Inhibitory Effects of Active Vitamin D Preparations on PTH Secretion in Rats

NOBUO KUGAI*, YOSHINOBU KOIDE, SATOSHI KIMURA**, AND KAMEJIRO YAMASHITA

Institute of Clinical Medicine, University of Tsukuba, Sakura-mura, Ibaraki-ken 305; *The Third Department of Internal Medicine, National Defense Medical College, Tokorozawa-shi, Saitama-ken 359; **Endocrinology Division, National Cancer Center Research Institute, Tsukiji, Tokyo 104

Abstract

To study the effects of various vitamin D preparations on PTH secretion, serum calcium and urinary excretion of cAMP were monitored in conscious perfused rats, and the influences of a bolus iv injection of the preparations on these parameters were examined. Three hours after the administration of 0.25 μg/kg (0.6 nmol/kg) of 1α, 24(OH)2D3, the urinary excretion of cAMP decreased to a level compatible with that of parathyroidectomized (PTX) rats (50% of initial value; p < 0.05) with no change in the concentration of serum calcium (total and ionized). In PTX rats supplemented with bovine PTH (1 U/h), the vitamin D preparation showed no significant effects either on the urinary excretion of cAMP or on serum calcium. These effects were rather specific for active vitamin D preparations, i.e. 1α, 25(OH)2D3 (0.25 μg/kg) and 1αOHD3 (1.25–6.25 μg/kg). However, 24, 25(OH)2D3 (up to 25 μg/kg) had no significant effect on these parameters. These results suggest that, in rats, active vitamin D preparations specifically inhibit PTH secretion without causing a significant increase in the serum calcium concentration, reflecting a direct feedback mechanism between active vitamin D metabolite and the parathyroid glands.

It has been shown that metabolic conversion of vitamin D is required to exhibit its biologic effects (DeLuca and Shnoes, 1976; Norman and Henry, 1974). PTH, as a principal trophic factor, regulates the renal production of the dihydroxylated metabolites of vitamin D, 1α, 25-dihydroxycholecalciferol [1α, 25(OH)2D3] (DeLuca, 1972). On the other hand, the mode of action of vitamin D metabolites has been shown to be analogous to that of classic steroid hormone (Norman, 1979). Assuming a similar feedback mechanism between classic steroid hormone and pituitary trophic hormone, the dihydroxylated metabolites of vitamin D may regulate the activity of the parathyroid gland. Nuclear and cytoplasmic binding components for 1α, 25(OH)2D3 tentatively have been identified on cell-free extracts of parathyroid glands (Brumberg et al., 1975; Henry and Norman, 1975; Hughes and Haussler, 1978). These findings raise the possibility of the existence of a short-loop feedback system involving the metabolites of vitamin D and the secretion of PTH. Such a feedback loop theoretically could serve as
a complement to the negative feedback loop on the parathyroid gland involving calcium and PTH secretion. A number of studies on the effects of vitamin D metabolites on PTH secretion have been carried out both in vivo and in vitro. However, the results of these studies are conflicting. The dihydroxylated metabolites are reported to suppress (Chertow et al., 1975; Chertow et al., 1980; Dietel et al., 1979), stimulate (Canterbury, 1978), or to have no effect (Golden et al., 1980; Llach et al., 1977; Oldham et al., 1979) on PTH secretion. One of the possible reasons for these controversial results is the difference in the radioimmunoassay used to assess the PTH secretion rate. The heterogeneity of PTH exists among different animal species and has been also established in the parathyroid venous effluent (Flueck et al., 1977; Mayer et al., 1979) as well as in the peripheral circulation (Berson and Yalow, 1968) of single animal species. Furthermore, in in vitro studies, nonspecific PTH leakage from cells, or tissues could be another source of error.

In previous report, avoiding these technical and confusing heterogeneity problems in iPTH determination, we had developed a kind of in vivo bioassay system to estimate the PTH secretion from the rate of urinary excretion of cAMP and had demonstrated an inhibitory effect of dihydroxylated vitamin D metabolite, 1α, 25(OH)2D3, and an active preparation of vitamin D, 1α-hydroxycholecalciferol (1αOHD3) on PTH secretion in rats (Kugai et al., 1981a, b).

To further evaluate the specificity of the inhibitory effect of vitamin D preparations on PTH secretion, the effects of another active vitamin D preparation, 1α, 24(R)-dihydroxycholecalciferol [1α, 24(OH)2D3] on PTH secretion were examined in rat, and compared with those of other vitamin D preparations, 1α, 25(OH)2D3, 1αOHD3, and 24(R), 25-dihydroxycholecalciferol [24, 25 (OH)2D3]. A single iv injection of active vitamin D preparations, 1α, 24(OH)2D3 (0.25 μg/kg), 1α, 25(OH)2D3 (0.25 μg/kg), and 1αOHD3 (1.25–6.25 μg/kg) but not 24, 25 (OH)2D3 (up to 25 μg/kg) reduced the urinary excretion of cAMP without causing a significant rise in the level of serum calcium. These results suggest that, in rats, active vitamin D preparations specifically inhibit PTH secretion through a somewhat direct feedback mechanism rather than a rise in the serum calcium concentration.

Materials and Methods

A) Animals

Male Sprague-Dawley rats, weighing about 200 g, were used. They had been maintained on tap water and a standard stock diet containing adequate vitamin D, calcium, and phosphorus, and were fasted overnight before surgery.

B) Continuous Perfusion Experiments

Under ether anesthesia, the surgery for the perfusion study was performed as described previously (Kugai et al., 1981a, b). Briefly, after indwelling a cystostomy catheter (Intramedic PE 260, Clay Adams, Parsippany, N. J.), and heparinized cannula (Intramedic PE 10) in blood vessels (femoral artery and vein), the animal was placed in a Ballman cage.

Surgical parathyroidectomy was performed on some animals concurrently. The animal was continuously (3 ml/h) infused with a defined solution (222 mM glucose, 5 mM CaCl2, 5 mM MgCl2, 20 mM NaCl, and 2.5 mM KCl) through the cannulated femoral vein. In some experiments, bovine PTH (bPTH; TCA powder, Inolex Laboratories, Chicago, IL; 250 U/mg) was used as a supplement to the solution and infused into the parathyroidectomized (PTX) rats. After an equilibration period of 16 h, vehicle (50 μl of 99.5 % ethanol), or vitamin D preparation [1α, 24(OH)2D3; Teijin Pharmaceutical Co., Tokyo, 1αOHD3, 1α, 25(OH)2D3, and 24, 25(OH)2D3; Chugai Pharmaceutical Co., Tokyo, Japan] was administered through the cannulated femoral vein. Blood samples were obtained through the cannulated femoral artery immediately before and 6, 10, and 24 h after the administration. Urine was collected hourly with a fraction collector. Total calcium in the serum
was measured by atomic absorption spectrophotometry (AA 620, Shimadzu, Tokyo, Japan). Ionized calcium in the blood was estimated by a selective calcium ion flow-through electrode (SS 20, Orion Research Inc., Cambridge, MA). Urine samples were stored at -20°C until assayed for cAMP by radioimmunoassay (Cailla et al., 1973; Honma et al., 1977).

Results

Effects of 1α, 24(OH)₂D₃ in intact rats

The effects of a single iv injection of 0.25 µg/kg (0.6 nmol/kg) of 1α, 24(OH)₂D₃ on the serum calcium concentration and urinary excretion of cAMP in intact rats are shown in Fig. 1 (left panel). Serum calcium showed no significant change. Ionized calcium measured in a different series of rats showed no significant change at 6 h (initial, 2.26 ± 0.17 meq/l; 6 h, 2.28 ± 0.18 meq/l, n=4). The initial urinary excretion of cAMP ranged from 6.70 to 10.22 nmol/h. Three hours after the injection of 1α, 24(OH)₂D₃, the urinary excretion of cAMP showed a significant decrease which was sustained for more than 10 h, then recovered toward 24 h.

After the injection of the vehicle, serum calcium and urinary excretion of cAMP showed little and probably non specific change (Fig. 1, right panel).

![Fig. 1. Effects of 1α, 24(OH)₂D₃ (left) and vehicle alone (right) on serum calcium and urinary excretion of cAMP in intact rats. The animals were constantly (3 ml/h) infused with solution (222 mM glucose, 5 mM CaCl₂, 5 mM MgCl₂, 20 mM NaCl, and 2.5 mM KCl). 1α, 24(OH)₂D₃ (0.25 µg/kg) or its vehicle (0.05 ml ethanol) was administered intravenously as indicated by an arrow. The time after the administration of 1α, 24(OH)₂D₃ or its vehicle is indicated on the abscissa. Values are the mean ± SD (n=6). Values which are significantly different from the initial value according to Duncan's multiple range test are indicated (*, p<0.05).]
Effects of 1α, 24(OH)2D3 in PTX rats

The response of serum calcium and urinary excretion of cAMP to an injection of 0.25 μg/kg of 1α, 24(OH)2D3 was studied in PTX rats (Fig. 2). The mean initial serum calcium concentration in these animals was 6.12 ± 0.09 mg/dl, which was significantly less than that in intact rats (p < 0.01). After the injection of 1α, 24(OH)2D3, the mean serum calcium level rose gradually until 24 h, when it increased to the normocalcemic range. The mean initial urinary excretion of cAMP was 2.51 ± 0.84 nmol/h, which was significantly less than that in intact rats (p < 0.01). The mean urinary excretion of cAMP showed little change after the administration of 1α, 24(OH)2D3.

Effects of 1α, 24(OH)2D3 on the urinary cAMP response to PTH infusion

To determine if 1α, 24(OH)2D3 could alter the renal response to PTH, the effects of 1α, 24(OH)2D3 on urinary excretion of cAMP were also studied in PTX rats supplemented with bPTH (1 U/h; Fig. 3). After the equilibration period of 16 h with bPTH infusion, the mean serum concentration of calcium was 9.85 ± 0.31 mg/dl which was not different from that in intact rats. After the injection of 1α, 24(OH)2D3, the mean serum calcium level did not change significantly at 6 h and increased slightly but significantly at 10 h. The mean initial urinary excretion of cAMP was 6.65 ± 0.39 nmol/h which was not significantly different from that in intact rats. After the injection of 1α, 24(OH)2D3, no significant change in...
Table 1. Effects of vitamin D preparations on serum calcium and urinary excretion of cAMP in intact rats.

<table>
<thead>
<tr>
<th>Agent and dose (μg/kg)</th>
<th>Serum Ca</th>
<th>urinary cAMP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 (mg/dl)</td>
<td>6 h (mg/dl)</td>
</tr>
<tr>
<td></td>
<td>0 (nmol/h)</td>
<td>6 h (nmol/h)</td>
</tr>
<tr>
<td>1α, 25(OH)₂D₃ (0.25)</td>
<td>10.2±0.35</td>
<td>9.7±0.30</td>
</tr>
<tr>
<td>1αOHD₃ (1.25)</td>
<td>8.8±1.04</td>
<td>8.9±1.13</td>
</tr>
<tr>
<td></td>
<td>9.4±0.35</td>
<td>9.2±1.06</td>
</tr>
<tr>
<td>24, 25(OH)₂D₃ (2.5)</td>
<td>9.5±0.40</td>
<td>9.2±0.87</td>
</tr>
</tbody>
</table>

Experiments were performed as described in Fig. 1. Values are the the mean±SD of data from three or four rats. (%) refers to percent change from 0 to 6 h. *significant decrease from initial value by paired Student’s t-test (p<0.05).

The urinary excretion of cAMP was observed.

**Effects of various vitamin D preparations in intact rats**

The effects of various vitamin D preparations on the mean serum calcium and the urinary excretion of cAMP at 6 h after the single iv injection in intact rats are summarized in Table 1. The injection of either 1α, 25(OH)₂D₃ (0.25 μg/kg) or 1αOHD₃ (1.25–6.25 μg/kg) reduced the urinary excretion of cAMP without causing a significant increase in the serum calcium concentration. However, 24, 25(OH)₂D₃ had no significant effects on these parameters at tested doses (2.5 and 25 μg/kg) (Table 1 and Fig. 4).

**Discussion**

The present study in rats indicates that the administration of 1α, 24(OH)₂D₃, like 1α, 25(OH)₂D₃ or 1αOHD₃, decreased the urinary excretion of cAMP without causing a significant rise in the serum calcium concentration. Since the measurement of urinary excretion of cAMP had constituted an in vivo bioassay of circulating bioactive PTH (Schmidt-Gayk and Roher, 1973), the change in urinary excretion of cAMP seems to reflect mainly an acute alteration in parathyroid function. Our findings, i.e. 1) the low basal urinary excretion of cAMP in PTX rats, and 2) its recovery to the normal range following constant infusion of bPTH at a dosage estimated to be equivalent to endogenous hormone secretion (Takahashi et al., 1978), again support the specificity to PTH of this measurement. Because 1α, 24(OH)₂D₃ did not change the urinary excretion of cAMP in PTX rats infused with exogenous bPTH, it is concluded that 1α, 24(OH)₂D₃...
does not alter the renal cAMP response to PTH. While \( \alpha \), \( \alpha \alpha \), \( \alpha \alpha \alpha \), \( \alpha \alpha \alpha \alpha \) showed no significant decrease in urinary excretion of cAMP in PTX rats or in PTX rats receiving bPTH supplement, the same dose of \( \alpha \), \( \alpha \alpha \), \( \alpha \alpha \alpha \), \( \alpha \alpha \alpha \alpha \) induced a significant decrease in urinary excretion of cAMP in intact rats. Thus, it is concluded that \( \alpha \), \( \alpha \alpha \), \( \alpha \alpha \alpha \), \( \alpha \alpha \alpha \alpha \) decreased the urinary excretion of cAMP by inhibiting PTH secretion.

The serum calcium level did not change significantly after the administration of \( \alpha \), \( \alpha \alpha \), \( \alpha \alpha \alpha \), \( \alpha \alpha \alpha \alpha \) in intact rats. Ionized calcium also did not change significantly 6 h after the administration of the preparation in intact rats. The gradual increase in the serum calcium concentration after the administration of the agent to PTX rats indicates the possibility of a feedback mechanism involving hypercalcemia on PTH secretion in parathyroid intact animals. However, in PTX rats kept normocalcemic with the bPTH supplement, the hypercalcemic effect of \( \alpha \), \( \alpha \alpha \), \( \alpha \alpha \alpha \), \( \alpha \alpha \alpha \alpha \) was not demonstrated until 10 h and disappeared toward 24 h. Therefore, it is likely that \( \alpha \), \( \alpha \alpha \), \( \alpha \alpha \alpha \), \( \alpha \alpha \alpha \alpha \) acutely (at least until 6 h) inhibited PTH secretion in rats, and this inhibition developed not through the elevation of the serum calcium concentration but in a somewhat more direct manner.

In previous reports, using \( \alpha \), \( \alpha \alpha \), \( \alpha \alpha \alpha \), \( \alpha \alpha \alpha \alpha \) and \( \alpha \alpha \alpha \alpha \), we had demonstrated a similar inhibitory effects of these vitamin D preparations on PTH secretion as \( \alpha \), \( \alpha \alpha \), \( \alpha \alpha \alpha \), \( \alpha \alpha \alpha \alpha \) in bovine parathyroid culture. Using classical biological parameters for vitamin D effects, \( \alpha \), \( \alpha \alpha \) has been reported to be one tenth less active than \( \alpha \), \( \alpha \alpha \), \( \alpha \alpha \alpha \), \( \alpha \alpha \alpha \alpha \) and twice as active as \( \alpha \alpha \alpha \alpha \) (Smith et al., 1982). Since \( \alpha \), \( \alpha \alpha \), \( \alpha \alpha \alpha \), \( \alpha \alpha \alpha \alpha \) exerted more sustained effects than \( \alpha \), \( \alpha \alpha \), \( \alpha \alpha \alpha \), \( \alpha \alpha \alpha \alpha \) in suppressing PTH secretion and in elevating the serum calcium concentration (Kugai et al., 1981 a, b), the apparent discrepancy in the relative effectiveness of these two preparations might be due to differences in methods used in assessing the different biological parameters at different time points. It has also been mentioned that the shorter duration of action of \( \alpha \), \( \alpha \alpha \), \( \alpha \alpha \alpha \), \( \alpha \alpha \alpha \alpha \) than \( \alpha \), \( \alpha \alpha \), \( \alpha \alpha \alpha \), \( \alpha \alpha \alpha \alpha \) must be kept in mind to estimate biological potency (Smith et al., 1982).

A time lag of 3-4 h between the administration of these agents and the marked decrease in the urinary excretion of cAMP, along with the observation of the binding of these agents to the cytosol receptor protein in the parathyroid gland, suggests that the effect of vitamin D preparations was exhibited through a mechanism of action similar to that of other steroid hormones. A similar time lag between the administration of vitamin D metabolites and the decrease in PTH secretion was also reported both in vivo and in vitro (Chertow et al., 1975; Chertow et al., 1980). Assuming that \( \alpha \), \( \alpha \alpha \), \( \alpha \alpha \alpha \), \( \alpha \alpha \alpha \alpha \) is at least as active as \( \alpha \), \( \alpha \alpha \), \( \alpha \alpha \alpha \), \( \alpha \alpha \alpha \alpha \) in a near physiological range in its acute suppressing effect on PTH secretion, these findings are consistent with the observation of Chertow et al. (Chertow et al., 1975) that \( \alpha \), \( \alpha \alpha \), \( \alpha \alpha \alpha \), \( \alpha \alpha \alpha \alpha \) at physiological dose (0.9 nmol/kg, ip) decreased the iPTH concentration with no significant rise in serum calcium concentration in rats.

In bovine parathyroid culture, \( \alpha \), \( \alpha \alpha \), \( \alpha \alpha \alpha \), \( \alpha \alpha \alpha \alpha \) has been reported to be at least 100 times more potent than \( \alpha \), \( \alpha \alpha \), \( \alpha \alpha \alpha \), \( \alpha \alpha \alpha \alpha \) in suppressing PTH secretion (Chertow et al., 1980). In the present study, a relatively high dose of \( \alpha \), \( \alpha \alpha \), \( \alpha \alpha \alpha \), \( \alpha \alpha \alpha \alpha \), up to 100 times
1α, 25(OH)₂D₃, also did not significantly decrease the urinary excretion of cAMP. Considering the 24, 25(OH)₂D₃ to 1α, 25(OH)₂D₃ concentration ratio in the plasma of normal subjects under physiological conditions, the inhibitory effect on PTH secretion seems to be rather specific for the active vitamin D preparations, 1α, 25(OH)₂D₃ and its derivatives, 1αOHD₃ and 1α, 24(OH)₂D₃.

Thus, our findings support the concept of the existence of a feedback mechanism between physiological vitamin D metabolite with biological activity, 1α, 25(OH)₂D₃ and the parathyroid gland. This feedback control system with an active vitamin D metabolite, along with that of serum calcium, could serve as a physiologically significant regulator in calcium homeostasis.

References


