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$24,25(OH)_2D_3$ Enhances the Calcemic Effect of $1,25(OH)_2D_3$ in PTX Rats (42444)

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Abstract. The effect of $24,25(OH)_2D_3$ on $1,25(OH)_2D_3$-induced hypercalcemia was studied in parathyroidectomized (PTX) rats for 10 days. Serum (S) and urinary Ca excretion ($U_{Ca}V$) 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 $1,25(OH)_2D_3$ + $24,25(OH)_2D_3$. Our results show that (i) $24,25(OH)_2D_3$ alone does not increase $S_{Ca^{2+}}$ in PTX rats, (ii) 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 $U_{Ca}V$, (iii) combined administration of $1,25(OH)_2D_3$ + $24,25(OH)_2D_3$ reduces the rise in urinary excretion of Ca^{2+} compared with that of rats receiving $1,25(OH)_2D_3$ alone for 10 days, and (iv) these alterations are independent of parathyroid hormone.

In a previous study from this laboratory $24,25(OH)_2D_3$ has been shown to enhance the hypercalcemic response to $1,25(OH)_2D_3$, but $24,25(OH)_2D_3$ alone did not change serum calcium concentration in intact rats (1). The enhancement of hypercalcemia by $24,25(OH)_2D_3$ in the presence of $1,25(OH)_2D_3$ was not paralleled by similar changes in urinary excretion of calcium. It was speculated, therefore, that the hypercalcemic effect of $24,25(OH)_2D_3$ under the above circumstances could at least partly be attributed to its hypocalciuric effect. $25(OH)D_3$ did not cause changes similar to those observed with $24,25(OH)_2D_3$ when given in combination with $1,25(OH)_2D_3$. The present study was designed to characterize further the hypercalcemic and possible hypocalciuric effect of $24,25(OH)_2D_3$ in the presence of $1,25(OH)_2D_3$ and to clarify the role of parathyroid hormone in this response.

Materials and Methods. White male rats of the Hebrew University strain, weighing 200–250 g, were studied. Twenty-eight 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 underwent parathyroidectomy by cauterization under ether anesthesia, and were divided into four groups: (a) control rats receiving the vehicle 1,2 propanediol sc only, (b) rats receiving a daily sc injection of 54 ng $1,25(OH)_2D_3$ in 1,2 propanediol, (c) rats receiving $24,25(OH)_2D_3$ in the same dose and same manner, and (d) rats receiving $1,25(OH)_2D_3$ + $24,25(OH)_2D_3$ in the same dose and same manner.

Urine output and food intake were measured at 24-hr intervals for 10 consecutive days, and blood was drawn at the end of 10 days. Urine was drained into plastic bags attached to the bottom frame of the metabolic cages. The collecting bags had two compartments separated by fine perforations that allowed passage of urine into the lower compartment and trapped the feces in the upper one; thus there was a complete separation of urine from the feces. This arrangement minimizes the contamination and evaporization of the urine. When the collection was completed, the lower tip of the urine compartment was cut with scissors and the urine was drained into a calibrated cylinder. Food intake was measured by preweighing the food container and weighing it again after 24 hr. The food container is designed to enable its detachment from the metabolic cage and also collection of the crumbs of the purina pellets in a special attached drawer. Ca^{2+}, P_i, and creatinine were measured by an automated technique using the Gilford computer-directed analyzer system 3500 (Gilford, Oberlin, Ohio).

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 effects of $1,25(OH)_2D_3$, $24,25(OH)_2D_3$, and their combination on serum Ca^{2+} levels and urinary Ca^{2+} excretion by PTX rats after 10 days are depicted in Fig.

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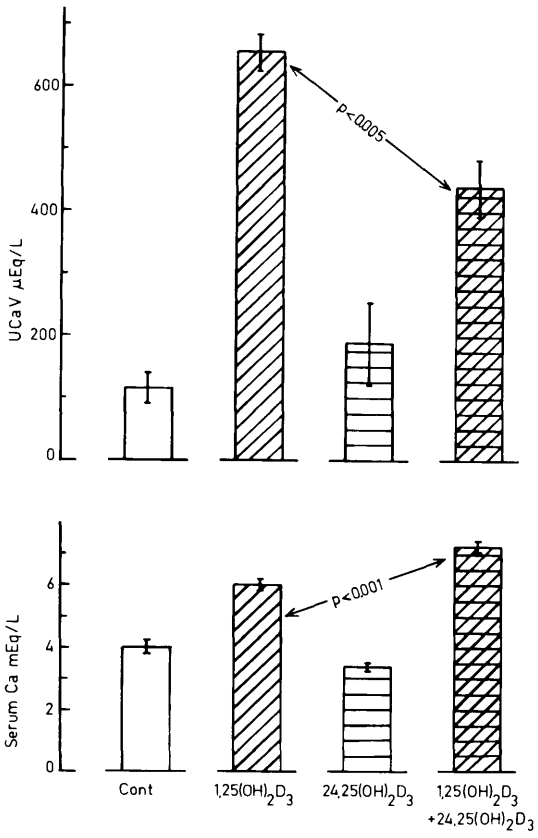

FIG. 1. The effect of $1,25(OH)_2D_3$, $24,25(OH)_2D_3$, or $1,25(OH)_2D_3$ + $24,25(OH)_2D_3$ on urinary excretion and serum concentration of calcium in PTX rats after 10 days. Each bar represents the mean of seven determinations.

1. After 10 days $1,25(OH)_2D_3$ + $24,25(OH)_2D_3$ induced a significantly greater increase in serum Ca^{2+} than $24,25(OH)_2D_3$ alone ($P < 0.001$). The level of serum Ca^{2+} reached by $1,25(OH)_2D_3$ alone in PTX rats was not different from the level reached in intact rats (1). $24,25(OH)_2D_3$ alone did not increase serum Ca^{2+}. With $1,25(OH)_2D_3$ there was a marked increase in urinary Ca^{2+} excretion after 10 days. With $1,25(OH)_2D_3$ + $24,25(OH)_2D_3$ urinary Ca^{2+} excretion was significantly less than with $1,25(OH)_2D_3$ alone ($P < 0.005$).

Table I shows food intake, water consumption, urine output and phosphate excretion, and serum creatinine in control rats and rats treated with vitamin D derivatives for 10 days. Food intake did not vary among the four groups studied. Water consumption was increased in the groups treated with $1,25(OH)_2D_3$ or the combination, but there was no difference between these two groups. Phosphate excretion increased with $1,25(OH)_2D_3$ and $1,25(OH)_2D_3$ + $24,25(OH)_2D_3$ but there was no difference between them.

Serum creatinine was increased significantly with $1,25(OH)_2D_3$, but there was no difference in serum creatinine between the rats treated with $1,25(OH)_2D_3$ or the combination.

Discussion. The results of the present study demonstrate that $24,25(OH)_2D_3$ enhances the hypercalcemic response to $1,25(OH)_2D_3$ in PTX rats, but $24,25(OH)_2D_3$ alone does not increase serum calcium concentration. Similar response has been demonstrated by us in intact rats (1). In our previous study (1), decreased calcium excretion by rats given $1,25(OH)_2D_3$ + $24,25(OH)_2D_3$ compared with that of rats given $1,25(OH)_2D_3$ was observed only after 24 hr when the increment in serum calcium was still similar with $1,25(OH)_2D_3$ + $24,25(OH)_2$-

TABLE I. FOOD INTAKE, WATER CONSUMPTION, URINE OUTPUT, PHOSPHATE EXCRETION, AND SERUM CREATININE IN CONTROL RATS AND RATS TREATED WITH VITAMIN D DERIVATIVES FOR 10 DAYS

	Control	$1,25(OH)_2D_3$	$24,25(OH)_2D_3$	$1,25(OH)_2D_3$ + $24,25(OH)_2D_3$
Food intake (g)	22.9 ± 1.1	21.4 ± 1.3	22 ± 1.7	22.4 ± 1.2
Water consumption (ml)	25.9 ± 1.9	39.4 ± 3.1*	22.1 ± 0.7	42.8 ± 4.0*
Urine output (ml)	19.1 ± 1.9	24.4 ± 1.7	17.9 ± 3.4	25.1 ± 1.9
Phosphate excretion (μeq/24 hr)	216 ± 38	715 ± 105*	200 ± 57	741 ± 49*
Serum creatinine (μmol/liter)	64.4 ± 2.0	74.0 ± 3.0*	67.8 ± 1.5	69.2 ± 1.7
n	7	7	7	7

* $P < 0.025$ compared to control.

D_3 and $1,25(OH)_2D_3$. After 5 days when serum calcium was already significantly higher with the combination compared with that of rats given $24,25(OH)_2D_3$ alone, urinary excretion of calcium was identical in both groups. These results were interpreted by us as a tendency toward increased reabsorption of Ca^{2+} in the renal tubules of rats treated with the combination compared with that of rats given $1,25(OH)_2D_3$ alone.

In the present study, after 10 days when serum calcium with the combination was significantly higher than that with $1,25(OH)_2D_3$ alone, the hypercalciuria with the combination was markedly and significantly attenuated compared with that of rats given $1,25(OH)_2D_3$ alone ($P < 0.005$). The decrease in urinary excretion of calcium due to $1,25(OH)_2D_3$ + $24,25(OH)_2D_3$ compared with that of rats given $1,25(OH)_2D_3$ alone may not be ascribed to a decrease in filtered calcium because serum creatinine did not vary among these groups and indicates similar and normal renal function. These results strengthen the contention that $24,25(OH)_2D_3$ in the presence of $1,25(OH)_2D_3$ may induce a hypocalciuric effect, through increased Ca^{2+} reabsorption by the renal tubule. This effect seems to be independent of parathyroid hormone because it was demonstrated in PTX rats.

Increased intestinal calcium absorption as an explanation of the observed hypercalcemic response of $24,25(OH)_2D_3$ in the presence of $1,25(OH)_2D_3$ may be ruled out for several reasons. First, even though $24,25(OH)_2D_3$ has been shown to enhance intestinal absorption of calcium (3, 4), the amount required for such an action is 20 times greater than the dose of $1,25(OH)_2D_3$ with a similar effect. Second, if the response to $24,25(OH)_2D_3$ would be accrued from increased intestinal absorption, one would expect a commensurate increase in urinary excretion of calcium rather than hypocalciuria as observed in the present study. And third, if the response to $24,25(OH)_2D_3$ were derived from increased intestinal absorption, one would expect an increase in serum calcium after the administration of $24,25(OH)_2D_3$ alone; however, the only single change observed with $24,25(OH)_2D_3$ alone was a decrease in serum calcium after 10 days.

The increase in serum calcium brought about by $1,25(OH)_2D_3$ and $1,25(OH)_2D_3$ + $24,25(OH)_2D_3$ may stimulate calcitonin secretion. The increase in calcitonin may directly stimulate urinary excretion of calcium. Thus the hypercalciuria seen in these groups of rats may be ascribed at least in part to increased calcitonin levels. The decreased excretion of calcium observed with the administration of $1,25(OH)_2D_3$ + $24,25(OH)_2D_3$ compared with the administration of $1,25(OH)_2D_3$ alone, however, cannot be ascribed to changes in calcitonin, because calcitonin would be expected to be higher in the group receiving $1,25(OH)_2D_3$ + $24,25(OH)_2D_3$, and this in turn would increase rather than decrease calcium excretion.

A hypocalciuric effect of $24,25(OH)_2D_3$ was demonstrated in the present and in a previous study (1) in intact and PTX rats, whereas, Pavlovitch *et al.* (2) demonstrated that $24,25(OH)_2D_3$ suppressed the hypercalcemia induced by acute bilateral nephrectomy. These results and ours suggest that $24,25(OH)_2D_3$ plays a role in the conservation of body calcium. In nephrectomized rats, $24,25(OH)_2D_3$ induces a hypocalcemic effect, possibly by storing calcium in the bone. In normal rats, $24,25(OH)_2D_3$ is hypocalciuric and in this manner induces the conservation of calcium.

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