Resistance to the Phosphaturic and Calcemic Actions of Parathyroid Hormone during Phosphate Depletion: *PREVENTION BY 1,25-DIHYDROXYVITAMIN D$_3$*

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Recent observations indicate that in thyroparathyroidectomized (TPTX) rats fed a low (0.2 g/100 g) phosphorus diet, the tubular phosphaturic response to parathyroid hormone (PTH) remains markedly blunted even when it is assessed at normal or high plasma concentration and filtered load of inorganic phosphate (Pi). Because 1,25-dihydroxyvitamin D$_3$ [1,25(OH)$_2$D$_3$] decreases the tubular capacity to reabsorb Pi when chronically administered to TPTX rats, we have studied whether this vitamin D$_3$ metabolite could specifically increase the phosphaturic response to PTH in phosphate-deprived animals. The results show that in Vitamin D-replete TPTX rats fed a low (0.2 g/100 g) phosphorus diet, 1,25(OH)$_2$D$_3$ (2 × 13 pmol/d i.p. for 7 d) markedly enhanced the acute tubular phosphaturic response to PTH (2.5 IU/h i.v.) without affecting the action of the peptide hormone on Ca reabsorption and cyclic-3′,5′-AMP excretion. The influence of 1,25(OH)$_2$D$_3$ on the phosphaturic response to PTH could not be ascribed to an increased plasma concentration and(or) filtered load of Pi during the administration of the peptide hormone. However, it could be, at least in part, related to the elevation in the basal level of plasma Pi which was observed in the 1,25(OH)$_2$D$_3$-treated animals. The results also indicate that 1,25(OH)$_2$D$_3$ significantly enhanced the calcemic response to PTH, which was blunted in these conditions of phosphate deprivation. Unlike 1,25-(OH)$_2$D$_3$, 25-hydroxyvitamin D$_3$ did not unmask the […]

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PREVENTION BY 1,25-DIHYDROXYVITAMIN D_3

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A B S T R A C T Recent observations indicate that in thyroparathyroidectomized (TPTX) rats fed a low (0.2 g/100 g) phosphorus diet, the tubular phosphaturic response to parathyroid hormone (PTH) remains markedly blunted even when it is assessed at normal or high plasma concentration and filtered load of inorganic phosphate (Pi). Because 1,25-dihydroxyvitamin D_3 [1,25(OH)$_2$D$_3$] decreases the tubular capacity to reabsorb Pi when chronically administered to TPTX rats, we have studied whether this vitamin D_3 metabolite could specifically increase the phosphaturic response to PTH in phosphate-deprived animals. The results show that in Vitamin D-replete TPTX rats fed a low (0.2 g/100 g) phosphorus diet, 1,25(OH)$_2$D$_3$ (2 × 13 pmol/d i.p. for 7 d) markedly enhanced the acute tubular phosphaturic response to PTH (2.5 IU/h i.v.) without affecting the action of the peptide hormone on Ca reabsorption and cyclic-3',5'-AMP excretion. The influence of 1,25(OH)$_2$D$_3$ on the phosphaturic response to PTH could not be ascribed to an increased plasma concentration and(or) filtered load of Pi during the administration of the peptide hormone. However, it could be, at least in part, related to the elevation in the basal level of plasma Pi which was observed in the 1,25(OH)$_2$D$_3$-treated animals. The results also indicate that 1,25(OH)$_2$D$_3$ significantly enhanced the calcemic response to PTH, which was blunted in these conditions of phosphate deprivation. Unlike 1,25-(OH)$_2$D$_3$, 25-hydroxyvitamin D_3 did not unmask the phosphaturic effect of PTH in phosphate-depleted animals, even when given in doses 100 times larger. Thus,

1,25(OH)$_2$D$_3$ displays a selective and powerful activity in preventing the occurrence of tubular resistance to the phosphaturic action of PTH during Pi depletion. This finding suggests the existence of an important interaction between dietary Pi, 1,25(OH)$_2$D$_3$, and PTH in the homeostasis of phosphate.

INTRODUCTION

Restriction in the supply of dietary inorganic phosphate (Pi)[1] has been shown to blunt the acute tubular phosphaturic response to parathyroid hormone (PTH) in dogs (1). The same phenomenon was later observed in thyroparathyroidectomized (TPTX) rats (2, 3). Although the nature of this phenomenon remains obscure, it may be related to the fact that phosphate deprivation enhances the tubular capacity of the kidney to reabsorb Pi in TPTX rats (4, 5).

Recently, clearance studies have shown that chronic administration of small doses of 1,25-dihydroxyvitamin D_3 [1,25(OH)$_2$D$_3$] to TPTX rats hinders the effect of phosphate deprivation on the overall tubular Pi transport capacity (6).

In the present work, we have investigated whether chronic administration of 1,25(OH)$_2$D$_3$ could prevent the blunting of the phosphaturic response to PTH in phosphate-deprived TPTX rats. Because preliminary studies indicated that in our experimental conditions phosphate depletion also reduced the calcemic effect

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[1] *Abbreviations used in this paper:* cAMP, cyclic-3',5'-AMP; [Ca]$_{Pl}$, plasma Ca concentration; FEPi, fractional excretion of inorganic phosphate; GFR, glomerular filtration rate; HPD, high phosphorus diet; LPD, low phosphorus diet; 1,25 (OH)$_2$D$_3$, 1,25-dihydroxyvitamin D_3; 25(OH)D$_3$, 25-hydroxyvitamin D_3; Pi, inorganic phosphate; [Pi]$_{Pl}$, plasma Pi concentration; PTH, parathyroid hormone; TPTX, thyroparathyroidectomized; Ur$_{Ca}$V, urinary Ca excretion; Ur$_{cAMP}$V, urinary cAMP excretion; Ur$_{Na}$V, urinary Na excretion; Ur$_{Pi}$V, urinary Pi excretion; UrV, urinary excretion.

of PTH, the influence of 1,25(OH)$_2$D$_3$ on this response was also investigated.

METHODS

Preparation of the animals. Male Wistar rats that weighed 160–180 g, raised on a commercial chow (Altromin 1314, Altrogge, Lage, Lippe, West Germany) containing 1.2 g/100 g phosphorus (P), 1.1 g/100 g calcium (Ca), and 180 IU/100 g vitamin D$_3$, were selected for the study. 7 d before the renal study, the animals were pair-fed low (0.2 g/100 g) and high (1.2 g/100 g) P diets prepared as previously described (6). Ca content was 1.1 g/100 g in both diets. During this period, and in the first series of experiments, one group of rats fed the low P diet (LPD) received 1,25(OH)$_2$D$_3$ intraperitoneally at the dose of 13 pmol/25 μl ethanol twice daily. The last injection of 1,25(OH)$_2$D$_3$ was administered 1 h before the renal study. The other two groups of animals fed either a LPD or high P diet (HPD) received intraperitoneal injections of the ethanol vehicle. In a second series of experiments, the influence of 25-hydroxyvitamin D$_3$ [25(OH)D$_3$] was compared with that of 1,25(OH)$_2$D$_3$ in rats fed LPD. 25(OH)D$_3$ was given intraperitoneally in doses of 13, 130, 1,300, and 13,000 pmol/25 μl ethanol twice daily. In a third series of experiments, the chronic influence of 1,25(OH)$_2$D$_3$, administered as mentioned above, on the tubular phosphaturic response to parathyroid hormone in rats fed the 0.2-g/100 g P diet was compared with the effect of raising the P content of the diet from 0.2 to 0.3 g/100 g. This latter diet was selected because it provided a basal level of plasma Pi similar to that observed in the 1,25(OH)$_2$D$_3$-treated animals fed the 0.2-g/100 g P diet.

2 d before the renal study, all rats were TPTX as previously reported (6). The food was withdrawn 24 h before starting the clearance measurements.

Clearance experiments. The general methodology of the clearance measurement in conscious rats has been described earlier (4, 6). In the present study, the experiments were started between 9 and 10 a.m. and lasted until 3–4 p.m. A first dose of 0.4 μCi of [methoxy-³H]inulin (New England Nuclear, Boston, Mass.) and 12.8 mg of unlabeled inulin (Fluka A. G., Basel, Switzerland) dissolved in isotonic saline was injected intravenously in a volume of 0.4 ml/rat. A 0.15-M NaCl solution containing 50 μCi/100 ml of [methoxy-³H]inulin and 1 g/100 ml of unlabeled inulin was then infused at a rate of 4 ml/h into a tail vein throughout the experiment. After an equilibration period of 60–90 min, a first clearance period (control 1) was made by collecting urine for 45 min and sampling blood at the end of this period. Then, PTH (TCA powder, Inolex Pharmaceutical Div., Inolex Corp., Park Forest South, Ill., 248 IU/mg) was administered. Each rat received within 1 min a first dose of 2.5 IU of PTH diluted in 0.5 ml of 0.15-M NaCl i.v. PTH was then added to the isotonic saline-inulin infusion in a concentration calculated to deliver 2.5 IU/h. 90 min later, a second 45-min urine collection was started. Blood samples were taken before and after this clearance period. The PTH infusion was then stopped and replaced by the initial solution. After 30 min of equilibration, a third urine specimen (45 min) was collected (control 2), after which a final blood sample was taken. The equilibration period of 90 min after the onset of PTH infusion was selected purposely because preliminary experiments have shown that after this lapse of time the initial differences in plasma concentration and filtered load of Pi, either as a result of the dietary Pi intake or of 1,25(OH)$_2$D$_3$ treatment, were negligible.

Urine specimens of the first series of experiments were collected in ice to allow cyclic AMP determination. In one experiment of the first series, urinary pH was measured after collecting urine specimens under xylol.

Adequacy of thyroparathyroidectomy. After the clearance study, vitamin D$_3$ metabolites treatment was stopped, and all animals were fed with the commercial chow mentioned above. 2–3 wk later, plasma Ca concentration ([Ca]$_{Pl}$) was determined after a 24-h fasting period. Only results of animals displaying a value of [Ca]$_{Pl}$ $\leq$1.88 mM (7.5 mg/100 ml) have been considered for this study.

Analytical methods. Diets were analyzed after incineration of samples and dissolution of the ash in 0.1 N HCl. Urinary volume (UrV) was determined by weighing. The activity of [³H]inulin in plasma and urine was counted in a liquid scintillation spectrometer. Pi was determined spectrophotometrically as phosphomolybdate after reduction with 10% ascorbic acid solution. Ca was measured by complexometric titration with EGTA (Corning Calcium Analyzer 940, Corning Glass Works, Scientific Products Div., Corning, N. Y.). Na concentration was obtained by flame photometry (EEL, flame photometer, Evans Electroselenium, Ltd., Halstead, Essex, England). Urinary cyclic-3′,5′-AMP (cAMP) was measured by a competitive binding assay with a commerical kit (cyclic AMP assay kit, The Radiochemical Centre, Amersham, England.) Urinary pH was determined with a combination glass electrode.

Statistical analysis. The experimental data are expressed as mean values±SEM. The significance of the differences between groups was assessed by the two-sided Student's t test. Paired sample analysis was used for testing the effect of PTH within each group.

RESULTS

Phosphaturic response. As expected, the phosphaturic response to PTH was markedly influenced by the phosphate intake of the animals (Table I, Fig. 1) Indeed, the mean (±SEM) rise in the fractional excretion of Pi (FEPi) was $\cong$ 10 times larger in the rats fed HPD as compared with that observed in the LPD group (HPD: ΔFEPi = +0.216±0.018, n = 8; LPD: ΔFEPi = +0.019±0.006, n = 8, P < 0.001) (Fig. 1).

The chronic administration of 1,25(OH)$_2$D$_3$ (2 × 13 pmol/d i.p. for 7 d) to LPD rats had a striking influence on the phosphaturic response to PTH. As illustrated in Fig. 1, 1,25(OH)$_2$D$_3$ treatment produced a response similar to, or even larger than, that recorded in the HPD group (ΔFEPi = +0.351±0.016, n = 7). The influence of 1,25(OH)$_2$D$_3$ on the phosphaturic response to PTH in LPD rats could not be ascribed to an increased plasma concentration and(or) filtered load of Pi. Indeed, during the PTH infusion, the mean (±SEM) plasma Pi was quite similar in the two LPD groups (Table I), and filtered load of Pi averaged 3.93±0.03 and 3.82±0.28 μmol/min in the animals with and without 1,25(OH)$_2$D$_3$ treatment, respectively.

The effect of the chronic administration of 25(OH)D$_3$ on the acute phosphaturic response to PTH in LPD rats was compared with that of 1,25(OH)$_2$D$_3$. The response was assessed at a similar plasma Pi concentration ([Pi]$_{Pl}$) (Fig. 2). In this series of experiments, the marked effect of 1,25(OH)$_2$D$_3$ given in doses of 2 × 13 pmol/d for 7 d was reproduced (Fig. 2). In sharp contrast,

H. J. Gloor, J-P. Bonjour, J. Caverzasio, and H. Fleisch

TABLE I

TABLE I
Response to PTH in TPTX Rats: Influence of Dietary Pi and 1,25(OH)₂D₃

	$[Pi]_{Pi}$	$Ur_{Pi}V$	$Ur_{cAMP}V$	$[Ca]_{Pi}$	$Ur_{Ca}V$	C_{In}	$Ur_{Na}V$
	mM	*nmol/ml GFR*	*pmol/ml GFR*	*mM*	*nmol/ml GFR*	*ml/min*	*μmol/ml GFR*
Dietary P: 1.2 g/100 g; ethanol vehicle; $n = 8$; body wt = 168±1 g							
Control 1	4.09±0.09	245±46	41±6	1.37±0.02	44±9	1.38±0.09	2.30±0.47
PTH	3.17±0.09*	875±52*	91±3*	1.60±0.04*	3±1*	1.39±0.06	3.22±0.30‡
Control 2	3.11±0.01	619±38§	53±3"	1.72±0.07	44±8"	1.36±0.07	6.12±0.39"
Dietary P: 0.2 g/100 g; ethanol vehicle; $n = 8$; body wt = 169±4 g							
Control 1	2.81±0.18	15±14	38±5	2.04±0.12	223±42	1.26±0.12	2.77±0.51
PTH	2.93±0.12	71±17‡	87±7*	2.14±0.13	31±11*	1.30±0.08	2.55±0.38
Control 2	2.96±0.07	79±21	43±7"	2.05±0.12	143±26§	1.26±0.12	4.74±0.35"
Dietary P: 0.2 g/100 g; 1,25(OH)₂D₃ treatment; $n = 7$; body wt = 165±2 g							
Control 1	3.37±0.05	36±17	28±3	2.09±0.06	220±17	1.34±0.07	2.37±0.39
PTH	2.77±0.07*	996±32*	76±7*	2.66±0.09*	66±17*	1.41±0.09	3.48±0.38‡
Control 2	2.63±0.04	179±32"	40±2"	2.72±0.08	242±42§	1.35±0.04	5.71±0.32"

1,25(OH)₂D₃ was administered at a dose of 2 × 13 pmol/d i.p. during the 7 d preceding the renal study. PTH was infused at the dose of 2.5 IU/h i.v. C_{In}, clearance of inulin.
Values represent mean±SEM.
n = number of animals.
* $P < 0.001$ as compared with the "control 1" period.
‡ $P < 0.05$ as compared with the "control 1" period.
§ $P < 0.01$ as compared with the "PTH" period.
" $P < 0.001$ as compared with the "PTH" period.

identical doses of 25 (OH)D₃ had no such effect. Furthermore, 25(OH)D₃ did not restore the phosphaturic response to PTH even when given in doses 100-fold larger (2 × 1,300 pmol/d) than 1,25(OH)₂D₃. However, a response to PTH could be elicited when 25 (OH)D₃ was administered at 2 × 13,000 pmol/d. Note that this latter amount was at the limit of toxicity, as increasing the dose by one order of magnitude under the same conditions provoked the death of all treated animals. Thus, 1,25(OH)₂D₃ is much more potent that 25(OH)D₃ in enhancing the phosphaturic response to PTH in phosphate-depleted animals. In agreement with the former experiment presented in Table I and in Fig. 1, the marked influence of 1,25(OH)₂D₃ pretreatment on the phosphaturic response to PTH was not associated with a higher plasma concentration or filtered load of Pi during the infusion of the peptide hormone.

However, the effect of 1,25(OH)₂D₃ was associated with a slightly, but significantly ($P < 0.05$), higher level of plasma Pi during the period (control 1) preceding the administration of PTH (Table I). Therefore, the 1,25(OH)₂D₃-induced enhancement of the phosphaturic response to PTH could be related to the in-

creased phosphatemia recorded during basal conditions. Feeding rats a 0.3- instead of a 0.2-g/100 g P diet increased the base-line $[Pi]_{Pi}$ to the level observed in the 1,25(OH)₂D₃-treated animals. This regimen (0.3 g/100 g P) elicited a larger phosphaturic response than that observed in a group of rats pair-fed with a 0.2-g/100 g P diet (Table II). This response remained, however, significantly ($P < 0.001$) smaller than that monitored in the 1,25(OH)₂D₃-treated animals pair-fed with the 0.2-g/100 g P diet.

cAMP response. As shown in Table I, urinary cAMP excretion ($Ur_{cAMP}V$)/ml glomerular filtration rate (GFR) measured before the administration of PTH did not significantly differ among the three experimental groups. This result confirms previous observations that neither the dietary amount of Pi (2) nor 1,25(OH)₂D₃ administration (6) alters the basal rate of $Ur_{cAMP}V$ in TPTX rats. The increase in $Ur_{cAMP}V$/ml GFR in response to PTH was very similar in the three groups, contrasting with the marked difference in the augmentation of phosphate excretion. This dissociation between the increase in phosphate and cAMP excretion in response to PTH is illustrated in Fig. 1.
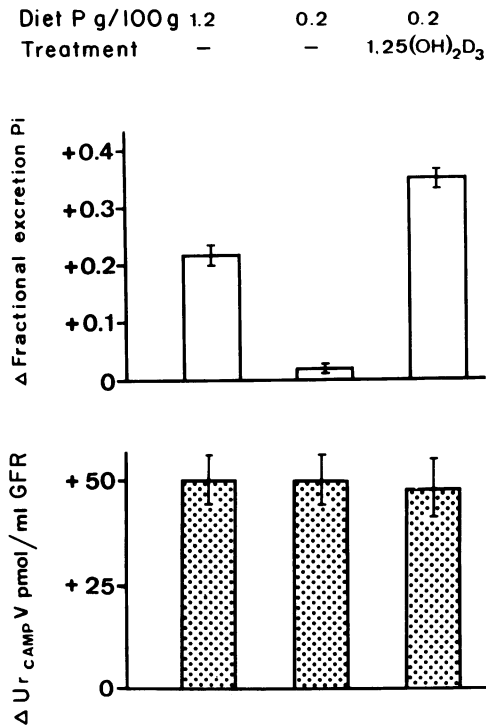
1,25-Dihydroxyvitamin D_3 and Resistance to Parathyroid Hormone Actions 373

Diet P g/100g 1.2   0.2   0.2
Treatment   —   —   1.25(OH)₂D₃

FIGURE 1 Increase in FEPi and in cAMP excretion in response to PTH in TPTX rats: Influence of dietary Pi and 1,25(OH)$_2$D$_3$. Each column corresponds to the rise ($\Delta \pm$SEM) in FEPi and cAMP excretion (Ur$_{cAMP}$V/ml GFR) during PTH (2.5 IU/h) infusion. Rats were fed either a 1.2- or 0.2-g/100 g P diet. 1,25(OH)$_2$D$_3$ (2 × 13 pmol/d i.p.) was administered during the 7 d preceding the PTH infusion. See Table I for further details.

Hypocalciuric response. The HPD rats as well as the two LPD groups responded to PTH by reducing their urinary Ca excretion (Ur$_{Ca}$V/ml GFR, Table I). In LPD rats, pretreatment with 1,25(OH)$_2$D$_3$ did not modify the hypocalciuric response to PTH. Thus, chronic administration of 1,25(OH)$_2$D$_3$, like dietary Pi, alters the tubular action of PTH which affects phosphate, but not calcium. In all three groups, the hypocalciuric response to PTH disappeared rapidly after stopping the PTH infusion.

Calcemic response. Before the PTH infusion (control 1), plasma Ca was, as expected, significantly lower in HPD than in LPD rats (Table I). In LPD rats, plasma Ca did not change whether or not the animals had received 1,25(OH)$_2$D$_3$ treatment. In response to PTH infusion, the increase in plasma Ca was significantly ($P < 0.05$) greater in HPD ($\Delta \pm$SEM) = +0.23±0.03 mM) than in the LPD rats (Δ = +0.10±0.04 mM) which did not receive 1,25(OH)$_2$D$_3$. In this latter group the calcemia barely rose above the base-line value recorded during control 1. By contrast, a conspicuous increase in the plasma Ca in LPD rats treated with 1,25(OH)$_2$D$_3$ was noted during the PTH infusion (Δ =

+0.57±0.05 mM). After discontinuing the PTH administration, the rise in plasma Ca was maintained both in HPD (Δ control 2 − 1 = +0.35±0.06 mM) and in LPD plus 1,25(OH)$_2$D$_3$ (Δ control 2 − 1 = +0.63±0.06 mM). In contrast, the small calcemic response observed in LPD rats without 1,25(OH)$_2$D$_3$ treatment rapidly disappeared after stopping the PTH infusion (Δ control 2 − 1 = +0.01±0.07 mM). Thus, although the magnitude and the duration of PTH-induced reduction in Ur$_{Ca}$V/ml GFR were similar in both LPD groups, a substantial and prolonged rise in plasma Ca was observed solely in the animals pretreated with 1,25-(OH)$_2$D$_3$.

GFR, urinary Na excretion (Ur$_{Na}$V), and urine pH. The modulation of the phosphaturic response to PTH by dietary Pi or 1,25(OH)$_2$D$_3$ was not associated with alteration in GFR (Table I).

No difference in Ur$_{Na}$V/ml GFR was found between the three groups during control 1. The rise in urinary Pi excretion (U$_{Pi}$V) observed in the HPD and LPD groups treated with 1,25(OH)$_2$D$_3$ was associated with a parallel increase in Ur$_{Na}$V/ml GFR. In the latter two groups, the increase in the amount of pmoles of sodium excreted under PTH was <1.5-fold that of phosphate. Therefore, the increase in sodium excretion certainly did not exceed the amount required for maintaining the electroneutrality of urine. After cessation of the PTH infusion, and increase in Ur$_{Na}$V/ml GFR was noted in all three experimental groups. Such a rise probably resulted from a progressive expansion of the extracellular volume as a result of the constant infusion of isotonic saline.
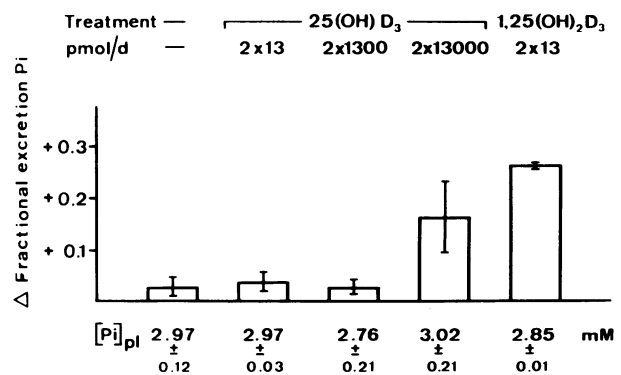
Finally, during PTH infusion, urinary pH (mean±

Treatment —   ┌─────── 25(OH) D₃ ───────┐ 1.25(OH)₂D₃
pmol/d   —   2x13   2x1300   2x13000   2x13

[Pi]$_{pl}$ 2.97   2.97   2.76   3.02   2.85   mM
             ±   ±   ±   ±   ±
             0.12   0.03   0.21   0.21   0.01

FIGURE 2 Increase in FEPi in response to PTH in TPTX rats fed a LPD: Influence of 25(OH)D$_3$ as compared with that of 1,25(OH)$_2$D$_3$. Each column corresponds to the rise ($\Delta \pm$SEM) in FEPi during PTH infusion (2.5 IU/h). Rats were fed a LPD (0.2 g/100 g). 25(OH)D$_3$ or 1,25(OH)$_2$D$_3$ was administered during the 7 d preceding the PTH infusion. [Pi]$_{Pl}$($\pm$SEM) corresponds to the mean [Pi]$_{Pl}$ determined before and during the PTH infusion. The number of rats was five for the untreated group and three for each treated group.

374   H. J. Gloor, J-P. Bonjour, J. Caverzasio, and H. Fleisch

TABLE II
Relation between Basal Plasma Pi Level and Phosphaturic Response to PTH in TPTX Rats

	$[Pi]_{Pi}$	FEPi	$[Pi]_{Pi}$	FEPi	$[Pi]_{Pi}$	FEPi
	mM	%	mM	%	mM	%
Before PTH	2.90±0.18	0.03±0.01	3.34±0.08	0.81±0.44	3.27±0.14	0.02±0.01
During PTH	3.18±0.23	4.86±1.51	3.21±0.13	36.00±2.43	3.15±0.12	14.18±1.12
Δ		+4.83±1.51		+35.19±2.50*‡		+14.16±1.12*
Dietary P, g/100 g	0.2		0.2		0.3	
Treatment	—		1,25(OH)₂D₃		—	
n	5		7		5	

1,25(OH)$_2$D$_3$ was administered at a dose of 2 × 13 pmol/d i.p. during the 7-d preceding the study. PTH was infused at the dose of 2.5 IU/h i.v.
Values represent mean±SEM.
n = number of animals.
* $P < 0.001$ as compared with the untreated group fed the 0.2-g/100 g P diet.
‡ $P < 0.001$ as compared with the untreated group fed the 0.3-g/100 g P diet.

SEM) was similar in all three experimental groups: HPD, 5.90±0.20; LPD 6.13±0.25; LPD plus 1,25-(OH)$_2$D$_3$, 5.98±0.14.

DISCUSSION

The present study indicates that phosphate deprivation does not blunt the acute phosphaturic response to PTH when rats are chronically supplemented with small doses of 1,25(OH)$_2$D$_3$. The marked enhancement of the phosphaturic response to PTH in the 1,25(OH)$_2$D$_3$-treated animals cannot be ascribed to an elevation in the plasma concentration and(or) filtered load of Pi at the time the tubular effect of the peptide hormone was recorded. It is unlikely that the slightly higher initial plasma Pi level observed after 1,25(OH)$_2$D$_3$ treatment in LPD rats could in itself account for the dramatic increase in the phosphaturic response to PTH. Indeed, a previous study (7) has shown that the phosphaturic response to PTH in phosphate-depleted TPTX rats remains conspicuously blunted during a Pi infusion, which acutely increased the plasma Pi to the level observed in phosphate-replete TPTX counterparts. It is, however, conceivable that 1,25(OH)$_2$D$_3$ given during phosphate restriction prevented the renal resistance to the phosphaturic action of PTH by its long-term action on Pi metabolism (8). The small increase in the basal plasma Pi level observed after 1,25(OH)$_2$D$_3$ treatment in LPD rats may in fact reflect such a long-term alteration in Pi metabolism. When a similar elevation in the basal plasma Pi level was achieved by providing more Pi in the diet (Table II), an enhancement in the phosphaturic response to PTH was also observed. However, the magnitude of the response remained significantly inferior to that recorded after treating the animals with 1,25(OH)$_2$D$_3$. Thus, the action of 1,25(OH)$_2$D$_3$ appears to be only partly related to its long-term effect on the plasma Pi level. This implies the existence of an additional mechanism of action of 1,25(OH)$_2$D$_3$ which, nevertheless, may still be related to an alteration in Pi metabolism. 1,25(OH)$_2$D$_3$ might modulate the phosphaturic response to PTH by interfering with the distribution of Pi between either the extra- and intracellular spaces or the different cellular compartments. The exploration of such a possibility will require the development of techniques allowing the accurate measurement of Pi within the various intracellular pools.

In sharp contrast to the effect observed after the administration of 1,25(OH)$_2$D$_3$, treatment with 25-(OH)D$_3$ does not influence this response unless it is administered in huge and thus nearly toxic doses. A recent study has revealed the existence of a striking difference between the activity of 1,25(OH)$_2$D$_3$ and that of its precursor 25(OH)D$_3$ in decreasing the tubular capacity to reabsorb Pi when these vitamin D$_3$ metabolites are chronically administered to TPTX rats (6). This suggests that the capability of enhancing the phosphaturic response to PTH is closely related to that of reducing the capacity to reabsorb Pi. In the present work, FEPi determined in the phosphate-depleted TPTX rats during the period preceding the PTH infusion was not significantly higher in the group treated with 1,25(OH)$_2$D$_3$ than in the nontreated animals. However, it is important to note that the FEPi, measured at endogenous or slightly elevated $[Pi]_{Pi}$ in phosphate-depleted rats, does not reflect the actual Pi reabsorptive capacity. Indeed, in similar experimental conditions, 1,25(OH)$_2$D$_3$ given to phosphate-depleted TPTX rats has been shown to significantly enhance FEPi when

determined at high, but not at low, plasma Pi concentrations (6).

The data reported in the present work raises the question of whether or not the powerful effect of 1,25-$(OH)_2D_3$ on the phosphaturic response to PTH when given at the dose of 13 pmol twice daily has a physiological relevance. From previous studies (9–11), evidence has been obtained that the same amount of 1,25$(OH)_2D_3$ administered to TPTX rats fed a HPD can be considered a substitution dose for the reduced endogenous production of the metabolite that follows the removal of the parathyroid glands (12, 13). However, the same consideration cannot be applied when these doses of 1,25$(OH)_2D_3$ are given to TPTX rats fed a LPD. Indeed, in these animals the production (14) and the plasma level (13) of 1,25$(OH)_2D_3$ can be expected to be increased as compared with their TPTX counterparts fed a HPD. Therefore, the blunted phosphaturic response to PTH observed in phosphate-depleted rats cannot be ascribed to a lack of 1,25$(OH)_2D_3$. On the contrary, our results would suggest that in these animals the increase in the endogenous production of 1,25$(OH)_2D_3$ tends to counteract the dramatic effect of phosphate depletion on the tubular phosphaturic response to PTH.

The present investigation also shows that by varying the dietary Pi supply and treating the animals with 1,25$(OH)_2D_3$, the tubular action of PTH on phosphate can be completely dissociated from its effect on cAMP excretion and calcium reabsorption. Thus 1,25$(OH)_2D_3$, like dietary Pi, appears to selectively affect the tubular phosphaturic response to PTH and seems to do this independently of the adenyl cyclase system.

It is interesting that the same kind of renal response to PTH after 1,25$(OH)_2D_3$ treatment has been observed in one particular clinical situation. Indeed, in patients with pseudohypoparathyroidism of type II (15), the phosphate, but not the cAMP response to PTH is impaired. In this condition, 1,25$(OH)_2D_3$ has also been shown to restore the phosphaturic response to the peptide hormone without affecting the rise in $Ur_{cAMP}V$ (16).

Our experiments do not permit us to understand the mechanism whereby 1,25$(OH)_2D_3$ influences the phosphaturic response to PTH. It is difficult to believe that the effect of 1,25$(OH)_2D_3$ might be related to the influence of the metabolite on plasma calcium (17). In the present work, 1,25$(OH)_2D_3$ did not increase the level of plasma Ca as determined before the PTH infusion (Table I). However, during the administration of the peptide hormone, plasma Ca rose significantly in phosphate-depleted rats that were treated with 1,25$(OH)_2D_3$. It has been suggested (18) that an acute elevation in plasma Ca may potentiate the phosphaturic response to PTH in TPTX dogs. However, this effect has not been observed in parathyroidectomized rats (19). Furthermore,

acute calcium infusion has been shown to decrease rather than to increase FEPi in parathyroidectomized rats (20). Therefore, it appears unlikely that the elevation in plasma Ca during PTH infusion is the main factor responsible for the enhanced phosphaturia that we have observed in the 1,25$(OH)_2D_3$-treated rats.

In our experiments, the elevation of plasma Ca in response to PTH was somewhat blunted after phosphate deprivation. To our knowledge such an influence of the dietary phosphate supply on the calcemic effect of PTH has not yet been reported. Interestingly, an enhanced calcemic response to the peptide hormone can be observed when phosphate-depleted TPTX rats are treated with 1,25$(OH)_2D_3$. The rise in the calcemia does not seem to be of intestinal or renal origin. The animals were fasted overnight, and the increase in the tubular reabsorption of calcium was similar whether or not they had received the 1,25$(OH)_2D_3$ treatment. It is more likely that the elevation in the calcemia during and immediately after PTH infusion results from a greater mobilization of calcium from bone in the animals supplemented with 1,25$(OH)_2D_3$. The fact that the calcemic response was maintained after discontinuing the infusion of PTH and after the disappearance of its hypocalciuric effect sustains this assertion. It has been known for several years that in vitamin D-deficient animals the skeleton is resistant to the Ca-mobilizing action of PTH (21, 22). More recently, evidence has been presented indicating that the PTH-induced mobilization of calcium from bone requires the presence of 1,25$(OH)_2D_3$ to be fully expressed (23). Other evidence along this line has been obtained by Massry et al. (24), who showed that in uremic dogs, 1,25$(OH)_2D_3$ administration partially restores the blunted calcemic response to PTH. Finally, in patients with pseudohypoparathyroidism, 1,25$(OH)_2D_3$ has been shown to normalize the impaired calcemic response to PTH (16, 25), a phenomenon which had previously been observed with pharmacological doses of vitamin D (26, 27).

Thus, in addition to phosphate-depleted animals, in several experimental and pathological situations where the phosphaturic and(or) calcemic response to PTH is impaired, 1,25$(OH)_2D_3$ has the ability to normalize this response. The relation between these situations and phosphate depletion is not at all apparent at this stage of our knowledge. More information about the basic mechanisms regulating the translocation of Pi and Ca at the kidney and bone levels is needed before the nature of the interrelationship between the action of PTH and 1,25$(OH)_2D_3$ on these processes can be fully appreciated.

REFERENCES

1. Harter, H. R., A. Mercado, W. E. Rutherford, H. Rodriguez, E. Slatopolsky, and S. Klahr. 1974. Effects of phosphate depletion and parathyroid hormone on renal glucose reabsorption. *Am. J. Physiol.* 227: 1422–1427.

2. Steele, T. H., J. L. Underwood, B. A. Stromberg, and C. A. Larmore. 1976. Renal resistance to parathyroid hormone during phosphorus deprivation. *J. Clin Invest.* 58: 1461–1464.

3. Bonjour, J-P., U. Tröhler, R. Mühlbauer, C. Preston, and H. Fleisch. 1977. Is there a bone-kidney link in the homeostasis of inorganic phosphate (Pi)? *In* Phosphate Metabolism. S. G. Massry and E. Ritz, editors. Plenum Publishing Corporation, New York. 81: 319–322.

4. Tröhler, U., J-P. Bonjour, and H. Fleisch. 1976. Inorganic phosphate homeostasis. Renal adaptation to the dietary intake in intact and thyroparathyroidectomized rats. *J. Clin. Invest.* 57: 264–273.

5. Steele, T. H. and H. F. DeLuca. 1976. Influence of dietary phosphorus on renal phosphate reabsorption in the parathyroidectomized rats. *J. Clin. Invest.* 57:867–874.

6. Bonjour, J-P, C. Preston, and H. Fleisch. 1977. Effect of 1,25-dihydroxyvitamin D_3 on the renal handling of Pi in thyroparathyroidectomized rats. *J. Clin. Invest.* 60: 1419–1428.

7. Bonjour, J-P., U. Tröhler, C. Preston, and H. Fleisch. 1978. Parathyroid hormone and renal handling of Pi: Effect of dietary Pi and diphosphonates. *Am. J. Physiol.* 234: F497–F505.

8. DeLuca, H. F., and K. Schnoes. 1976. Metabolism and mechanism of action of vitamin D. *Annu. Rev. Biochem.* 45: 631–666.

9. Bonjour, J-P., H. Fleisch, and U. Trechsel. 1977. Calcium absorption in diphosphonate-treated rats: Effect of parathyroid function, dietary calcium and phosphorus. *J. Physiol. (Lond.).* 264: 125–139.

10. Rizzoli, R., H. Fleisch, and J-P. Bonjour. 1977. Effect of thyroparathyroidectomy on calcium metabolism in rats: Role of 1,25-dihydroxyvitamin D_3. *Am. J. Physiol.* 233: E160–E164.

11. Rizzoli, R., H. Fleisch, and J-P. Bonjour. 1977. Role of 1,25-dihydroxyvitamin D_3 on intestinal phosphate absorption in rats with a normal vitamin D supply. *J. Clin. Invest.* 60: 639–647.

12. Garabedian, M., M. F. Holick, H. F. DeLuca, and I. T. Boyle. 1972. Control of 25-hydroxycholecalciferol metabolism by parathyroid gland. *Proc. Natl. Acad. Sci. U. S. A.* 69: 1673–1676.

13. Hughes, M. R., P. F. Brumbaugh, and M. R. Haussler. 1975. Regulation of serum $1\alpha,25$-dihydroxyvitamin D_3 by calcium and phosphate in the rat. *Science (Wash. D. C.).* 190: 578–579.

14. Tanaka, Y., and H. F. DeLuca. 1973. The control of 25-hydroxyvitamin D metabolism by inorganic phosphorus. *Arch. Biochem. Biophys.* 154: 566–574.

15. Drezner, M., F. A. Neelon, and H. E. Lebovitz. 1973. Pseudohypoparathyroidism type II: A possible defect in the reception of the cyclic AMP signal. *N. Engl. J. Med.* 289: 1056–1060.

16. Brickman, A. S., A. W. Norman, and J. W. Coburn. 1977. Vitamin D in pseudohypoparathyroidism. *In* Vitamin D. Biochemical, Chemical and Clinical Aspects Related to Calcium Metabolism. A. W. Norman, K. Schaefer, J. W. Coburn, H. F. DeLuca, D. Fraser, H. G. Grigoleit, and D. V. Herrath, editors. Walter DeGruyter, New York. 867–876.

17. Massry, S. G., R.M. Friedler, and J. W. Coburn. 1973. Excretion of phosphate and calcium. Physiology of their renal handling and relation to clinical medicine. *Arch. Intern. Med.* 131: 828–859.

18. Cuche, J. L., C. E. Ott, G. R. Marchand, J. A. Diaz-Buxo, and F. G. Knox. 1976. Intrarenal calcium in phosphate handling. *Am. J. Physiol.* 230: 790–796.

19. Popovtzer, M. M., J. B. Robinette, K. M. McDonald, and C. K. Kuruvila. 1975. Effect of Ca^{++} on renal handling of $PO_4^=$: evidence for two reabsorptive mechanisms. *Am. J. Physiol.* 229: 901–906.

20. Amiel, C., H. Kuntziger, S. Couette, C. Coureau, and N. Bergounioux. 1976. Evidence for a parathyroid hormone-independent calcium modulation of phosphate transport along the nephron. *J. Clin. Invest.* 57: 256–263.

21. Rasmussen, H., H. DeLuca, C. Arnaud, C. Hawker, and M. von Stedingk. 1963. The relationship between vitamin D and parathyroid hormone. *J. Clin. Invest.* 42: 1940–1946.

22. Arnaud, C., H. Rasmussen, and C. Anast. 1966. Further studies on the interrelationship between parathyroid hormone and vitamin D. *J. Clin. Invest.* 45: 1955–1964.

23. Garabedian, M., Y. Tanaka, M. F. Holick, and H. F. DeLuca. 1974. Response of intestinal calcium transport and bone calcium mobilization to 1,25-dihydroxyvitamin D_3 in thyroparathyroidectomized rats. *Endocrinology.* 94: 1022–1027.

24. Massry, S. G., R. Stein, J. Garty, A. I. Arieff, J. W. Coburn, A. W. Norman, and R. M. Friedler. 1976. Skeletal resistance to the calcemic action of parathyroid hormone in uremia: Role of $1,25(OH)_2D_3$. *Kidney Int.* 9: 467–474.

25. Metz, St. A., D. J. Baylink, M. Hughes, M. R. Haussler, and R. P. Robertson. 1977. Selective deficiency of 1,25-dihydroxycholecalciferol. A cause of isolated skeletal resistance to parathyroid hormone. *N. Engl. J. Med.* 297: 1084–1090.

26. Stögmann, W., and J. A. Fischer. 1975. Pseudohypoparathyroidism. Disappearance of the resistance to parathyroid extract during treatment with vitamin D. *Am. J. Med.* 59: 140–144.

27. Suh, S. M., D. Fraser, and S. W. Kooh. 1970. Pseudohypoparathyroidism: Responsiveness to parathyroid extract induced by vitamin D_2 therapy. *J. Clin. Endocrinol. Metab.* 30: 609–614.