The Influence of $24,25(OH)_2D_3$ on the Calcemic Effect of $1,25(OH)_2D$ in Rats with Chronic Renal Failure Is Parathyroid Hormone Dependent (43103)

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Abstract. **Previous studies from our laboratory have shown that $24,25(OH)_2D_3$ attenuates the calcemic effect of $1,25(OH)_2D_3$ in rats with reduced renal mass. This study was undertaken to clarify the role of parathyroid hormone in this response. Adult rats (n = 27) with reduced renal mass after parathyroidectomy with an initial plasma calcium of 3.7 ± 0.1 mEq/liter were divided into four groups: (i) control rats and rats treated with (ii) $24,25(OH)_2D_3$, (iii) $1,25(OH)_2D_3$, and (iv) both 1,25 and $24,25(OH)_2D_3$. After 4 days significant hypercalcemia was seen in PTX animals receiving $1,25(OH)_2D_3$ alone or in combination with $24,25(OH)_2D_3$. Plasma calcium in the combined therapy rats (7.42 ± 0.22 mEq/liter) was significantly higher than in those treated with $1,25(OH)_2D_3$ alone (6.68 ± 0.22 mEq/liter, $P < 0.05$). After 8 days, plasma calcium was higher in the rats treated with $1,25(OH)_2D_3$ but was of same magnitude in those treated with $1,25(OH)_2D_3$ alone or in combination with $24,25(OH)_2D_3$. In contrast, in a subset of rats (n = 35) with reduced renal mass but intact parathyroid glands similarly treated with the vitamin D metabolites, a blunted calcemic response was seen after the combination of $1,25(OH)_2D_3$ with $25,25(OH)_2D_3$ administration alone.**

These results show that in rats with reduced renal mass, $24,25(OH)_2D_3$ attenuates the calcemic effect of $1,25(OH)_2D_3$ only in the presence of intact parathyroid glands. The different calcemic responses to 1,25 or combined 1,25 and $24,25(OH)_2D_3$ in intact or parathyroidectomized rats with chronic renal insufficiency may result from different interaction between the vitamin D metabolites and the parathyroid hormone, presumably at the level of bone. [P.S.E.B.M. 1990, Vol 195]

Previous studies from our laboratory have shown that $24,25(OH)_2D_3$ attenuates the calcemic effect of $1,25(OH)_2D_3$ in rats with reduced renal mass (1, 2). $24,25(OH)_2D_3$ alone did not affect plasma calcium. The blunting of the hypercalcemic effect of $1,25(OH)_2D_3$ by $24,25(OH)_2D_3$ was not explained by changes in urinary calcium (1) or by alterations in the intestinal absorption of calcium (2).

During $24,25(OH)_2D_3$ administration, a significant suppression of plasma iPTH was reported in patients with chronic renal failure and in uremic dogs (3, 4). Thus, we speculated that $24,25(OH)_2D_3$ attenuates the calcemic effect of $1,25(OH)_2D_3$ via a parathyroid hormone related mechanism. The present study was undertaken to clarify the role of parathyroid hormone in this mechanism.

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Materials and Methods

The experiments were performed in white male rats of the Hebrew University strain weighing 200–250 g. The animals were fed a standard pellet chow, with a calcium and phosphorus content of 0.78% and 0.51% of 100 g dry wt, respectively, and rank tap water *ad libitum*.

The reduction of renal mass was induced by 5/6 nephrectomy as described previously (1). Four weeks after surgery the rats were placed in metabolic cages. We have recently shown that at this stage after 5/6 nephrectomy, significant secondary hyperparathyroidism is established. Thus, a 60% increase in parathyroid hormone (PTH) levels and histologic findings of increased bone resorption are presented in rats with re-

duced renal mass as compared with rats with intact kidneys, age and weight method. These results were published elsewhere (5). One day before the study, 52 rates underwent parathyroidectomy, by cauterization under ether anesthesia. After 24 hr, plasma calcium was checked in a venous blood sample in each animal. Twenty-seven animals were found to be hypocalcemic, with a plasma calcium level of 3.7 ± 0.1 mEq/liter. These rats were considered parathyroidectomized (PTX) and were selected for the study.

The PTX rat were divided into four groups: (i) rats receiving 54 ng of $1,25(OH)_2D_3$ in 1,2-propanediol as a single daily subcutaneous injection (n = 8); (ii) rats receiving $24,25(OH)_2D_3$ in the same dose and manner (n = 5); (iii) rats receiving both $1,25(OH)_2D_3$ (54 ng) and $24,25(OH)_2D_3$ (54 ng) in the same manner (n = 8); and (iv) control rats receiving the vehicle 1,2-propanediol only (n = 6). The dosages of 1,25 and $24,25(OH)_2D_3$ were chosen similarly to those used in our previous experiments (1).

Venous blood samples and 24-hr urine collections were obtained at the beginning of the study (Day 0) and on the first, fourth, and eighth days of vitamin D metabolite or vehicle treatment. All blood and urine samples were checked for calcium, phosphorus, and creatinine. An additional subset of nonparathyroidectomized rats with chronic renal failure (n = 35) were subdivided into four similar groups and treated with $1,25(OH)_2D_3$ (n = 9), $24,25(OH)_2D_3$ (n = 7), 1,25 + $24,25(OH)_2D_3$ (n = 11), or propanediol (n = 8) for 8 days. A venous blood sample was drawn from these animals at the end of the experiment and checked for calcium, phosphorus, and creatinine.

Plasma and urinary calcium determinations were performed using an atomic absorption spectrophotometer. Inorganic phosphate and creatinine were measured by an automated technique using the Gilford computer-directed analyzer system 3500 (Gilford, Oberlin, OH).

Vitamin D metabolites were a gift from Hoffman-La Roche and Co. (Basel, Switzerland). Results are presented as mean ± SE. Statistical significance of the data was evaluated by using one-way analysis of variance and the unpaired t test.

Results

Twenty-four hours after the administration of the various metabolites of vitamin D to PTX rats, a mild calcemic effect was seen in animals receiving $1,25(OH)_2D_3$ alone or in combination with $24,25(OH)_2D_3$. In these rats, plasma calcium averaged 4.77 ± 37 and 4.76 ± 0.19 mEq/liter, respectively, as compared with control and $24,25(OH)_2D_3$-treated animals in which plasma calcium was 2.95 ± 0.61 and 3.06 ± 0.43 mEq/liter ($P < 0.05$).

The effects of $1,25(OH)_2D_3$, $24,25(OH)_2D_3$ alone, and in combination on plasma calcium levels and urinary calcium excretion by PTX rats after 4 days of treatment are depicted in Figure 1. Significant hypercalcemia was seen in both the $1,25(OH)_2D_3$ and the $1,25(OH)_2D_3$-$24,25(OH)_2D_3$ groups. Plasma calcium, however, in the animals on combined therapy (7.49 ± 0.22 mEq/liter) was significantly higher than in those treated with $1,25(OH)_2D_3$ alone (6.68 ± 0.22 mEq/liter; $P < 0.05$). Plasma calcium level was similar in control rats and in rats treated with $24,25(OH)_2D_3$. The increments in plasma calcium in the $1,25(OH)_2D_3$ and the combined therapy groups were associated with a marked increase in urinary calcium excretion. urinary calcium excretion was not significantly different between the $1,25(OH)_2D_3$ and the combined $1,25(OH)_2D_3$-$24,25(OH)_2D_3$ groups.

Figure 2 shows the effects after 8 days of $1,25(OH)_2D_3$, $24,25(OH)_2D_3$ alone, and in combination on plasma calcium and urinary calcium excretion in PTX rats and on plasma calcium in animals with chronic renal failure and intact parathyroid glands. After 8 days, plasma calcium was 4.29 ± 0.17 mEq/liter in control and 4.39 ± 0.2 mEq/liter in $24,25(OH)_2$-treated PTX animals. Significant hypercalcemia was seen in both 1,25 and in 1,25 + $24,25(OH)_2D_3$-treated rats.

Plasma calcium was slightly, but not significantly, higher in the PTX group with combined therapy than in the PTX animals treated with $1,25(OH)_2D_3$ alone. The rise in plasma calcium was accompanied by a marked increase in urinary calcium excretion, which was of similar magnitude after $1,25(OH)_2D_3$ or combined 1,25 and $24,25(OH)_2D_3$ therapy.

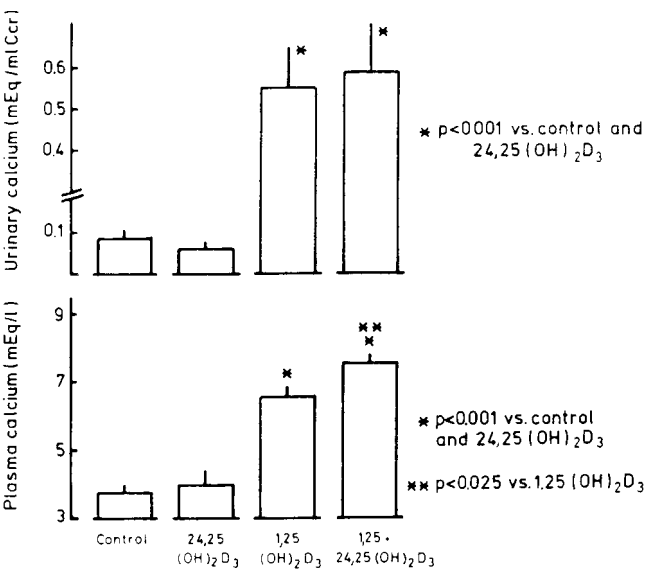

Figure 1. The effects of $1,25(OH)_2D_3$, $24,25(OH)_2D_3$, and $1,25(OH)_2D_3$ + $24,25(OH)_2D_3$ on plasma calcium level and urinary excretion of calcium in PTX rats with reduced renal mass after 4 days. * $P < 0.001$ vs control and $24,25(OH)_2D_3$; ** $P < 0.025$ vs $1,25(OH)_2D_3$.

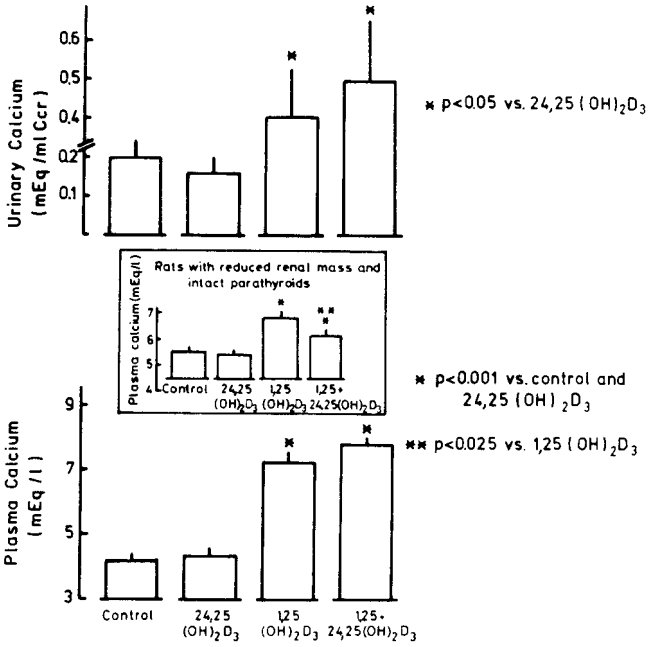

Figure 2. The effects of $1,25(OH)_2D_3$, $24,25(OH)_2D_3$, and $1,25(OH)_2D_3$ + $24,25(OH)_2D_3$ on plasma calcium level and urinary excretion of calcium in PTX rats with reduced renal mass after 8 days. In the inner square, plasma calcium level in a subset of nonparathyroidectomized rats with reduced renal mass which were divided into four similar groups and treated in the same dose and manner with the vitamin D metabolites or propanediol. * $P < 0.05$ vs $24,25(OH)_2D_3$ (upper panel) and $P < 0.001$ vs control and $24,25(OH)_2D_3$ (inner square and lower panel); ** $P < 0.025$ vs $1,25(OH)_2D_3$ (inner square).

In contrast, in a subset of rats with chronic renal insufficiency and intact parathyroid glands treated with the same amounts of vitamin D (Fig. 2, inner square), plasma calcium was lower in those treated with the combination 1,25 + $24,25(OH)_2D_3$ (6.19 ± 0.18 mEq/liter) than in rats treated with $1,25(OH)_2D_3$ alone (6.86 ± 0.22 mEq/liter, $P < 0.025$). In this subset, again, $24,25(OH)_2$ administration alone did not affect plasma calcium.

Table I shows the body weight, the water consumption, urine volume, plasma creatinine, creatinine clearance, and fractional excretion of phosphate in the PTX rats treated with vitamin D derivatives for 8 days. Body weight and water consumption tended to be lower in all $1,25(OH)_2D_3$-treated animals, while the urine flow was greatest after combined 1,25 + $24,25(OH)_2D_3$ administration. Plasma creatinine increased and the creatinine clearance decreased significantly in the hypercalcemic animals, but there were no differences between the rats treated with $1,25(OH)_2D_3$ alone or in combination with $24,25(OH)_2D_3$. The fractional excretion of phosphate increased similarly with both $1,25(OH)_2D_3$ or combined 1,25 + $24,25(OH)_2D_3$ therapy.

Discussion

This study shows that in rats with reduced renal mass $24,25(OH)_2D_3$ attenuates the calcemic effect of $1,25(OH)_2D_3$ only in the presence of intact parathyroid tissue. In parathyroidectomized rats with chronic renal insufficiency, $24,25(OH)_2$ administration enhanced the calcemic response to $1,25(OH)_2D_3$ after 4 days of combined administration. After 8 days of treatment, plasma calcium in the rats treated with $1,25(OH)_2D_3$ alone was similar to that in rats receiving the combination of $1,25(OH)_2D_3$ and $24,25(OH)_2D_3$. $24,25(OH)_2D_3$ alone did not significantly affect plasma calcium in parathyroidectomized rats with reduced renal mass.

The response of PTX rats with renal insufficiency to the vitamin D metabolites is similar to that observed previously in our laboratory in animals with normal renal function and intact parathyroids or after parathyroidectomy. In these animals, an enhanced calcemic effect was found after combined administration of 1,25 and $24,25(OH)_2D_3$. The enhancement of the calcemic effect of $1,25(OH)_2D_3$ by $24,25(OH)_2D_3$ in rats with normal renal function was attributed to a decrease in the urinary excretion of calcium (6, 7).

The interaction of 1,25 and $24,25(OH)_2D_3$ and its effect on plasma calcium in rats with reduced renal mass with and without parathyroid glands is not well understood.

The difference in the calcemic response to the combination of vitamin D metabolites in parathyroidectomized rats with reduced renal mass if compared with rats with intact parathyroids also cannot be attributed to variations in the urinary calcium excretion which was similar in animals receiving 1,25 alone or in combination with $24,25(OH)_2D_3$ to changes in the intestinal absorption of calcium. We have previously shown (2) that $24,25(OH)_2D_3$ administration does not modify the fractional absorption of ^{45}Ca in animals with chronic renal failure. Even though the experiments described above were performed in animals with intact parathyroids, we have no reason to assume that the response will be different in similar but PTX animals.

Taken together, our previous and present data support a role for bone as a possible interaction site between PTH and vitamin D metabolites, particularly $24,25(OH)_2D_3$. The different calcemic effects of 1,25 and 1,25 combined with $24,25(OH)_2D_3$ observed in our experiments in intact versus PTX animals with reduced renal mass may indicate different bone responses to the metabolites mentioned above, in the presence and absence of PTH.

Both PTH and vitamin D play a crucial role in bone metabolism. PTH, a potent resorption hormone, had been recently shown to affect also bone formation (8–10). It is generally accepted that $1,25(OH)_2D_3$ stimulates osteoclastic bone resorption and promotes bone mineralization (8, 11). Although the biologic role of $24,25(OH)_2$ is controversial, in the last decade, however, an important role in bone formation was attributed to $24,25(OH)_2D_3$ (12).

Table I. Body Weight, Water Consumption, Urine Output, Fractional Excretion of Phosphate, Plasma Creatinine, and Creatinine Clearance in PTX Rats with Reduced Renal Mass Treated with Vitamin D Derivatives for 8 Days

	Control	$24,25(OH)_2D_3$	$1,25(OH)_2D_3$	$1,25 + 24,25(OH)_2D_3$
Body weight (g)	275 ± 18.2	271 ± 11	245 ± 14	237 ± 16
Water consumption (ml/24 hr)	32.2 ± 6.9	33 ± 6.7	38 ± 5.9	32 ± 1.9
Urine output (ml/24 hr)	18.7 ± 3	18.72 ± 1.4	27.4 ± 4.3	27.4 ± 2.9[a]
Fractional excretion of phosphate (CP/CCr)	0.017 ± 0.005	0.032 ± 0.011	0.313 ± 0.022[b]	0.305 ± 0.04[b]
Plasma creatinine (μmol/liter)	80 ± 3.9	77.3 ± 1.6	100.9 ± 9.6[c]	101.4 ± 3.7[b]
Creatinine clearance (ml/min)	0.79 ± 0.03	0.77 ± 0.03	0.52 ± 0.08[d]	0.43 ± 0.05[e]
n	6	5	8	8

[a] $P < 0.025$ vs $24,25(OH)_2D_3$.
[b] $P < 0.005$ vs control and $24,25(OH)_2D_3$.
[c] $P < 0.05$ vs $24,25(OH)_2D_3$.
[d] $P < 0.01$ vs control and $24,25(OH)_2D_3$.
[e] $P < 0.001$ vs control and $24,25(OH)_2D_3$.

Little is known by certain investigators on the need for the role of parathyroid hormone in the modulation of the skeletal actions or vitamin D metabolites. Several lines of evidence suggest that parathyroid hormone may influence both the resorptive and bone-forming effects of vitamin D metabolites.

Thus, in the study of Pavlovitch *et al.* (13) different responses by 1,25 and $24,25(OH)_2D_3$ were elicited in bilaterally nephrectomized rats with intact parathyroids and in parathyroidectomized animals. In animals with parathyroids, $24,25(OH)_2D_3$ administration was associated with a blunting of the calcemic effect of $1,25(OH)_2D_3$ and a reduction in the osteoclastic count. In parathyroidectomized rats, however, $24,25(OH)_2$ did not affect the calcemic effect of $1,25(OH)_2D_3$ and did not modify the count of osteclasts (13). These experiments show first that $24,25(OH)_2D_3$ may alter bone resorption in bilaterally nephrectomized rats with intact parathyroids given $1,25(OH)_2D_3$; and, second, that this effect is different in animals lacking parathyroid hormone.

Endo *et al.* (14), in an *in vitro* culture system using chicken embryo femora, showed that PTH is essential for the calcification of bone. Maximal stimulation of bone calcification was seen in their experiment with the combination of 1,25 and $24,25(OH)_2D_3$ in the presence of PTH, as compared with media containing both metabolites but lacking PTH (13).

In a recent study performed in vitamin D replete rats, the bone mineral apposition rate which decreased by 50% after PTX could be restored to normal by treatment with PTH but not with $24,25(OH)_2D_3$ alone. Simultaneous treatment with PTH and $24,25(OH)_2D_3$ led to a greater increase in bone mineral apposition rate than with PTH alone (15).

All of the data reported above support the concept that $24,25(OH)_2D_3$ plays a critical role in the regulation of bone formation and/or mineralization and demonstrate interactions among PTH, $1,25(OH)_2D_3$, and $24,25(OH)_2D_3$ in bone.

Our study showing that $24,25(OH)_2D_3$ blunts the calcemic effects of $1,25(OH)_2D_3$ in rats with chronic renal insufficiency and intact parathyroids, but not after parathyroidectomy, emphasizes the importance of PTH in the action of $24,25(OH)_2D_3$ and point to the bone as the site of interaction. The nature of the skeletal effects of $24,25(OH)_2D_3$ and its relationships with $1,25(OH)_2D_3$ and PTH remain to be elucidated.

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