Effect of 1,25-Dihydroxyvitamin D₃ on the Renal Handling of P₁ in Thyroparathyroidectomized Rats

J-P. Bonjour, C. Preston, and H. Fleisch, Department of Pathophysiology, University of Berne, 3010 Berne, Switzerland

Abstract The kidney adapts its tubular capacity to transport inorganic phosphate (P₁) according to the dietary supply of P₁ in both intact and thyroparathyroidectomized (TPTX) rats. However, in TPTX rats the capability of the renal tubule to adapt to a high P₁ diet is diminished. In TPTX rats the production of the active vitamin D₃ metabolite, 1,25-dihydroxyvitamin D₃ [1,25-(OH)₂D₃], is also reduced. 1,25-(OH)₂D₃ has been shown to have a marked effect on P₁ metabolism. Therefore the question arises whether the deficient production of 1,25-(OH)₂D₃ contributes to the alteration of the tubular transport of P₁ 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 x 13 pmol/day of 1,25-(OH)₂D₃, a dose which was shown to just normalize the decreased intestinal absorption of Ca and P₁ in TPTX rats. The capacity of tubular P₁ transport was then assessed by measuring the fractional excretion of P₁ (FEP₁) at increasing plasma P₁ concentration ([P₁]ₚ) obtained by acute infusion of P₁. The results show that in SHAM rats fed either P diet, 1,25-(OH)₂D₃ has no effect on the renal handling of P₁. In TPTX rats fed 1.2 g/100 g P diet, 1,25-(OH)₂D₃ increases FEP₁ over a wide range of [P₁]ₚ. In TPTX rats fed a 0.2 g/100 g P diet, 1,25-(OH)₂D₃ does not alter FEP₁ up to a [P₁]ₚ of 3.0–3.5 mM, but does increase it at higher [P₁]ₚ. In fact, on both diets TPTX rats supplemented with 1,25-(OH)₂D₃ appear to have the same renal handling of P₁ as SHAM counterparts. The effect of 1,25-(OH)₂D₃ 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 D₃ in doses of 2 x 130 or 2 x 1,300 pmol/day i.p. did not increase FEP₁.

In conclusion, 1,25-(OH)₂D₃ administered in physiological amounts to TPTX rats restores to normal the capability of the renal tubule to excrete P₁ and to adapt to large variation in dietary P₁. The results suggest that 1,25-(OH)₂D₃ plays an important role in the regulation of the renal handling of P₁ and that the chronic change in the tubular capacity to transport P₁ after TPTX may be due to the decreased formation of 1,25-(OH)₂D₃.

Introduction The kidney responds to variations in the dietary intake of inorganic phosphate (P₁) by changing its tubular capacity to transport P₁ (1–3). This adaptive 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-P₁ 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 P₁ (FEP₁) (4–7). This effect can be observed within the first 3 h after the surgical procedure (7). However, after the acute phase, FEP₁...
increases up to a steady-state value within 24–48 h after PTX (7, 8). This rise in FEPi could well be interpreted as an adaptive response tending to normalize the handling of Pi. In chronic PTX or TPTX animals FEPi 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 Pi 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 D3 (25-OH-D3) into 1,25-dihydroxyvitamin D3 [1,25-(OH)2D3] (10). This latter metabolite influences markedly Pi homeostasis (11). Therefore, in TPTX animals the reduced production (10) and plasma level of 1,25-(OH)2D3 (12) could contribute to the chronic alteration in the tubular capacity to transport Pi.

In order to investigate this problem, we have studied the renal handling of Pi in sham-operated (SHAM) and TPTX rats supplemented or not with 1,25-(OH)2D3. The study was made under both low-P and high-P diet. 1,25-(OH)2D3 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 Pi 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)2D3 production which occurs in these animals (10). Therefore the minimal administered amount of 1,25-(OH)2D3 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 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 D3 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. Six 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 synthesized 1,25-(OH)2D3 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-OH-D3 on the renal handling of Pi was studied instead of 1,25-(OH)2D3. TPTX rats fed the 1.2 g/100 g P experimental diet received i.p. 14 injections of 25-OH-D3 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 mmol (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 insulin, i.e., 0.4 µCi of [methoxy-3H] insulin (New England Nuclear, Boston, Mass.) with 12.8 µg of unlabeled insulin (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-3H] insulin and 1 g/100 ml of unlabeled insulin and increasing amount of Pi, 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 Pi, at stepwise increasing doses (45-min equilibrations followed by 30-min urine collection periods), 1.0 µmol Pi/min (period II), 2.0 µmol Pi/min (period III), and 3.0 µmol Pi/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-3H] insulin (Ca) and Pi (Cp) were calculated for each period by the standard formula. The filtered load of phosphate (FLPi = [Pi]n × Cn), the absolute (UVPi) and fractional excretion of Pi (UVPi/FLPi) × 100), the absolute tubular reabsorption of phosphate (TRPi = FLPi - UVPi), and the fractional excretion of sodium (FENA = UVA/FL.Na) were calculated likewise. No correction was made for incomplete ultrafiltrability of plasma Pn, since previous studies have shown that similar P loading did not alter significantly the ultrafiltrable fraction of P (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)2D3 treatment as mentioned above. Finally, in TPTX rats with or without 1,25-(OH)2D3 administration, the renal handling of Pi, 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 [3H]inulin in plasma and urine

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-glycolmonomethylether, 400 ml; naphthalene, 80 g; and Butyl-PBD [2-(4-tert-butyl-phenyl)-5-(4-biphenyl[1,3,4-oxazidazol] (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, 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 in-dent’s t test.

**RESULTS**

In SHAM rats, the chronic administration of 1,25-(OH)₂D₃ (2 × 1 pmol/day i.p. for 7 days) does not appear to have any significant influence on the renal handling of P, under either diet, as shown in Table I and Fig. 1. Fig. 1 also shows that in intact rats the dietary-

<table>
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<th>TRPᵢ</th>
<th>Cᵢ</th>
<th>FENᵢ</th>
<th>[P₃]</th>
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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)₂D₃, 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)₂D₃, respectively. The indicated body weight was measured on the day of the clearance study. Plasma [P₃], phosphatemia; UVPᵢ, urinary excretion of phosphate; TRPᵢ, net tubular reabsorption of phosphate; Cᵢ, clearance of inulin; FENᵢ, fractional excretion of sodium.

* 2 × 13 pmol/day i.p. given during the 7 days preceding the renal study.
induced change in the transport capacity that has been previously described (1) is not affected by the chronic administration of $1,25-(\text{OH})_2\text{D}_3$. However, $1,25-(\text{OH})_2\text{D}_3$ 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 ($2.39 \pm 0.14$ vs. $1.89 \pm 0.09$ mM, $P < 0.01$, Table I).

In TPTX rats fed a 1.2 g/100 g P diet (Fig. 2), the administration of $1,25-(\text{OH})_2\text{D}_3$ produces a much greater capacity to excrete $P_i$ than in nontreated TPTX animals. At similar $[P_i]_m$ (as for instance at 3.3 mM) FEP, was markedly increased ($P < 0.001$) in the TPTX rats treated with $1,25-(\text{OH})_2\text{D}_3$ (Table V). In fact, the rats supplemented with $1,25-(\text{OH})_2\text{D}_3$ display the same capacity to transport $P_i$ as their sham-operated and pair-fed counterparts. This marked effect of the $1,25-(\text{OH})_2\text{D}_3$ 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, $1,25-(\text{OH})_2\text{D}_3$ only influences $P_i$ excretion significantly above a $[P_i]_m$ 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/ml glomerular filtration) was significantly ($P < 0.01$) enhanced in the animals treated with $1,25-(\text{OH})_2\text{D}_3$ (Table II). Again the relationship between plasma $P_i$ and FEP, appears to be very similar in SHAM and TPTX rats supplemented with $1,25-(\text{OH})_2\text{D}_3$ (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 $1,25-(\text{OH})_2\text{D}_3$. $1,25-(\text{OH})_2\text{D}_3$ 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, $1,25-(\text{OH})_2\text{D}_3$ given to TPTX rats fed the low-P diet enhances significantly the plasma level of $P_i$ measured in the same conditions ($2.15 \pm 0.07$ vs. $1.45 \pm 0.12$ mM, $P < 0.001$, Table II). Fig. 4 illustrates this opposite effect of $1,25-(\text{OH})_2\text{D}_3$ 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 $1,25-(\text{OH})_2\text{D}_3$ 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, $1,25-(\text{OH})_2\text{D}_3$ treatment had no consistent effect on urine pH (Table III).

To investigate whether the adenylate cyclase system was involved in the $1,25-(\text{OH})_2\text{D}_3$ effect, we determined urinary excretion of cyclic AMP in groups of SHAM and TPTX rats with or without $1,25-(\text{OH})_2\text{D}_3$ 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 $1,25-(\text{OH})_2\text{D}_3$. Thus the increase in the capacity to excrete $P_i$ in TPTX rats receiving $1,25-(\text{OH})_2\text{D}_3$ 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 1](https://doi.org/10.1172/JCI108903)

**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 $1,25-(\text{OH})_2\text{D}_3$ (2 x 13 pmol/day i.p. for 7 days). Other data concerning these four groups of rats are presented in Table I.
of 1,25-(OH)\(_2\)D\(_3\) (2 x 13 pmol/day i.p. for 7 days) on the renal handling of Pi of TPTX rats fed a 1.2 g/100 g P diet was studied under marked ECVE. FEPi was measured at a similar [Pi]n in TPTX rats receiving an isotonic saline solution infused at 20 ml/h. As shown in Table V, the effect of 1,25-(OH)\(_2\)D\(_3\) on the renal handling of Pi 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)\(_2\)D\(_3\) abolished the difference in plasma calcium between SHAM and TPTX rats, when assessed during the infusion of isotonic saline. Under acute Pi infusion, the fall in plasma calcium was smaller in SHAM than in TPTX + 1,25-(OH)\(_2\)D\(_3\), 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 Pi infusion was not higher in the 1,25-(OH)\(_2\)D\(_3\)-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 x 13 pmol/day of 25-OHD\(_3\) given for 7 days did not decrease the capacity of the tubule to reabsorb Pi. As shown in Fig. 5, a dose of 25-OHD\(_3\) 10 times higher (2 x 130 pmol/day) had no significant influence on the renal handling of Pi. A dose of 25-OHD\(_3\) 100 times higher (2 x 1,300 pmol/day) might, if anything, enhance the net tubular reabsorption of Pi. However, the effect of this dose on FEPi was not observed at the endogenous or at the highest plasma Pi 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 x 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].

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_i 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

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<td>Ethanol vehicle</td>
<td>1,25-(OH)_2D_3</td>
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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)₂D₃ Treatment

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</tr>
<tr>
<td>1</td>
<td>134.6±1.8</td>
<td>68.0±11.3§</td>
<td>47.3±14.4§</td>
</tr>
<tr>
<td>2</td>
<td>140.6±8.7</td>
<td>59.0±17.6†</td>
<td>58.7±7.0§</td>
</tr>
<tr>
<td>3</td>
<td>108.0±8.5</td>
<td>52.3±4.7§</td>
<td>46.5±6.0§</td>
</tr>
</tbody>
</table>

All values are means±SEM. No statistically significant difference was found between TPTX + ethanol and TPTX + 1,25-(OH)₂D₃.

* 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.

The mechanism whereby 1,25-(OH)₂D₃ affects the renal handling of Pi does not seem to be related to a change in tubular acidification. Indeed, the overall alteration in the renal handling of Pi is not associated with any consistent change in the urinary pH. Nor can the change in the renal handling of Pi 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 Pi transport as that observed in TPTX rats supplemented with 1,25-(OH)₂D₃ (Table V). Furthermore, the change in the renal handling of Pi 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)₂D₃ (Table III), and the increased calcium 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)₂D₃.

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 Pi excretion. Another observation (29) was that acute hypocalcemia was associated with an augmentation in the urinary output of Pi. Chronic elevation of plasma calcium by i.v. infusion in patients with hypoparathyroidism (30) resulted in an increase in Pi excretion.

TABLE V
Maintenance of the Influence of 1,25-(OH)₂D₃ Treatment on the Renal Handling of Phosphate in TPTX Rats Undergoing Marked Saline Diuresis

<table>
<thead>
<tr>
<th>Treatment</th>
<th>i.v. infusion, 4 ml/h</th>
<th>i.v. infusion, 20 ml/h</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>Ethanol vehicle</td>
<td>1,25-(OH)₂D₃*</td>
</tr>
<tr>
<td>Plasma [Pi]</td>
<td>3.3±0.2</td>
<td>3.3±0.1</td>
</tr>
<tr>
<td>FEPi, %</td>
<td>3.1±0.1</td>
<td>34.3±2.01</td>
</tr>
<tr>
<td>FENa, %</td>
<td>3.0±0.4</td>
<td>5.2±0.51</td>
</tr>
</tbody>
</table>

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 [Pi] 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 (mmolliter) in SHAM and TPTX Rats with or without 1,25-(OH)2D3 Treatment

<table>
<thead>
<tr>
<th>Infused P, ( \mu )mol/min</th>
<th>Dietary P: 1.2 g/100 g</th>
<th>Dietary P: 0.2 g/100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SHAM + ethanol vehicle (9)</td>
<td>SHAM + 1,25-(OH)2D3* (8)</td>
</tr>
<tr>
<td></td>
<td>2.33±0.04</td>
<td>2.25±0.03</td>
</tr>
<tr>
<td></td>
<td>2.05±0.03</td>
<td>2.03±0.07</td>
</tr>
</tbody>
</table>

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, 0) and last (infused P, 3 \( \mu \)mol/min) clearance period (see Tables I and II).

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

\( \dagger P < 0.001; \ddagger P < 0.01; \mathit{P} < 0.05, \) all with respect to the sham-operated group receiving the ethanol vehicle.

\( \mathit{P} < 0.001 \) with respect to the thyroparathyroidectomized group receiving the ethanol vehicle.

increase in the renal P, clearance. Therefore, the 1,25-(OH)2D3-induced change in the renal handling of P, could be related to the chronic effect of the D3 metabolite on the level of plasma calcium. However, it should be noticed that any influence of plasma calcium on the renal handling of P, 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)2D3 treatment did not prevent them from excreting a urine virtually free of P, up to a plasma P, level of about 3.0 mM. Furthermore, above this plasma P, level, the TPTX rats receiving 1,25-(OH)2D3 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)2D3 on the renal handling of P, in the TPTX rats fed the high-P diet, it cannot explain the results obtained in the TPTX animals fed the low-P diet.

The present study does not permit the assessment of whether 1,25-(OH)2D3 influences directly or indirectly the renal transport of P, 1,25-(OH)2D3 can mobilize P, 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)2D3 on P, metabolism, since the magnitude of the renal response depends upon the P, status of the animals. However, the effect of 1,25-(OH)2D3 on the intestinal P, 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)2D3 on the renal handling of P, contrasts with the changes observed after the acute administration of the metabolite. Acute injection or infusion of 1,25-(OH)2D3 to dogs (32) or rats (33) has been shown to depress the fractional excretion of P, In the rat (33), this effect was elicited.
by a rather larger dose of 1,25-(OH)₂D₃, 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)₂D₃ requires the presence of the parathyroid glands (33). In both dogs (32) and rats (33) the precursor of 1,25-(OH)₂D₃, namely 25-OHD₃, 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 Pi elicited by 25-OHD₃ or 1,25-(OH)₂D₃ parallels alterations in calcium and sodium excretion (32, 34). The reduced Pi 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 D₂ metabolites does not appear to be specific for the tubular Pi transport system, and 1,25-(OH)₂D₃ 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)₂D₃ nor any other vitamin D metabolites are required to ensure a complete tubular reabsorption of Pi in response to a restriction in the dietary supply of Pi.

The marked influence of 1,25-(OH)₂D₃, given chronically in small amounts, on the renal handling of Pi of TPTX rats may bear some relevance to previous reports showing that large doses of vitamin D can lead to an increased Pi excretion in patients with hypoparathyroidism (35) or in PTX animals (36, 37). Thus, Albright and Reifenstein (35) found that 400,000 U of vitamin D₂ given to a hypoparathyroid patient led to an increase in urinary Pi, which was associated with a decrease in serum Pi. Similar results were found by Crawford et al. (36) in PTX rats, wherein the administration of 10,000–100,000 U of vitamin D₂/day (625–6250 nmol/day) increased the fractional excretion of Pi 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 D₂ i.m., or 6 or more days after the administration of 30,000 U daily, the urinary excretion of Pi 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)₂D₃ 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)₂D₃ might be needed to alter the renal transport of Pi. In fact, preliminary experiments in our laboratory indicate that doses of 25-OHD₃ as high as 2 x 13,000 pmol/day i.p. also tend to normalize the renal handling of Pi 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)₂D₃ (72–96 pmol/kg per day, a dose very similar to that used in the present study) also promotes a decrease in the plasma Pi level while it concomitantly increases the renal clearance of Pi. This suggests that in humans as in rats the physiological role of 1,25-(OH)₂D₃ on the renal handling of Pi 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 Pi load of the organism. The process whereby 1,25-(OH)₂D₃ interacts, directly or indirectly, with the tubular Pi transport deserves to be further explored.

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REFERENCES


