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Structural Determinants of the Renal Tubular Activity of Vitamin D_3 Derivatives: Studies with 1α-Hydroxy, 24*R*,25-Dihydroxy, and 1α,24*R*,25-Trihydroxy Vitamin D_3 (40315)

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Previous studies from this and other laboratories have documented an acute affect of vitamin D_3 and its major metabolites on renal tubular electrolyte transport (1, 2). The infusion of the biologically active metabolites of vitamin D_3, 25-hydroxyvitamin D_3 (25-hydroxycholecalciferol, 25-HCC) and 1,25-dihydroxyvitamin D_3 (1,25-dihydroxycholecalciferol, 1,25-DHCC), have been shown to produce an enhancement of phosphate, calcium, and sodium reabsorption both in the dog and rat (1–6). Recently, substantial progress has been made in the identification and biochemical synthesis of other naturally occurring vitamin D metabolites as well as structural analogs. These advancements have provided us with the opportunity to study the renal tubular effects of additional metabolites and analogs of the parent compound and to identify the structural requirements of these vitamin D_3 derivatives with regard to their transport actions. The data demonstrate that in order for an antiphosphaturia to occur, the derivative must contain a hydroxyl group in the 1 position. Furthermore, no effect on calcium or sodium transport is evident unless the compound possesses a 25-hydroxyl group which is sterically unhindered.

Methods. Acute clearance studies were performed in female mongrel dogs weighing 16 to 23 kg which had been thyroparathyroidectomized (TPTX) at least 48 hr prior to the experiment. Details of the surgical procedures and clearance technique have been reported elsewhere (1, 3). Completeness of parathyroidectomy was verified by comparing the serum calcium concentration 2 to 4 days postoperatively to those just before the procedure. Animals with at least a 30% reduction in serum calcium were selected for study. Thyroid replacement was accomplished by oral administration of 0.1 to 0.2 mg of synthyroid (Flint) daily. The animals were fasted and thirsted for 16 hr before the study and received 5 U of vasopressin (Pitressin tannate in oil, Parke, Davis and Company) the evening prior to the study. The dogs were anesthetized with 25 mg/kg of sodium pentobarbital with subsequent intermittent supplemental doses as required. A cuffed endotracheal tube was inserted and the animals were ventilated with a Harvard respirator. Catheters were inserted into a hindlimb vein for infusion of saline and into the external jugular vein for blood sampling. Priming doses of inulin and *p*-aminohippurate (PAH) were injected and a sustaining infusion of these substances was administered at a rate of 1 ml/min in physiological saline solution. Aqueous vasopressin was added to the solution in an amount calculated to deliver 20 mU/min. Volume expansion was performed by infusing a 0.9% saline solution containing 1.0 to 1.5 mEq/liter of calcium gluconate. The total amount of saline infused was approximately 2.5% of animal body weight, after which urinary losses were replaced by adjusting the rate of infusion so that the expansion was sustained. Urine collections were begun and after a steady state was achieved, one of the following experimental maneuvers was performed. In five dogs (group A, control animals), 0.25 ml of the vehicle (propylene glycol) was injected intravenously as a bolus. The experiment was then continued for approximately 2 hr during which 10 to 12 clearance periods of approximately 10 min each were obtained. In five other dogs (group B) 0.625 μg of 1α-hydroxyvitamin D_3 (1α-hydroxycholecalciferol, 1α-HCC)[1] dissolved in 0.25 ml of propylene glycol was given according to the same protocol as described for the control group. Group C consisted of five dogs which were

[1] The 1α-HCC utilized in this study was generously supplied by Dr. Jack Hinman, Upjohn Company, Kalamazoo, Mich.

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given 0.625 μg of 24*R*,25-dihydroxyvitamin D_3 (24*R*,25-dihydroxycholecalciferol, 24*R*, 25-DHCC)[2] dissolved in 0.25 ml of propylene glycol. The animals in experimental group D received 0.625 μg of 1α,24*R*,25-THCC dissolved in 0.25 ml of propylene glycol. Blood was drawn at the beginning of the study, at the plateau of each steady state, before each experimental maneuver, and every 30 min throughout the study. Blood and urine were analyzed for inulin, PAH, phosphorus, calcium, and sodium by methods previously described from this laboratory (1). Serum ultrafiltrates were obtained by centrifuging serum handled anaerobically through CF-50 centrifuge cones (Amicon Corp., Lexington, Mass.). Statistical evaluation of the data was performed by paired *t* test.

Results. Table I summarizes the data obtained in the control experiments (group A) as well as those in which the synthetic analog of vitamin D, 1α-HCC (group B) or the vitamin D metabolites, 24*R*,25-DHCC (group C) or 1α,24*R*,25-THCC (group D), were administered intravenously. In the control animals the intravenous administration of propylene glycol did not cause any changes either in the absolute urinary excretion of these ions or in their percentage excretion rates (Figs. 1–3, group A, Table I). Neither were renal hemodynamics nor serum ultrafilterable calcium concentration (SUF_{Ca}) altered in any consistent manner. The acute administration of 0.625 μg of 24*R*,25-DHCC had no effect on either the absolute or percentage excretion rates of phosphate, calcium, or sodium (group C, Table I). The mean delta $\%E_{PO_4}$ was $-3.6 \pm 3.9\%$ ($P > 0.40$). However, both 1α,24*R*,25-THCC and 1α-HCC, when given in the same amount (0.625 μg), induced a significant decline (by 28 and 30%, respectively) in the percentage excretion of phosphate ($P < 0.01$, < 0.05). The mean changes in phosphate excretion were -6.6 ± 1.6 and $-4.7 \pm 1.4\%$, respectively. These decrements were accompanied by reductions in the absolute excretion rates of phosphate of 18 and 30%, respectively, which were also statistically significant ($P < 0.05$, < 0.02, Fig. 1). No significant change in either absolute or percentage calcium or sodium excretion was observed in the animals receiving 1α,24*R*,25-THCC and 1α-HCC (groups C and D, Table I, Figs. 2 and 3). Glomerular filtration rate and effective renal plasma flow as measured by the clearances of inulin and PAH, respectively, were unaltered. There was no statistically significant change in the level of serum sodium concentration in any of the groups. SUF_{Ca} decreased slightly but consistently in group C (from 1.76 ± 0.08 to 1.70 ± 0.07 mmole/liter) after 24*R*,25-DHCC was administered. This change did not affect either filtered load or excretion rate. In all of the other groups there was no significant change in either SUF_{Ca} or SUF phosphate.

Discussion. Previous studies of the biological activities of 24*R*,25-DHCC and 1α, 24*R*,25-THCC have been limited to an evaluation of these substances in the skeleton and gastrointestinal tract. Furthermore, not only is there only a single study of the effects of 1α-HCC on the kidney (8) but none of the studies involving these compounds have been performed in the dog. The vitamin D_3 derivatives utilized in this study were evaluated, therefore, with the following objectives in mind. First, it was our intention to attempt to establish their respective capacities to alter renal electrolyte transport and to compare these experimental observations with those previously obtained with 25-HCC and 1,25-DHCC (1, 2). Second, the availability of these agents provided us with the opportunity to investigate what might be the structural determinants of vitamin D metabolites as regards their ability to alter electrolyte reabsorption at the renal tubular level.

These newly described derivatives of the parent vitamin have recently been shown to have substantial activity in stimulating intestinal calcium and phosphate transport (9–12). They are also active in elevating serum calcium and phosphorus and in the mobilization of calcium from bone in rachitic rats (12). However, in the latter systems, the response to 24*R*,25-DHCC occurs after a considerable time lag (13). Furthermore, nephrectomy and a high calcium diet abolished the effect of 24*R*,25-DHCC on intestinal calcium transport (9, 13). This finding suggests that renal conversion of this metabolite to 1α,24*R*,25-THCC or some other more polar metabolite

[2] Kindly provided by Dr. Milan Uskokovic, Roche Laboratories, Nutley, N.J.

TABLE I. EFFECT OF VITAMIN D METABOLITES ON ELECTROLYTE EXCRETION AND RENAL HEMODYNAMICS.[a]

	A ($N = 5$) Control		B ($N = 5$) 1α-HCC		C ($N = 5$) 24*R*,25-DHCC		D ($N = 7$) 1,24*R*,25-THCC	
	C	E	C	E	C	E	C	E
C_{In} (ml/min)	49.7 ± 6.0	47.5 ± 5.0	86.4 ± 5.6	87.8 ± 6.5	71.44 ± 1.6	67.5 ± 3.5	63.96 ± 7.6	69.4 ± 6.1
	P = NS		P = NS		P = NS		P = NS	
C_{PAH} (ml/min)	133.6 ± 22.5	138.0 ± 20.8	263.6 ± 34.6	269.0 ± 32.5	273.7 ± 32.6	258.3 ± 23.6	146.7 ± 20.5	122.8 ± 22.5
	P = NS		P = NS		P = NS		P = NS	
$\%E_{PO4}$	19.4 ± 4.0	20.3 ± 4.2	15.6 ± 2.4	10.9 ± 2.5	20.5 ± 3.7	16.8 ± 3.0	23.9 ± 3.3	17.3 ± 2.3
	P = NS		$P < 0.05$		P = NS		$P < 0.01$	
$\%E_{Ca}$	8.4 ± 2.0	8.9 ± 1.3	13.4 ± 4.3	12.1 ± 3.6	10.5 ± 2.6	10.0 ± 2.1	10.6 ± 1.9	9.9 ± 1.8
	P = NS		P = NS		P = NS		P = NS	
$\%E_{Na}$	9.0 ± 2.2	10.6 ± 1.6	14.0 ± 4.0	13.7 ± 3.6	12.8 ± 2.7	13.0 ± 2.4	10.9 ± 1.5	10.5 ± 1.5
	P = NS		P = NS		P = NS		P = NS	
SUF_{Ca} (μEq/liter)	2.03 ± 0.05	2.08 ± 0.02	1.87 ± 0.13	1.89 ± 0.15	1.81 ± 0.16	1.79 ± 0.17	1.76 ± 0.07	1.70 ± 0.07
	P = NS		P = NS		P = NS		$P < 0.05$	

[a] Abbreviations: 1α-HCC, 1α-hydroxyvitamin D_3; 24*R*,25-DHCC, 24*R*,25-dihydroxyvitamin D_3; 1α,24*R*,25-THCC, 1α,24*R*,25-trihydroxycholecalciferol. *N*, number of studies. C and E, control and experimental phases of the experiment, respectively. C_{In} and C_{PAH}, glomerular filtration rate and effective renal plasma flow as estimated by the clearances of inulin and *p*-aminohippurate, respectively. $\%E_P$, $\%E_{Ca}$, and $\%E_{Na}$, % excretion rates of phosphate, calcium, and sodium, respectively. SUF_{Ca}, serum ultrafilterable calcium concentration.

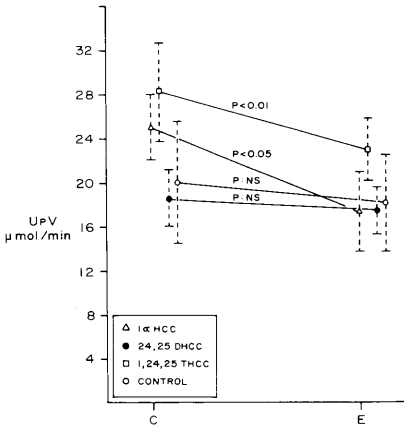

FIG. 1. The effects of vitamin D_3 metabolites and 1α-HCC on the absolute excretion rate of phosphate. Note the significant reduction in phosphate excretion following the administration of both 1α-HCC and 1α,24*R*,25-THCC. 24*R*,25-HCC was without effect on urinary phosphate excretion. Data points represent the mean values (±SE) for all dogs before (C) and after (E) administration of these compounds.

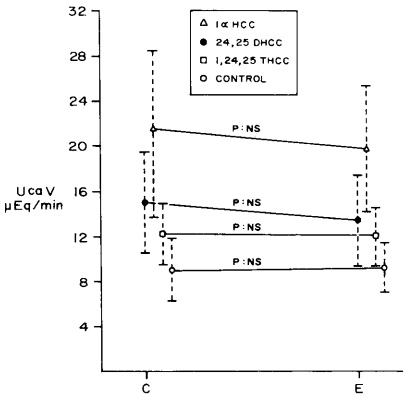

FIG. 2. The effect of vitamin D_3 metabolites and 1α-HCC on urinary calcium excretion. All three compounds, 24R,25-DHCC, 1α,24*R*,25-THCC, and 1α-HCC, were without effect on the renal tubular handling of calcium.

is required for its biological activity to be expressed (13). Indeed, radioactive 24*R*,25-DHCC has been shown to be further metabolized *in vivo* as well as *in vitro* to 1α,24*R*,25-THCC (14). It seems likely that hydroxylation at the 1 position is a prerequisite step in the metabolism of 24*R*,25-DHCC for this compound to become biologically active.

Our results on renal phosphate excretion confirm the observations obtained in the intestinal transport system that hydroxylation

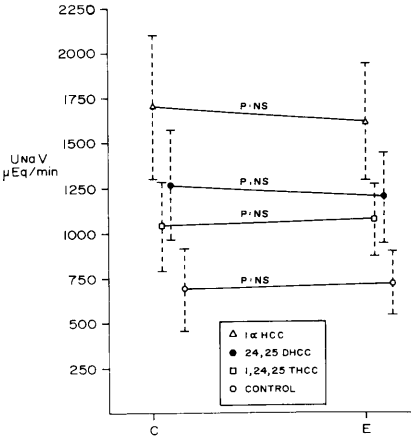

FIG. 3. The effects of vitamin D_3 metabolites on the urinary excretion of sodium. Note that none of the agents (1α-HCC, 24*R*,25-DHCC, or 1α,24*R*,25-THCC) changed urinary sodium excretion.

at the 1 position is necessary for the manifestation of its biological activity. Thus, a common feature of all the compounds which in the present study effected a reduction in phosphate excretion, was the presence of the 1α-hydroxy configuration. Since glomerular filtration rate and effective renal plasma flow, as well as the filtered load of phosphorus were unchanged (Table I), it is reasonable to conclude that the changes observed were due to a direct action of these compounds on the renal tubule. Furthermore, we conclude from these findings that the action of vitamin D metabolites on the tubular transport of phosphate depends upon the presence of the 1α-hydroxyl configuration. Since, in earlier studies from this laboratory, 25-HCC was likewise very effective in reducing phosphate excretion (1), we infer that the latter metabolite was converted *in vivo* to another vitamin D_3 derivative containing a 1α-hydroxyl group. This most probably means the formation of 1α,25-DHCC or some other "tissue active" substance, as yet unidentified (2). The fact that 25-HCC does not act immediately on renal electrolyte transport (1) and requires the "permissive" effect of either parathyroid hormone (4, 7) or vasopressin (6) for its renal tubular effects to become evident, are supportive of this thesis.

Perhaps the most important synthetic analog of 1α,25-DHCC currently available, at least from a therapeutic standpoint, is 1α-

HCC. This compound is almost as potent as 1α,25-DHCC in stimulating intestinal calcium transport in the chicken (15) and has approximately two to five times the activity of vitamin D_3 on calcification of the skeleton and in stimulating gut absorption of calcium in the rat (16). It has recently been reported that the intravenous administration of 1α-HCC to the rachitic rat produces an enhancement of intestinal calcium absorption within 1 hr of its infusion (17). This extremely rapid onset of action suggested to the investigators that 1α-HCC might act directly on the cell membrane transport of calcium. However, studies by Zerwekh *et al.* (18) suggest that the action of 1α-HCC requires prior conversion to 1α,25-DHCC. In addition, it has been demonstrated that tritiated 1α,25-DHCC appears in the intestine and bone within 2 hr after intravenous administration of 1α-[6-^{3}H]hydroxy vitamin D_3 (19). The design of the acute clearance studies presented in this report was such that observations were made for only 2 hr following the administration of the vitamin D_3 derivatives. Thus, since further metabolic conversion of both 1α-HCC and 24*R*,25-DHCC appears to require longer than 2 hr, we presume that the observed changes in renal transport were due to the action of the unchanged compounds. As regards the effects of 1α-HCC, our results confirm the observations of Pechet and Hesse (8) and Toffolon *et al.* (17) that 1α-HCC has a very rapid onset of action. Of course, we cannot rule out the possibility of some (more rapid) metabolism of these substances to an as yet unidentified "tissue-active" metabolite or metabolites.

Unlike 25-HCC and 1,25-DHCC (1, 2) none of the vitamin D_3 derivatives examined in the current study (1α-HCC, 24*R*,25-DHCC, or 1α,24*R*,25-THCC) were effective in altering either sodium or calcium excretion when given acutely. While no explanation of these observations is conclusively provided by the data, the findings could be explained as follows. The 1α-hydroxylated compounds (1α-HCC and 1α,24*R*,25-THCC) may act at different sites within the nephron or other receptor molecules than those affected by 25-HCC and 1α,25-DHCC. Alternatively, it may be that in order for a compound to alter calcium and sodium reabsorption, it must have a hydroxyl group in the 25 position or in both the 1 and 25 positions. Furthermore, it appears that the 25-hydroxyl grouping must also be sterically unhindered. Indeed, Stern *et al.* recently presented evidence that 24*R*,25-DHCC was rather less potent than either 25-HCC or 24*R*-HCC in its ability to effect bone resorption (20). They proposed that solubility factors, steric hindrance, or an excess of hydrophilic groups in this region may explain the decreased activity of this compound. Further study will be required to elucidate which of the above postulated mechanisms (or others) explain the experimental observations.

Summary. The acute effects of 24*R*,25-DHCC, 1α,24*R*,25-THCC, and 1α-HCC on the renal handling of phosphate, calcium, and sodium were evaluated in the TPTX dog which had been mildly volume expanded and infused with vasopression to establish a phosphaturia. Both 1α-HCC and 1α,24*R*,25-THCC when given intravenously in a dosage of 0.625 μg produced a significant decrease in urinary phosphate excretion. Percentage phosphate excretion decreased by 30 and 28%, respectively ($P < 0.05$, < 0.01). Since there was no alteration in renal hemodynamics or in the filtered load of this ion, the data suggest a direct action of these compounds on renal tubular transport mechanisms. No change in the urinary excretion of either calcium or sodium was observed following the administration of the two vitamin D_3 derivatives. 24*R*,25-DHCC was without effect on the renal handling of all three ions.

When previous experimental findings regarding the renal actions of 25-HCC and 1α,25-DHCC are considered, the data suggest that the 1-hydroxyl grouping is required for the metabolites of vitamin D to influence phosphate transport at the renal tubular level. It appears that a sterically unhindered 25-hydroxyl group is necessary in order for the vitamin D_3 derivatives to act on the reabsorption of either calcium or sodium.

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