The Acute Renal Tubular Effects of 1,25-Dihydroxycholecalciferol (36781)

JULES B. PUSCHETT, PEDRO C. FERNANDEZ, IAIN T. BOYLE, RICHARD W. GRAY, JOHN L. OMDAHL, AND HECTOR F. DELUCA

University of Pennsylvania Medical Service, Veterans Administration Hospital and the University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104; and the Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin Madison, Wisconsin 53706

It has now been established that the acute effect of vitamin D administration on the kidney is the enhancement of phosphate, sodium and calcium reabsorption (1). Furthermore, the availability of the 25-hydroxylated form of vitamin D_3, now identified as the major circulating biologically active derivative formed in the liver (2), has permitted the employment of a physiologic, rather than pharmacologic dose of the vitamin in evaluating its renal actions. While 25-hydroxycholecalciferol (25-HCC) has been demonstrated to be approximately 400 times as potent as the parent compound in inducing alterations in renal electrolyte transport and in shortening the lag time of onset of this effect from that noted with cholecalciferol (CC), it did not act immediately, nor was it possible to detect an effect with very small amounts of this substance (1). These observations prompted the search for a vitamin D metabolite which might prove to be that derivative which is active directly on the renal tubular cell without further metabolic conversion. Such a "tissue active" substance would be expected to act quite rapidly, to possess greater potency than other forms of the vitamin, and to induce changes in the renal tubular handling of ionic substances when administered in minute amounts.

Recently, intensive investigative effort has resulted in the isolation of a polar derivative of 25-HCC which is formed in the kidney (3, 4) and which possesses the ability to enhance markedly the uptake of calcium from the gut contents (5, 6). This report describes the results of an investigation into the possibility that this substance, subsequently identified as 1,25-dihydroxycholecalciferol (1, 25-DHCC) (7, 8), might also be effective in altering phosphate, calcium and sodium handling by the kidney. In addition, an attempt was made to evaluate whether or not 1, 25-DHCC is also that form of the vitamin which exerts a direct effect on renal tubular transport, at the tissue level.

Methods. Twelve acute clearance studies, the details of which have been previously described (1), were performed in thyroparathyroidectomized (TPTX) mongrel dogs weighing 16–24 kg. Absence of the parathyroids was verified by a postoperative fall in the serum calcium concentration of at least 30% or the development of tetany, or both. Thyroid hormone replacement was provided as synthroid (0.1 mg) daily. Because of the difficulties encountered in accurately analyzing urinary phosphate in TPTX animals, mild to moderate phosphaturia was first induced by means of the infusion of 25–30 ml/kg physiological saline solution (containing 3.5–4.5 mEq/liter calcium gluconate) and the volume expansion was then maintained by matching urinary flow with the infusion rate. Once a steady state had been achieved, one of two maneuvers was then accomplished: in 6 control animals, propylene glycol (1 ml) was given intravenously and urine and blood collections were continued while the volume expansion was sustained; in 6 animals, 25 U (0.625 μg) of 1,25-DHCC, diluted in 1 ml of propylene glycol was given intravenously. Blood was drawn at the beginning of each steady state plateau, before each experimental maneuver and approximately every 30–45 min through-

TABLE I. Effects of 1,25-Dihydroxycholecalciferol on Renal Electrolyte Transport.[a]

Study no.		C_{In} (ml/min)	C_{PAH} (ml/min)	U_PV (μM/min)	C_P/C_{In}	SUF_P (mM/liter)	$U_{Na}V$ (μEq/min)	C_{Na}/C_{In}	$U_{Ca}V$ (μEq/min)	C_{Ca}/C_{In}	SUF_{Ca} (mEq/liter)
A. Control studies											
D 85	C[b]	56.7	184	30.3	.288	1.86	1445	.192	19.9	.204	1.70
	E[b]	58.2	177	31.0	.287	1.86	1642	.204	22.9	.228	1.73
D 86	C	56.9	127	24.0	.197	2.15	822	.102	11.9	.051	4.09[c]
	E	72.2	116	24.0	.172	1.92	1069	.104	16.1	.050	4.49[c]
D 87	C	72.6		33.8	.244	1.92	908	.086	20.7	.085	3.37
	E	73.5		35.3	.278	1.73	961	.091	21.8	.101	2.94
D 88	C	69.7		20.4	.118	2.48	375	.038	7.0	.055	1.84
	E	61.7		17.3	.129	2.18	391	.046	5.8	.051	1.87
D 89	C	60.3	107	21.2	.208	1.69	879	.108	18.7	.083	3.79
	E	57.8	101	21.0	.210	1.73	801	.105	15.4	.073	3.68
D 93	C	74.5	179	19.0	.111	2.29	636	.060	8.0	.072	1.50
	E	70.7	192	22.0	.130	2.40	654	.066	9.1	.086	1.51
Means	C	65.1	149	24.8	.194	2.07	844	.098	14.4	.092	2.44
± SE		3.3	19	2.4	.028	0.12	145	.022	2.5	.023	0.47
	E	65.7	147	25.1	.201	1.97	920	.103	15.2	.098	2.35
		3.0	22	2.8	.029	0.11	174	.022	2.8	.027	0.41
p		>.80	>.70	>.80	>.50	>.25	>.20	>.05	>.50	>.30	>.90

TABLE I (continued)

Study no.		C_{In} (ml/min)	C_{PAH} (ml/min)	U_PV (μM/min)	C_P/C_{In}	SUF_P (mM/liter)	$U_{Na}V$ (μEq/min)	C_{Na}/C_{In}	$U_{Ca}V$ (μEq/min)	C_{Ca}/C_{In}	SUF_{Ca} (mEq/liter)
B. + U 1,25-DHCC											
D 80	C	72.1	151	13.4	.111	1.65	1038	.099	11.9	.106	1.56
	E	81.1	108	8.6	.66	1.62	812	.072	9.4	.074	1.57
D 81	C	68.8	106	36.7	.220	2.37	475	.045	4.2	.038	1.62
	E	69.8	104	19.9	.117	2.25	373	.037	3.1	.030	1.47
D 82	C	85.0		26.3	.145	2.19	750	.064	7.6	.026	3.53
	E	90.1		21.8	.109	2.20	435	.034	5.3	.021	2.86
D 83	C	64.3	169	30.6	.200	2.36	936	.101	16.0	.078	3.26
	E	66.3	187	15.5	.106	2.21	878	.096	14.6	.044	3.75
D 90	C	60.6	117	30.5	.272	1.86	1382	.155	23.9	.140	2.83
	E	60.4	98	24.8	.195	2.10	1009	.111	19.5	.095	3.70
D 91	C	49.1		19.2	.182	2.14	553	.079	6.1	.076	1.64
	E	46.8		9.1	.081	2.12	317	.049	3.9	.049	1.69
Means	C	66.7	136	26.1	.188	2.10	856	.091	11.6	.077	2.41
± SE		4.9	15	3.5	.023	0.12	137	.016	3.0	.017	0.37
	E	69.1	124	16.6	.112	2.09	637	.067	9.3	.052	2.51
		6.2	21	2.7	.018	0.10	121	.013	2.7	.011	0.44
p		>.25	>.40	<.01	<.05	>.80	<.01	<.02	<.005	<.02	>.60

[a] Abbreviations: C_{In} = glomerular filtration rate (clearance of inulin); C_{PAH} = effective renal plasma flow (clearance of PAH); U_PV, $U_{Na}V$, $U_{Ca}V$ = absolute excretion rates of phosphate, sodium and calcium, respectively; C_P/C_{In}, C_{Na}/C_{In}, and C_{Ca}/C_{In} = the fraction of filtered phosphate, sodium, or calcium (respectively) appearing in the urine; SUF_P, SUF_{Ca} = ultrafilterable serum phosphate and calcium concentrations, respectively.

[b] C, E = means of two to four consecutive urine collection periods during the control phase of the study (C) and at the time of peak effect of the infused metabolite (E). In the case of the group A studies, in which no effect was noted, collection periods were selected at times utilized for the group B studies (80–120 min after the beginning of the infusion).

[c] In this study, total, rather than ultrafilterable serum calcium was determined. The values for this experiment were therefore not utilized in obtaining the means.

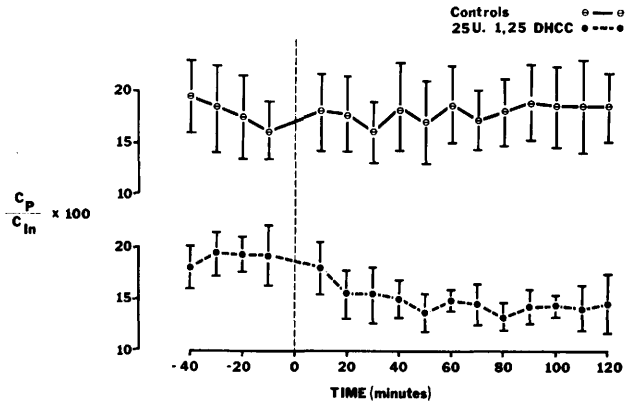

FIG. 1. Time course of the effects of sustained volume expansion (controls, upper panel) and 25 U of 1,25-DHCC (lower panel) on the percentage of filtered phosphate excreted ($C_P/C_{In} \times 100$). Values shown are the means (±SE) for the clearance periods obtained at the times indicated. At time zero, either the vehicle (propylene glycol) or 1,25-DHCC was given.

out the study. Blood and urine specimens were analyzed for inulin, para-aminohippurate (PAH), phosphate, calcium and sodium by methods previously described (1). Determinations of the serum levels of calcium and phosphate were performed on ultrafiltered samples handled anaerobically (1)

The [26, 27] ^{3}H-25-HCC (9) was prepared by the method of Suda, DeLuca and Hallick (9). The 1,25-DHCC employed in the study was isolated from an *in vitro* chick kidney incubation system described by Gray, Boyle and DeLuca (4), as modified by Boyle *et al.* (10).

Results. In Table I are summarized the experimental observations obtained in both the control animals (group A) and those receiving 25 U (0.625 μg) of 1,25-DHCC (group B). Sustained volume expansion resulted in the lack of any significant change in the transport of phosphate, calcium or sodium, or in renal hemodynamics. Hematocrit was likewise unaffected. However, 1, 25-DHCC induced a mean decline of 40, 32 and 26% in the fractional excretion rates of phosphate, calcium and sodium, respectively, accompanied by depresssions of absolute excretion rates for these ions of 36, 26 and 20%, respectively. Furthermore, this effect of the agent was invariable (Table I, group B). In pilot studies, 5 U of 1,25-DHCC was found to be without such an effect. Glomerular filtration rate and effective renal plasma flow were unaltered, nor was there any significant change in serum ultrafilterable calcium or phosphate concentration.

An analysis of the time course of the action of 1,25-DHCC on prosphate excretion is depicted in Fig. 1. In the control animals, maintenance of the modest volume expansion employed resulted in no consistent alteration in the percentage of filtered phosphate which was excreted. However, the effect of 1,25-DHCC on this parameter can be seen to have begun in about 20–30 min, with a peak response developing in about 80–120 min.

Discussion. The concept that there might exist several metabolites of vitamin D which have differing degrees of potency on the various end organs involved in calcium and phosphate homeostasis has evolved from investigative work aimed at uncovering the metabolic products of the vitamin, their tissue localization and sites of biological action. It now seems likely that 25-HCC is the major circulating (or "humoral") form of vitamin D and that further biochemical conversion of this substance is required before the characteristic effects of the vitamin can become manifest. The most extensively studied of these metabolites of 25-HCC is 1,25-DHCC. This compound has been shown to shorten the lag time between its administration and the onset of an increase in calcium transport by the small intestine from that which is required for

vitamin D_3 or 25-HCC to act (6, 11). Furthermore, in these studies, 1,25-DHCC was demonstrated to possess much greater potency in this regard than either of the latter two substances (6, 11). Investigations directed toward establishing the mechanism of action of vitamin D in the promotion of intestinal calcium transport have resulted in the observation that, while this effect on the part of CC and 25-HCC is blocked by the prior administration of actinomycin D, no such suppression by this inhibitor of protein synthesis attends the action of 1,25-DHCC (12). These experiments, as well as more recent investigations utilizing tritiated 1, 25-DHCC (13) strongly suggest that 1, 25-DHCC and not a further metabolite of this compound, is the vitamin D derivative directly responsible for enhancing intestinal calcium transport.

As regards the actions of vitamin D on the skeleton, previous studies have documented that 25-HCC can act directly to mobilize bone mineral (14), However, more recent evidence suggests the likelihood that 1, 25-DHCC is not only more active than 25-HCC in this respect, but that it is also more rapidly effective in inducing bone resorption (15, 16). Holick, Garabedian and DeLuca (17) have also demonstrated in nephrectomized rats that 1,25-DHCC, but *not* 25-HCC promotes the mobilization of calcium from bone, thus strongly suggesting that 1,25-DHCC is the metabolically active form in this system, at least at physiological doses.

With the perspective of these recent developments in the study of vitamin D actions, it is possible to tentatively interpret the findings obtained in the present studies. 1,25-DHCC does possess the capacity to alter renal-electrolyte transport, as do CC and 25-HCC (1). Moreover, this effect is a direct one on *tubular* ionic transfer, since alterations in filtered load and renal hemodynamics were eliminated as contributing factors to the changes noted. However, its effect on fractional phosphate excretion (mean decline of 40%) is less than that induced by an equivalent dose (25 U) of 25-HCC (mean fall of 66%) and about the same as that obtained with 400 times as much vitamin D_3 (25%) (1). The fact that the onset of its effect is noted to occur more rapidly (20–30 min) than that of either 25-HCC (30–40 min) or CC (50–60 min) (1), suggests that at least part of the time lag in the physiologic response of the kidney to the administration of vitamin D is the result of the necessity for the parent compound to be converted to 25-HCC, and then to 1,25-DHCC. Unfortunately, data regarding the effect of pretreatment with actinomycin D on the renal tubular transport effects of 1,25-DHCC are not yet available. On the basis of the studies reported in this communication, we propose that 1,25-DHCC is probably *not* the primary kidney-active vitamin D derivative for the following reasons: first, its potency in altering renal tubular transport was less than that of 25-HCC; second, it did not act immediately; and third, it did not induce any changes in phosphate excretion when given in small doses (5 U), a situation similar to that noted for 25-HCC (1), although it is possible that lower doses of *both* agents might have proved effective in D-deficient animals. Therefore, the search continues for the final vitamin D metabolite primarily responsible for the acute enhancement of phosphate, calcium and sodium reabsorption, at the renal "tissue" level.

Summary. 1,25-Dihydroxycholecaliferol (1,25-DHCC) is a metabolite of vitamin D produced in the kidney from the major circulating biologically active form of the vitamin, 25-hydroxycholecalciferol (25-HCC) and has been demonstrated to be the derivative of cholecalciferol (CC) primarily responsible for the initiation of calcium transport from the gut contents. Because previous studies have shown that both CC and 25-HCC act acutely to enhance phosphate, sodium and calcium reabsorption in the kidney, the effect of 1,25-DHCC was evaluated in clearance studies performed in mildly expanded thyroparathyroidectomized dogs. In a dose of 25 U (0.625 μg), 1,25-DHCC induced an invariable fall in both fractional and absolute phosphate excretion (mean declines of 40 and 36%, respectively, $p < .05$ and $< .01$). Fractional sodium and calcium excretion also de-

creased by mean values of 26 and 36%, respectively ($p < .02$). Renal hemodynamic changes and alterations in serum ultrafilterable calcium and phosphate concentrations did not occur, providing evidence for a direct tubular effect of the metabolite. A comparison of the action of 1,25-DHCC on phosphate excretion, to that obtained previously with CC and 25-HCC revealed the following: First, whereas the former metabolite further shortened (to 20–30 min) the time required for the onset of the renal action of CC (50–60 min) and 25-HCC (30–40 min), it did not act immediately; and secondly, an equivalent dose of 25-HCC had a greater effect on phosphate excretion than did 1,25-DHCC in this investigation.

The authors are indebted to Mrs. Sheila Strom, Mrs. Harriet Gaudiosi, Mrs. Carla Young and Mrs. Diane Sylk for invaluable technical assistance. Dr. Puschett is a Clinical Investigator of the Veterans Administration. This work was supported, in part, by United States Public Health Service Grant Nos. AM-14708-02 and AM-15512. Address requests for reprints to: Dr. J. B. Puschett, Room A819, VA Hospital, Philadelphia, PA 19104.

1. Puschett, J. B., Moranz, J., and Kurnick, W., J. Clin. Invest. **51**, 373 (1972).

2. Deluca, H. F., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **28**, 1678 (1969).

3. Fraser, D. R., and Kodicek, E., Nature (London) **228**, 674 (1970).

4. Gray, R., Boyle, E., and DeLuca, H. F., Science **172**, 1232 (1971).

5. Haussler, M. R., Myrtle, J. F., and Norman, A. W., J. Biol. Chem. **243**, 4055 (1968).

6. Haussler, M. R., Boyce, D. W., Littledike, E. T., and Rasmussen, H., Proc. Nat. Acad. Sci. U.S.A. **68**, 177 (1971).

7. Holick, M. F., Schnoes, H. K., and DeLuca, H. F., Proc. Nat. Acad. Sci. U.S.A. **68**, 803 (1971).

8. Holick, M. F., Schnoes, H. K., DeLuca, H. F., Suda, T., and Cousins, R. J., Biochemistry **10**, 2799 (1971).

9. Suda, T., DeLuca, H. F., and Hallick, R. B., Anal. Biochem. **43**, 139 (1971).

10. Boyle, I. T., Miravet, L., Gray, R. W., Holick, M. F., and DeLuca, H. F., Endocrinology **90**, 605 (1972).

11. Omdahl, J., Holick, M., Suda, T., Tanaka, Y., and DeLuca, H. F., Biochemistry **10**, 2935 (1971).

12. Tanaka, Y., DeLuca, H. F., Omdahl, J., and Holick, M. F., Proc. Nat. Acad. Sci. U.S.A. **68**, 1286 (1971).

13. Frolik, C. A., and DeLuca, H. F., Arch. Biochem. Biophys. **147**, 143 (1971).

14. Trummel, C. L., Raisz, L. G., Blunt, J. W., and DeLuca, H. F., Science **163**, 1450 (1969).

15. Tanaka, Y., and DeLuca, H. F., Arch. Biochem. Biophys. **146**, 574 (1971).

16. Raisz, L. G., Trummel, C. L., Holick, M. F., and DeLuca, H. F., Science **175**, 768 (1972).

17. Holick, M. F., Garabedian, M., and DeLuca, H. F., Science **176**, 1146 (1972).

Received May 22, 1972. P.S.E.B.M., 1972, Vol. 141.