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Influence of Vitamin D Status and Chronic Administration on the Renal Tubular Effects of 1,25-Dihydroxy Vitamin D_3 (41708)

HELEN GEORGAKI AND JULES B. PUSCHETT

Renal-Electrolyte Divisions, Department of Medicine, Presbyterian-University and Veterans Administration Hospitals and the University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15261

Abstract. Previous studies from this laboratory have demonstrated that the acute infusion of the vitamin D_3 derivative, 1,25-dihydroxy vitamin D_3 ($1,25(OH)_2D_3$) is antiphosphaturic when administered with small "permissive" amounts of parathyroid hormone (PTH). To determine the effect of chronic administration of this metabolite, studies were performed in both vitamin D-deficient (−D) and vitamin D-fed (+D) rats which had been pretreated with $1,25(OH)_2D_3$ for 6 days and then infused with either the metabolite alone, the metabolite with a small "permissive" dose of parathyroid hormone (PTH) or neither. The results indicate that in the −D animals, $1,25(OH)_2D_3$ infusion alone results in antiphosphaturia without the presence of PTH. However, in the +D rats, PTH was required, despite $1,25(OH)_2D_3$ pretreatment, for the acute infusion of the metabolite to enhance phosphate reabsorption. Thus, both vitamin D and parathyroid status are important in determining the effect of $1,25(OH)_2D_3$ on the renal tubular transport of phosphate.

Considerable information has now accumulated on the subject of the renal transport effects of vitamin D. In a series of studies published from this laboratory, an antiphosphaturic action of the vitamin and its biologically active metabolites has been demonstrated in both dog and rat (1–6). All of these experiments were performed acutely (that is, over several hours), and the investigations in rats were all performed in D-deficient animals (5, 6). It was determined that either parathyroid hormone (PTH) or exogenous antidiuretic hormone (ADH) is required for the enhancement of phosphate reabsorption induced by the D-metabolites, 25-hydroxy vitamin D_3 ($25(OH)D_3$) and 1,25-dihydroxy vitamin D_3 ($1,25(OH)_2D_3$) to become manifest. In addition, the amount of the metabolite required to cause this alteration in phosphate transport has been gradually lowered, so that an acute action of $1,25(OH)_2D_3$ has now been seen with as little as 0.6 IU (equivalent to 36 pmol or 15 ng) of the metabolite (7), which may, perhaps, be considered a "physiological" dose in the rat (8, 9).

The current studies were performed to expand our observations to include more chronic administration of the metabolite. Accordingly, studies were performed in both D-deficient and D-replete weanling rats given $1,25-(OH)_2D_3$ for 6 days prior to an acute clearance study. The resultant data indicate that the vitamin D status of the animals and chronic administration of $1,25(OH)_2D_3$ have profound and important effects on the capacity of $1,25(OH)D_3$ to induce an antiphosphaturia. Furthermore, these factors modulate the PTH "permissive" effect on the nephron.

Methods and Materials. Studies were performed in six groups of vitamin D-deficient, weanling rats obtained from the Holtzman Co. (Madison, Wisc.). The animals were placed on a diet (Teklad Mills, Chagrin Falls, Ohio) containing 0.40% calcium and 0.43% phosphorous including vitamins A, B, C, E, and K but no vitamin D (−D). The rats were divided into two groups, one of which received the diet just described which, however, was supplemented with 25 IU of vitamin D/day. We determined what amount of diet each rat was to eat per day (approx 15–20 g) and added sufficient vitamin D_2 to the +D diet such that 15–20 g of diet contained approximately 25–30 IU of vitamin D. The rats were housed in a room devoid of both natural and ultraviolet light for 2 weeks prior to entry into the study. Both the −D and +D rats were then subjected to one of three protocols: (i) The animals were injected with either 0.4 cc saline or propylene glycol intraperitoneally (ip) for 6 days. On the seventh day they underwent a continuous perfusion study as described previously (5, 6) based on the technique originally described by Cotlove (10). The animals were anesthe-

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tized with ether, thyroparathyroidectomy (TPTX) was performed, a bladder catheter placed, and an infusion containing 4% glucose, 10 m*M* calcium, 20 m*M* sodium, 2.5 m*M* potassium, and 2 m*M* magnesium, as the respective chloride salts, and inulin in a concentration of 2 mg/ml was begun via a tail vein. The animals were then allowed to recover from the anesthesia and were placed in restraining cages. After a 14-hr equilibration period, a 4-hr control period was obtained followed by a 6-hr experimental period. In this group of rats, utilized as time controls, only the vehicle (propylene glycol or saline) was given during the experimental period. Urine was collected every hour during the control and experimental periods. At the end of the study, the rats were sacrificed and blood was taken by aortic puncture for the determination of total calcium, phosphate, and inulin. These determinations, as well as analyses of urine for phosphate and inulin were performed by methods previously described from this laboratory (5, 6).

(ii) In a second group of both −D and +D rats, 1,25 dihydroxy vitamin D_3(1,25-$(OH)_2D_3$)[1] was given in a dose of 13 pmole ip twice daily for 6 days. The rats then underwent a continuous perfusion study during the last 6 hr of which (see above for experimental design) they received 26 pmole of the metabolite intravenously. (iii) In a third group of both −D and +D rats, the animals were again primed by a daily injection of a total of 26 pmole/day of $1,25(OH)_2D_3$, in two divided doses. On the seventh day, after the control period urines had been obtained, they received both 26 pmole of $1,25(OH)_2D_3$ over 6 hr as well as 0.2 U of bovine parathyroid hormone (highly purified 1-84 PTH, Inolex, Inc., Park Forest South, Ill.)/hr for 6 hr, at a rate of 0.15 cc/hr.

Each animal was utilized as its own control. The grouped data for urinary excretion before and after each of the three experimental maneuvers was therefore analyzed statistically utilizing Student's *t* test for dependent variables. The blood values obtained at the end of the study were analyzed by an independent *t* test comparing the control group to each of the experimental maneuvers. Animals with widely fluctuating excretion rates of inulin (presumably reflecting large variations in glomerular filtration rate) were excluded from consideration.

To compare the effects of the −D and +D diet regimen on serum calcium and phosphorous values, two to three rats were randomly selected from several batches of newly arriving weanlings, after the animals had been on their respective diets for at least 2 weeks (and not more than 3 weeks). These rats therefore did not undergo any of the pretreatment or infusion schedules but were utilized to determine the effects of the diet itself.

Results. The serum calcium and phosphate values obtained after 2–3 weeks on the 0.40% calcium and 0.43% phosphate diet are presented in Table I. While a wide disparity was evident between groups for mean serum calcium concentration related to vitamin D administration: 6.6 ± 0.2 mg% (−D) vs 9.5 ± 0.7 mg% (+D), $P < 0.001$, there was no real difference in serum phosphate concentration: 8.8 ± 0.4 mg% for −D and 8.6 ± 0.2 mg% for +D ($P > 0.70$). These observations are similar to those originally published by Steenbock and Herting (11), both with regard to the absolute levels obtained as a result of dietary influence and as concerns the major effect of vitamin D on serum calcium with either a minor or no effect on serum phosphorous.

In Table II are presented the results obtained when D-deficient animals were infused either with $1,25(OH)_2D_3$, 26 pmole over 6 hr, or with the metabolite plus 0.2 U PTH/hr for 6 hr, or with only the vehicles which were employed to solubilize the PTH and $1,25(OH)_2D_3$

TABLE I. COMPARISON OF SERUM CALCIUM AND PHOSPHATE VALUES IN VITAMIN D-FED AND DEFICIENT RATS[a]

Group	*N*	S_{Ca} (mg%)	S_P (mg%)
−D	15	6.6 ± 0.2	8.8 ± 0.4
+D	11	9.5 ± 0.7	8.6 ± 0.2
P		<0.001	NS

[1] Kindly supplied by Dr. Milan Uskokovic, Hoffman-LaRoche, Inc., Nutley, N.J.

[a] Abbreviations: S_{Ca} and S_P = serum concentrations of calcium and phosphate, respectively.

TABLE II. EFFECTS OF THE CHRONIC ADMINISTRATION OF 1,25-DIHYDROXY VITAMIN D_3 ON RENAL TUBULAR FUNCTION AND SERUM CALCIUM AND PHOSPHATE LEVELS IN RESPONSE TO THE METABOLITE IN VITAMIN D-DEFICIENT, THYROPARATHYROIDECTOMIZED RATS[a]

			Results of infusion study (Day 7)					
			$U_{In}V$ (μg/min)		U_PV (μg/min)		Serum	
Group	N	Pretreatment	C	E	C	E	Ca (mg%)	P (mg%)
1	6	Propylene glycol or saline ip BID × 6 days; infusion study (Day 7): propylene glycol or saline	9.5 ± 1.0	8.4 ± 0.7	13.2 ± 2.6	13.4 ± 3.4	5.9 ± 0.2	6.7 ± 0.4
		P[b]	NS[c]		NS		<0.005	<0.001
2	6	13 pmole $1,25(OH)_2D_3$ BID ip × 6 days; infusion study (Day 7): 26 pmole $1,25(OH)_2D_3$ over 6 hr	9.9 ± 0.7	11.7 ± 1.0	11.1 ± 1.6	6.5 ± 2.0	9.8 ± 0.2	8.8 ± 0.3
		P	NS		<0.01		<0.001	<0.001
3	8	13 pmol $1,25(OH)_2D_3$ BID × 6 days; infusion study (Day 7): 26 pmole $1,25(OH)D_3$ over 6 hr + 0.2 U PTH/hr × 6 hr	8.6 ± 0.4	10.6 ± 0.8	7.0 ± 1.0	8.4 ± 0.8	9.8 ± 0.2	9.2 ± 0.2
		P	NS		NS			

[a] Abbreviations: N = number of animals, $U_{In}V$, U_PV = absolute excretion rates of inulin and phosphate, respectively. C, E = control and experimental phases of the experiment, respectively. Ca, P = calcium, phosphate, respectively. PTH = parathyroid hormone, $1,25(OH)_2D_3$ = 1,25 dihydroxy vitamin D_3. Values given are means ± SE.

[b] P values compare control with experimental values.

[c] NS = not statistically significant.

(saline and propylene glycol, respectively). All animals had been pretreated with 26 pmole of $1,25(OH)_2D_3$ injected intraperitoneally for 6 days. There were no changes in inulin excretion ($U_{In}V$) from control to experimental phases of the studies in any of the three experimental groups. Neither was there any difference in absolute phosphate excretion (U_PV) in time control rats. However, when 26 pmole of $1,25(OH)_2D_3$ was infused over 6 hr, U_PV fell from 11.1 ± 1.6 to 6.5 ± 2.0 μg/min, a reduction of 41% from control phase levels ($P < 0.01$). Furthermore, when the action of PTH was superimposed upon that of the metabolite in these −D rats primed with $1,25(OH)_2D_3$, the antiphosphaturic effect was lost: the mean control phase value of 7.0 ± 1.0 μg/min did not differ from that obtained after the metabolites and hormone had been administered: 8.4 ± 0.8 μg/min. ($P > 0.30$).

The serum values for calcium and phosphorous obtained at the end of the experiments in these three groups of −D rats are shown in table II. As expected, $1,25(OH)_2D_3$ pretreatment as well as its infusion restored serum calcium to normal (9.8 ± 0.2 mg.% in each case, vs the hypocalcemic control value of 5.9 ± 0.2 mg%, $P < 0.005$ and <0.001 for groups 2 and 3, respectively, Table III). Furthermore, serum phosphate was likewise "normalized" in these animals to 8.8 ± 0.3 and 9.2 ± 0.2 mg.% in groups 2 and 3, respectively, from the hypophosphatemic control level of 6.7 ± 0.4 mg.% ($P < 0.001$, in each case). Thus, in −D rats, $1,25(OH)_2D_3$ restored serum calcium and phosphate values to levels generally seen in animals fed this diet (0.40% calcium, 0.43% phosphorous).

The infusion of the metabolite with and without PTH into D-replete rats produced results which differed importantly from those obtained in the −D animals. Again, as in the −D rat studies, there were no alterations in glomerular filtration rate as determined from $U_{In}V$ in any of the three experimental groups (Table III). Furthermore, as in the −D studies, the infusion of either saline or propylene glycol resulted in no changes in U_PV, demonstrating the stability of the preparation. However, as opposed to those studies performed in the −D rats, the infusion of the metabolite itself, after 6 days of pretreatment, did not result in any alteration in phosphate excretion (4.8 ± 0.9 → 3.1 ± 0.6, $P > 0.20$). However, when PTH was infused simultaneously with the metabolite, an antiphosphaturia did occur (group 3, Table III). In these rats, mean U_PV fell from 9.4 ± 1.3 to 4.9 ± 0.6 μg/min ($P < 0.025$), a reduction of 48%. Because there was a numerical decline in U_PV in the +D rats given $1,25(OH)_2D_3$ pretreatment, although it did not reach statistical significance, an additional eight rat studies were performed. In this supplemental group, mean U_PV was 5.5 ± 1.2 μg/min in the control phase and 6.0 ± 1.2 μg/min in the experimental phase of the experiments ($P > 0.60$), verifying the original data. Furthermore, $U_{In}V$ did not change: 7.2 ± 1.1 → 7.6 ± 1.1 μg/min ($P > 0.40$).

In these D-fed animals, serum calcium levels were all in the 9–10 mg% range in all three groups of rats, and did not differ from each other (Table III). However, both in the group given $1,25(OH)_2D_3$ alone and in those rats which also received PTH infusion, the mean serum phosphate value was significantly lower than that of the control group (8.4 ± 0.2 mg%): 5.1 ± 0.5 mg% in group 2 ($P < 0.001$), and 6.5 ± 0.1 mg% in group 3 ($P < 0.001$).

Discussion. Previous studies of the renal tubular actions of vitamin D metabolites have demonstrated an antiphosphaturia in both D-deficient (7–15) and D-replete animals (1–4, 12–14) both in our own laboratory and those of other workers. Furthermore, we have previously confined our experimental observations to the acute infusion of the biologically active metabolites so that the complicating effects of the metabolites on bone and gut could be avoided. We and others have now demonstrated that in both dogs and rats, either PTH or ADH can serve as permissive substances for the antiphosphaturic effects of the vitamin D_3 metabolites $25(OH)D_3$ and $1,25(OH)_2D_3$ to become manifest (1, 3, 4–7, 13, 14).

In this report, we have extended our observations to include animals pretreated with $1,25(OH)_2D_3$ in a dose which is considered to represent a "physological" amount (8, 9). That is, it is a dose which corrects the serum calcium concentration in TPTX (8) and D-deficient rats (9). We found that in the setting of D-deficiency, pretreatment with $1,25(OH)_2D_3$ removed the requirement for PTH as a permissive agent. Thus, acute phosphaturia re-

TABLE III. EFFECTS OF THE CHRONIC ADMINISTRATION OF 1,25-DIHYDROXY VITAMIN D_3 ON THE RENAL TUBULAR FUNCTION AND SERUM CALCIUM AND PHOSPHATE VALUES IN RESPONSE TO THE METABOLITE IN VITAMIN D-FED, THYROPARATHYROIDECTOMIZED RATS[a]

			Infusion study results					
			$U_{In}V$ (μg/min)		U_PV (μg/min)		Serum	
Group	N	Pretreatment	C	E	C	E	Ca (mg%)	P (mg%)
1	8	0.4 cc saline or propylene glycol ip BID × 6 days; infusion study (Day 7): saline or propylene glycol	8.9 ± 0.9	11.3 ± 0.6	7.9 ± 1.6	7.3 ± 1.6	9.9 ± 0.4	8.4 ± 0.2
		P	NS		NS		NS	<0.001
2	8	$1,25(OH)_2D_3$, 13 pmole ip BID × 6 days; infusion study (Day 7): 26 pmole $1,25(OH)_2D_3$ over 6 hr, iv	9.4 ± 0.9	9.1 ± 0.8	4.8 ± 0.9	3.1 ± 0.6	9.8 ± 0.2	5.1 ± 0.5
		P	NS		NS		NS	<0.001
3	6	$1,25(OH)_2D_3$, 13 pmole, ip BID × 6 days; infusion study (Day 7): $1,25(OH)_2D_3$, 26 pmole over 6 hr + 0.2 U PTH/hr × 6 hr, iv	10.5 ± 0.4	11.5 ± 1.3	9.4 ± 1.3	4.9 ± 0.6	9.4 ± 0.2	6.5 ± 0.1
		P	NS		<0.025			

[a] Abbreviations as in Table II.

sulted when $1,25(OH)_2D_3$ was given alone, in contrast to our prior studies (5–7) and those of Popovtzer (14). Therefore, repair of D-deficiency with $1,25(OH)_2D_3$ appears to be an additional "permissive" maneuver, allowing the metabolite to promote phosphate conservation when it is then given in an acute clearance experiment (see Table II). Furthermore, when D-repletion with $1,25(OH)_2D_3$ was accomplished, the introduction of PTH in a dose which is not usually phosphaturic in the −D setting (5, 6), provided the hormone with an apparently off-setting effect on phosphate transport from that of the metabolite, leading to no net change in phosphate excretion (Table II, group 3). The phenomenon of renal (and other target organ) resistance to the action of PTH in the absence of vitamin D has been described on a number of occasions previously (5, 6, 16–25) both in the experimental (5, 6, 16–22) and clinical literature (23–25).

The data obtained in the vitamin D-replete animals are strikingly different from those provided by the −D studies. Thus, in D-fed animals pretreated with $1,25(OH)_2D_3$, PTH *was* required for the antiphosphaturic effects of the metabolite to be expressed (Table III). These results, therefore, greatly resemble those obtained in −D rats in which pretreatment with the D metabolite was not performed (5–7) and in dogs which were not D-depeleted (1, 3). Accordingly, the demonstration of an antiphosphaturia after the acute infusion of $1,25(OH)_2D_3$ into D-fed rats appears to have the same requirement (that is, the presence of PTH) as is the case in −D rats. The data further indicate that pretreatment with $1,25(OH)_2D_3$ is not a substitute for the administration of the parent vitamin, raising the question as to whether other vitamin D metabolites are involved (as well) in these vitamin D–PTH interactions.

Although the antiphosphaturia associated with vitamin D metabolite administration has now been reported from several laboratories, our studies differ from those of Bonjour *et al.* (26). In their studies, the administration of $1,25(OH)_2D_3$ acutely into either −D or +D rats pretreated with the metabolite produced either no change in phosphate excretion or a phosphaturia. However, their studies differ from those presented here in three crucial ways: First, PTH was not infused simultaneously, although some studies were performed in intact (sham-operated) rats. Second, many of the rats in their study were phosphate-infused, a maneuver which lowers the tubular maximum for phosphate and could easily overcome any antiphosphaturic effect of the D metabolites. Third, their animals were on much different intakes of calcium and phosphorous than were the rats utilized in the present study.

In summary, the data show that $1,25(OH)_2D_3$ is antiphosphaturic in both −D and +D rats. However, in the former case, chronic $1,25(OH)_2D_3$ administration is required as a permissive substance. In the latter experimental circumstance, PTH acts as the "permissive" agent, similar to studies previously reported (5, 6).

Provision of either vitamin D (Table I) or $1,25(OH)_2D_3$ (Table III) repaired the hypocalcemia induced by the D-deficient diet utilized in this study (Table I). Furthermore, the chronic administration of $1,25(OH)_2D_3$ "normalized" the serum phosphate concentration (Table II). However, when the administration of $1,25(OH)_2D_3$ was superimposed upon animals which were already D-repleted, serum phosphate levels fell (Table III) presumably reflecting the deposition of calcium phosphate in bone, since phosphate did not appear in increased quantities in the urine (Table III). These observations, therefore, provide additional evidence that the effect of vitamin D on bone, as on the kidney, may involve more than one metabolite or action of the parent vitamin.

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