Comparison of acute effects of 1.25- and 24.25-dihydroxy-vitamin D₃ in normal subjects

G. Heynen¹, F. Cornet¹, P. Franchimont¹, S. Gaspar¹, G. Plomteux², G. Cession-Fossion, R. G. Russell⁴ and J. A. Kanis⁴

Radioimmunoassay Laboratory¹, Faculty of Medicine, University of Liège, Belgium, Department of Clinical Chemistry², University of Liège, Belgium, Department of Physiology³, University of Liège, Belgium, Department of Human Metabolism and Clinical Biochemistry⁴, University of Sheffield, England

Abstract. Effects of small iv doses of 1.25-dihydroxy- and 24.25-dihydroxy-vitamin D₃ (1 μg) were studied in 10 normal subjects. Injection of 1.25 (OH)₂D₃ was associated with small but significant increases in plasma calcium and phosphate but plasma levels of immunoreactive parathyroid hormone (iPTH) and calcitonin (iCT) did not change. The administration of 24.25 (OH)₂D₃ was associated with comparable increases in plasma calcium and a small and transient decrease in plasma iPTH. Plasma levels of iCT did not change. 24.25 (OH)₂D₃ also significantly increased glomerular filtration rate and decreased the urinary excretion of noradrenaline, in contrast to 1.25 (OH)₂D₃ which had no effect on these variables. The rapid infusion of calcium significantly decreased levels of iPTH. We conclude that small doses of 1.25 (OH)₂D₃ and 24.25 (OH)₂D₃ have little, if any, direct effect on the secretion of PTH and CT in man.

In many experimental systems, 1.25-dihydroxy-vitamin D₃ (1.25(OH)₂D₃) is the metabolite of vitamin D with the greatest biological activity. Physiological doses of this metabolite (less than 1 μg/day) increase the intestinal absorption of calcium and phosphorus and promote the remineralisation of bone in dietary deficiency of vitamin D and in osteomalacia due to several causes (Deluca & Schnoes 1976; Norman & Henry 1974). A further metabolite of vitamin D is 24.25-dihydroxyvitamin D₃ (24.25(OH)₂D₃), the daily production of which is quantitatively similar to that of 1.25(OH)₂D₃ (Mawer et al. 1975). The physiological role of 24.25(OH)₂D₃ is unclear, but several observations suggest that this metabolite is not without biological activity. Thus, small doses (1–10 μg/day) increase total body retention of calcium in both normal subjects and in patients with chronic renal failure (Kanis et al. 1978). In experimental models, 24.25(OH)₂D₃ stimulates the synthesis of proteoglycans in rabbit cartilage in vitro, and indeed chondrocytes may be a site for extrarenal synthesis of 24.25(OH)₂D₃ (Corvol et al. 1978; Garabedian et al. 1978). The presence of 24.25(OH)₂D₃ may also be necessary for normal mineralization of bone (Ornoy et al. 1978).

The secretion of parathyroid hormone (PTH) and of calcitonin (CT) appears to be primarily regulated in man by the prevailing plasma concentration of calcium (Arnaud et al. 1971; Franchimont & Heynen 1976). However, their secretion rates may also be controlled by other factors such as the adrenergic system (Fischer et al. 1973; Krukeja et al. 1979; Metz et al. 1978) and possibly by vitamin D or its metabolites, particularly 24.25(OH)₂D₃ (Canterbury et al. 1978; Care et al. 1977; Chertow et al. 1975).

The role of these metabolites in the control of the secretion of PTH and CT has not been adequately documented in physiological situations in man. For that reason, we decided to study the acute effects of the iv injection of both 1.25(OH)₂D₃ and 24.25(OH)₂D₃ on PTH and calcitonin secretion in normal man. Since the secretion of both PTH and CT appears to be modified by catecholamines, we...
also studied the urinary excretion rate of both adrenaline and noradrenaline following these infusions.

**Patients and Methods**

**Patients**

Ten male subjects (age 21 to 25 years) were studied under fasting conditions. All patients had normal plasma concentrations of calcium, phosphate, CT and PTH. Each patient received an infusion of 1.25(OH)2D3 and 24.25(OH)2D3 separated by a minimum period of 10 days. The order of administration of the infusions was randomised. Each patient gave informed consent for the study, which had prior approval of the local ethical Committee.

After an overnight fast an indwelling venous catheter was inserted into an antecubital vein for the withdrawal of venous blood and the subject emptied his bladder. Each subject was studied for a control period of 1 h and thereafter received an infusion of either 1.25(OH)2D3 or 24.25(OH)2D3. One µg of the metabolite was dissolved in 1 ml of absolute ethanol and was mixed with 10 ml intralipid. This was infused over 10 min into a forearm vein with the aid of an electric perfusion pump. Subjects were studied for 2 h thereafter. Each subject received 200 ml of distilled water at the start of the test and at hourly intervals. Three 1 h collections of urine were made and plasma samples taken at the start, midway, and the end of the 1 h control period, and 60 and 120 min after the start infusion. In addition, arterial blood pressure and pulse rate was measured in 4 of the subjects at intervals of 30 min.

In order to further characterise the assay for PTH an additional 9 normal subjects received an iv infusion of calcium under fasting conditions. These subjects received 50 µmoles/kg of calcium (as calcium gluconate) as a rapid iv injection. Plasma was withdrawn from the opposite forearm for estimation of calcium and iPTH before and at frequent intervals up to 1 h.

Plasma and urine concentrations of calcium (Gitelman 1967), phosphorus (Hurst 1964; Kraml 1966) and of creatinine (Chasson et al. 1961) were determined by autoanalyser. Plasma levels of immunoreactive PTH (iPTH) were measured by radioimmunoassay using an antiserum with predominantly carboxyterminal specificity. Normal values have a logarithmic distribution, with a mean 2.16 mIU/ml. The coefficient of variation (within-assay) was less than 10% for concentrations of iPTH between 1 and 9 mIU/ml (Heynen et al. 1979). Plasma levels of immunoreactive calcitonin (ICT) were measured by a radioimmunoassay using techniques previously described (Heynen & Franchimont 1974). Normal values of ICT show a logarithmic distribution with a mean value of 191 pg/ml and a range of 60 to 960 pg/ml

### ACUTE EFFECTS OF VITAMIN D METABOLITES IN 10 NORMAL SUBJECTS

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Baseline</th>
<th>0 h</th>
<th>1 h</th>
<th>2 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca m mol/l</td>
<td>2.4 ± 0.2</td>
<td>2.3 ± 0.1</td>
<td>2.2 ± 0.1</td>
<td>2.1 ± 0.1</td>
</tr>
<tr>
<td>P m mol/l</td>
<td>1.6 ± 0.1</td>
<td>1.5 ± 0.1</td>
<td>1.4 ± 0.1</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>PTH m IU/ml</td>
<td>3 ± 0.5</td>
<td>2.5 ± 0.5</td>
<td>2.0 ± 0.5</td>
<td>1.5 ± 0.5</td>
</tr>
<tr>
<td>CT log₁₀ pg/ml</td>
<td>2.6 ± 0.2</td>
<td>2.4 ± 0.2</td>
<td>2.2 ± 0.2</td>
<td>2.0 ± 0.2</td>
</tr>
</tbody>
</table>

**Fig. 1.**

Acute effects of vitamin D metabolites in 10 normal subjects. Both 1.25(OH)2D3 and 24.25(OH)2D3 increased plasma levels of calcium (mean ± se, P < 0.05). There was a small but consistent decrease in plasma iPTH (P < 0.05) following the injection of 24.25(OH)2D3 which was not observed after 1.25(OH)2D3.

620
Table 1.
Mean values (±SEM) of TmP, fasting urinary calcium (CaE), urinary adrenaline and noradrenaline, respectively, during the control period, 1 and 2 after the acute iv infusion of 1 µg or 1.25- and 24.25-dihydroxycholecalciferol.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>1 h</th>
<th>2 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.25</td>
<td>24.25</td>
<td>1.25</td>
</tr>
<tr>
<td>TmP (mmol/l GFR)</td>
<td>1.62 (0.11)</td>
<td>1.71 (0.07)</td>
<td>1.63 (0.13)</td>
</tr>
<tr>
<td>CaE (µmol/l GFR)</td>
<td>24.1 (3.7)</td>
<td>29 (8)</td>
<td>35.5 (7)</td>
</tr>
<tr>
<td>Adrenaline (µmol/µmolCr)</td>
<td>4.07 (0.82)</td>
<td>4.91 (0.9)</td>
<td>5.07 (0.9)</td>
</tr>
<tr>
<td>Noradrenaline (µmol/µmolCr)</td>
<td>16.1 (2.39)</td>
<td>20 (2.11)</td>
<td>15.79 (2.26)</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min)</td>
<td>155 (14)</td>
<td>121 (8)</td>
<td>150 (16)</td>
</tr>
</tbody>
</table>

Significantly different from control value * P < 0.05, ** P < 0.02.

(95% confidence limits on log transformed values). The coefficient of variation (within-assay) was less than 5% for values between 100 and 1000 pg/ml.

Urinary catecholamines were measured by a fluorometric technique (Anton & Sayre 1962). Normal values for subjects in the sitting position are 16.7 ± 1.8 µmol/µmol of creatine for noradrenaline and 4.26 ± 0.6 µmol/µmol of creatine in the case of adrenaline (Juchmes et al. 1976).

Urinary excretion of calcium was expressed as mmol/l of glomerular filtrate (Peacock et al. 1969). The maximum tubular re-absorption of phosphate was estimated from plasma and urine concentration of phosphorus and creatinine (Bijvoet & Morgan 1971) and expressed per unit of glomerular filtration rate (TmP/GFR).

The significance of differences between means was calculated by means of Student's t-test for paired values. For the purpose of comparing plasma values before and during the infusion of the vitamin D metabolites, the mean of the three plasma values taken before infusion was used.

Results

Effects of 1.25(OH)\textsubscript{2}D\textsubscript{3}

Intravenous injection of 1.25(OH)\textsubscript{2}D\textsubscript{3} was associated with a small but significant increase in plasma concentration of calcium and phosphate, whereas plasma levels of iPTH and iCT did not change (Fig. 1). There were also no significant changes in urinary excretion of adrenaline or noradrenaline, in the creatinine clearance nor in the TmP/GFR. Fasting urinary calcium excretion increased from 24.1 µmol ± 3.7 µmol/l to 35.5 µmol ± 7 µmol/l (mean ± se), but this change was not significant (Table 1). Arterial blood pressure and heart rate did not change throughout the infusion.

Effects of 24.25(OH)\textsubscript{2}D\textsubscript{3}

The injection of 1 µg of 24.25(OH)\textsubscript{2}D\textsubscript{3} was associated with a comparable increase in plasma calcium as that seen following 1.25(OH)\textsubscript{2}D\textsubscript{3}. One hour following the injection of 24.25(OH)\textsubscript{2}D\textsubscript{3}, plasma levels of iPTH were significantly lower than those before injection (Fig. 1). The degree of change, however, was very small and values of iPTH obtained 2 h after injection were not significantly different from those at the start of the test. Plasma levels of iCT, urinary excretion of calcium and TmP/GFR did not change (Fig. 1, Table 1).

In contrast to the effects of 1.25(OH)\textsubscript{2}D\textsubscript{3}, the injection of 24.25(OH)\textsubscript{2}D\textsubscript{3} was associated with a significant increase in the glomerular filtration rate and a significant decrease in the urinary excretion of noradrenaline (Table 1). Urinary excretion of adrenaline, arterial blood pressure and heart rate did not change throughout the test.

Effects of intravenous infusion of calcium

The rapid infusion of calcium resulted in a significant fall in plasma levels of iPTH from the 15th min up and to the 30th min following infusion (Fig. 2).

Discussion

Variations in plasma Ca concentrations induced by EDTA or calcium infusions are capable of changing plasma iPTH and iCT levels within a period of 2 h in normal subjects (Franchimont & Heynen 1976). By analogy, the purpose of our study was to investigate if the vitamin D metabolites could also acutely modify these hormone levels. In fact, small
Acute effects of rapid calcium chloride infusion (50 \textmu mol/kg) into 9 normal subjects. PTH concentrations (mIU/ml) significantly decrease ($P < 0.05$) from 15 min up to 45 min after the injection. Ca concentrations were significantly higher from 5 min up to 60 min. But, at this time, PTH concentration was not significantly different from the values of the control period (–30 min to 0 min).

In contrast, experimental observations in vivo and in vitro suggest that these two dihydroxylated metabolites of vitamin D may have direct effects on the secretion of PTH. Thus, receptors for 1.25(OH)$_2$D$_3$ have been identified in parathyroid tissue (Brumbaugh et al. 1975), but there is controversy as to whether 1.25(OH)$_2$D$_3$ increases or decreases the secretion of PTH (Chertow et al. 1975; Dietel et al. 1977; Magliola et al. 1979). Several experimental models suggest that 24.25(OH)$_2$D$_3$ may directly suppress the secretion of PTH acutely and during its long-term administration (Canterbury et al. 1978, 1980; Care et al. 1977).

In man, secondary hyperparathyroidism is commonly reversed by the administration of 1.25(OH)$_2$D$_3$, but this is almost invariably associated with an increase in plasma calcium which may account for the suppression of PTH. The lack of effect of 1.25(OH)$_2$D$_3$ on PTH secretion observed in this study is in agreement with several other observations (Tanaka et al. 1979; Golden et al. 1979). Large doses of 24.25(OH)$_2$D$_3$ in rat have
been reported to decrease plasma iPTH (Nko et al. 1981). In the present study a small but transient suppression of iPTH occurred, though this was less marked than that noted with the infusion of calcium. The daily endogenous production rate for 24.25(OH)2D3 in man is in the order of 1 to 2 μg/day. The dose used in the present study might be considered physiological provided it was infused over a 24 h period. It is likely, therefore, that the acute injection of 1 μg of 24.25(OH)2D3 was associated with supraphysiological levels of 24.25-(OH)2D3 and the transient and modest suppression of PTH noted may have been a pharmacological rather than physiological effect.

The effects of vitamin D metabolites on the secretion of calcitonin have been less intensively studied. Both 1.25(OH)2D3 and 1 alpha (OH)D3 appear to increase plasma levels of iCT within days in normal subjects and patients with Paget's disease (Heynen et al. 1977) and within weeks in patients with chronic renal failure (Kanis et al. 1979; Chantraine et al. 1979). As in the case of PTH secretion, it is difficult to be sure whether these effects are direct or mediated indirectly, perhaps by an increase in the level of plasma calcium. The present results suggest that neither 1.25(OH)2D3 nor 24.25(OH)2D3 directly influence the secretion rate for calcitonin.

Control studies of the effects of intralipid alone were not undertaken. Despite this, it appears that 1.25(OH)2D3 and 24.25(OH)2D3 have differing effects on the glomerular filtration rate. Thus, the infusion of 24.25(OH)2D3 resulted in a significant and sustained increase in glomerular filtration rate associated with a fall in the urinary excretion of noradrenaline but 1.25(OH)2D3 was without effect. This suggests that 24.25(OH)2D3 can modify the sympathetic tone of the kidney or other tissues, and may account for some of the differences between effects of this metabolite in vitro and in vivo.

Both 1.25(OH)2D3 and 24.25(OH)2D3 increase plasma levels of calcium, though it is difficult to be sure that this was an effect of the metabolites since no control studies were undertaken. They were, however, undertaken in the fasting state suggesting that the increment in plasma calcium was due to either increased renal tubular re-absorption of calcium or calcium release from bone. In the case of 1.25(OH)2D3 the small rise in plasma phosphate and the rise in urinary calcium excretion rate is compatible with the latter hypothesis. It is interesting that the rise in plasma calcium following 24.25(OH)2D3 was not associated with a marked rise in fasting urinary calcium excretion rates, raising the possibility that 24.25(OH)2D3 may have increased renal tubular re-absorption for calcium.

Acknowledgments

The authors wish to acknowledge the Belgian F.R.S.M., the Wellcome Trust and the National Kidney Research Fund for their support. They are most grateful to Professor J. Lecomte and Mr. A. Adam for their help in various parts of this work. G. Heynen is Chargé Recherche au F.N.R.S.

References


Received on December 14th, 1980.