Vitamin D Deficiency and Renal Calcium Transport in the Rat

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Abstract. To examine the role of vitamin D in the renal tubular handling of calcium, clearance studies were performed in three groups of rats: group A rats fed a standard vitamin D-deficient diet (Ca 0.45%, P 0.3%) for 6 wk, were hypocalcemic with secondary hyperparathyroidism; group B rats fed the same diet as in group A but with high calcium (Ca 1.4%) and 20% lactose, were normocalcemic and without secondary hyperparathyroidism; group C rats fed the same diet as in group A but supplemented with 25 U of vitamin D_3 orally twice a week, were normocalcemic, vitamin D-replete, and euparathyroid. After thyroparathyroidectomy (TPTX), each rat was infused intravenously with an electrolyte solution that contained a fixed concentration of calcium (0–30 mM) with or without parathyroid hormone (PTH; 0.75 or 2.5 U/h) at a rate of 3 ml/h. Urinary calcium excretion and serum calcium concentrations were measured between 16 and 19 h of the infusion, and the apparent threshold of calcium excretion was determined.

The threshold of calcium excretion was lower in vitamin D-deficient TPTX rats (groups A and B) than in vitamin D-replete TPTX rats (group C), and not different between group A and group B. Administration of PTH at a dose of 0.75 U/h increased the threshold of calcium excretion by ~0.6 mM in group C, but did not alter the threshold either in group A or group B. Administration

of a higher dose of PTH (2.5 U/h) raised the threshold similarly in both group A and group B to the extent comparable with that in group C, when it was given 0.75 U/h of PTH. These results demonstrate that the renal threshold of calcium excretion is decreased in the vitamin D-deficient rats independent of the secondary hyperparathyroidism, and that the higher dose of PTH was necessary to raise the calcium threshold in vitamin D-deficient rats. Thus, present study indicates the presence of dual effects of vitamin D on renal tubular handling of calcium; the one is to facilitate renal calcium reabsorption and the other is to enhance the responsiveness of the tubule to PTH.

Introduction

The vitamin D endocrine system is central to the regulation of calcium homeostasis (1). Extensive studies examining the biological effects of vitamin D in the intestine and bone have established that vitamin D promotes the intestinal calcium absorption (2) and increases the resorption of bone (3). However, it has not been clear whether vitamin D, or one or more of its metabolites, exerts a direct effect upon the renal tubular transport of calcium. In contrast to the situation with vitamin D, it is now generally accepted that parathyroid hormone (PTH)[1] has a direct and physiologically important action on the distal tubular reabsorption of calcium (4–7).

Studies in man as well as in experimental animals which dealt with the effects of vitamin D on renal calcium transport demonstrated that vitamin D has a hypocalciuric effect (8, 9), a calciuric effect (10, 11), or no effect at all (4, 5, 12). These differing conclusions appear to arise from several reasons: differences in the vitamin D status of animals (4, 9), differences in doses and kinds of vitamin D metabolites administered (4, 9), changes in the serum calcium concentration and filtered

1. *Abbreviations used in this paper:* GF, glomerular filtrate; PTH, parathyroid hormone; TPTX, thyroparathyroidectomy.

load of calcium (13), and differences in the status of the function of PTH (10), a hormone that has a direct action on the renal tubular calcium reabsorption. Since one or more of these factors was not controlled or taken into account in most studies, it is often difficult to determine whether or not vitamin D has any direct action on the renal tubular transport of calcium.

In an effort to critically examine this question, we have investigated in rats the renal handling of calcium in situations in which the vitamin D status, the PTH status, and the serum calcium concentration were independently controlled. The data of the present study indicate that vitamin D not only facilitates renal tubular calcium reabsorption but also enhances the effect of PTH on tubular calcium reabsorption.

Methods

Animals and diet. Male weanling rats (Wistar strain) were divided into four groups and raised on different diet, described below, for 6 wk in the absence of daylight or fluorescent light. In group A (vitamin D-deficient standard), animals were fed a standard synthetic vitamin D-deficient diet that contained 0.45% of calcium and 0.3% of phosphorus (14). In group B (vitamin D-deficient with high calcium), animals were fed the same vitamin D-deficient diet as in group A for 2 wk. During the next 4 wk they were fed on the same diet but with 1.4% calcium and with the substitution of 20% lactose for an equivalent amount of glucose. In group C (vitamin D-replete standard), animals were fed the standard vitamin D-deficient diet, which was supplemented with 25 U (0.625 μg) of vitamin D_3 orally twice a week. In group D (vitamin D-replete with high calcium), animals were raised by the same dietary regimen as in group B except for supplementation with 25 U of vitamin D_3 orally twice a week. Daily food consumption by individual rats were measured in one series of feeding for the last 4 wk: they were 14.0±0.74, 14.1±0.77, 15.6±0.67, and 15.3±0.22 g/d (mean±SE; n = 8 for each group) for groups A, B, C, and D, respectively. The animals had free access to distilled water throughout the 6 wk of feeding.

Clearance studies. Renal clearance studies were performed as described in detail in a previous report (15) with minor modifications. In short, the rats underwent surgical thyroparathyroidectomy (TPTX) or sham operation under anesthesia with intraperitoneal hexobarbital (100 mg/kg of body weight). The femoral vein, femoral artery, and urinary bladder were cannulated for the purpose of infusion, blood sampling, and urine collection, respectively. Shortly after the cannulation, sterilized nutrient solution, which contained 20 mM NaCl, 5 mM MgCl₂, 2.5 mM KCl, 0.1% bovine serum albumin, 4% glucose, and graded concentrations (0-30 mM) of CaCl₂, was infused at a constant rate of 3 ml/h with or without addition of bovine PTH (TCA powder, Wilson Laboratories, Chicago, IL). The maximal CaCl₂ concentration in each set of experiments was adjusted so that serum calcium concentrations did not exceed 2.75 mM. Doses of PTH, 0.75 and 2.5 U/h, were chosen because they represent the estimated endogenous PTH secretion rates in both vitamin D-replete and vitamin D-deficient rats, respectively (16). Each rat was infused with a single concentration of calcium throughout the period of infusion, and each experimental subgroup consisted of at least five animals that were given the same concentration of calcium infusion. Hence, each data point represents the results obtained in at least five separate animals.

After 16 h of infusion, when blood and urinary electrolyte values had been stabilized, urine samples were collected hourly for 3 h. Urine volumes were estimated gravimetrically. Blood samples of 0.5 ml were

drawn immediately after surgical procedure and at midpoint of the second urine collection. In preliminary experiments with hourly blood sampling, we ascertained that serum calcium levels did not change appreciably during the 3 h of urine collection. Therefore, only one blood sample was drawn at the midpoint of the 3-h clearance study, in order to minimize hemodynamic changes (17). Sera were separated as soon as possible and urine and serum samples were kept frozen at −20°C until assay. In some animals, additional blood specimen was obtained for blood gas analysis.

Analytical techniques. Urine and serum samples were analyzed for calcium and sodium by atomic absorption spectrophotometry, for inorganic phosphate by the method of Goldenberg (18), and for creatinine according to Bonsnes and Taussky (19). Serum total protein was measured by the method of Lowry et al. (20). Arterial blood pH was measured with a radiometer pH meter (model ABL2, Radiometer, Copenhagen, Denmark). Urinary cyclic AMP was measured by a radioimmunoassay method using Yamasa cyclic AMP assay kit (Yamasa Shōyu Co., Chōshi, Chiba, Japan).

Calculations and statistics. Urinary calcium, creatinine, sodium, and cyclic AMP were the mean values measured in the three hourly urine samples. Urinary excretion of calcium was expressed as micromole per 100 ml of glomerular filtrate (GF) by multiplying the urine calcium/creatinine ratio by serum creatinine concentration. This calculation standardized the calcium excretion for variations in size of the rats and corrected for changes in the filtered load that were unrelated to changes in serum concentrations of diffusible calcium. Some of the data calculated were pooled according to a continuous series of 0.25-mM changes of serum calcium concentrations in order to present the results more concisely (4). Renal threshold of calcium excretion was defined as a serum calcium concentration at which calcium appeared in the urine. This value was determined as the intercept on the serum calcium axis of the line that was derived from the least square regression analysis of the linear portion of the relationships between urinary excretion and serum concentration of calcium (21). All results are given as mean±SE, and analyzed by unpaired t test.

Results

Influence of dietary manipulation on serum and urine chemistries

Serum concentrations of calcium, inorganic phosphate, and total protein before TPTX and the start of infusion are shown in Table I. The serum calcium levels of rats in group A were significantly ($P < 0.001$) lower than those in the other three groups. The serum calcium levels were not different among groups B, C, and D. The serum phosphate concentrations of rats in group A were slightly but significantly ($P < 0.05$) lower than those in groups C and D. The serum phosphate concentrations of rats in group B were significantly ($P < 0.001$) lower than those of groups A, C, and D. The serum total protein levels were comparable among the four groups. The urinary cyclic AMP excretion rates of sham-operated rats that were infused with calcium-free medium for 16 h were significantly ($P < 0.001$) higher in group A than in the other three groups, among which the differences were insignificant (Table I).

These data suggest that the animals in group A were in a state of vitamin D deficiency that was accompanied by secondary

Table I. Serum and Urine Chemistries in Four Groups of Rats Fed on the Different Diet

	Group A	Group B	Group C	Group D
Diet	D(−)standard	D(−)high Ca	D(+)standard	D(+)high Ca
[Ca]s (mM)*	1.35±0.05 (31)§	2.38±0.03 (50)	2.33±0.03 (31)	2.43±0.08 (7)
[Pi]s (mM)*	3.04±0.16 (24)||	2.15±0.07 (52)§	3.51±0.10 (28)	3.90±0.26 (6)
TP (g/dl)*	7.69±0.28 (25)	7.11±0.18 (30)	7.44±0.19 (25)	7.07±0.24 (7)
[cAMP]u (nmol/h)‡	21.96±1.55 (6)§	12.40±1.59 (6)	13.89±1.44 (6)	13.25±2.00 (6)

Values are presented as mean±SE with the number of animals in parentheses. * Serum was obtained before TPTX and the start of infusion. ‡ Urine was obtained 16–19 h after the start of infusion with a calcium-free electrolyte solution into sham-operated rats. § $P < 0.001$, as compared with the other three groups. || $P < 0.05$, as compared with group C and group D. D(−)standard, vitamin D-deficient standard diet, which contains 0.45% calcium and 0.3% phosphorus; D(−)high Ca, vitamin D-deficient diet supplemented with high calcium (1.4% calcium, 0.3% phosphorus) and 20% lactose; D(+)standard, vitamin D-replete standard diet, whose composition is the same as that of D(−)standard except for supplementation with vitamin D_3; D(+)high Ca, vitamin D-replete diet with high calcium content, whose composition is the same as that of D(−)high Ca except for supplementation with vitamin D_3; [Ca]s, serum total calcium concentration; [Pi]s, serum inorganic phosphate concentration as phosphorus; TP, serum total protein concentration; and [cAMP]u, urinary cyclic AMP excretion rate.

hyperparathyroidism, and that the development of secondary hyperparathyroidism was successfully prevented in spite of vitamin D deficiency in group B by adding 1.4% calcium and 20% lactose to the vitamin D-deficient diet. In the vitamin D-replete state, the addition of calcium and lactose had no influence on the parameters shown in Table I. Therefore, group D was not studied further.

Difference in the threshold of calcium excretion between vitamin D-deficient and vitamin D-replete TPTX rats

As summarized in Table II, serum calcium concentration and urinary calcium excretion increased in each group of TPTX rats with increasing calcium concentrations of the infusate. Fig. 1 shows the relationships between the urinary calcium excretion and serum calcium concentrations among the three groups of rats. The urinary calcium excretion is consistently lower in vitamin D-replete rats than in vitamin D-deficient rats over a wide range of serum calcium concentrations. The apparent threshold of calcium excretion occurs at a serum calcium concentration of ∼1.5 mM in the vitamin D-replete rats (group C), and 1.0 mM in the vitamin D-deficient rats (groups A and B). The results obtained in vitamin D-deficient rats supplemented with high calcium and lactose in the diet (group B) were superimposable to those in group A, though the rats in group B showed higher serum calcium concentrations than those in group A with equivalent amounts of calcium infusion (Table II). These results indicate that the threshold of calcium excretion is influenced by vitamin D status independent of PTH.

Effects of PTH infusion

Hypercalcemic effect. Infusion of PTH at a dose of 0.75 U/h caused no significant change in serum calcium levels in vitamin D-deficient TPTX rats (data not shown). However, a higher

Table II. Effect of Graded Amounts of Calcium Infusion on Thyroparathyroidectomized Rats

Group	[Ca]i	[Ca]s	[Ca]u	Ccr	C$_{Na}$
	mM	mM	μmol/100 ml GF	ml/h	ml/h
A	0	0.94±0.03	2.28±0.15	29.7±1.6	0.179±0.024
	5	0.99±0.03	3.88±0.28	32.9±1.9	0.196±0.012
	10	1.24±0.10	6.23±0.55	36.9±1.9	0.218±0.028
	15	1.40±0.08	12.63±1.28	34.6±2.9	0.226±0.030
	20	1.55±0.06	19.25±1.05	33.1±2.1	0.184±0.017
	25	2.01±0.04	22.95±1.83	39.0±2.1	0.239±0.032
	30	2.34±0.12	41.58±3.08	38.0±3.1	0.222±0.024
B	0	1.51±0.03	13.00±1.25	32.4±3.9	0.173±0.015
	5	1.92±0.11	19.35±2.05	35.4±6.3	0.181±0.022
	10	2.00±0.06	21.58±1.23	38.6±1.9	0.210±0.025
	15	2.41±0.12	37.00±2.60	46.7±2.3	0.176±0.021
	20	2.64±0.06	56.68±3.90	43.4±1.7	0.166±0.025
C	0	1.35±0.05	1.18±0.05	44.7±3.1	0.244±0.034
	5	1.62±0.04	1.98±0.13	42.0±3.1	0.288±0.061
	10	1.90±0.06	8.63±0.55	49.8±1.7	0.237±0.040
	15	2.02±0.10	11.93±1.15	48.4±8.3	0.206±0.044
	20	2.19±0.07	15.63±1.33	52.3±3.9	0.282±0.039
	25	2.37±0.05	26.05±2.48	52.0±7.4	0.235±0.058

Values are expressed as mean±SE. Each experimental subgroup consisted of 5–6 animals which were infused with a solution of single concentration of $CaCl_2$ throughout the period of experiment. [Ca]i, calcium concentration of the infusion medium; [Ca]s, serum total calcium concentration; [Ca]u, urinary calcium excretion; Ccr, endogenous creatinine clearance; and C$_{Na}$, sodium clearance.
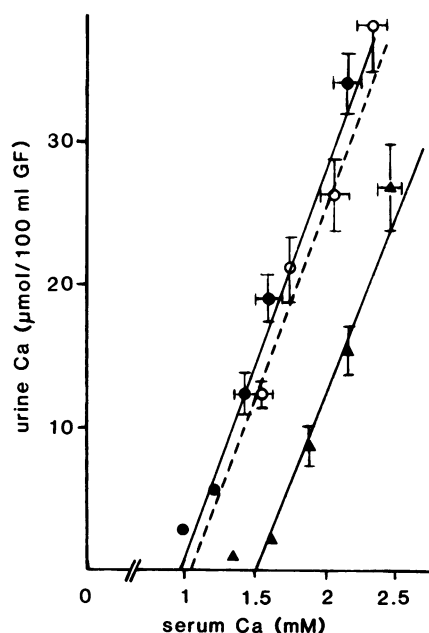
Figure 1. Relationships between urinary calcium excretion and serum calcium concentration among three groups of thyroparathyroidectomized rats. Serum concentration and urinary excretion of calcium were determined 16–19 h after continuous infusion of an electrolyte solution containing 0–30 mM of $CaCl_2$. Each point represents the data pooled according to a continuous series of 0.25-mM changes of serum calcium concentration. Horizontal bars indicate standard error of mean serum calcium concentration, and vertical bars indicate standard error of mean urinary calcium excretion. The lines were derived from the regression analysis of the linear portion of data (see Methods). (●): Group A rats fed vitamin D-deficient standard diet. (○): Group B rats fed vitamin D-deficient diet containing high calcium and lactose. (▲): Group C rats fed vitamin D-replete standard diet. For any given serum calcium level, the urinary calcium excretion was significantly lower in vitamin D-replete rats (group C) than in vitamin D-deficient rats (groups A and B). Thus, the apparent serum calcium threshold determined as an intercept of the regression line on serum calcium axis was higher in vitamin D-replete rats (~1.5 mM) than in vitamin D-deficient rats (~1.0 mM). There was no significant difference in the calcium threshold between group A and group B.

dose (2.5 U/h) of PTH raised serum calcium by 0.24–0.72 mM from the levels seen in the TPTX state (Tables II and III). In vitamin D-replete TPTX rats (group C), 0.75 U/h of PTH increased serum calcium by 0.48–0.64 mM (Tables II and III), and 2.5 U/h of PTH caused a marked hypercalcemia >2.75 mM. Thus, the hypercalcemic action of PTH was evident in vitamin D-deficient rats either with (group A) or without (group B) preceding secondary hyperparathyroidism. However, PTH was less effective in vitamin D-deficient rats than in vitamin D-replete rats.

Effect on the renal calcium threshold. Infusion of PTH at a dose of 0.75 U/h to vitamin D-deficient TPTX rats did not

alter the threshold of calcium excretion (data not shown). When vitamin D-deficient TPTX rats (group A) were given 2.5 U/h of PTH, the threshold of calcium excretion increased from ~1.0–1.6 mM (Fig. 2 *A*). A similar shift in the calcium threshold was observed in response to 2.5 U/h of PTH in vitamin D-deficient rats that were supplemented with high calcium and lactose (group B) (Fig. 2 *B*). When vitamin D-replete TPTX rats (group C) were infused with 0.75 U/h of PTH, the threshold of calcium excretion increased from 1.5 mM to ~2.1 mM (Fig. 2 *C*). Infusion of 2.5 U/h of PTH into vitamin D-replete TPTX rats further raised the threshold: however, the exact value could not be determined because of hypercalcemia >2.75 mM that was observed in this group of rats. These results indicate that the effect of PTH on renal tubular calcium reabsorption is exerted in both vitamin D-deficient and vitamin D-replete rats. The striking difference between vitamin D-deficient and vitamin D-replete states is that the dose of PTH required to exhibit a comparable effect on calcium reabsorption is higher in the vitamin D-deficient rats, and that the threshold of calcium excretion in the presence of PTH is lower in vitamin D-deficient animals.

Effects of experimental managements on the parameters other than calcium

The body weights at the time of clearance studies were lower in vitamin D-deficient rats than in vitamin D-replete rats (group A, 234.5±7.2 g, n = 39; group B, 239.1±5.2 g, n = 61; group C, 274.6±5.1 g, n = 43).

Serum total protein and creatinine concentrations were almost identical among experimental subgroups, as was arterial blood pH (data not shown). Intravenous calcium load and/or PTH infusion were not associated with any consistent change in the creatinine clearance and sodium clearance (Tables II and III). The differences in creatinine clearance among three groups of rats on the different dietary regimens seem to result from the differences in the size of the rats, because the creatinine clearance generally parallelled the body weight (data not shown).

Discussion

It is well known that small variations in the serum concentration and filtered load of calcium lead to a marked change in the absolute and fractional excretion of calcium (6). Therefore, the assessment of the effect of any factor on renal calcium transport must take into account the simultaneous changes in serum calcium concentration. With regard to PTH action, stimulatory effects of PTH on tubular calcium reabsorption have been amply demonstrated in several studies by determining urinary calcium excretions at different serum calcium concentrations (4, 6). However, the effect of vitamin D on renal calcium transport has not been studied systematically by determining urinary calcium excretion over a wide range of serum calcium concentrations. Our study for the first time demonstrates the presence of a direct influence of vitamin D status on tubular reabsorption

Table III. Effect of PTH Plus Graded Amounts of Calcium Infusion on Thyroparathyroidectomized Rats

Group (PTH dose)	[Ca]i	[Ca]s	[Ca]u	Ccr	C_{Na}
	mM	*mM*	*μmol/100 ml GF*	*ml/h*	*ml/h*
A	0	1.18±0.08	2.65±0.28	35.7±2.7	0.238±0.012
(2.5 U/h)	5	1.56±0.12	3.18±0.25	36.2±2.6	0.190±0.023
	10	1.89±0.12	7.70±0.68	41.3±2.9	0.225±0.029
	15	2.02±0.15	13.25±1.58	42.1±1.3	0.313±0.027
	20	2.14±0.21	22.55±2.20	34.4±1.9	0.194±0.025
	25	2.30±0.23	28.60±2.30	43.7±2.1	0.254±0.085
B	0	1.88±0.19	10.83±1.38	31.9±1.6	0.180±0.015
(2.5 U/h)	5	2.31±0.17	23.65±2.45	40.7±2.0	0.232±0.026
	10	2.72±0.11	37.90±3.15	33.9±3.4	0.196±0.022
C	0	1.95±0.13	0.85±0.13	47.6±2.3	0.256±0.026
(0.75 U/h)	5	2.10±0.04	2.05±0.35	52.1±2.6	0.260±0.056
	10	2.54±0.16	19.05±1.55	42.0±2.7	0.206±0.023

Values are expressed as mean±SE. Each experimental subgroup consisted of 5–6 animals which were infused with a solution of single concentration of $CaCl_2$ and PTH throughout the period of experiment. See legend to Table II for abbreviation list.

of calcium. Thus, our data clearly show that not only PTH but also vitamin D stimulate the renal tubular reabsorption of calcium (Figs. 1 and 2).

When TPTX vitamin D-deficient rats (group A) were compared with TPTX vitamin D-replete rats, the urinary calcium excretion was greater in the vitamin D-deficient rats than in the vitamin D-replete rats at any given level of serum calcium (Fig. 1). Thus, the difference in the renal handling of calcium between vitamin D-deficient and vitamin D-replete rats is due to a difference in the apparent threshold of calcium excretion. The decrease in the threshold of calcium excretion in vitamin D-deficient rats might be caused by vitamin D-deficiency per se, or by preceding PTH excess due to secondary hyperparathyroidism. In order to differentiate these possibilities, a group of rats (group B) were fed a vitamin D-deficient diet that was supplemented with high calcium and lactose, a dietary regimen

known to maintain normocalcemia in spite of vitamin D-deficiency (22). The absence of severe secondary hyperparathyroidism in the group B rats was confirmed by a low urinary cyclic AMP excretion rate which was comparable with that in vitamin D-replete rats (Table I). The results obtained in group B rats demonstrated that the threshold of calcium excretion was reduced and was almost identical to that in group A (Fig. 1). These observations suggest that the decrease in the tubular reabsorption of calcium in vitamin D-deficient rats is not due to preceding PTH excess followed by a sudden withdrawal of the hormone by TPTX, but due to vitamin D deficiency per se, and that vitamin D has a direct effect on the renal tubular transport of calcium.

In agreement with observations by others (4, 5), administration of a physiological dose of PTH induced an increase in the renal calcium threshold in vitamin D-replete rats (Fig. 2
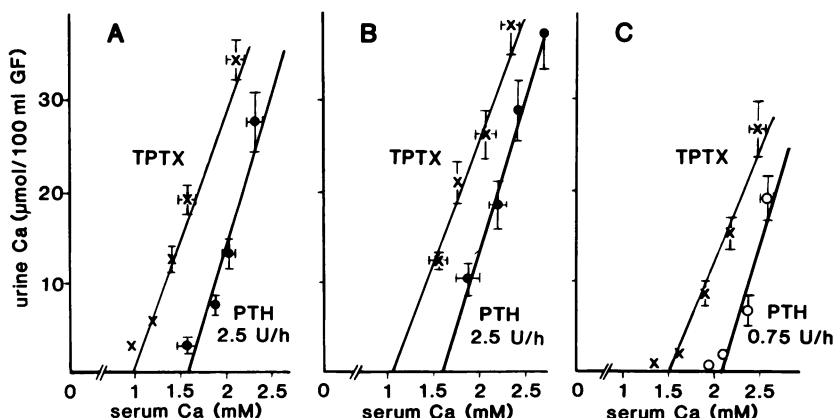

Figure 2. The effects of PTH infusion on the renal handling of calcium among three groups of TPTX rats. The data are presented as described in the legend to Fig. 1. PTH was delivered at 2.5 U/h (•) on groups A and B rats, and 0.75 U/h (o) on group C rats. *A, B,* and *C* illustrate the results in group A, group B, and group C, respectively. The enhancement of calcium reabsorption by PTH is shown as the shifts of the lines to the right in each group. The striking difference exists between vitamin D-deficient (groups A and B) and vitamin D-replete (group C) rats in the doses of PTH required to induce a comparable shift in the calcium threshold. x, data of TPTX rats in each group (from Fig. 1).

C). In contrast, PTH, at a dose that was effective in vitamin D-replete rats, failed to induce any change in the calcium threshold in TPTX vitamin D-deficient animals. However, a much higher dose of PTH (2.5 U/h) induced a rise in the threshold of calcium excretion in TPTX vitamin D-deficient rats (group A). These observations raised the question as to which of the two factors, vitamin D deficiency per se or possible down regulation of PTH action due to secondary hyperparathyroidism, caused the resistance to PTH in vitamin D deficiency. Answer to this question was provided by the results obtained in the vitamin D-deficient rats without secondary hyperparathyroidism (group B). The renal calcium reabsorption in response to PTH in group B was similarly decreased to that in group A (compare Fig. 2 *B* with 2 *A* and 2 *C*). Thus, the resistance to PTH results from the deficiency of vitamin D, and not from prior secondary hyperparathyroidism.

The present results cannot be explained on the basis of alterations in the fraction of ultrafiltrable calcium among experimental groups. Although the concentration of ultrafiltrable calcium was not directly measured in the present study, there was no significant difference in arterial blood pH and serum total protein concentration among experimental groups. Therefore, it may be assumed that any change in the total concentration of serum calcium reflects a change in the concentration of ultrafiltrable calcium, as shown by Hugi et al. (4).

Among the known factors that might cause an increase in urinary calcium excretion, an excess of mineralocorticoids, starvation, and carbohydrate load are unlikely explanations for the relative hypercalciuria seen in vitamin D-deficient rats. The urine volume was almost identical to the volume of the electrolyte solution that was infused during the period in all experiments. Also, a constant infusion of an electrolyte solution that contained glucose provided the same caloric intake to all the animals during the experiments.

We did not observe any significant difference in the amount of food consumed in each group. In addition, a restriction of food intake by one-third in vitamin D-replete rats did not change the renal threshold of calcium excretion (unpublished observation). These data argue against the possibility that the decrease in caloric and nutritional intake, as well as the parallel reduction in the intake of calcium and phosphorus, was responsible for the decrease in the renal threshold of calcium excretion in vitamin D-deficient rats.

It has been reported that vitamin D deficiency is often accompanied by metabolic acidosis, which is a condition known to cause hypercalciuria (23). In our experiments, however, the arterial blood pH measured in vitamin D-deficient rats at the end of the clearance studies showed no evidence for systemic acidosis. Therefore, the decrease in the threshold of calcium excretion in the vitamin D-deficient rats cannot be attributed to metabolic acidosis.

The renal handling of calcium is known to be influenced by the transport of other ions, especially sodium ions (24). Costanzo et al. (9) demonstrated an enhancement by vitamin D of calcium transport relative to sodium transport in the kidney of vitamin D-deficient rats. They manipulated sodium clearance without changing serum calcium levels. In contrast, our experiments were performed under the condition of a fixed sodium load in order to minimize the influence of changes in sodium transport. Though the sodium clearance in our study varied somewhat within and between experimental groups, the variation was small and not proportionate to the change in calcium excretion. Hence, if we expressed urinary calcium excretion relative to sodium clearance, the results and conclusion would be the same as obtained using creatinine excretion as the reference.

Renal transport of calcium is also known to be affected by phosphate transport. It has been reported that phosphate depletion is associated with hypercalciuria (25, 26) and with resistance to the actions of PTH to stimulate tubular reabsorption of calcium (25), to inhibit tubular reabsorption of phosphate (27), and to raise serum calcium (28). The hypercalciuria in phosphate depletion has been attributed to the increase in the filtered load of calcium, which is due to accompanying hypercalcemia, and to the decrease in the tubular reabsorption, which is due to functional hypoparathyroidism (25, 29). In addition, Coburn and Massry (25) have suggested that hypophosphatemia per se alters renal handling of calcium and reduces the responsiveness to PTH. The mechanism through which phosphate depletion affects the action of PTH on renal calcium transport remains to be defined. In this context, we considered the possible contribution of the phosphate depletion in vitamin D deficiency to the decrease in the threshold of calcium excretion. It is well known that a certain degree of phosphate depletion may develop in vitamin D deficiency (30). In our experiments, however, severe hypophosphatemia did not develop in the rats fed vitamin D-deficient diet that contained 0.3% of phosphorus (group A). It is not apparent from our data whether the difference in phosphate metabolism between the vitamin D-deficient and vitamin D-replete rats contributed to the altered tubular transport of calcium. However, since animals in groups A and B had markedly different serum phosphate concentrations, but nearly identical calcium thresholds, it is unlikely that alterations in phosphate metabolism were responsible for the change in the calcium threshold.

In summary, the present study demonstrates that the vitamin D deficiency leads to a decrease in renal tubular calcium reabsorption both in the absence and in the presence of PTH, and that the vitamin D deficiency decreases the effect of PTH to stimulate tubular reabsorption of calcium. However, our study does not address the questions of which metabolite or metabolites of vitamin D is responsible for the enhancement of tubular calcium reabsorption, nor on which portion or portions of the nephron vitamin D acts to regulate calcium transport.

Acknowledgments

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