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24,25$(OH)_2D_3$ Attenuates the Calcemic Effect of 1,25$(OH)_2D_3$ in Rats with Reduced Renal Mass (42585)

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Abstract. The present study was undertaken to evaluate the effect of 24,25$(OH)_2D_3$ on serum calcium concentration in rats with reduced renal mass. Adult 5/6 nephrectomized male rats were divided into four groups: (i) control rats, (ii) rats treated with 1,25$(OH)_2D_3$, (iii) rats treated with 24,25$(OH)_2D_3$, and (iv) rats treated with 1,25$(OH)_2D_3$ and 24,25$(OH)_2D_3$. After 4 days, serum calcium in the 1,25$(OH)_2D_3$-treated group was 7.13 ± 0.32 meq/liter ($P < 0.001$ vs control). With the combination of 1,25$(OH)_2D_3$ and 24,25$(OH)_2D_3$ serum calcium was higher than that in control, 6.25 ± 0.5 meq/liter ($P < 0.001$ vs control), but lower than that in rats receiving 1,25$(OH)_2D_3$ alone ($P < 0.05$). No change in serum calcium was seen in animals treated with 24,25$(OH)_2D_3$ alone. On the eighth day serum calcium in the 1,25$(OH)_2D_3$-treated group, 6.52 ± 0.25, was higher than in the 1,25$(OH)_2D_3$ + 24,25$(OH)_2D_3$ group, 5.87 ± 0.17 meq/liter, $P < 0.05$, $P < 0.001$ vs control. In both 1,25$(OH)_2D_3$- and 1,25$(OH)_2D_3$ + 24,25$(OH)_2D_3$-treated rats, hypercalciuria of similar magnitude occurred on the fourth and eighth day of treatment. No change in urinary calcium was seen in the control and 24,25$(OH)_2D_3$-treated rats. Thus, in 5/6 nephrectomized rats combined administration of 1,25$(OH)_2D_3$ and 24,25$(OH)_2D_3$ attenuates the calcemic response to 1,25$(OH)_2D_3$ without changes in urinary calcium excretion. These observations suggest that the effect of 24,25$(OH)_2D_3$ on serum calcium is different in 5/6 nephrectomized rats as compared to normal rats, in which an augmentation of serum calcium was observed following administration of both vitamin D metabolites. The effect of 24,25$(OH)_2D_3$ on serum calcium in rats with reduced renal mass may result from a direct effect of 24,25$(OH)_2D_3$ on the bone.

1,25$(OH)_2D_3$, the active vitamin D metabolite, exerts its calcemic action by stimulating the intestinal transport and skeletal mobilization of calcium in normal individuals and laboratory animals as well as in uremic patients or animals with experimental renal disease (1, 2). In contrast, administration of 24,25$(OH)_2D_3$ alone does not alter plasma calcium (2–4). Clinical and experimental evidence suggests that 24,25$(OH)_2D_3$ may enhance bone mineralization with no detectable change in plasma calcium level (2–5). Furthermore, a single dose of 24,25$(OH)_2D_3$ had been shown to attenuate the calcemic effect of 1,25$(OH)_2D_3$ and to suppress hypercalcemia in acutely uremic rats after bilateral nephrectomy (6).

A previous study in our laboratory, however, showed that combined administration of 1,25- and 24,25$(OH)_2D_3$ to normal rats results in enhanced hypercalcemia, as compared with administration of 1,25$(OH)_2D_3$ alone. It was suggested that the augmentation of the calcemic effect of 1,25$(OH)_2D_3$ in normal rats might be caused by a hypocalciuric effect of 24,25$(OH)_2D_3$ (7).

The present study was undertaken to evaluate the effect of the administration of 24,25$(OH)_2D_3$ and its interaction with 1,25$(OH)_2D_3$ in rats with reduced renal mass with respect to plasma calcium and urinary calcium excretion.

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

The reduction of renal mass was induced by 5/6 nephrectomy. The first step, 2/3 right nephrectomy, was followed by removal of the contralateral kidney 1 week later. In this model, after 3 weeks the creatinine clearance averaged 0.5–0.6 ml/min (about 60%) of the normal range and the fractional excretion of phosphate was 25–35% (higher than in normal rats). Four weeks after the second nephrec-

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tomy the animals were housed in metabolic cages for several days for acclimatization. At the beginning of the study (Day 0) the rats with reduced renal mass were divided into four groups: (i) rats receiving a daily subcutaneous injection of 54 ng of $1,25(OH)_2D_3$ in 1,2-propanediol (in preliminary experiments this dosage of $1,25(OH)_2D_3$ was found to induce hypercalcemia in rats); (ii) rats receiving $24,25(OH)_2D_3$ in the same dose and manner; (iii) rats receiving both $1,25(OH)_2D_3$ and $24,25(OH)_2D_3$ in the same dose and manner; and (iv) control rats receiving the vehicle 1,2-propanediol only. Samples (0.5 ml) of venous blood were drawn from each rat and 24-hr urine collections were performed at the beginning of the study (Day 0) and on the first, fourth, and eighth days of vitamin D metabolites and/or vehicle treatment.

All blood and urine samples were checked for calcium, phosphorus, and creatinine. Serum and urinary calcium determinations were performed using an atomic absorption spectrophotometer.

Inorganic phosphate was determined spectrophotometrically as phosphomolybdate after reduction with 70% ascorbic acid.

Creatinine was determined by the picric acid method. These solutes were measured with an automated technique using the Gilford 3500 computer-directed analyzer system (Gilford; Oberlin, Ohio).

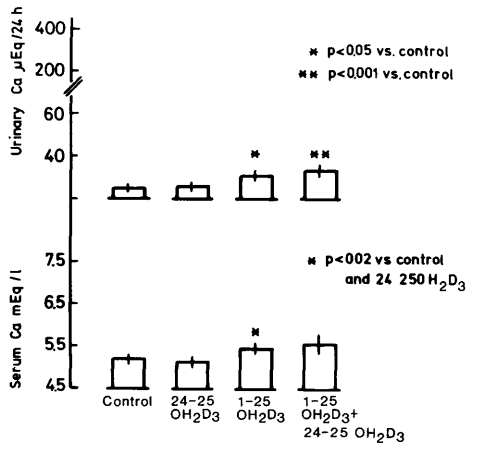

FIG. 1. The effects of $1,25(OH)_2D_3$, $24,25(OH)_2D_3$, and $1,25(OH)_2D_3$ + $24,25(OH)_2D_3$ on serum concentration and urinary excretion of calcium in rats with reduced renal mass after 24 hr.

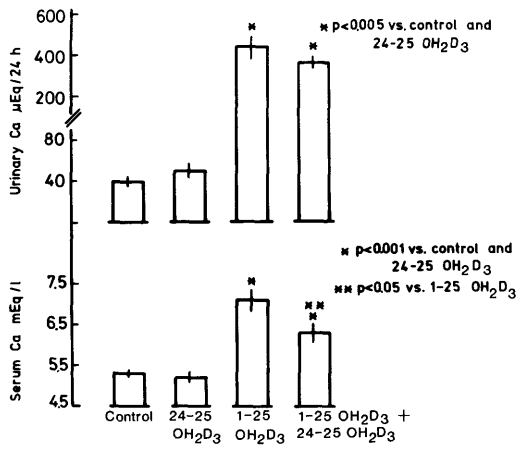

FIG. 2. The effects of $1,25(OH)_2D_3$, $24,25(OH)_2D_3$, and $1,25(OH)_2D_3$ + $24,25(OH)_2D_3$ on serum concentration and urinary excretion of calcium in rats with reduced renal mass after 4 days.

Vitamin D metabolites were a gift from Hoffman–LaRoche and Co. (Basel, Switzerland). Results are presented as means ± SE. The statistical significance was evaluated using analysis of variance and the paired and unpaired Student *t* test.

Results. The serum calcium level and the urinary excretion of calcium in control and experimental animals after 24 hr of administration of the various vitamin D metabolites are shown in Fig. 1. The serum calcium level in the $24,25(OH)_2D_3$-treated group was similar to the level of the control animals. A mild calcemic effect was seen in both $1,25(OH)_2D_3$- and $24,25(OH)_2D_3$-treated animals. Thus, serum Ca in the $1,25(OH)_2D_3$ group was 5.48 ± 0.07 meq/liter as compared to 5.16 ± 0.09 and 5.08 ± 0.12 meq/liter in the control and $24,25(OH)_2D_3$-treated animals, respectively ($P < 0.05$). The calcium level in the combined 1,25- and $24,25(OH)_2D_3$ group, while higher than in control animals (5.57 ± 0.17 meq/liter), was not significantly different. A mild but significant calciuric effect was seen in both $1,25(OH)_2D_3$ and combined 1,25- and $24,25(OH)_2D_3$ groups.

Figure 2 shows the serum levels and urinary calcium excretion in the four groups of animals after 4 days of treatment with vitamin D metabolites. Serum calcium remained un-

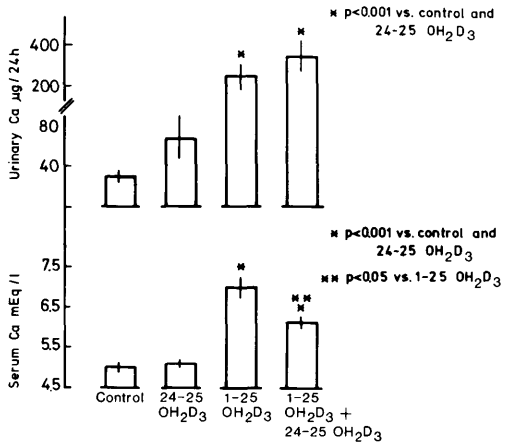

FIG. 3. The effects of 1,25(OH)$_2$D$_3$, 24,25(OH)$_2$D$_3$, and 1,25(OH)$_2$D$_3$ + 24,25(OH)$_2$D$_3$ on serum concentration and urinary excretion of calcium in rats with reduced renal mass after 8 days.

changed in the control and 24,25(OH)$_2$D$_3$ animals. In contrast, significant hypercalcemia was seen in the 1,25(OH)$_2$D$_3$ and in the combined group. Serum calcium, however, in the combined therapy animals (6.27 ± 0.15 meq/liter) was lower than that in 1,25(OH)$_2$D$_3$ (7.13 ± 0.32 meq/liter, $P < 0.05$).

After 8 days of administration, hypercalcemia persisted in both 1,25(OH)$_2$D$_3$ and 1,25(OH)$_2$D$_3$ + 24,25(OH)$_2$D$_3$ groups (Fig. 3). Again, serum calcium was lower in animals receiving both metabolites than in those receiving 1,25(OH)$_2$D$_3$ alone (5.93 ± 0.20 vs 6.82 ± 0.029 meq/liter, $P < 0.05$).

The urinary calcium excretion was significantly increased in the hypercalcemic groups. The increased urinary calcium excretion was seen on Days 4 and 8 of the experiment. No difference, however, was seen between the 1,25(OH)$_2$D$_3$ and the combined 1,25(OH)$_2$D$_3$ and 24,25(OH)$_2$D$_3$ groups.

The plasma creatinine, creatinine clearance, and the phosphate clearance did not change significantly during the experimental period. Table I shows the effect of vitamin D metabolites on fractional excretion of phosphate (C_p/C_{Cr}) in the four groups of animals. C_p/C_{Cr} remained stable in the control and 24,25(OH)$_2$D$_3$ groups. On the eighth day C_p/C_{Cr} in the groups receiving 1,25(OH)$_2$D$_3$ or the combined therapy was slightly higher than in 24,25(OH)$_2$D$_3$-treated rats, but similar to that of the control animals.

The rats treated with 1,25(OH)$_2$D$_3$ or the combination drank significantly more than did those from the other two experimental groups. Daily food intake did not differ between the four groups studied.

Discussion. The results of the present study show that as previously demonstrated in normal animals, the administration of 1,25(OH)$_2$D$_3$ significantly increases serum calcium level and augments urinary calcium excretion. The administration of 24,25-(OH)$_2$D$_3$ alone was not associated with

TABLE I. THE EFFECT OF VITAMIN D METABOLITES ON FRACTIONAL EXCRETION OF PHOSPHATE (C_p/C_{Cr}) IN 5/6 NEPHRECTOMIZED RATS

	Day			
Metabolite	0	1	4	8
1,25(OH)$_2$D$_3$	25.53 ± 2.31 (n = 16)	24.9 ± 2.11 (n = 9)	33.50 ± 3.64 (n = 7)	33.39 ± 2.87* (n = 16)
24,25(OH)$_2$D$_3$	22.90 ± 1.94 (n = 10)	33.53 ± 5.97 (n = 8)	27.30 ± 2.39 (n = 9)	22.44 ± 3.03 (n = 8)
1,25(OH)$_2$D$_3$ + 24,25(OH)$_2$D$_3$	25.91 ± 3.86 (n = 15)	29.23 ± 4.91 (n = 6)	29.07 ± 2.41 (n = 6)	40.66 ± 3.83* (n = 14)
Control	21.98 ± 2.79 (n = 12)	32.70 ± 3.84 (n = 6)	25.33 ± 3.17 (n = 6)	32.05 ± 3.81 (n = 7)

Note. n, number of rats.
* $P < 0.02$ vs 24,25(OH)$_2$D$_3$

changes in serum calcium or the urinary excretion of calcium. In contrast, combined $1,25(OH)_2D_3$ and $24,25(OH)_2D_3$ resulted in significant hypercalcemia and hypercalciuria.

After the first day of treatment serum calcium rose similarly higher in the groups receiving $1,25(OH)_2D_3$ or both metabolites. After 4 and 8 days, however, serum calcium was significantly lower in the animals receiving the combined therapy as compared with those receiving $1,25(OH)_2D_3$ alone.

The urinary excretion of calcium increased in both groups receiving $1,25(OH)_2D_3$ therapy and remained unchanged in the control and $24,25(OH)_2D_3$-treated animals. There was no difference in the urinary calcium excretion between the animals receiving $1,25(OH)_2D_3$ and those receiving both 1,25- and $24,25(OH)_2D_3$.

The attenuation of the calcemic effect of $1,25(OH)_2D_3$ by $24,25(OH)_2D_3$ in rats with reduced renal mass is similar to the results reported by Pavlovitch *et al.* in anephric rats after 16 hr of combined treatment with both metabolites (6). It is also consistent with the clinical studies in hemodialysis patients in whom $24,25(OH)_2D_3$ diminished plasma calcium (3). In contrast, this effect is opposite to that reported in normal rats in which $24,25(OH)_2D_3$ was shown to enhance the calcemic response to $1,25(OH)_2D_3$ (7).

The understanding of the mechanism of attenuation of the calcemic effects of $1,25(OH)_2D_3$ by $24,25(OH)_2D_3$ requires an evaluation of several proposed effects of the metabolites. An increased renal excretion of calcium by $24,25(OH)_2D_3$ as a possible explanation can be ruled out for several reasons. First, in the animals treated with $24,25(OH)_2D_3$ alone no significant change in urinary calcium excretion was seen during the experiment. Second, there was no difference in the urinary calcium excretion between the animals treated with $1,25(OH)_2D_3$ and those treated with both 1,25- and $24,25(OH)_2D_3$ despite different serum calcium levels. It is worthwhile to mention that in normal rats, $24,25(OH)_2D_3$ was shown to diminish the urinary excretion of calcium, when administered together with $1,25(OH)_2D_3$.

A diminished intestinal absorption of calcium due to $24,25(OH)_2D_3$ treatment as a possible mechanism of the attenuation of hypercalcemia is possible but unlikely since $24,25(OH)_2D_3$ was shown to increase the calcium absorption in uremic patients, and in normal and vitamin D-deficient rats (2, 4, 5, 8).

Previous investigations reported suppression of plasma iPTH during therapy with $24,25(OH)_2D_3$ in patients with chronic renal failure and in uremic dogs (9, 10). Thus, a direct effect on 24,25-dihydroxycholecalciferol on the parathyroid glands could be the underlying mechanism of the diminished hypercalcemia of 1,25 by $24,25(OH)_2D_3$ seen in the rats with reduced renal mass. A parathyroid hormone-mediated effect, however, would be expected to induce changes in urinary phosphate excretion. In our experiments, no change in fractional excretion of phosphate was seen after $24,25(OH)_2D_3$ administration. A mild but similar phosphaturic effect was seen in the last day of follow-up in both groups receiving $1,25(OH)_2D_3$ and combined 1,25- and $24,25(OH)_2D_3$. Therefore, a parathyroid hormone-mediated effect seems unlikely in these experiments. Furthermore, $1,25(OH)_2D_3$ rather than $24,25(OH)_2D_3$ has been shown recently to suppress PTH synthesis (11).

Another possible explanation of the variance between the effects of combined treatment in the rats with reduced renal mass as compared with intact animals will imply an alteration of the degradative metabolism of $1,25(OH)_2D_3$ by $24,25(OH)_2D_3$. In that case the circulating $1,25(OH)_2D_3$ levels would be actually reduced in the animals treated with both metabolites. Since no measurement of circulating vitamin D metabolites was performed, this possibility cannot be entirely ruled out.

A direct action of $24,25(OH)_2D_3$ on bone is an acceptable explanation of the attenuation of the calcemic effect of $1,25(OH)_2D_3$ by the metabolite. This effect is consistent with those previously reported in *in vivo* and *in vitro* experiments, in which it was suggested that $24,25(OH)_2D_3$, under certain conditions, may act as an anabolic hormone of the bone (9, 12).

The nature of the direct effect of $24,25(OH)_2D_3$ on bone is not clearly understood. Pavlovitch *et al.* had shown that pre-

treatment with $24,25(OH)_2D_3$ prevents the increase in the number of osteoclasts usually observed in bones of bilateral nephrectomized rats with intact parathyroid glands. In parathyroidectomized animals in that study, however, despite a similar decrease of the calcemic effect of $1,25(OH)_2D_3$ by $24,25(OH)_2D_3$, no change was seen in the number of osteoclasts (6).

Another possible mechanism would be an indirect effect of $24,25(OH)_2D_3$ via calcitonin. Calcitonin administration was shown to inhibit osteoclastic bone response, while $24,25(OH)_2D_3$ enhanced plasma calcitonin level in nephrectomized rats and in vitamin D-deficient pigs (6, 13, 14). In a previous study $1,25(OH)_2D_3$ was also shown to increase plasma calcitonin similarly to $24,25(OH)_2D_3$ (13). Recent evidence, however, showed that $1,25(OH)_2D_3$ selectively suppressed calcitonin synthesis determined as mRNA and a mild inhibitory effect was also seen with $24,25(OH)_2D_3$ (15).

Thus, if calcitonin would be affected by the vitamin D metabolites, its effect would be difficult to determine in the groups treated with $1,25(OH)_2D_3$ or with combined 1,25- and $24,25(OH)_2D_3$.

A puzzling observation is the opposite effect of $24,25(OH)_2D_3$ in rats with reduced renal mass as compared with normal animals treated with $1,25(OH)_2D_3$. This difference could be related to the original vitamin D status of the experimental animals. Despite the fact that the endogenous plasma level of $1,25(OH)_2D_3$ was not measured in our experiments, it is probable that because of the decrease in renal mass the animals had been mildly $1,25(OH)_2D_3$ deficient. Recent reports stressed the simulating effects of $24,25(OH)_2D_3$ on bone and cartilage mineralization and the synergistic effects of 1,25- and $24,25(OH)_2D_3$ on bone development in the rats fed a vitamin D deficient diet (16). This interaction between these two metabolites could be more pronounced in rapid bone turnover as is present in uremic rats.

In conclusion, our study demonstrates that $24,25(OH)_2D_3$ attenuates the calcemic effect of $1,25(OH)_2D_3$ probably by a bone-mediated mechanism. Whether this is a direct effect, calcitonin mediated, or dependent on the original vitamin D status of the experimental animals remains to be determined.

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