The Acute Effect of 25-Hydroxycholecalciferol on Renal Handling of Phosphorus

EVIDENCE FOR A PARATHYROID HORMONE–DEPENDENT MECHANISM

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A B S T R A C T The acute effect of i.v. and direct intrarenal arterial infusion of 25-hydroxycholecalciferol (25HCC) and 1,25-dihydroxycholecalciferol (1,25-DHCC) on renal handling of phosphorus was evaluated in the following groups of rats: (a) intact animals, (b) parathyroidectomized (PTX) hypocalcemic rats, (c) PTX rats in which normocalcemia was maintained with calcium supplements and (d) PTX animals in which urinary phosphorus was augmented by (i) i.v. sodium phosphate, (ii) expansion of the extracellular fluid volume with normal saline, and (iii) i.v. parathyroid hormone (PTH). Clearances of inulin (C_{In}), phosphorus (C_P), and fractional clearances of phosphorus (C_P/C_{In}) of the experimental groups were compared with those of the corresponding control groups, and the clearances of the infused kidneys with those of the contralateral kidneys.

In intact animals, i.v. 25HCC decreased C_P/C_{In} from 0.29±0.04 (mean ±SE) to 0.19±0.04, and i.v. 1,25-DHCC decreased C_P/C_{In} from 0.25±0.04 to 0.15±0.02. The intrarenal infusion of both 25HCC and 1,25DHCC into intact animals failed to produce a unilateral change; however, it decreased C_P/C_{In} bilaterally. i.v. and intrarenal infusions of 25HCC or 1,25DHCC in PTX hypocalcemic and normocalcemic rats, and i.v. infusions of 25HCC in PTX rats receiving either sodium phosphate

or normal saline, all failed to produce significant changes in C_P/C_{In}. In contrast, 24HCC given i.v. to PTX animals receiving exogenous PTH was associated with a significant fall in C_P/C_{In}, from 0.34±0.08 to 0.13±0.02. These results indicate that 25HCC enhances tubular reabsorption of phosphorus in rats, only in the presence of either endogenous or exogenous circulating PTH, but not in its absence and thus imply a PTH-dependent mechanism of 25HCC action on the kidney. This effect does not appear to be related to the conversion of 25HCC into 1,25DHCC, since the latter fails to affect tubular reabsorption of phosphorus in PTX rats.

INTRODUCTION

The effect of vitamin D on renal handling of phosphorus has been the subject of numerous investigations (1-7). The main difficulty encountered in interpreting changes in urinary excretion of phosphorus has been related to the calcemic action of the vitamin which, per se, by suppressing parathyroid hormone secretion, might, in an indirect fashion, alter renal handling of phosphorus (8, 9). Thus, the enhanced tubular reabsorption of phosphorus associated with administration of vitamin D to patients with osteomalacia (6, 7) and to rachitic animals with intact parathyroid glands (1) could be accounted for either by inhibition of parathyroid hormone secretion or by a direct tubular action of the vitamin. In contrast, the phosphaturic action of large doses of vitamin D observed in hypoparathyroid humans (2) and in thyroparathyroidectomized dogs (4) has been either attributed to a direct effect of the steroid on renal reabsorption of phosphorus (4) or to

an indirect effect mediated by the increase in serum calcium (10). However, the effect of the latter is rather complex, and an increase in serum calcium may lead either to an increase or a decrease in the urinary excretion of phosphorus independently of variations in parathyroid glands' activity (10, 11). In addition, the level of calcium has been implicated as an important factor, determining the response of the bone to vitamin D (12). The concentration of calcium may also play an important role in other actions of vitamin D. In a recent study, a direct renal action of 25-hydroxy-cholecalciferol (25HCC)[1] independent of parathyroid hormone secretion was suggested; however, the interdependence between the vitamin and the hormone was not explored in great detail (5). The present study was designed to investigate the effect of 25HCC on renal handling of phosphorus with an attempt to characterize the relationships between the action of the steroid and the level of parathyroid hormone, and between the serum concentration of calcium and the basal excretion rate of phosphorus in the urine.

METHODS

White female Sprague-Dawley rats (200–300 g) fed Purina pellet chow (Livestock and Poultry Feeds, Ralston Purina Co., St. Louis, Mo.) diet with tap water *ad lib.* were studied.

Clearance studies

The clearance studies were performed in all animals at the same part of the day between 8:00 a.m. and 4:00 p.m. After the induction of anesthesia with an intramuscular injection of sodium pentobarbital (40 mg/kg body weight), the animals were placed on heated operating boards and a tracheostomy tube was inserted. Rectal temperature was monitored by means of a thermistor and telethermometer (Yellow Springs Instrument Co., Yellow Springs, Ohio). The femoral artery and vein were exposed through an inguinal incision and PE-20 tubings (Clay Adams, Div. of Becton, Dickinson, and Co., Parsippany, N. J.) were inserted into each vessel. The arterial line was used for monitoring mean arterial blood pressure with a Statham strain gauge, model 23AA (Statham Instruments, Inc., Oxnard, Calif.) and for the collection of blood samples. The venous line was extended to a syringe mounted on a variable speed continuous infusion pump (Harvard Apparatus Co., Inc., Millis, Mass.). The urinary bladder was exposed and a funnel shaped end of a PE-240 tubing was introduced through an incision at the fundus for urine collections. The incision was closed tightly with a purse string suture which excluded a major portion of the vesical lumen and thus eliminated most of the dead space. The technique of renal artery catheterization which was employed in the experiments with intrarenal arterial infusion was described in detail in previous reports (13, 14). The technique of

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[1] *Abbreviations used in this paper:* C_{In}, clearance of inulin; C_P, clearance of inulin phosphorus; C_{Na}, clearance of sodium; 1,25DHCC, 1,25-dihydroxycholecalciferol; 25HCC, 25-hydroxycholecalciferol; PTH, parathyroid hormone; PTX, parathyroidectomized.

measuring inulin clearances (C_{In}) and sodium clearances (C_{Na}) in rats, and the analytical procedures used in our laboratory have been described in detail in a recent report (14). All plasma and urine specimens were analyzed for inulin (15) and phosphorus, and at least two plasma specimens obtained at the beginning and the end of each experiment were analyzed for calcium. Inorganic phosphorus was measured utilizing the methodology developed by the American Monitor Co. (American Monitor Patent No. 3-547-586). Calcium was determined with Perkin-Elmer Atomic Absorption Spectrophotometer model 290 (Perkin-Elmer Corp., Norwalk, Conn.). The fractional phosphorus clearance was determined by factoring clearance of phosphorus (C_P) by C_{In}. Before administration both 25HCC and 1,25-dihydroxycholecalciferol (1,25DHCC) were dissolved in plasma obtained from rats similar to those that were studied with the vitamin. The infusion rate of plasma in all experiments by each route of administration was 0.3 ml/100 g per h. In the control groups that did not receive 25HCC or 1,25DHCC, the corresponding vehicle was added to the infused plasma in a volume equal to that given to the experimental groups.

Experimental groups

Group 1a. In six rats with intact parathyroid glands after four control clearance periods, 0.1 µg/100 g per h of 25HCC was given intravenously over five collection periods. Six additional rats treated in an identical manner but not receiving 25HCC served as control.

Group 1b. In four rats with intact parathyroid glands after four control clearances, 0.02 µg/100 g per h of 25HCC were infused directly into the left renal artery during five collection periods. During the control collections, the intrarenal arterial infusion delivered plasma with the vehicle.

Group 2a. Parathyroidectomy was performed 2 days before the clearance studies, using previously described techniques (16). In six parathyroidectomized (PTX) rats after four control clearances 0.15–0.30 µg/100 g per h of 25HCC was given intravenously over five collection periods. Six additional PTX rats treated in a similar manner but not receiving 25HCC served as control.

Group 2b. In four PTX rats after four control collections, 0.02–0.10 µg/100 g per h of 25HCC were infused directly into the left renal artery over five collection periods. During control collection, plasma obtained from similar PTX rats was infused directly into the left renal artery.

Group 3. Four PTX rats received oral supplements of calcium as 50 mg/100 ml of elemental calcium in drinking water. In addition, they were receiving daily 10 mg of calcium as calcium chloride by subcutaneous injection. During the clearance studies, the sustaining infusion was supplemented with calcium chloride, resulting in a concentration of calcium of 20 mg/100 ml in the infusate. After four control collections, 0.3 µg/100 g per h of 25HCC was given intravenously over five clearance periods. Four additional PTX control rats, receiving calcium supplements, were treated in a similar fashion, but were not infused with 25HCC.

Group 4a. In six PTX rats after three control collections, isotonic neutral sodium phosphate was infused intravenously at the rate of 6 mg/100 g per h (0.2 mM/100 g per h) of elemental phosphorus. After three additional clearance periods, as the phosphorus infusion continued, i.v. infusion of 0.3 µg/100 g per h of 25HCC was

started and was given for four collection periods. An additional group of six PTX rats which were treated in an identical fashion but did not receive 25HCC served as control.

Group 4b. Four PTX rats were treated in an identical fashion as described in group 4a; however, sodium phosphate was infused at a slower rate, 3 mg/100 g per h (0.1 mM/100 g per h).

Group 5. In five PTX rats after three control collections, normal saline was infused intravenously at the rate of 0.1 ml/100 g per min. After three additional clearance periods as the saline infusion continued, i.v. infusion of 0.3 μg/100 g per h of 25HCC was started and was given for four collection periods. An additional group of five PTX rats which were treated in an identical fashion but did not receive 25HCC served as control.

Group 6. In six PTX rats after three control collections, i.v. infusion of 1 U/100 g per h of parathyroid extract (Eli Lilly and Co., Indianapolis, Ind.) was begun. After four additional periods, as the parathyroid extract infusion continued, i.v. infusion of 0.1 μg/100 g per h 25HCC was started and was given for four collection periods. Additional groups of six PTX rats which were treated in an identical fashion but did not receive 25HCC served as control.

Group 7a. In four rats with intact parathyroid glands after four control clearances, i.v. infusion of 0.1 μg/100 g per h of 1,25DHCC was started and continued for four periods. An additional four rats which were treated in a similar fashion but did not receive 1,25DHCC served as control.

Group 7b. In four rats with intact parathyroid glands after four control clearances, intrarenal arterial infusion of 0.1 μg/100 g per h of 1,25DHCC was started and continued for an additional four periods.

Group 8a. In six PTX rats after four control collections, i.v. infusion of 1,25DHCC 0.1 μg/100 g per h was started and continued over four periods. An additional group of six PTX rats which were treated in a similar fashion but did not receive 1,25DHCC served as control.

Group 8b. In three PTX rats after four control collections, intrarenal arterial infusion of 0.1 μg/100 g per h of 1,25DHCC was begun and continued for an additional four periods.

In all studies the individual animals in the experimental and in the corresponding control groups were littermates, matched by age, sex, weight, and feeding time; both the experimental and the matched control rats were studied on the same day and during the same hours, under identical conditions.

The analysis of the results was based on the comparison of the experimental with the respective control groups for the corresponding clearance periods, using the paired Student's *t* test. The results in the studies with direct intrarenal arterial infusion were evaluated by comparing values obtained on the ipsilateral side with those on the contralateral side.

RESULTS

The effect of i.v. 25HCC on fractional clearance of phosphorus (C_P/C_{In}) in rats with intact parathyroid glands (group 1a) is depicted in Fig. 1. The results represent the mean±SE for both the control and the experimental groups, during the control periods and during the infusion of plasma without or with 25HCC, in
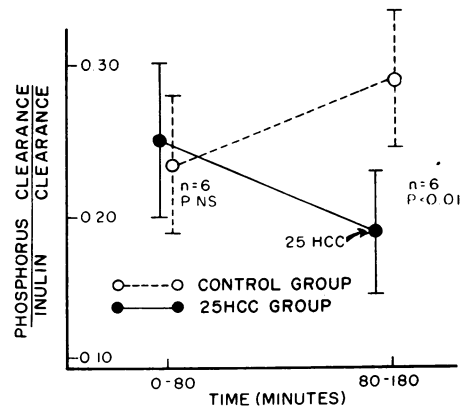

FIGURE 1 The effect of i.v. 25HCC on renal handling of phosphorus in rats with intact parathyroid glands. *P* refers to the difference between the control and experimental group.

the control and the experimental groups, respectively. The mean±SE of C_P/C_{In} was calculated from the averages of the control and the vitamin infusion periods. There was no difference in C_P/C_{In} between the control and the experimental groups during the four control periods (0–80 min). During five periods (80–180 min) in which 25HCC was given intravenously to the experimental group, there was a marked decrease in C_P/C_{In}; whereas in the control group there was an increase, resulting in a highly significant discrepancy between the two groups. The fall in C_P/C_{In} became apparent 20–40 min after the beginning of 25HCC infusion. These changes were not associated with significant variations in C_{In} and serum concentrations of calcium and phos-

TABLE I

Representative Experiment Showing the Response to i.v. 25HCC in a Rat with Intact Parathyroid Glands

Time	V*	C_{In}	S_{Ca}‡	S_P	C_P/C_{In}
min	*μl/min*	*μl/min*	*mg/100 ml*	*mg/100 ml*	
0	Prime with inulin 5 mg/100 g and continue sustaining infusion delivering inulin 0.17 mg/min per 100 g in 5% dextrose in water at the rate of 0.040 ml/min per 100 g				
0–60	Equilibration period				
60–80	107	3,990	9.2	2.4	0.27
80–100	190	3,700		2.6	0.31
100–120	120	3,800		2.5	0.25
120–140	135	3,960	9.0	2.7	0.26
	Begin intravenous infusion of 25HCC 0.1 μg/100 g per h				
140–160	125	4,000	8.9	2.8	0.19
160–180	120	3,560		2.8	0.14
180–200	80	3,660		2.9	0.12
200–220	85	3,650		3.0	0.11
220–240	100	5,560	8.4	3.2	0.11

* Urine volume.
‡ Concentration of calcium in serum.
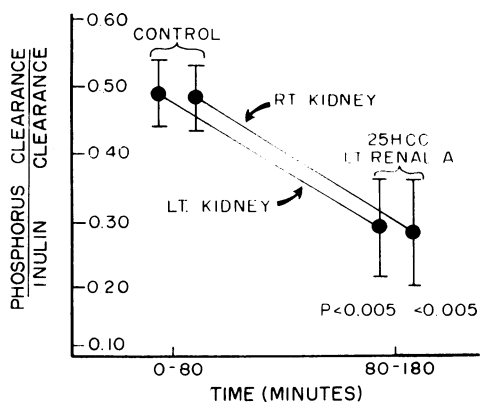§ Concentration of inorganic phosphorus in the serum.

FIGURE 2 The effect of 25HCC infusion directly into left renal artery (LT. Renal A.), with separate urine collection from both the right (RT.) and the left (LT.) kidneys, on renal handling of phosphorus in rats with intact parathyroid glands. P refers to the difference between the control and the 25HCC infusion collection periods on both sides.

phorus. A representative experiment showing the response to i.v. 25HCC in a rat with intact parathyroid glands is shown in Table I.

In group 1b (Fig. 2) there was no difference in C_P/C_{In} between both kidneys during the control periods (0–80 min) and during the infusion of 25HCC into the left renal artery (80–180 min). During the infusion periods, there was a marked but equal decrease in C_P/C_{In} on both sides. There were no significant changes in C_{In} and serum calcium and phosphorus concentrations during the whole study.

Although there were no changes in serum concentrations of calcium during the administration of 25HCC significant enough to implicate secondary changes in parathyroid hormone secretion in the fall in C_P/C_{In}, the possibility that a change in the rate of parathyroid hormone secretion, in the presence of parathyroid glands, played a role in the decline could not be ruled out with certainty. To further clarify this question, PTX rats were studied. In each animal that entered the study, the success of parathyroidectomy was ascertained by a drop in serum calcium to a level of 6 mg/100 ml or less.
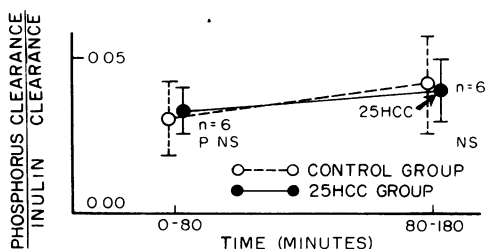

FIGURE 3 The effect of i.v. infusion of 25HCC on renal handling of phosphorus in PTX rats with hypocalcemia.
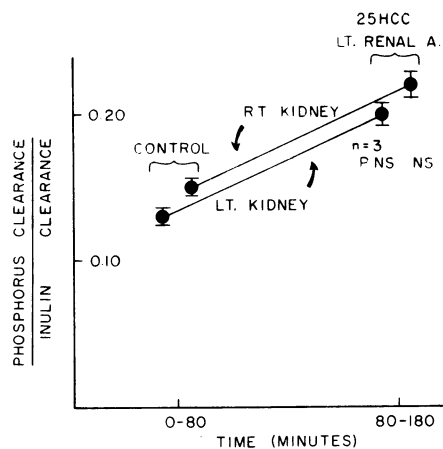

FIGURE 4 The effect of 25HCC infusion directly into left renal artery on renal handling of phosphorus in PTX rats.

The effect of i.v. 25HCC on C_P/C_{In} in PTX hypocalcemic rats (group 2a), compared with the effect on a control group of PTX animals who were not receiving 25HCC is shown in Fig. 3. There was no significant difference in C_P/C_{In} between the two groups before (0–80 min) and during 25HCC infusion (80–180 min), indicating a lack of effect of 25HCC on renal handling of phosphorus in PTX rats. Similarly no response was noticeable when 25HCC was infused directly into the left renal artery in group 2b (Fig. 4). In both the 2a and b groups, there were no significant changes in C_{In} and serum concentrations of calcium and phosphorus throughout the experiment.

In group 3 after a drop in serum calcium below 6 mg/100 ml after PTX, serum calcium was raised and maintained at the range of 8.2–10.0 mg/100 ml in both the control and the experimental groups, before and throughout the acute experiments. There was no difference in C_P/C_{In}, during the control periods (Fig. 5), between the control and the experimental groups, nor during the in-
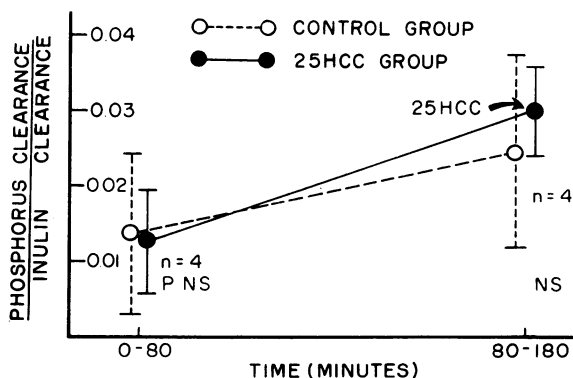

FIGURE 5 The effect of i.v. infusion of 25HCC on renal handling of phosphorus in PTX rats with normocalcemia.

fusion of 25HCC into the experimental group. C_{In} and serum concentrations of calcium and phosphorus did not change significantly throughout the study.

In group 4a the constant infusion of sodium phosphate was associated with a rise in serum phosphorus from 5.20 ± 0.16 (mean $\pm$ SE) to 17.30 ± 1.10, and from 5.00 ± 0.35 to 16.13 ± 1.60 mg/10 ml in the control and the experimental groups, respectively. Fig. 6 depicts the variations in C_P/C_{In} in both groups, during the control period (0–60 min), during sodium phosphate infusion (60–100 min), during phosphate with 25HCC infusion in the experimental group, and during phosphate infusion (without 25HCC) in the control group (100–180 min). During the combined phosphate and 25HCC infusion periods in the experimental group, there was no significant difference in C_P/C_{In} compared to the control group. Both groups exhibited a progressive increase in phosphorus excretion reaching a mean maximum of C_P/C_{In} of about 50%. There were no significant changes in C_{In} throughout the study. There was a slight decrease in serum calcium which was not significant. Similarly in group 4b, in which C_P/C_{In} was increased by a smaller fraction, 25HCC had no effect on tubular reabsorption of phosphorus (Table II). In group 5 saline infusion was associated with considerable increases in both C_P/C_{In} and C_{Na}/C_{In}; however 25HCC did not affect these changes (Table III).
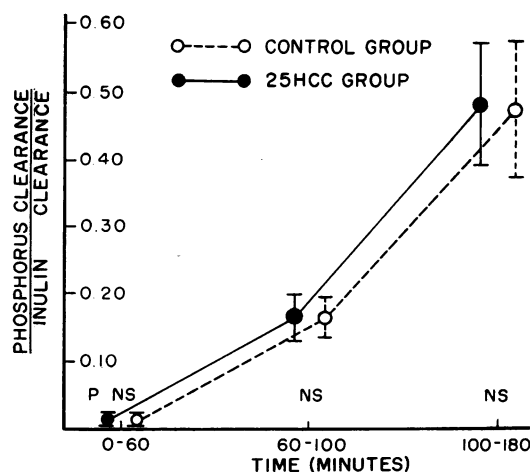

FIGURE 6 The effect of i.v. 25HCC on renal handling of phosphorus in PTX rats receiving sodium phosphate (NaP) infusion. NaP was started after 60 min, 25HCC was infused in the experimental group after 100 min.

The sequential changes in C_P/C_{In} in group 6, both in the control and in the experimental animals, are depicted in Fig. 7. There was no difference in C_P/C_{In} between the two groups during the control period (0–60 min), nor during the infusion of parathyroid extract (60–140 min); however there was a progressive increase in C_P/C_{In} in both groups. The addition of 25HCC

TABLE II

The Effect of 25HCC on Renal Handling of Phosphorus in PTX Animals Receiving Sodium Phosphate Infusion (P-INF)

		C_{In}			U_PV			C_P/C_{In}			C_{Na}/C_{In}			S_P	
	Base-line*	P-INF‡	P-INF +25 HCC§	Base-line	P-INF	P-INF +25 HCC	Base-line	P-INF	P-INF +25 HCC	Base-line	P-INF	P-INF +25 HCC	Base-line	P-INF	P-INF +25 HCC
		μl/min			μg/min									mg/100 ml	
Experimental rats															
1	1,492	1,650	1,490	2.51	6.60	18.62	0.0280	0.0500	0.125	0.0010	0.0040	0.0080	6.0	8.0	10.0
2	940	1,060	1,015	0.16	4.81	17.54	0.0020	0.0420	0.135	0.0020	0.0050	0.0110	8.8	10.8	12.8
3	1,500	1,065	1,350	5.60	10.96	24.01	0.0450	0.1050	0.156	0.0050	0.0250	0.0210	8.3	9.8	11.4
4	1,277	1,010	1,160	3.79	6.44	12.06	0.0430	0.0750	0.105	0.0090	0.0040	0.0090	6.9	8.5	9.9
Mean	1,302	1,196	1,253	3.01	7.20	18.06	0.0295	0.0680	0.1302	0.0042	0.0095	0.0122	7.5	9.2	11.0
±SE	±131	±150	±104	1.14	1.30	2.45	±0.0084	±0.0141	±0.0106	±0.0017	±0.0050	±0.0030	±0.2	±0.6	±0.7
Control rats															
1	1,020	1,060	1,500	2.51	4.29	17.79	0.0390	0.0460	0.105	0.0090	0.0080	0.0090	6.3	8.8	11.3
2	1,470	1,060	1,060	6.77	16.31	17.84	0.0490	0.1620	0.165	0.0018	0.0015	`0.0130	9.4	9.5	10.2
3	1,028	944	1,360	0.65	1.00	19.99	0.0090	0.0100	0.105	0.0060	0.0080	0.0160	7.1	10.6	14.0
4	989	1,135	790	0.34	2.02	9.99	0.0050	0.0200	0.115	0.0020	0.0170	0.0200	6.9	8.9	11.0
Mean	1,126	1,049	1,175	2.57	6.05	16.40	0.0255	0.0595	0.1225	0.0047	0.0086	0.0145	7.4	9.5	11.6
±SE	±114	±40	158	1.48	3.53	2.20	±0.0105	±0.0309	±0.0143	±0.0016	±0.0031	±0.0023	±0.7	±0.4	±0.8
	NS‖	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

* Average of three clearance periods before starting P-INF (0–60 min).
‡ Average of three clearance periods after starting P-INF (60–120 min).
§ Average of four clearance periods 60 min after starting P-INF, during which 25HCC was given to the experimental animals; the control animals received the vehicle only (120–200 min).
‖ Denotes comparison between corresponding study periods of the experimental and control groups.

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TABLE III

The Effect of 25HCC on Renal Handling of Phosphorus in PTX Animals Undergoing Extracellular Fluid Volume Expansion with Saline Infusion (ECVE)

	C_{In}			C_{Na}/C_{In}			U_PV			C_P/C_{In}			S_P		
	Base-line*	ECVE‡	ECVE +25 HCC§	Base-line	ECVE	ECVE +25 HCC	Base-line	ECVE	ECVE +25 HCC	Base-line	ECVE	ECVE +25 HCC	Base-line	ECVE	ECVE +25 HCC
	μl/min						*μg/min*						*mg/100 ml*		
Experimental rats															
1	700	1,013	912	0.0045	0.0800	0.1420	0.78	15.55	17.46	0.0120	0.1600	0.2200	9.3	9.6	8.7
2	840	1,423	1,699	0.0012	0.1150	0.1900	0.41	3.66	8.82	0.0045	0.0220	0.0530	10.8	11.7	9.8
3	972	1,264	1,035	0.0032	0.0810	0.1570	0.79	4.46	12.20	0.0100	0.0350	0.1420	8.1	10.1	8.3
4	748	1,409	1,260	0.0120	0.0500	0.1030	4.42	24.47	25.17	0.0650	0.1930	0.2220	9.1	9.0	9.0
5	694	1,030	1,060	0.0250	0.0550	0.1240	4.72	7.50	9.23	0.1050	0.1400	0.1300	6.8	5.2	6.7
Mean	790	1,227	1,193	0.0092	0.0762	0.1404	2.22	11.12	14.58	0.0393	0.0644	0.1534	8.8	9.1	8.5
±SE	±44	±83	±153	±0.0042	±0.0110	±0.0129	0.96	3.94	3.06	±0.0190	±0.0231	±0.0311	±0.6	±1.1	±0.5
Control rats															
1	800	800	800	0.0040	0.0680	0.1380	0.30	6.73	15.36	0.0035	0.0850	0.2000	10.9	9.9	9.6
2	740	900	900	0.0012	0.0630	0.1620	0.51	3.02	6.05	0.0120	0.0550	0.1120	5.8	6.1	6.0
3	900	1,480	1,460	0.0120	0.0690	0.0950	1.45	17.70	17.74	0.0170	0.1300	0.1350	9.5	9.2	9.0
4	500	1,590	1,240	0.0120	0.0940	0.1280	4.09	17.69	16.74	0.0900	0.1250	0.1450	9.1	8.9	9.0
5	700	850	1,100	0.0020	0.0650	0.1790	0.97	1.91	7.50	0.0150	0.0250	0.0750	9.2	9.0	9.1
Mean	728	1,124	1,100	0.0062	0.0716	0.1384	1.46	9.41	12.67	0.0275	0.0840	0.1334	8.9	8.6	8.5
±SE	±63	±150	±111	±0.0023	±0.0052	±0.0132	0.69	3.47	2.45	±0.0150	±0.0191	±0.0192	±0.9	±0.6	±0.6
	NS‖	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

* Average of three clearance periods before starting ECVE (0–60 min).
‡ Average of three clearance periods after starting ECVE (60–120 min).
§ Average of four clearance periods, 60 min after starting ECVE, during which 25HCC was given to the experimental animals; the control animals received the vehicle only (120–200 min).
‖ Denotes comparison between corresponding study periods of the experimental and control groups.

to the parathyroid extract infusion in the experimental group (140–220 min) led to a significant decrease in C_P/C_{In} in this group; whereas in the control group which received only parathyroid hormone, C_P/C_{In} continued to rise, resulting in a large discrepancy in C_P/C_{In} between the two groups. These changes were not associated with significant variations in C_{In} and serum concentrations of calcium and phosphorus. This observation demonstrated that 25HCC may decrease C_P/C_{In} in PTX animals, provided that exogenous hormone is present in the circulation.

The effect of 1,25DHCC on C_P/C_{In} in animals with intact parathyroid glands (group 7a) is shown in Fig. 8. There was no difference in C_P/C_{In} between the control
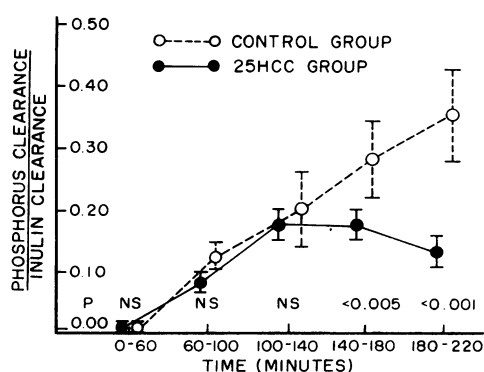

FIGURE 7 The effect of i.v. 25HCC on renal handling of phosphorus in PTX rats receiving constant infusion of parathyroid hormone (PTH). PTH was started after 60 min, 25HCC was started in the experimental group after 140 min. P refers to the difference between corresponding collection periods in the control and the experimental group.
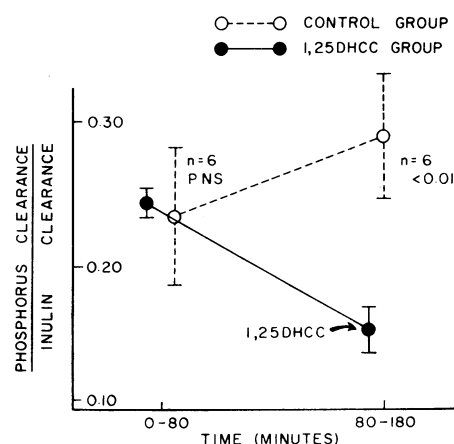

FIGURE 8 The effect of i.v. 1,25DHCC on renal handling of phosphorus in rats with intact parathyroid glands. P refers to the difference between corresponding collection periods in the control and the experimental groups.

TABLE IV

Representative Experiment Showing the Response to i.v. 1,25DHCC in a Rat with Intact Parathyroid Glands

Time	V*	C_{In}	S_{Ca}‡	S_P§	C_P/C_{In}
min	*μl/min*	*μl/min*	*mg/100 ml*	*mg/100 ml*	
0	Prime with inulin 5 mg/100 g and continue sustaining infusion delivering inulin 0.17 mg/min per 100 g in 5% dextrose in water at the rate of 0.040 ml/min per 100 g				
0–60	Equilibration period				
60–80	50	2,500	9.6	5.1	0.190
80–100	50	2,850		5.0	0.192
100–120	65	2,820		5.2	0.203
120–140	100	2,130	9.5	5.0	0.392
	Begin intravenous infusion of 1,25DHCC 0.1 μg/100 g per h				
140–160	135	2,790		5.2	0.100
160–180	163	2,800		5.3	0.095
180–200	120	2,460		5.0	0.129
200–220	83	2,760	9.4	5.2	0.100

* Urine volume.
‡ Concentration of calcium in serum.
§ Concentration of inorganic phosphorus in the serum.

and the experimental groups during the control periods (0–80 min). During four periods (80–160 min) in which 1,25DHCC was given intravenously to the experimental group, there was a marked decrease in C_P/C_{In} in the experimental animals; whereas in the control animals there was an increase resulting in a highly significant discrepancy in C_P/C_{In} between the two groups. The fall in C_P/C_{In} became apparent 20–60 min after the beginning of 1,25DHCC infusions. These changes were not associated with significant changes in C_{In} and serum concentrations of calcium and phosphorus. A representative experiment showing the response to i.v. 1,25DHCC in a rat with intact parathyroid glands is shown in Table IV.
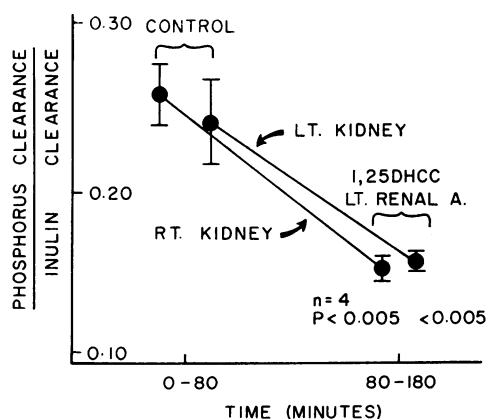

FIGURE 9 The effect of 1,25DHCC infusion directly into left renal artery on renal handling of phosphorus in rats with intact parathyroid glands. P refers to the difference between the control and 1,25DHCC infusion periods in both kidneys.
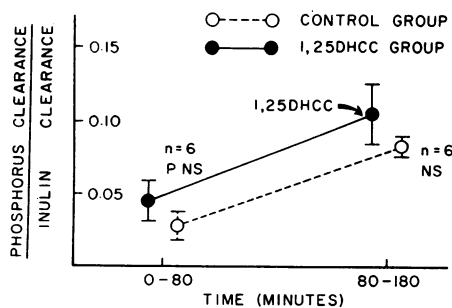

FIGURE 10 The effect of i.v. 1,25DHCC on renal handling of phosphorus in PTX rats. P refers to the difference between the corresponding collection periods in both groups.

In group 7b (Fig. 9), there was no difference in C_P/C_{In} between the two kidneys during the control periods (0–80 min) and during the infusions of 1.25DHCC into the left renal artery (80–160 min). During the infusion of 1,25DHCC there was a marked but equal decrease in C_P/C_{In} on both sides. There were no significant changes in C_{In} and serum concentrations of calcium and phosphorus during the study. The effect of i.v. 1,25-DHCC on C_P/C_{In} in PTX rats (group 8a) compared with the effect on a control group of rats not receiving 1,25DHCC is shown in Fig. 10. There was no significant difference in C_P/C_{In} between the two groups before (0–80 min) and during 1,25DHCC infusion (80–160 min), suggesting a lack of effect of 1,25DHCC on renal handling of phosphorus in PTX rats. Similarly no response was elicited when 1,25DHCC was infused directly into the left renal artery in group 8b (Fig. 11). In both the 8a and b groups there were no significant changes in C_{In} and serum concentrations of calcium and phosphorus throughout the study.

DISCUSSION

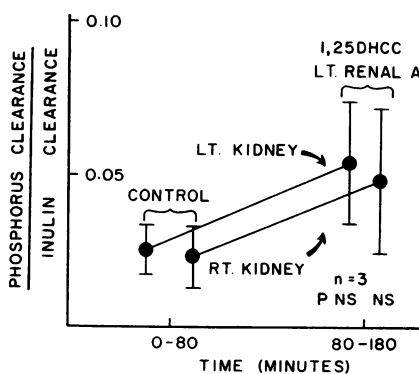The results of the present study demonstrate that 25HCC enhances fractional reabsorption of phosphorus in the

FIGURE 11 The effect of 1,25DHCC infusion directly into left renal artery on renal handling of phosphorus in PTX rats. P refers to the difference between the control and 1,25DHCC infusion periods in both kidneys.

presence of either endogenous or exogenous parathyroid hormone and imply a parathyroid hormone-dependent mechanism of action of 25HCC on the kidney. Since the observed changes in C_P/C_{In} were not associated with significant variations in glomerular filtration rate or serum concentration of inorganic phosphorus, it may be concluded that 25HCC produced a net increase in tubular reabsorption of phosphorus.

Our observations agree with a previous study which demonstrated a lack of acute effect of vitamin D on tubular reabsorption of phosphorus in PTX animals. The authors of the cited report considered the calcium-dependent secretory activity of parathyroid glands as the underlying mechanism for the increase in tubular reabsorption of phosphorus after the administration of vitamin D (4). In contrast, our study demonstrates an acute renal effect of vitamin D in the absence of parathyroid glands, although it required the presence of parathyroid hormone. Our study provides detailed evidence regarding the effect of 25HCC, but not regarding that of 1,25DHCC.

In keeping with our observations, previous studies demonstrated an increase in tubular reabsorption of phosphorus, after the administration of vitamin D to patients with osteomalacia before a detectable rise in serum calcium became apparent (17). Similarly in the present study the increase in tubular reabsorption of phosphorus during the administration of 25HCC (and 1,25DHCC) to intact rats was not associated with significant changes in serum calcium concentration. Should the renal response to vitamin D be secondary to suppression of parathyroid hormone, a rise in serum calcium would be expected to precede or coincide with the increase in tubular reabsorption of phosphorus.

The nature of the association between 25HCC and parathyroid hormone with regard to the observed changes in C_P/C_{In} is not clearly defined by the present study. Adequate parathyroid function maintains normal serum calcium. Under certain circumstances the metabolic action of 25HCC may be controlled by the level of calcium (12). To examine the possibility that hypocalcemia could be responsible for the lack of response to 25HCC in PTX rats, serum calcium was raised deliberately and maintained at normal range in group 3. However, sustained normocalcemia failed to restore the renal response to 25HCC in the PTX rats. Furthermore, subsequent experiments demonstrated that administration of exogenous parathyroid hormone (group 6) restored the renal response to 25HCC, even though it did not correct the hypocalcemia.

The failure of 25HCC to produce a noticeable decrease in C_P/C_{In} in PTX rats could be due to the pre-existing low excretion rate of phosphorus. Parathyroid hormone could have restored the renal response to 25HCC by increasing urinary excretion of phosphorus.

Previous studies suggested that the phosphaturic response to extracellular fluid volume expansion with normal saline in rats was mainly mediated by changes in parathyroid gland activity and was minimal or absent in acutely PTX rats (18). We were able to augment urinary excretion of phosphorus in rats subjected to PTX two days before, by expanding the extracellular fluid volume with saline. Even though the amount of excreted phosphorus reached 15% or more of its filtered load, this increase failed to restore the renal response to 25HCC. Similarly we were unable to see a response to 25HCC in PTX rats in which C_P/C_{In} was increased with i.v. sodium phosphate.

Recent studies demonstrated that the presence of parathyroid hormone is necessary for the conversion of 25-HCC into a 1,25DHCC in the kidney (19, 20). The latter compound has been shown to be the active form of vitamin D which acts directly on the gut and on the bone (21–24). Should 1,25DHCC also be the form of vitamin D which acts directly on the kidney, parathyroid hormone could be necessary for the renal action of 25-HCC because of its role in coverting 25HCC into 1,25DHCC. To test this possibility, 1,25DHCC was given to PTX rats, both intravenously and by a direct infusion into one renal artery, but had no apparent effect on tubular reabsorption of phosphorus. However, the administration of 1,25DHCC to rats with intact parathyroid glands increased significantly fractional reabsorption of phosphorus.

The question whether 25HCC and/or 1,25DHCC directly or indirectly affects tubular reabsorption of phosphorus (in the presence of parathyroid hormone) is not answered by the results of the present study. The lack of unilateral response during intrarenal arterial infusion of both 25HCC and 1,25DHCC does not support a direct action, but certainly does not exclude it. With regard to the observations made during volume expansion, a word of caution is warranted: one cannot exclude the possibility that the phosphaturic effect of volume expansion procedure could have influenced the capacity of 25HCC to reduce phosphorus excretion.

Several alternatives regarding the role of parathyroid hormone in the renal response to vitamin D are worth comment. First, parathyroid hormone may play a permissive role in the renal response to vitamin D, similar to the role ascribed to vitamin D in facilitating the action of parathyroid hormone on the bone (25, 26). Next, the presence of parathyroid hormone may be necessary for converting 25HCC and/or 1,25DHCC into an additional yet unknown compound(s) which act(s) directly on the renal tubule. And finally, vitamin D may act only on one component of inorganic phosphorus transport in kidney which has been described to be selectively sensitive to parathyroid hormone (27, 28). In the absence of parathyroid hormone this transport sys-

tem may be operating already at its maximal capacity precluding any additional effect of vitamin D on phosphorus reabsorption.

ACKNOWLEDGMENTS

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