown below. (1: 1 α ,25(OH)₂D₃ - 0.5 μ g/day, 7 days), (3: 1 α -OH-D₃ - 10 μ g/day, 14 days), (5c: 1 α ,25(OH)₂D₃ - 0.25 μ g/day, 4 days), (6: 1 α -OH-D₃ - 0.5 μ g/day, 4 m), (8: 1 α -OH-D₃ - 3 μ g/day and phosphorus 2 g/day, 4 m), (10: 1 α -OH-D₃ - 0.5 μ g/day, alkali- 10 mEq/kg/day and phosphorus 1 g/day, 10 days)

2) hPTH-(1-34) stimulation test

Synthetic active 1-34 fragment of human PTH [hPTH-(1-34), (Toyo Jozo Co.)] was infused over a five-minute period at 1000 h and 1300 h (except on three occasions when PTH was infused at nine-hour intervals, see Table 1) at a dose of 100 U/m² (maximum dose 100 U). This dose (100 U) of hPTH-(1-34) was already proved to be almost equivalent to 200 USP U of bovine parathyroid extract (Parathormone, PTE, Eli Lilly Co.) with regard to phosphaturic and cAMP

responses (Igarashi *et al.*, 1982). Blood for 1,25(OH)₂D measurement was drawn before and at three, six, nine and 24 hours after the first hPTH-(1-34) infusion. Hourly urine before and three hours after the first hPTH-(1-34) infusion was collected, and urinary P, Cre and cAMP were measured.

3) Dibutyl cAMP infusion test

In the two-year-old patient with PHP-I, infusion of 2.5 mg/kg (over one hour) of dibutyl cAMP was undertaken (Table 1, Case 5b).

4) Assay procedures and control values

Serum 1,25(OH)₂D was extracted from serum samples (1.5–2 ml) by dichloromethane, isolated and purified by high-pressure liquid chromatography (Hitachi 635A) employing Zorbax SIL and Zorbax ODS and was quantified using a chick embryonic cytosol receptor (Yamasa Syoyu Co.) (Eisman *et al.*, 1976; Dokoh *et al.*, 1978). The extraction rate and detection limit were $77 \pm 6\%$ ($m \pm SD$) and 2 pg/tube, respectively. The intra-assay coefficient of variation was 10% (40 pg/ml, $n=6$). The normal 1,25(OH)₂D values in children and adults were 30–80 pg/ml and 30–70 pg/ml, respectively (Yasuda *et al.*, 1983). Serum 25-OHD was measured by competitive protein binding assay, and the normal value was 14–50 ng/ml (Kitasato biochemical laboratories). Serum immunoreactive (i) PTH was measured with an Eiken-RIA-Kit, which mainly detects the carboxyl-terminal portion of PTH.

Urinary cAMP was measured with a Yamasa-RIA-Kit. Basal urinary cAMP excretion in controls in our laboratory was 138 ± 106 nmol/m²/hr ($m \pm SD$, $n=4$) in infants, 203 ± 47 ($n=6$) in children and 160 ± 20 ($n=6$) in adults, while the increase in urinary cAMP excretion in response to hPTH-(1-34) was 14–176-fold (range, mean = 88-fold, $n=4$) in infants, 21–94 fold (mean = 60-fold, $n=6$) in children and 34–86-fold (mean = 55-fold, $n=6$) in adults (Yasuda *et al.*, 1983). The increase of urinary cAMP in response to hPTH-(1-34) in PHP-I was less than 10-fold (Igarashi *et al.*, 1982; Unakami *et al.*, 1982; Yasuda *et al.*, 1983).

Serum Ca, P, and Cre and urinary P and Cre were measured by the standard autoanalyzer technique. $\Delta P(2h)$ is the difference between urinary phosphorus excretion before and after two hours of hPTH-(1-34) infusion. Most cases of HP showed a $\Delta P(2h)$ value of more than 35 mg

(or 35 mg/m² if the body surface area of the patient was less than 1 m²), and most cases of PHP did not (Igarashi *et al.*, 1982; Unakami *et al.*, 1982; Yasuda *et al.*, 1983).

Results

Serum Ca, P, iPTH and 25-OHD at the time of the PTH stimulation test are shown in Table 1. All subjects had normal 25-OHD values. All controls showed significant increases in serum 1,25(OH)₂D [43 ± 3.8 ($m \pm SEM$, $n=5$, before), 53 ± 4.3 (three hours after the first hPTH-(1-34) infusion), 65 ± 7.7 (six hours), and 66 ± 4.4 (nine hours) pg/ml] (Fig. 1). The maximum increment in serum 1,25(OH)₂D in normal controls

Fig. 1. Serum 1,25(OH)₂D in normal controls in response to hPTH-(1-34) infusions. hPTH-(1-34) infusion is indicated by the arrows. Maximal increment in serum 1,25(OH)₂D was 25 ± 4.0 pg/ml ($m \pm SEM$, $n=5$). □: Normal serum 1,25(OH)₂D value in children (30–80 pg/ml).

PTH-deficient hypoparathyroidism

Fig. 2. Serum $1,25(\text{OH})_2\text{D}$ in PTH-deficient hypoparathyroidism in response to hPTH-(1-34) infusions. For Case No. see Table 1.

Table 2. Phosphaturic and urinary cAMP responses to hPTH-(1-34) or dibutyl cAMP infusion.

Case No.	Phosphaturia		cAMP response	
	$\Delta\text{P}(2\text{h})$ mg	% TRP basal-after the PTH	basal nmol/m ² /h	-fold increase
1	99 ^c	90-32	117	100
2	109	97-80	124	27
3	102	97-73	114	154
4	22	90-81	146	71
5a	17 ^c	84-70	51	3.1
5b	74 ^c	92-69		
5c	22 ^c	84-67	94	2.1
6	24	86-72	142	3.9
7	20 ^c	68-45	260	42
8	12 ^c	76-69	121	54
9	23 ^c	85-59	116	16
10	10	45-39	188	16

Case numbers correspond to those in Table 1. $\Delta\text{P}(2\text{h})$ is expressed as mg/m² in patients (c) with a body surface area of less than 1 m². For $\Delta\text{P}(2\text{h})$, normal basal urinary cAMP excretion and increase in it to hPTH-(1-34), see the text. As a HP, phosphaturic response to hPTH-(1-34) in case 4 was slightly diminished, which was partly due to its low serum phosphate level.

Pseudohypoparathyroidism

Fig. 3. Serum $1,25(\text{OH})_2\text{D}$ in PHP-I in response to hPTH-(1-34) infusions or dibutyl cAMP infusion. Dibutyl cAMP infusion (2.5 mg/kg in an hour) is indicated in the figure. For dbc and Case No. see Table 1. There was no or diminished response of $1,25(\text{OH})_2\text{D}$ in a patient with PHP-I (Case 5a°, 5c°) that showed a definite increase after 2.5 mg/kg dibutyl cAMP infusion. In a patient with normocalcemic PHP-I (Case 6), a definite increase of $1,25(\text{OH})_2\text{D}$ in response to hPTH-(1-34) was shown. The symbol ° represents the same patient.

was 25 ± 4.0 pg/ml ($m \pm \text{SEM}$, $n=5$).

Although the basal serum $1,25(\text{OH})_2\text{D}$ value was low in all but one patient (case 4) with HP, it increased significantly in all and was within the normal range (Fig. 2). The maximum increment in serum $1,25(\text{OH})_2\text{D}$ in HP was 30 ± 3.8 pg/ml ($m \pm \text{SEM}$, $n=4$). Phosphaturic and urinary cAMP responses to hPTH-(1-34) in these patients are shown in Table 2.

There was no response or only diminished response of serum $1,25(\text{OH})_2\text{D}$ to hPTH-(1-34)

Renal tubular disorders

Fig. 4. Serum 1,25(OH)₂D in XLH (○—○), Lowe's synd. (●—●) and Fanconi's synd. (●---●) in response to hPTH-(1-34) infusions. For Case No. see table 1.

infusions in a patient with PHP-I [2y: before treatment (case 5a) basal 17 pg/ml → peak 24, after cessation of treatment (case 5c) basal 20 pg/ml → peak 19] (Table 1, Fig. 3). This patient's cAMP responses to hPTH-(1-34) were also diminished (Table 2). Her phosphaturic response to hPTH-(1-34) tended to be low compared with that of HP (Table 2). But she showed a definite increase in 1,25(OH)₂D [(case 5b) basal 20 pg/ml → peak 88] and prominent phosphaturia [% TRP 92 → 69, ΔP(2h) 74 mg/m²] after the infusion of 2.5 mg/kg of dibutyl cAMP (Table 2, Fig. 3). A patient with almost normocalcemic PHP-I (aged 34, case 6) had a high serum 1,25(OH)₂D basal value of 74 pg/ml (normal adult value 30–70 pg/ml), which increased further to 128 pg/ml after hPTH-(1-34) infusions despite deficient cAMP response (3.9-fold increase, Table 2, Fig. 3).

Although one patient with XLH had a low basal 1,25(OH)₂D value of 25 pg/ml (case 7), almost normal increases of 1,25(OH)₂D in response to hPTH-(1-34) infusions were observed in connection with this disorder (case 7; basal 25 pg/ml → peak 57; case 8; basal 50 pg/ml → peak 83) (Fig. 4). Such patients also showed phosphaturic response to hPTH-(1-34) infusion (, but it is difficult to evaluate the degrees of phosphaturia induced by PTH infusion in these hypophosphatemic patients) (Table 2).

The patient with Lowe's syndrome (case 9) had a normal serum 1,25(OH)₂D value of 80 pg/ml, which increased to 111 pg/ml after hPTH-(1-34) infusions in the state of compensated metabolic acidosis (arterial blood gas: pH 7.39, HCO₃ 18.3 mEq/l, BE – 5.2 mEq/l, PCO₂ 30 mmHg) (Fig. 4). Although the patient with primary Fanconi's syndrome (case 10) had a subnormal basal 1,25(OH)₂D value of 30 pg/ml, it increased to 68 pg/ml after hPTH-(1-34) infusions in the state of metabolic acidosis (arterial blood gas: pH 7.34, HCO₃ 17.2 mEq/l, BE – 7.2 mEq/l, PCO₂ 36 mmHg) (Fig. 4). Her cAMP response to hPTH-(1-34) was slightly diminished, and phosphaturic response seemed to be absent [% TRP 45 → 39, P/Cre (mg/mg) basal 1.17 → peak 1.21, ΔP(2h) 10 mg] (Table 2).

Discussion

1,25(OH)₂D production after stimulation with bovine parathyroid extract (PTE) was recently reported in normal controls (Eisman *et al.*, 1979; Prince *et al.*, 1983) and various conditions, such as hypoparathyroid disorders (Lambert *et al.*, 1980; Markowitz *et al.*, 1982; Unakami *et al.*, 1982), vitamin D dependency type I (Aarskog *et al.*, 1983) and XLH (Lyles and Drezner, 1982). Eisman *et al.*, (1979) reported that an 80% increase in plasma 1,25(OH)₂D was noted in normal controls after two successive PTE infusions

(eight hours apart) with the peak value at eight to 40 hours after the first infusion. Other investigators (Markowitz *et al.*, 1982; Aarskog *et al.*, 1983; Prince *et al.*, 1983) reported a slightly rapid increase in serum $1,25(\text{OH})_2\text{D}$ measurable at three or four hours after PTE infusion. The response of serum $1,25(\text{OH})_2\text{D}$ after hPTH-(1-34) infusions in normal controls suggests that the response of serum $1,25(\text{OH})_2\text{D}$ is almost identical or slightly less in magnitude and time course with that induced by PTE and that this stimulation test is an adequate method for the evaluation of $1,25(\text{OH})_2\text{D}$ production because of the significant increase in serum $1,25(\text{OH})_2\text{D}$ in all normal controls as well as HP. Recently Unakami *et al.*, (1982) reported the response of serum $1,25(\text{OH})_2\text{D}$ after a single infusion of 67 to 100 U of hPTH-(1-34), and it seemed to be slightly lower than that reported by the present authors.

The increase in serum $1,25(\text{OH})_2\text{D}$ in HP is considered to be almost identical with that of normal controls. In addition, the data suggest that the decreased $1,25(\text{OH})_2\text{D}$ in HP is due to deficient PTH secretion (Markowitz *et al.*, 1982). This might also imply that the stimulation of renal 25-OHD- 1α -hydroxylase activity to hPTH-(1-34) measured by the increase in serum $1,25(\text{OH})_2\text{D}$ was not exaggerated in HP (Unakami *et al.*, 1982). With regard to phosphaturic response to PTH, hyper-responsiveness was noted in HP, supposedly due to a lack of basal stimulation by PTH (Igarashi *et al.*, 1982; Yasuda *et al.*, 1983). It may be the hyperphosphatemia in HP that partly diminishes the response of 1α -hydroxylase activity to PTH in HP. Fukase *et al.* (1982) reported that an *in vitro* serum-free renal cortical cell culture system high phosphate in medium diminished 25-OHD- 1α -hydroxylase activity.

Prominent phosphaturia and an increase in serum $1,25(\text{OH})_2\text{D}$ were seen after dibutyryl cAMP infusion in a patient with

PHP-I, but not after hPTH-(1-34) infusions (Lambert *et al.*, 1980; Unakami *et al.*, 1982). These data suggest that the renal mechanism for the response to PTH is intact distal to the renal adenylate cyclase in PHP-I and diminished response to PTH in PHP-I is due to diminished cAMP synthesis response to PTH (Yamaoka *et al.*, 1981; Unakami *et al.*, 1982). But our investigation also showed that normocalcemic PHP-I with normal serum phosphate has a normal $1,25(\text{OH})_2\text{D}$ productive capacity in response to hPTH-(1-34) despite diminished cAMP production. The mechanism of this finding remains to be elucidated. However in normocalcemic PHP-I, normocalcemia is considered to be maintained by both high PTH and $1,25(\text{OH})_2\text{D}$ levels (our patient, aged 34, iPTH 1.0 ng/ml, and $1,25(\text{OH})_2\text{D}$ 74 pg/ml) (Drezner *et al.*, 1979). In this study, it was shown that $1,25(\text{OH})_2\text{D}$ production to PTH might differ considerably in each case in PHP-I.

There is considerable controversy with regard to serum $1,25(\text{OH})_2\text{D}$, renal 1α -hydroxylase activity and the metabolism of $1,25(\text{OH})_2\text{D}$ in XLH. These issues are very important because of the improvement in therapy for XLH after the introduction of $1\alpha,25(\text{OH})_2\text{D}_3$ and $1\alpha\text{-OH-D}_3$ into clinical use. Glorieux *et al.*, (1980) reported that combined therapy with phosphate and $1\alpha,25(\text{OH})_2\text{D}_3$ for XLH was sufficient to normalize serum $1,25(\text{OH})_2\text{D}$ and to promote trabecular bone mineralization, neither of which was seen during combined therapy with ergocalciferol and phosphate. Seino *et al.*, (1980) reported that high doses of $1\alpha\text{-OH-D}_3$ alone in XLH were very effective in increasing serum $1,25(\text{OH})_2\text{D}$, in curing rickets and in decreasing renal phosphate leak. These results suggest that deficient $1,25(\text{OH})_2\text{D}$ in XLH exacerbates the existing rickets of osteomalacia, which is due mainly to renal phosphate leak. Lyles and Drezner (1982) and Lobaugh and Drezner (1983) reported that decreased serum $1,25(\text{OH})_2\text{D}$ in XLH or XLH mice was due to decreased renal

1 α -hydroxylase activity. Seino *et al.*, (1982) reported increased catabolism of 1,25(OH)₂D in XLH mice and suggested that reduced or normal serum 1,25(OH)₂D for the state of hypophosphatemia in XLH was due in part to this increased catabolism. Seino *et al.* (1983) also reported increased renal 25-OHD-1 α -hydroxylase activity in a patient with XLH. From Seino's observations and our results, it is suggested that renal 1 α -hydroxylase activity in XLH is not reduced, but that there may be abnormalities in its stimulation in the state of hypophosphatemia (Insogna *et al.*, 1983) and in catabolism of 1,25(OH)₂D in XLH.

Demonstration of almost normal 1,25(OH)₂D production to hPTH-(1-34) in patients with Lowe's syndrome and Fanconi's syndrome suggests that alkali therapy is more important than the administration of active vitamin D metabolites in the management of these renal tubular disorders with metabolic acidosis and normal glomerular function. Very recently, Chesney *et al.*, (1984) reported that a basal 1,25(OH)₂D value in patients with renal tubular acidosis was normal, which may support the above statement. But Kawashima *et al.*, (1982) reported that metabolic acidosis induced attenuation of PTH stimulation of 1 α -hydroxylase activity in the proximal convoluted tubule, which was partly due to reduced stimulation of PTH-dependent adenylate cyclase in vitamin-D-deficient rats. Therefore, further research is required to evaluate the vitamin D metabolism in these renal tubular disorders in relation to various stages of the diseases. And 1,25(OH)₂D production in chronic renal failure should be evaluated with regard to a more rational basis for vitamin D treatment.

Although recent reports of direct measurement of mammalian renal 1 α -hydroxylase activity are very interesting (Tanaka and DeLuca, 1981; Lobaugh and Drezner, 1983; Seino *et al.*, 1983), the PTH stimulation test is of great value because one can see

not only the response of the adenylate cyclase system, but also of the effector systems, such as the response of serum 1,25(OH)₂D, urinary phosphate excretion and serum Ca by this stimulation test.

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