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$24,25(OH)_2D_3$ Enhances the Calcemic Effect of $1,25(OH)_2D_3$ (42167)

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Abstract. The effect of $24,25(OH)_2D_3$ on $1,25(OH)_2D_3$-induced hypercalcemia was studied in normal rats. Serum (S) levels and urinary excretion of Ca^{2+} ($U_{Ca}V$) were measured in (a) control rats, (b) rats receiving a daily sc injection of 54 ng $1,25(OH)_2D_3$, (c) rats receiving $24,25(OH)_2D_3$ in the same dose and same manner, and (d) rats receiving $1,25(OH)_2D_3$ + $24,25(OH)_2D_3$. The animals were housed in metabolic cages and 24-hr urine specimens were collected. After 24 hr SCa^{2+} increased similarly with $1,25(OH)_2D_3$ and with $1,25(OH)_2D_3$ + $24,25(OH)_2D_3$, while $24,25(OH)_2D_3$ alone did not change SCa^{2+}. $U_{Ca}V$ after 24 hr increased significantly less ($P < 0.025$) with $1,25(OH)_2D_3$ + $24,25(OH)_2D_3$ than with $1,25(OH)_2D_3$ alone. After 5 days of $1,25(OH)_2D_3$, SCa^{2+} rose from 5.1 ± 0.15 to 6.29 ± 0.08 whereas $1,25(OH)_2D_3$ + $24,25(OH)_2D_3$ effected a greater increase in SCa^{2+} up to 6.63 ± 0.09 ($P < 0.01$). $24,25(OH)_2D_3$ alone did not change SCa^{2+}. $U_{Ca}V$ after 5 days of treatment rose similarly with $1,25(OH)_2D_3$ and with $1,25(OH)_2D_3$ + $24,25(OH)_2D_3$. After 10 days of $1,25(OH)_2D_3$ SCa^{2+} was 6.17 ± 0.15 meq/liter while with the combination SCa^{2+} rose to 6.74 ± 0.2 ($P < 0.025$). $24,25(OH)_2D_3$ alone did not change SCa^{2+}. These results show that (a) $24,25(OH)_2D_3$ alone does not alter SCa^{2+} in normal rats, (b) combined administration of $1,25(OH)_2D_3$ + $24,25(OH)_2D_3$ enhances the hypercalcemic response to $1,25(OH)_2D_3$ without a parallel increase in $U_{Ca}V$, and (c) it is suggested that the effect of $24,25(OH)_2D_3$ on serum Ca^{2+} level, at least partly, may result from its hypocalciuric effect.

The metabolite $1,25(OH)_2D_3$, the active form of vitamin D, is more potent than $25(OH)D_3$ both in enhancing the gut transport and in augmenting the skeletal mobilization of calcium (1, 2). In contrast to $25(OH)D_3$ and $1,25(OH)_2D_3$, both of which exhibit a calcemic action, $24,25(OH)_2D_3$ does not alter the serum calcium concentration (3, 4). Experimental evidence has been advanced implying that $24,25(OH)_2D_3$ may act to augment bone formation and bone mineralization (5–7).

In a previous study by Pavlovitch *et al.* (8) a single dose of $24,25(OH)_2D_3$ has been shown to suppress the hypercalcemia induced by acute bilateral nephrectomy and to attenuate the hypercalcemic effect of $1,25(OH)_2D_3$ in acutely nephrectomized rats.

The present study was undertaken to examine the effect of $24,25(OH)_2D_3$ on the hypercalcemia induced by $1,25(OH)_2D_3$ in intact rats.

Materials and Methods. White male rats of the Hebrew University strain weighing 200–250 g were studied.

Twenty-four rats of comparable age and weight were housed in metabolic cages and fed Purina pellet chow and tap water *ad libitum* for several days for acclimatization. On the first day of the study the rats were divided into four groups:

(1) Control rats receiving the vehicle 1,2 propanediol sc only.

(2) Rats receiving a daily sc injection of 54 ng $1,25(OH)_2D_3$ in 1,2 propanediol.

(3) Rats receiving $24,25(OH)_2D_3$ in the same dose and same manner.

(4) Rats receiving $1,25(OH)_2D_3$ + $24,25(OH)_2D_3$ in the same dose and same manner.

Urine output and food intake were measured at 24-hr intervals for 5 consecutive days and blood was drawn at the end of 1 and 5 days. In an additional group of 24 rats the same experiment was performed for 10 days and blood was drawn at the end of 10 days. Urinary data for 10 days are not presented. In a separate group of 24 rats a control experiment of the same design as detailed above was performed for 5 days except that $25(OH)D_3$ was used instead of $24,25(OH)_2D_3$. Blood was drawn at the end of 5 days.

Ca^{2+}, P_i, and creatinine excretion rates in 24-hr urine collections and in plasma were measured.

Calcium was determined by the *o*-cresolphthalein complexone method. Inorganic

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phosphate was determined spectrophotometrically as phosphomolybdate after reduction with 10% ascorbic acid. Creatinine was determined by the picric acid method. All these solutes were measured with an automated technique using the Gilford Computer-Directed Analyzer System 3500 (Gilford, Oberlin, Ohio). Serum albumin was measured by the albumin color reagent from Sigma Chemical Company.

Vitamin D metabolites were a gift of Hoffman–La Roche and Company, Basel, Switzerland. Results are presented as means ± SE and compared by the Student t test.

Results. The effect of $1,25(OH)_2D_3$ and $24,25(OH)_2D_3$ and their combination on serum Ca^{2+} levels and urinary Ca^{2+} excretion after 24 hr is depicted in Fig. 1. Already after 24 hr $1,25(OH)_2D_3$ caused a significant increase in serum Ca^{2+} level, $P < 0.005$. The same increase in serum Ca^{+} was observed with $1,25(OH)_2D_3$ + $24,25(OH)_2D_3$. $24,25(OH)_2D_3$ alone did not change serum Ca^{2+} levels. $1,25(OH)_2D_3$ brought about a threefold increase in urinary Ca^{2+} excretion from 42 to 132 μeq/24 hr. The combined administration

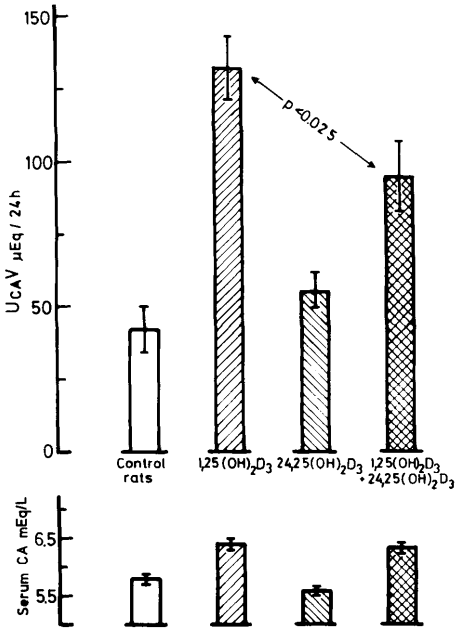

FIG. 1. The effect of $1,25(OH)_2D_3$, $24,25(OH)_2D_3$, and $1,25(OH)_2D_3$ + $24,25(OH)_2D_3$ on urinary excretion and serum concentration of calcium in intact rats after 24 hr.

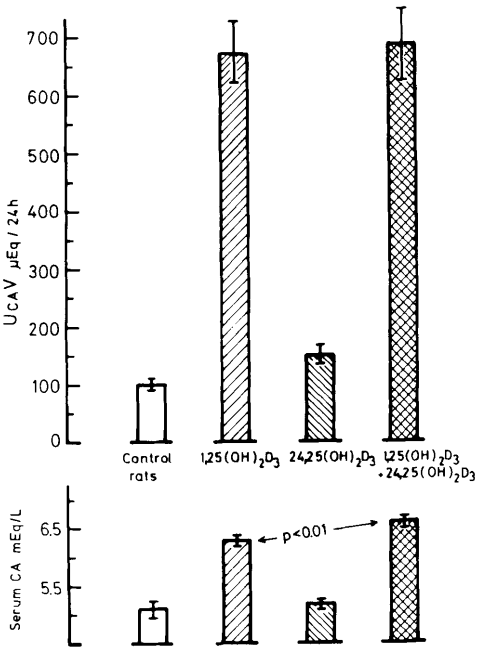

FIG. 2. The effect of $1,25(OH)_2D_3$, $24,25(OH)_2D_3$, and $1,25(OH)_2D_3$ + $24,25(OH)_2D_3$ on urinary excretion and serum concentration of calcium in intact rats after 5 days.

of $1,25(OH)_2D_3$ + $24,25(OH)_2D_3$ was also accompanied by an increase in urinary Ca^{2+} excretion, but this increase was significantly smaller than that observed with $1,25(OH)_2D_3$ alone ($P < 0.025$).

It should be noted that the fall in urinary Ca^{2+} excretion, caused by the addition of $24,25(OH)_2D_3$ to $1,25(OH)_2D_3$ compared to $1,25(OH)_2D_3$ alone, was not due to decreased serum Ca^{2+} level, which as mentioned above was the same as with the administration of $1,25(OH)_2D_3$ alone. $24,25(OH)_2D_3$ alone did not significantly change urinary Ca^{2+} excretion.

The effect of $1,25(OH)_2D_3$, $24,25(OH)_2D_3$, and their combination on serum Ca^{2+} levels and urinary Ca^{2+} excretion after 5 days is depicted in Fig. 2. After 5 days $1,25(OH)_2D_3$, as expected, caused a rise in serum Ca^{2+}. This increase was significantly augmented by the combined administration of $24,25(OH)_2D_3$ + $1,25(OH)_2D_3$ ($P < 0.01$). $24,25(OH)_2D_3$ alone did not change serum Ca^{2+} levels.

$1,25(OH)_2D_3$ brought about a marked increase in urinary Ca^{+} excretion. The combined administration of $24,25(OH)_2D_3$

+ $1,25(OH)_2D_3$ causes the same increase in urinary Ca^{2+} excretion as $1,25(OH)_2D_3$ alone in spite of the greater increase observed in serum Ca^{2+}.

The effect of $1,25(OH)_2D_3$, $24,25(OH)_2D_3$, and their combination on serum Ca^{2+} levels after 10 days is depicted in Fig. 3. Similarly to what occurred after 5 days, also after 10 days the increment in serum Ca^{2+} caused by the combination of $24,25(OH)_2D_3$ + $1,25(OH)_2D_3$ was significantly greater than that caused by $1,25(OH)_2D_3$ alone while $24,25(OH)_2D_3$ alone did not change serum Ca^{2+}. In a separate experiment two additional groups receiving either 54 ng/day of $25(OH)D_3$ or a combination of $1,25(OH)_2D_3$ + $25(OH)D_3$ were studied for 5 days. Serum calcium was measured after 5 days. The control value was 5.6 ± 0.1. With $25(OH)D_3$ alone serum calcium was 5.7 ± 0.1, not different from control. With $1,25(OH)_2D_3$ + $25(OH)D_3$ serum calcium was 6.8 ± 0.07, not different from $1,25(OH)_2D_3$ alone 6.8 ± 0.2.

No significant changes in creatinine clearance were observed after 24 hr or 5 days of treatment with vitamin D metabolites. In all cases the clearances ranged between 0.9 and 1.1 ml/min which represent normal values for rats of about 200 g.

Fractional excretion of phosphate after 24 hr and 5 days of vitamin D metabolite administration is shown in Table I. After 24 hr fractional excretion of phosphate was similar in all groups. After 5 days of treatment with $1,25(OH)_2D_3$ and with the combination of $1,25(OH)_2D_3$ + $24,25(OH)_2D_3$, marked and similar phosphaturia was observed ($P < 0.01$). $24,25(OH)_2D_3$ alone causes a mild but significant phosphaturic effect ($P < 0.05$). There were no measurable changes in serum levels of phosphate after 24 hr or 5 days of treatment with vitamin D metabolites.

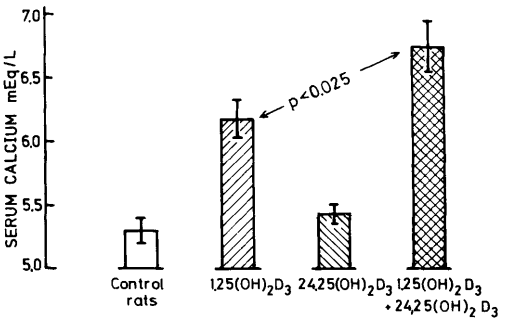

FIG. 3. The effect of $1,25(OH)_2D_3$, $24,25(OH)_2D_3$, and $1,25(OH)_2D_3$ + $24,25(OH)_2D_3$ on serum calcium concentration in intact rats after 10 days of treatment.

Serum albumin concentrations and daily food intake did not differ between the four groups studied.

Discussion. The results of the present study show that $24,25(OH)_2D_3$ enhances the hypercalcemic response to $1,25(OH)_2D_3$ while $24,25(OH)_2D_3$ alone does not change serum calcium concentration in intact rats.

After 5 and 10 days of vitamin D metabolite administration serum calcium concentration was significantly higher with the combination of $24,25(OH)_2D_3$ + $1,25(OH)_2D_3$ as compared to $1,25(OH)_2D_3$ alone while $24,25(OH)_2D_3$ did not change serum calcium concentration. Since serum albumin concentrations were identical in all groups studied, the rise observed in serum calcium could not be attributed to changes in albumin concentration. Urinary calcium excretion after 5 days in rats receiving $1,25(OH)_2D_3$ was similar to that in rats receiving the combination in spite of the differences in serum calcium concentration (Fig. 2) and the similarity in GFR. These results could be interpreted as reflecting a tendency toward increased tubular reabsorption of calcium in rats treated with the combination of $24,25(OH)_2D_3$ + $1,25(OH)_2D_3$ compared to $1,25(OH)_2D_3$ alone. This interpretation is supported by the results after 24 hr of vitamin D metabolite administration. After 24 hr serum calcium concentration increased similarly with $1,25(OH)_2D_3$ and $24,25(OH)_2D_3$ while urinary calcium excretion in rats receiving the combination rose significantly less than in those receiving $1,25(OH)_2D_3$ alone. The inhibition of PTH caused by the enhanced hypercalcemia could further contribute to increased Ca^{2+} excretion in the group treated by the combination of $24,25(OH)_2D_3$ + $1,25(OH)_2D_3$. Yet, Ca^{2+} excretion was similar in both groups.

The augmentation of the calcemic effect of $1,25(OH)_2D_3$ by $24,25(OH)_2D_3$ is contrary to the results shown by Pavlovitch *et al.* (8) in anephric rats after 16 hr of combined treatment with $24,25(OH)_2D_3$ + $1,25(OH)_2D_3$.

One could speculate that because of the presence of normal kidneys in our experiment $24,25(OH)_2D_3$ was converted to

TABLE I. FRACTIONAL EXCRETION OF PHOSPHATE (%) 24 hr AND 5 DAYS AFTER VITAMIN D METABOLITE ADMINISTRATION

	Control	$1,25(OH)_2D_3$	$24,25(OH)_2D_3$	$1,25(OH)_2D_3$ + $24,25(OH)_2D_3$
24 hr	5.6 ± 1.1	5.6 ± 1.35	6.4 ± 2.8	6.6 ± 1.2
5 Days	8.3 ± 1.0	25.7 ± 6.1	13.8 ± 2.1	24.0 ± 2.5

$1,24,25(OH)_3D_3$ and acted additively to $1,25(OH)_2D_3$. The arguments against this consideration are

(1) If $24,25(OH)_2D_3$ were converted to $1,24,25(OH)_2D_3$, one could expect an independent calcemic response similar to $1,25(OH)_2D_3$ which did not occur in our experiments (14, 15).

(2) The administration of $1,25(OH)_2D_3$ by itself inhibits directly the 1-hydroxylase and thus would inhibit the conversion of $24,25(OH)_2D_3$ to $1,24,25(OH)_3D_3$ (9).

(3) The increased calcium concentration induced by vitamin D metabolite treatment is known to inhibit PTH secretion which is an important determinant of 1-hydroxylation (10).

(4) $24,25(OH)_2D_3$ may suppress directly PTH secretion independent of changes in extracellular calcium concentrations (11–13).

Thus the enhancement by $24,25(OH)_2D_3$ of the hypercalcemia induced by $1,25(OH)_2D_3$ may be attributed to $24,25(OH)_2D_3$ itself rather than to its metabolite $1,24,25(OH)_3D_3$. Our present findings that $24,25(OH)_2D_3$ by itself does not change serum calcium concentration is in agreement with previous studies (3, 4, 8).

An increase in circulating PTH is known to increase tubular reabsorption of calcium. However, in the present study, the expected suppression of PTH due to enhanced hypercalcemia by the combination of $24,25(OH)_2D_3$ and $1,25(OH)_2D_3$ would tend to increase and not to decrease urinary Ca excretion. Thus changes in PTH cannot be considered as contributing to the observed changes in urinary calcium in relation to changes in serum calcium.

An additional factor needs consideration. Because alterations in calcitonin levels may in direct proportion affect urinary excretion of calcium, a possible rise in the level of the hormone following the observed rise in serum calcium could certainly increase the urinary excretion whereas in our experiment an opposite response, if any, was the case. Our results, however, are not sufficient to draw any conclusions regarding the role of calcitonin.

The combined treatment with $1,25(OH)_2D_3$ + $25(OH)D_3$ did not cause changes similar to those observed with $1,25(OH)_2D_3$ + $24,25(OH)_2D_3$. Thus, the administration of twice the total amount of vitamin D compounds could not play a role in the augmentation of the hypercalcemic effect of $1,25(OH)_2D_3$. Regardless of the mechanism underlying the augmentation of hypercalcemia it appears that it is specific to $24,25(OH)_2D_3$ since it could not be demonstrated with $25(OH)D_3$. The nature of this response remains unknown. However, an involvement of a renal hypocalciuric mechanism warrants further experiments.

1. DeLuca HF, Schnoes HK. Metabolism and mechanism of action of Vitamin D. Annu Rev Biochem **45**: 631–666, 1976.
2. Favus MJ. Vitamin D physiology. Med Clin North Amer **62**:1291–1317, 1978.
3. Llach F, Brickman AS, Singer FR, Coburn JW. 24,25 dihydroxycholecalciferol, a vitamin D sterol with qualitatively unique effects in uremic man. Metab Bone Dis Relat Res **2**:11–15, 1979.
4. Miravet L, Redel J, Qucille ML, Bordier P. The biological activity of synthetic 24,26-dihydroxycholecalciferol and 24,25-dihydroxycholecalciferol in vitamin D deficient rats. Calcif Tissue Res **21**:145–152, 1976.
5. Bordier P, Rasmussen H, Marie P, Miravet L, Quenis J, Ryckwaert A. Vitamin D metabolites and bone mineralization in man. J Clin Endocrinol Metab **46**: 284–294, 1978.
6. Kanis JA, Cundy T, Bartlett M, Smith R, Heyen G, Warner GT, Russell RGG. Is 24,25 dihydroxycholecalciferol a calcium regulating hormone in man? Brit Med J **1**:1382–1386, 1978.
7. Russell RGG, Kanis JA, Smith R, Adams ND, Bartlett M, Cundy T, Cochran M, Heynen G, Warner, GT.

Physiological and pharmacological aspects of 24,25 dihydroxycholecalciferol in man. Adv Exp Med Biol **103**:487–503, 1978.

8. Pavlovitch JH, Cournet-Witman G, Bourdeau A, Balsan S, Fischer JA, Heynen G. Suppressive effects of 24,25-dihydroxycholecalciferol on bone resorption induced by acute bilateral nephrectomy in rats. J Clin Invest **68**:803–810, 1981.
9. Cork DJ, Hansslen NR, Pitt MJ, Rizzardo E, Hesse RH, Pecket MH. 1 Hydroxyvitamin D_3: A synthetic sterol which is highly active in preventing rickets in the chick. Endocrinology **94**:1337–1345, 1974.
10. Henry HL, Midgett RJ, Norman AW. Regulation of 25-hydroxyvitamin D_3-1-hydroxylase in vivo. J Biol Chem **249**:7584–7592, 1974.
11. Care AD, Bates FL, Pickard DW, Peakock M, Tomlinson S, O'Riardau JLH, Nawen EB, Taylor CM, DeLuca HF, Norman AW. The effect of vitamin D metabolites and their analogues on the secretion of parathyroid hormone. Calcif Tissue Res **21**:142–146, 1977.
12. Canterbury JM, Lerman S, Chafdlin AJ, Henry H, Norman AW, Reiss E. Inhibition of parathyroid hormone secretion by 25-hyroxycholecalciferol and 24,25 dihydroxycholecalciferol in the dog. J Clin Invest **61**: 1375–1383, 1978.
13. Canterbury JM, Gavelass G, Bourgoignies JJ, Reiss E. Metabolic consequences of oral administration of 24,25 dihydroxycholecalciferol to uremic dogs. J Clin Invest **65**:571–576, 1980.
14. Haussler MR, McCain TA. Basic and clinical concepts related to vitamin D metabolism and action. N Engl J Med **297**:974–983, 1977.
15. Castillo L, Tanaka Y, DeLuca HF, Ikekawa N. On the physiological role of 1,24,25-trihydroxyvitamin D_3. Min Electrolyte Metab **1**:198–207, 1978.

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