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Research Article

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Effect of 1,25-Dihydroxyvitamin D_3 on the Renal Handling of P_i in Thyroparathyroidectomized Rats

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ABSTRACT The kidney adapts its tubular capacity to transport inorganic phosphate (P_i) according to the dietary supply of P_i in both intact and thyroparathyroidectomized (TPTX) rats. However, in TPTX rats the capability of the renal tubule to adapt to a high P_i diet is diminished. In TPTX rats the production of the active vitamin D_3 metabolite, 1,25-dihydroxyvitamin D_3 [1,25-$(OH)_2D_3$], is also reduced. 1,25-$(OH)_2D_3$ has been shown to have a marked effect on P_i metabolism. Therefore the question arises whether the deficient production of 1,25-$(OH)_2D_3$ contributes to the alteration of the tubular transport of P_i observed in chronically TPTX rats. In the present investigation, vitamin D-replete rats were sham operated (SHAM) or thyroparathyroidectomized and then pair fed diets containing either 0.2 or 1.2 g/100 g P for 7 days. During this period, groups of SHAM and TPTX rats received i.p. 2 × 13 pmol/day of 1,25-$(OH)_2D_3$, a dose which was shown to just normalize the decreased intestinal absorption of Ca and P_i in TPTX rats. The capacity of tubular P_i transport was then assessed by measuring the fractional excretion of P_i (FEP_i) at increasing plasma P_i concentration ($[P_i]_{Pl}$) obtained by acute infusion of P_i. The results show that in SHAM rats fed either P diet, 1,25-$(OH)_2D_3$ has no effect on the renal handling of P_i. In TPTX rats fed 1.2 g/100 g P diet, 1,25-$(OH)_2D_3$ increases FEP_i over a wide range of $[P_i]_{Pl}$. In TPTX rats fed a 0.2 g/100 g P diet, 1,25-$(OH)_2D_3$ does not alter FEP_i up to a $[P_i]_{Pl}$ of 3.0–3.5 mM, but does increase it at higher $[P_i]_{Pl}$. In fact, on both diets TPTX rats supplemented with 1,25-$(OH)_2D_3$ appear to have the same renal handling of P_i as SHAM counterparts. The effect of 1,25-$(OH)_2D_3$ was not associated with a change in urine pH or in urinary excretion of cyclic AMP and was maintained under marked extracellular volume expansion. It was associated with a rise in plasma calcium in the TPTX rats fed the high, but not the low, P diet. In TPTX rats fed 1.2 g/100 g P diet, 25-hydroxyvitamin D_3 in doses of 2 × 130 or 2 × 1,300 pmol/day i.p. did not increase FEP_i.

In conclusion, 1,25-$(OH)_2D_3$ administered in physiological amounts to TPTX rats restores to normal the capability of the renal tubule to excrete P_i and to adapt to large variation in dietary P_i. The results suggest that 1,25-$(OH)_2D_3$ plays an important role in the regulation of the renal handling of P_i and that the chronic change in the tubular capacity to transport P_i after TPTX may be due to the decreased formation of 1,25-$(OH)_2D_3$.

INTRODUCTION

The kidney responds to variations in the dietary intake of inorganic phosphate (P_i)[1] by changing its tubular capacity to transport P_i (1–3). This adaptative response can be observed in both intact and chronically thyroparathyroidectomized (TPTX) rats (1, 3). However, the capability of the renal tubule to adapt to a high-P_i diet is diminished in TPTX rats (1, 3). The reason for this reduced capability of adaptation after TPTX has not yet been established. TPTX or parathyroidectomy (PTX) causes a rapid and marked decrease in the fractional excretion of P_i (FEP_i) (4–7). This effect can be observed within the first 3 h after the surgical procedure (7). However, after the acute phase, FEP_i

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[1] *Abbreviations used in this paper:* C_{In}, clearance of [*methoxy*-^{3}H]inulin; D_{Pi}, clearance of inorganic phosphate; ECVE, extracellular volume expansion; FENa, fractional excretion of sodium; FEP_i, fractional excretion of inorganic phosphate; FLP_i, filtered load of inorganic phosphate; 25-OHD_3, 25-hydroxyvitamin D_3; 1,25-$(OH)_2D_3$, 1,25-hydroxyvitamin D_3; P_i, inorganic phosphate; $[P_i]_{Pl}$, plasma inorganic phosphate concentration; PTX, parathyroidectomy; SHAM, sham operated; TPTX, thyroparathyroidectomized; TRP_i, absolute tubular reabsorption of phosphate; UVP_i, absolute excretion of P_i; V, urinary volume.

increases up to a steady-state value within 24–48 h after PTX (7, 8). This rise in FEP_i could well be interpreted as an adaptive response tending to normalize the handling of P_i. In chronic PTX or TPTX animals FEP_i remains, nevertheless, lower than normal (4–7), in spite of the preservation of an operating adaptation mechanism (1, 2). This incomplete readjustment of the tubular capacity could be attributed to the disappearance of the direct and rapid effect of PTH on the tubular P_i transport, which is probably mediated through the adenylate cyclase system (9). However, studies on vitamin D metabolism indicate that removal of the parathyroid glands leads within 24 h to a conspicuous reduction in the renal conversion of 25-hydroxyvitamin D_3 (25-OHD_3) into 1,25-dihydroxyvitamin D_3 [1,25-$(OH)_2D_3$] (10). This latter metabolite influences markedly P_i homeostasis (11). Therefore, in TPTX animals the reduced production (10) and plasma level of 1,25-$(OH)_2D_3$ (12) could contribute to the chronic alteration in the tubular capacity to transport P_i.

In order to investigate this problem, we have studied the renal handling of P_i in sham-operated (SHAM) and TPTX rats supplemented or not with 1,25-$(OH)_2D_3$. The study was made under both low-P and high-P diet. 1,25-$(OH)_2D_3$ was given i.p. at the dose of 13 pmol twice daily for 7 days. This dose was chosen because previous studies have shown that it just normalizes but does not overcorrect the low intestinal Ca and P absorption of TPTX rats (13, 14). The influence of TPTX on intestinal Ca (15, 16) and P (14) absorption is very likely due to the decrease in the 1,25-$(OH)_2D_3$ production which occurs in these animals (10). Therefore the minimal administered amount of 1,25-$(OH)_2D_3$ which will normalize the intestinal calcium absorption may be considered a physiological dose, since it will substitute for the reduced endogenous production of the vitamin D_3 metabolite after the removal of the parathyroid gland.

METHODS

Male Wistar rats weighing 150–170 g and raised on a commercial chow food (Altromin 1314, Altrogge, Lage, Lippe, W. Germany) containing 1.2 g/100 g P, 1.1 g/100 g Ca, and 280 IU/100 g vitamin D_3, with free access to tap water, were used. In a first series of experiments, these vitamin D-replete rats were fed 14 days before the renal study an experimental diet containing the same amount of P and Ca, i.e., 1.2 g/100 g P and 1.1 g/100 g Ca. This diet was prepared by the addition of sodium phosphate and calcium gluconate to a basic diet (Altromin C-1730) containing 0.2 g/100 g P and 0.1 g/100 g Ca. 6 days after starting the experimental diet, the rats were either thyroparathyroidectomized or sham operated under ether anesthesia. Then during the 8 days preceding the renal study, the rats were either maintained under the experimental diet containing 1.2 g/100 g P and 1.1 g/100 g Ca or pair fed 0.2 g/100 g P and 1.1 g/100 g Ca prepared from the same basic food (Altromin C-1730). The sodium content was kept constant by adding NaCl to the low-P diet. The day after the surgical procedure, the rats were given a first i.p. injection of either 13 pmol of chemically synthetized 1,25-$(OH)_2D_3$[2] dissolved in 25 μl of 95% ethanol or the ethanol vehicle alone. This treatment was given during the next 6 days twice daily between 8 and 9 a.m. and 4 to 5 p.m. On the day of the renal study, the last injection was given at 8 a.m., 90 min before starting the equilibration period of the clearance experiment.

In a second set of experiments, the influence of 25-OHD_3[2] on the renal handling of P_i was studied instead of 1,25-$(OH)_2D_3$. TPTX rats fed the 1.2 g/100 g P experimental diet received i.p. 14 injections of 25-OHD_3 in doses of 130 and 1,300 pmol dissolved in 25 μl of 95% ethanol. A group of pair-fed animals were injected i.p. with the ethanol vehicle as described above.

In order to reduce the dead space of the urinary tract, in all experiments a subtotal cystectomy was done under ether anesthesia 48 h before the renal study, as previously described (1, 17). After the clearance study the treatment was stopped and all rats were returned to the usual lab chow for 15 days. After this period the animals were fasted overnight and a blood sample was taken from the tip of the tail. Only data of TPTX rats displaying a plasma calcium concentration below 1.88 mM (7.5 mg/100 ml) have been considered for this study.

Clearance experiments. In both series of experiments, the renal clearance study was started at the same time (9:30 a.m.). The general methodology of the clearance measurement in conscious rats has been described earlier (1, 17). For the present study, a first dose (priming) of inulin, i.e., 0.4 μCi of [*methoxy*-^{3}H] inulin (New England Nuclear, Boston, Mass.) with 12.8 mg of unlabeled inulin (Fluka A. G., Buchs, Switzerland) dissolved in 0.15 M NaCl was injected i.v. in a volume of 0.4 ml. Isotonic solutions containing 5 μCi/100 ml of [*methoxy*-^{3}H]inulin and 1 g/100 ml of unlabeled inulin and increasing amount of P_i was then infused at 4 ml/h with an Ismatec pump (Ismatec S.A., Zürich, Switzerland). The animals were first infused with 0.15 M NaCl for a 90-min equilibration period. Then a first urine collection period of 30 min (period I) was made, at the end of which a blood sample (a) was taken from a dorsal hind limb vein. The rats were then infused with P_i at stepwise increasing doses (45-min equilibrations followed by 30-min urine collection periods): 1.0 μmol P_i/min (period II), 2.0 μmol P_i/min (period III), and 3.0 μmol P_i/min (period IV). Blood samples (b, c, and d) were again taken immediately at the end of each urine collection period. The clearance of [*methoxy*-^{3}H]inulin (C_{In}) and Pi (C_{Pi}) were calculated for each period by the standard formula. The filtered load of phosphate ($FLP_i = [P_i]_{PI} \times C_{In}$), the absolute ($UVP_i$) and fractional excretion of P_i($UVP_i/FLP_i \times 100$), the absolute tubular reabsorption of phosphate ($TRP_i = FLP_i - UVP_i$), and the fractional excretion of sodium (FENa = UVNa/FLNa) were calculated likewise. No correction was made for incomplete ultrafiltrability of plasma P_i, since previous studies have shown that similar P_i loading did not alter significantly the ultrafiltrable fraction of P_i (1, 17).

In one series of experiments, urine was collected under xylol and pH was measured. In another series, the excretion rate of cyclic AMP was determined in SHAM or TPTX rats with or without 1,25-$(OH)_2D_3$ treatment as mentioned above. Finally, in TPTX rats with or without 1,25-$(OH)_2D_3$ administration, the renal handling of P_i was also studied under marked extracellular volume expansion by infusing the isotonic solution at 20 instead of 4 ml/h.

Analytical method. Urinary volume (V) was determined by weighing. The activity of [^{3}H]inulin in plasma and urine

[2] Kindly provided by F. Hoffmann-La Roche & Co., Basel, Switzerland.

was measured in a scintillation spectrometer. 20 μl of urine or 10 μl of plasma was added to vials containing 10 ml of a scintillation solution made of toluene, 600 ml; ethylene-glycomonoethylether, 400 ml; naphthalene, 80 g; and Butyl-PBD [2-(4-tert-butyl-phenyl)-5-(4-biphenylyl)-1,3,4-oxadizaol] (Ciba-Geigy Corp., Basel, Switzerland), 7.0 g. All aliquots were counted in duplicate. No difference in quenching was found between plasma and urine samples.

P_i was determined in plasma and urine and in the diets as phosphomolybdate after reduction with 10% ascorbic solution (18).

Plasma Ca concentration was measured by complexometric titration with ethyleneglycol-bis-(2-aminoethyl)-tetraacetic acid (Corning Calcium Analyzer 940). Na concentration in plasma and urine was determined by flame photometry (EEL, flame photometer, Evans Electroselenium Ltd., Halstead, England). The diets were analyzed after incineration of the samples and dissolution of the ash in 0.1 N HCl. The osmolality of solutions for infusion was measured with an Advanced Osmometer (model 3W, Advanced Instruments, Inc., Needham Heights, Mass.). Urinary cyclic AMP was determined by competitive binding assay using a commercial kit (cyclic AMP assay kit, The Radiochemical Centre, Amersham, England).

Statistical analysis. The experimental results are expressed as mean values ±SEM. The significance of the differences between groups were evaluated by two-sided Student's *t* test.

RESULTS

In SHAM rats, the chronic administration of 1,25-$(OH)_2D_3$ (2 × 13 pmol/day i.p. for 7days) does not appear to have any significant influence on the renal handling of P_i under either diet, as shown in Table I and Fig. 1. Fig. 1 also shows that in intact rats the dietary-

TABLE I

Renal Handling of Phosphate in SHAM Rats with or without 1,25-$(OH)_2D_3$ Treatment

	SHAM + ethanol vehicle (n = 9; body wt = 186±3 g)					SHAM + 1,25-$(OH)_2D_3$* (n = 6; body wt = 180±3 g)				
Infused P_i	$[P_i]$	UVP_i	TRP_i	C_{in}	FENa	$[P_i]$	UVP_i	TRP_i	C_{in}	FENa
μmol/min	*mM*	*nmol/ml GF*	*nmol/ml GF*	*ml/min*	*%*	*mM*	*nmol/ml GF*	*nmol/ml GF*	*ml/min*	*%*
				Dietary P: 1.2 g/100 g						
0	2.86 ±0.07	357 ±44	2,506 ±72	2.00 ±0.10	2.34 ±0.16	2.71 ±0.10	347 ±55	2,667 ±132	1.98 ±0.12	3.05 ±0.60
1	2.89 ±0.06	610 ±63	2,272 ±65	2.08 ±0.16	3.06 ±0.43	2.95 ±0.07	536 ±61	2,417 ±81	1.99 ±0.24	3.28 ±0.49
2	3.10 ±0.09	981 ±57	2,120 ±117	1.91 ±0.23	3.83 ±0.23	3.10 ±0.07	883 ±75	2,220 ±87	1.71 ±0.16	3.79 ±0.40
3	3.61 ±0.08	1,596 ±157	2,036 ±196	1.56 ±0.11	4.62 ±0.39	3.45 ±0.06	1,400 ±92	2,044 ±86	1.68 ±0.10	4.20 ±0.56
				Dietary P: 0.2 g/100 g						
	SHAM + ethanol vehicle (n = 9; body wt = 168±4 g)					SHAM + 1,25-$(OH)_2D_3$ (n = 5; body wt = 168±4 g)				
0	1.89 ±0.09	0.7 ±0.1	1,894 ±88	1.62 ±0.17	2.14 ±0.45	2.39 ±0.14	0.6 ±0.2	2,389 ±155	1.88 ±0.12	2.23 ±0.59
1	2.62 ±0.10	1.0 ±0.3	2,619 ±107	1.83 ±0.18	3.15 ±0.52	3.01 ±0.16	0.8 ±0.11	3,009 ±181	2.00 ±0.23	2.88 ±0.42
2	3.59 ±0.08	114.1 ±26	3,481 ±69	1.76 ±0.26	3.77 ±0.29	3.73 ±0.12	214.7 ±97	3,473 ±83	2.00 ±0.17	3.56 ±0.24
3	4.10 ±0.08	1,114.5 ±95	2,971 ±177	1.37 ±0.15	4.23 ±0.46	3.92 ±0.12	1,230.1 ±74	2,698 ±155	1.83 ±0.06	4.27 ±0.47

Values represent means±SEM. n = number of animals. Under the 1.2 g/100 g P diet, the mean food intake (dry weight) monitored during the 7-day period preceding the clearance study was 15.6±0.2 and 14.7±0.5 g/day (mean±SEM) in SHAM and SHAM + 1,25-$(OH)_2D_3$, respectively. Under the 0.2 g/100 g P diet, it was 15.4±0.6 and 14.5±1.2 g/day in SHAM and SHAM + 1,25-$(OH)_2D_3$, respectively. The indicated body weight was measured on the day of the clearance study. Plasma $[P_i]$, phosphatemia; UVP_i, urinary excretion of phosphate; TRP_i, net tubular reabsorption of phosphate; C_{in}, clearance of inulin; FENa, fractional excretion of sodium.

* 2 × 13 pmol/day i.p. given during the 7 days preceding the renal study.

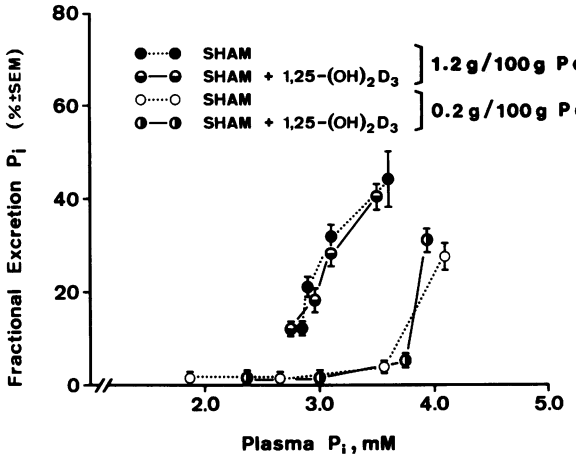

FIGURE 1 Fractional excretion of P_i (%) determined under acute i.v. sodium chloride and stepwise-increasing sodium phosphate infusion in sham-operated (SHAM) rats pair fed high- and low-P diet and treated or not with 1,25-$(OH)_2D_3$ (2 × 13 pmol/day i.p. for 7 days). Other data concerning these four groups of rats are presented in Table I.

induced change in the transport capacity that has been previously described (1) is not affected by the chronic administration of 1,25-$(OH)_2D_3$. However, 1,25-$(OH)_2D_3$ given to SHAM rats fed the low-P diet enhances significantly the level of plasma P_i measured during the 0.15 M NaCl infusion (2.39±0.14 vs. 1.89 ±0.09 mM, $P < 0.01$, Table I).

In TPTX rats fed a 1.2 g/100 g P diet (Fig. 2), the administration of 1,25-$(OH)_2D_3$ produces a much greater capacity to excrete P_i than in nontreated TPTX animals. At similar $[Pi]_{PI}$ (as for instance at 3.3 mM) FEP_i was markedly increased ($P < 0.001$) in the TPTX rats treated with 1,25-$(OH)_2D_3$ (Table V). In fact, the rats supplemented with 1,25-$(OH)_2D_3$ display the same capacity to transport P_i as their sham-operated and pair-fed counterparts. This marked effect of the 1,25-$(OH)_2D_3$ supplement in TPTX rats was not associated with any significant change in C_{In} and FENa (Table II). In TPTX rats fed a low-P(0.2 g/100 g) diet, 1,25-$(OH)_2D_3$ only influences P_i excretion significantly above a $[Pi]_{PI}$ of 3.0–3.5 mM (Fig. 3). Indeed, during the last period of clearance, when plasma P_i was similar in both groups, P_i excretion (UVP_i/ml glomerular filtration) was significantly ($P < 0.01$) enhanced in the animals treated with 1,25-$(OH)_2D_3$ (Table II). Again the relationship between plasma P_i and FEP_i appears to be very similar in SHAM and TPTX rats supplemented with 1,25-$(OH)_2D_3$ (Fig. 3). Thus in both high- and low-P_i diet the difference in the tubular capacity to transport P_i between SHAM and TPTX rats can be virtually abolished by the administration of these small doses of 1,25-$(OH)_2D_3$. 1,25-$(OH)_2D_3$ given to TPTX rats fed the high-P diet decreases significantly the phosphatemia determined during the 0.15 M NaCl infusion (2.66 ±0.07 vs. 3.32±0.15 mM, $P < 0.001$, Table II). In contrast, 1,25-$(OH)_2D_3$ given to TPTX rats fed the low-P diet enhances significantly the plasma level of P_i measured in the same conditions (2.15±0.07 vs. 1.45±0.12 mM, $P < 0.001$, Table II). Fig. 4 illustrates this opposite effect of 1,25-$(OH)_2D_3$ on the level of plasma P_i according to the prior dietary intake of P_i. Fig. 4 also shows that only the "hypo-" but not the "hyperphosphatemic" effect of 1,25-$(OH)_2D_3$ is associated with a change in the renal handling of P_i. Such a change could account for the decrease in the level of plasma P_i as described by Garabedian et al. (19).

Changes in urinary pH have been shown to be associated with overall alteration in the tubular handling of P_i (6). Our results are not likely to be due to this mechanism since in the TPTX rats fed either 1.2 or 0.2 g/100 g P diet, 1,25-$(OH)_2D_3$ treatment had no consistent effect on urine pH (Table III).

To investigate whether the adenylate cyclase system was involved in the 1,25-$(OH)_2D_3$ effect, we determined urinary excretion of cyclic AMP in groups of SHAM and TPTX rats with or without 1,25-$(OH)_2D_3$ treatment and pair fed the 1.2 g/100 g P diet. As shown in Table IV, the expected difference in cyclic AMP excretion between SHAM and TPTX rats was not modified by the administration of 1,25-$(OH)_2D_3$. Thus the increase in the capacity to excrete P_i in TPTX rats receiving 1,25-$(OH)_2D_3$ and fed a 1.2 g/100 g P diet was not associated with an increased excretion of cyclic AMP.

Since extracellular volume expansion (ECVE) can increase the fractional excretion of P_i (6), the influence

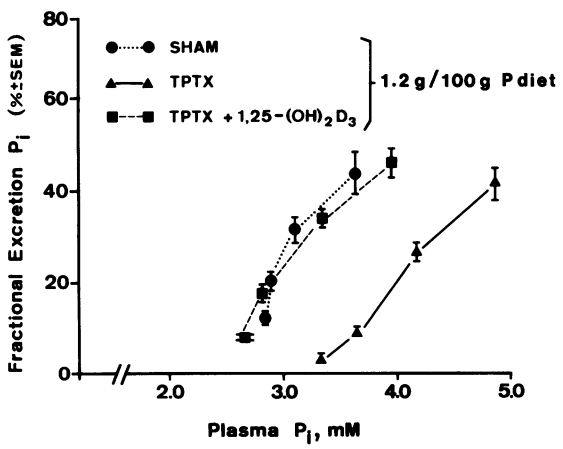

FIGURE 2 Fractional excretion of P_i (%) determined under acute i.v. sodium chloride and stepwise-increasing sodium phosphate infusion in sham-operated (SHAM), thyroparathyroidectomized (TPTX), and TPTX rats treated with 1,25-$(OH)_2D_3$ (2 × 13 pmol/day i.p. for 7 days). All rats were pair fed a 1.2 g/100 g P diet. Other data concerning these three groups of rats are presented in Table I (SHAM) and Table II [TPTX and TPTX + 1,25-$(OH)_2D_3$].

TABLE II

Renal Handling of P_i in TPTX Rats with or without 1,25-$(OH)_2D_3$ Treatment

Infused P_i	TPTX + ethanol vehicle (n = 11; body wt = 189±2 g)					TPTX + 1,25-$(OH)_2D_3$* (n = 14; body wt = 183±4 g)				
	$[P_i]$	UVP_i	TRP_i	C_{in}	FENa	$[P_i]$	UVP_i	TRP_i	C_{in}	FENa
μmol/min	*mM*	*nmol/ml GF*	*nmol/ml GF*	*ml/min*	*%*	*mM*	*nmol/ml GF*	*nmol/ml GF*	*ml/min*	*%*
				Dietary P: 1.2 g/100 g						
0	3.32 ±0.15	109 ±30	3,185 ±171	1.34 ±0.05	3.02 ±0.39	2.66 ±0.07	224 ±22	2,440 ±80	1.16 ±0.08	2.54 ±0.22
1	3.67 ±0.16	342 ±52	3,293 ±162	1.49 ±0.07	3.35 ±0.21	2.87 ±0.07	531 ±41	2,342 ±93	1.31 ±0.09	3.37 ±0.19
2	4.18 ±0.11	1,128 ±62	3,045 ±114	1.39 ±0.08	4.88 ±0.37	3.31 ±0.07	1,130 ±57	2,170 ±89	1.44 ±0.08	5.18 ±0.46
3	4.88 ±0.08	2,056 ±132	2,829 ±163	1.17 ±0.07	5.55 ±0.48	3.93 ±0.09	1,787 ±127	2,142 ±160	1.31 ±0.05	6.12 ±0.39
				Dietary P: 0.2 g/100 g						
	TPTX + ethanol vehicle (n = 8; body wt = 182±4 g)					TPTX + 1,25-$(OH)_2D_3$ (n = 8; body wt = 169±4 g)				
0	1.45 ±0.12	0.6 ±0.1	1,447 ±125	1.22 ±0.10	4.95 ±0.83	2.15 ±0.07	0.7 ±0.1	2,143 ±75	1.14 ±0.11	3.19 ±0.41
1	2.11 ±0.16	1.9 ±0.9	2,113 ±161	1.19 ±0.09	4.71 ±0.34	2.94 ±0.07	5.4 ±2.9	2,933 ±74	1.34 ±0.10	4.23 ±0.36
2	3.27 ±0.12	8.4 ±2.5	3,263 ±125	1.20 ±0.10	4.75 ±0.54	3.90 ±0.06	593.7 ±103	3,300 ±127	1.38 ±0.08	5.87 ±0.53
3	4.78 ±0.10	420.6 ±76.1	4,317 ±84	1.18 ±0.12	5.33 ±0.68	4.44 ±0.16	1,474 ±312	2,968 ±120	1.22 ±0.06	7.42 ±1.02

Values represent means±SEM. Under the 1.2 g/100 g P diet, the mean food intake (dry weight) monitored during the 7-day period preceding the clearance study was 14.6±0.8 and 13.8±0.4 g/day in TPTX and TPTX + 1,25-$(OH)_2D_3$, respectively. Under the 0.2 g/100 g P diet, it was 14.9±1.6 and 13.4±0.5 g/day in TPTX and TPTX + 1,25-$(OH)_2D_3$, respectively. See legend to Table I for further explanation.

of 1,25-$(OH)_2D_3$ (2 × 13 pmol/day i.p. for 7 days) on the renal handling of P_i of TPTX rats fed a 1.2 g/100 g P was studied under marked ECVE. FEP_i was measured at a similar $[Pi]_{PI}$ in TPTX rats receiving an isotonic saline solution infused at 20 ml/h. As shown in Table V, the effect of 1,25-$(OH)_2D_3$ on the renal handling of P_i was maintained under a conspicuous ECVE associated with a FENa of more than 15% in both groups.

Variation in plasma Ca has been shown to be associated with alteration in the renal handling of phosphate (6). The values of plasma calcium obtained at the end of the first and last clearance periods are presented in Table VI. In rats fed the 1.2 g/100 g P diet, 1,25-$(OH)_2D_3$ abolished the difference in plasma calcium between SHAM and TPTX rats, when assessed during the infusion of isotonic saline. Under acute P_i infusion, the fall in plasma calcium was smaller in SHAM than in TPTX + 1,25-$(OH)_2D_3$, so that the calcemia was significantly lower in the latter group during the last period of clearance (Table VI). In TPTX rats fed the 0.2 g/100 g P diet, plasma calcium assessed under either isotonic saline or P_i infusion was not higher in the 1,25-$(OH)_2D_3$-treated group than in the control group (Table VI).

The tubular response to long-term administration of various doses of 25-OHD_3 was studied in TPTX rats fed a 1.2 g/100 g P diet. Preliminary experiments indicated that administration of 2 × 13 pmol/day of 25-OHD_3 given for 7 days did not decrease the capacity of the tubule to reabsorb P_i. As shown in Fig. 5, a dose of 25-OHD_3 10 times higher (2 × 130 pmol/day) had no significant influence on the renal handling of P_i. A dose of 25-OHD_3 100 times higher (2 × 1,300 pmol/day) might, if anything, enhance the net tubular reabsorption of P_i. However, the effect of this dose on FEP_i was not observed at the endogenous or at the highest plasma P_i concentration.

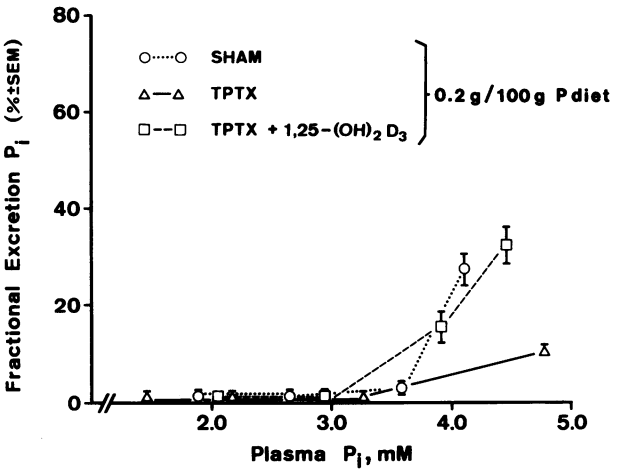

FIGURE 3 Fractional excretion of P_i (%) determined under acute i.v. sodium chloride and stepwise-increasing sodium phosphate infusion in sham-operated (SHAM), thyroparathyroidectomized (TPTX), and TPTX rats treated with 1,25-$(OH)_2D_3$ (2 × 13 pmol/day i.p. for 7 days). All rats were pair fed a 0.2 g/100 g P diet. Other data concerning these three groups of rats are presented in Table I (SHAM) and Table II [TPTX and TPTX + 1,25-$(OH)_2D_3$].

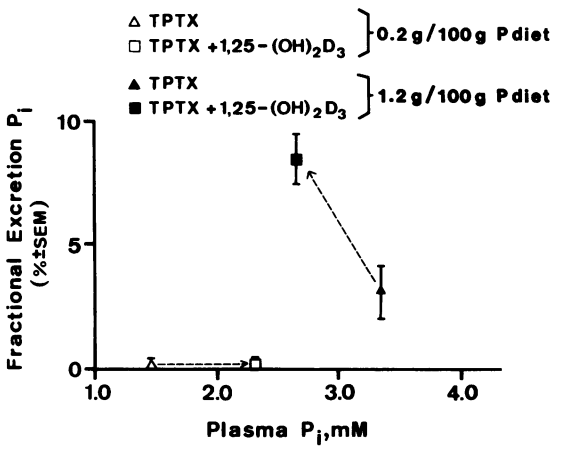

FIGURE 4 Influence of 1,25-$(OH)_2D_3$ treatment in TPTX rats fed low- or high-P diet. Under low-P diet, 1,25-$(OH)_2D_3$ (2 × 13 pmol/day i.p. for 7 days) increases $[P_i]_{PI}$ without altering FEP_i. In contrast, under high-P diet, 1,25-$(OH)_2D_3$ decreases $[P_i]_{PI}$ and enhances FEP_i. Data correspond to the values presented in Table II. They were obtained during the infusion of sodium chloride in the four groups of TPTX rats.

DISCUSSION

The present study demonstrates that in vitamin D-replete TPTX rats the chronic administration of a small dose of 1,25-$(OH)_2D_3$ has a profound effect on the renal handling of phosphate. Indeed, TPTX rats supplemented with doses of 1,25-$(OH)_2D_3$ which have been shown to just correct the decreased intestinal Ca and P absorption of these animals (13, 14) exhibit a very similar tubular capacity to transport P_i as do SHAM animals. The same doses of 1,25-$(OH)_2D_3$ given to vitamin D-replete intact rats have no apparent effect on the tubular P_i transport, although they can enhance the Ca (20) and P (14) intestinal absorption. Thus physiological doses of 1,25-$(OH)_2D_3$ only alter the renal handling of P_i in rats deprived of parathyroid hormone. Treatment with 25-OHD_3, even at 100 times larger doses, does not exert such an effect.

In TPTX rats the change in the fractional excretion of P_i observed after 1,25-$(OH)_2D_3$ treatment depends markedly upon the prior dietary intake of phosphorus and the level of plasma P_i at the time of the clearance measurement. In TPTX rats fed a 1.2 g/100 g P diet, administration of 1,25-$(OH)_2D_3$ increases the fractional excretion of P_i over a wide range of plasma P_i. However, in TPTX rats fed a low-P (0.2 g/100 g) diet, treatment with 1,25-$(OH)_2D_3$ does not interfere with the ability of the kidney to excrete a urine virtually free of P, up to a plasma P_i level of 3.0 to 3.5 mM. Thus, 1,25-$(OH)_2D_3$ in a dose which can be considered as an adequate substitution for the decreased endogenous production of 1,25-$(OH)_2D_3$ which occurs in TPTX rats (10) restores the full capability of the renal tubule to change its P_i transport capacity in response to variations in the dietary supply of P_i. Therefore it is conceivable that 1,25-$(OH)_2D_3$ plays a permissive role in the tubular adaptation to high-P_i intake, and that the diminished capability of the renal tubule for adapt-

TABLE III
Urine pH in TPTX Rats with or without 1,25-$(OH)_2D_3$ Treatment

Dietary P (*g/100 g*)	1.2	1.2	0.2	0.2
Treatment	Ethanol vehicle	1,25-$(OH)_2D_3$	Ethanol vehicle	1,25-$(OH)_2D_3$
n	4	3	4	3
Infused P_i, *μmol/min*				
0	7.14±0.14	6.74±0.39	7.36±0.12	7.25±0.27
1	6.53±0.13	6.66±0.05	7.29±0.13	7.25±0.21
2	6.38±0.06	6.73±0.19	7.38±0.10	7.00±0.05
3	6.75±0.08	6.89±0.11	7.02±0.12	7.04±0.04

All values are means±SEM.

TABLE IV

Urine Excretion of Cyclic AMP (pmol/min) in SHAM and TPTX Rats with or without 1,25-$(OH)_2D_3$ Treatment

Treatment	SHAM Ethanol vehicle	TPTX Ethanol vehicle	TPTX 1,25-$(OH)_2D_3$*
n	3	3	4
Infused P_i, *μmol/min*			
0	132.7±5.2	66.7±4.5‡	51.3±6.2‡
1	134.6±1.8	68.0±11.2§	47.3±14.4§
2	140.6±8.7	59.0±17.6‖	59.8±7.0§
3	108.0±8.5	52.3±4.7§	46.5±6.0§

All values are means±SEM. No statistically significant difference was found between TPTX + ethanol and TPTX + 1,25-$(OH)_2D_3$.

* 2 × 13 pmol/day i.p. given during the 7 days preceding the renal study.

‡ $P < 0.001$.

§ $P < 0.01$.

‖ $P < 0.02$ as compared with the corresponding value of the SHAM group.

ing to a high-P_i diet (1, 3) in chronically TPTX rats is due to the reduced production of 1,25-$(OH)_2D_3$ (10). Such a possibility would imply that parathyroid hormone may affect the tubular capacity to transport P_i by two distinct mechanisms: a rapid action mediated probably through the adenylate cyclase system (9), and a long-term influence by affecting the production of 1,25-$(OH)_2D_3$.

Our results confirm that 1,25-$(OH)_2D_3$ can exert opposite effects on the phosphatemia according to the phosphate status of the animals (19). On a low-P_i diet, 1,25-$(OH)_2D_3$ induces an elevation in plasma P_i which cannot be attributed to a change in the renal capacity to transport P_i but could be due to an increase in bone resorption (21, 22). On the other hand, the fall in the phosphatemia (19) which occurs in TPTX rats fed a normal or high-P diet could be due, in full or in part, to a change in the renal handling of P_i.

The mechanism whereby 1,25-$(OH)_2D_3$ affects the renal handling of P_i does not seem to be related to a change in tubular acidification. Indeed, the overall alteration in the renal handling of P_i is not associated with any consistent change in the urinary pH. Nor can the change in the renal handling of P_i be explained by an alteration in the tubular transport of sodium resulting from variations in ECVE. Indeed, in TPTX rats conspicuous changes in ECVE cannot produce such a prominent change in the tubular P_i transport as that observed in TPTX rats supplemented with 1,25-$(OH)_2D_3$ (Table V). Furthermore, the change in the renal handling of P_i observed under marked ECVE is associated with a significant increase in water excretion and an elevation in the fractional excretion of calcium which cannot be explained by alteration in filtered load (6). This was not the case for water excretion in our rats treated with 1,25-$(OH)_2D_3$ (Table III), and the increased calciuria observed can be entirely accounted for by the increased filtered load of calcium (23).

Although cyclic AMP excretion might not reflect in all circumstances the activity of the renal adenylate cyclase, our data suggest that the effect was not due to a stimulation of this system, since the rate of urine cyclic AMP excretion was not modified by the administration of 1,25-$(OH)_2D_3$.

Acute elevation of plasma calcium has been shown to be accompanied variously by an increase (24, 25) and a decrease (26–28) in the renal P_i excretion. Another observation (29) was that acute hypocalcemia was associated with an augmentation in the urinary output of P_i. Chronic elevation of plasma calcium by i.v. infusion in patients with hypoparathyroidism (30) resulted in an

TABLE V

Maintenance of the Influence of 1,25-$(OH)_2D_3$ Treatment on the Renal Handling of Phosphate in TPTX Rats Undergoing Marked Saline Diuresis

	i.v. infusion, 4 ml/h		i.v. infusion, 20 ml/h	
Treatment	Ethanol vehicle	1,25-$(OH)_2D_3$*	Ethanol vehicle	1,25-$(OH)_2D_3$*
n	11	14	5	5
Plasma [P_i]	3.3±0.2	3.3±0.1	3.2±0.1	3.2±0.2
FEP_i, %	3.1±0.1	34.3±2.0‡	7.0±1.1	31.0±4.1‡
FENa, %	3.0±0.4	5.2±0.5‡	19.3±1.0	17.2±1.8

All values are means±SEM. Animals were fed a 1.2 g/100 g P diet.

* 2 × 13 pmol/day i.p.

‡ $P < 0.001$ with respect to the corresponding group receiving the ethanol vehicle alone. For comparison, the results obtained at similar plasma [P_i] in rats infused at 4 ml/h are also presented. They correspond to the data presented in Table II and Fig. 2.

TABLE VI

Plasma Calcium (mmol/liter) in SHAM and TPTX Rats with or without 1,25-$(OH)_2D_3$ Treatment

Infused P_i	SHAM + ethanol vehicle (9)	SHAM + 1,25-$(OH)_2D_3$* (6)	TPTX + ethanol vehicle (11)	TPTX + 1,25-$(OH)_2D_3$* (14)
	Dietary P: 1.2 g/100 g			
μmol/min				
0	2.33±0.04	2.25±0.03	1.57±0.08‡	2.35±0.07§
3	2.05±0.03	2.03±0.07	1.08±0.04‡	1.66±0.05‡§
	Dietary P: 0.2 g/100 g			
	SHAM + ethanol vehicle (9)	SHAM + 1,25-$(OH)_2D_3$* (5)	TPTX + ethanol vehicle (8)	TPTX + 1,25-$(OH)_2D_3$* (8)
0	2.74±0.09	2.45±0.15	2.76±0.15	2.40±0.10‖
3	1.62±0.07	1.80±0.08	1.32±0.03¶	1.32±0.07¶

Values are mean±SEM. Number in parentheses = number of rats. Plasma calcium was determined in the blood samples taken at the end of the first (infused P_i: 0) and last (infused P_i: 3 μmol/min) clearance period (see Tables I and II).

* 2 × 13 pmol/day i.p. given during 7 days.

‡ $P < 0.001$; ¶ $P < 0.01$; ‖ $P < 0.05$, all with respect to the sham-operated group receiving the ethanol vehicle.

§ $P < 0.001$ with respect to the thyroparathyroidectomized group receiving the ethanol vehicle.

increase in the renal P_i clearance. Therefore, the 1,25-$(OH)_2D_3$-induced change in the renal handling of P_i could be related to the chronic effect of the D_3 metabolite on the level of plasma calcium. However, it should be noticed that any influence of plasma calcium on the renal handling of P_i will remain dependent upon the phosphorus status of the animals. Indeed, the chronic elevation of plasma calcium in TPTX rats fed a low-P diet with or without 1,25-$(OH)_2D_3$ treatment did not prevent them from excreting a urine virtually free of P_i, up to a plasma P_i level of about 3.0 mM. Furthermore, above this plasma P_i level, the TPTX rats receiving 1,25-$(OH)_2D_3$ exhibited a greater phosphaturia than their counterparts fed the same low-P diet (Table II, Fig. 3), although they did not present a higher plasma calcium level (Table VI). Therefore, although change in plasma calcium might well contribute to the effect of 1,25-$(OH)_2D_3$ on the renal handling of P_i in the TPTX rats fed the high-P diet, it cannot explain the results obtained in the TPTX animals fed the low-P diet.

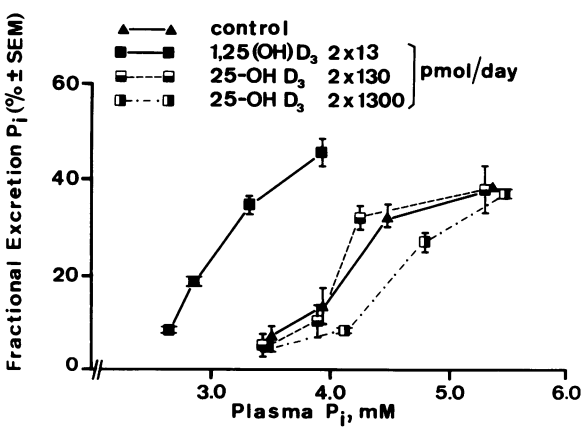

FIGURE 5 Fractional excretion of P_i (%) determined under acute i.v. sodium chloride and stepwise-increasing phosphate infusion in thyroparathyroidectomized (TPTX) rats treated or not with 25-OHD_3 (2 × 130 and 2 × 1,300 pmol/day i.p. for 7 days) and pair fed a 1.2 g/100 g P diet. The untreated group and the two groups treated with 25-OHD_3 consisted of four animals each. The data concerning 1,25-$(OH)_2D_3$, presented for comparison, belong to the group (n = 14) presented on Fig. 2 and Table II.

The present study does not permit the assessment of whether 1,25-$(OH)_2D_3$ influences directly or indirectly the renal transport of P_i. 1,25-$(OH)_2D_3$ can mobilize P_i from the gut (31) and probably also from the skeleton (21). Therefore, it is conceivable that the renal response is secondary to the extrarenal actions of 1,25-$(OH)_2D_3$ on P_i metabolism, since the magnitude of the renal response depends upon the P_i status of the animals. However, the effect of 1,25-$(OH)_2D_3$ on the intestinal P_i absorption is probably not large enough (14) to explain the dramatic change observed at the kidney level.

The chronic influence of small doses of 1,25-$(OH)_2D_3$ on the renal handling of P_i contrasts with the changes observed after the acute administration of the metabolite. Acute injection or infusion of 1,25-$(OH)_2D_3$ to dogs (32) or rats (33) has been shown to depress the fractional excretion of P_i. In the rat (33), this effect was elicited

by a rather larger dose of 1,25-$(OH)_2D_3$, 240 pmol/100 g body weight per h i.v. On a daily basis this dose is about 400 times larger than the amount used for the present study in 180-g TPTX rats. It is important to mention that in the rat the antiphosphaturic action of 1,25-$(OH)_2D_3$ requires the presence of the parathyroid glands (33). In both dogs (32) and rats (33) the precursor of 1,25-$(OH)_2D_3$, namely 25-OHD_3, appears to be more potent than its 1-hydroxylated derivative for promoting an antiphosphaturic response. Furthermore, in the dog the change in the fractional excretion of P_i elicited by 25-OHD_3 or 1,25-$(OH)_2D_3$ parallels alterations in calcium and sodium excretion (32, 34). The reduced P_i excretion is in fact associated with a fall 20 times larger in the absolute amount of sodium eliminated in the urine (32, 34). Thus, the acute antiphosphaturic effect of D_3 metabolites does not appear to be specific for the tubular P_i transport system, and 1,25-$(OH)_2D_3$ does not seem to be particularly active in eliciting such a response. Therefore, the physiological relevance of such an antiphosphaturic effect might be questioned. This is even more so in view of recent findings (22) demonstrating that neither 1,25-$(OH)_2D_3$ nor any other vitamin D metabolites are required to ensure a complete tubular reabsorption of P_i in response to a restriction in the dietary supply of P_i.

The marked influence of 1,25-$(OH)_2D_3$, given chronically in small amounts, on the renal handling of P_i of TPTX rats may bear some relevance to previous reports showing that large doses of vitamin D can lead to an increased P_i excretion in patients with hypoparathyroidism (35) or in PTX animals (36, 37). Thus, Albright and Reifenstein (35) found that 400,000 U of vitamin D_2 given to a hypoparathyroid patient led to an increase in urinary P_i which was associated with a decrease in serum P_i. Similar results were found by Crawford et al. (36) in PTX rats, wherein the administration of 10,000–100,000 U of vitamin D_2/day (625–6250 nmol/day) increased the fractional excretion of P_i for a given filtered load. Likewise, Ney et al. (37) found in vitamin D-deficient PTX dogs that 24 h after the administration of 100,000 U of vitamin D_2 i.m., or 6 or more days after the administration of 30,000 U daily, the urinary excretion of P_i increased about 10 times, while the filtered load remained constant. Therefore, it is quite possible that the effects described above pertain to the same mechanism as those we have observed under 1,25-$(OH)_2D_3$ treatment in TPTX rats receiving a normal supply of phosphate. As with the intestinal or bone response to vitamin D metabolites (11), much larger doses of the precursors of 1,25-$(OH)_2D_3$ might be needed to alter the renal transport of P_i. In fact, preliminary experiments in our laboratory indicate that doses of 25-OHD_3 as high as $2 \times 13{,}000$ pmol/day i.p. also tend to normalize the renal handling of P_i of TPTX rats.

Finally, our results obtained in TPTX rats are also consistent with a very recent clinical observation made in children with hypoparathyroidism (38) indicating that chronic treatment with small doses of 1,25-$(OH)_2D_3$ (72–96 pmol/kg per day, a dose very similar to that used in the present study) also promotes a decrease in the plasma P_i level while it concomitantly increases the renal clearance of P_i. This suggests that in humans as in rats the physiological role of 1,25-$(OH)_2D_3$ on the renal handling of P_i is not to stimulate the reabsorption of this ion, but to maintain the full capability of the renal tubule for adapting to variations in the P_i load of the organism. The process whereby 1,25-$(OH)_2D_3$ interacts, directly or indirectly, with the tubular P_i transport deserves to be further explored.

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