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Parathyroid hormone-stimulated calcium absorption in cTAL from vitamin D-deficient rabbits

JAMES E. BOURDEAU, CRAIG B. LANGMAN, and ROGER BOUILLON,
with the technical assistance of ANN L. THEISEN

Division of Renal Medicine, Michael Reese Hospital & Medical Center, and Department of Medicine, University of Chicago, Pritzker School of Medicine, and Division of Nephrology, Children's Memorial Hospital and Department of Pediatrics, Northwestern University Medical School, Chicago, Illinois, USA, and Laboratorium voor Experimentele Geneeskunde en Endocrinologie, Katholieke Universiteit Leuven, Leuven, Belgium

Parathyroid hormone-stimulated calcium absorption in cTAL from vitamin D-deficient rabbits. Cortical thick ascending limbs of Henle's loop were dissected from the kidneys of chronically vitamin D-deficient or -replete rabbits and perfused in vitro. Unidirectional transepithelial calcium fluxes from lumen to bath were measured with ^{45}Ca. The tubules were bathed in a solution containing 150 mM sodium and perfused with a solution containing 60 mM sodium to simulate conditions in the cortical thick ascending limb in vivo. Transepithelial voltages were equal across tubules from vitamin D-deficient and -replete rabbits. Likewise, baseline and parathyroid hormone-stimulated calcium fluxes were the same in tubules from the two groups. Because calcidiol and calcitriol were undetectable in the serum of the vitamin D-deficient rabbits, we suggest that neither of these endogenous vitamin D metabolites is essential in the regulation of calcium absorption in this portion of the rabbit nephron.

Parathyroid hormone (PTH) and calcitriol are the most important hormonal regulators of the plasma ionized-calcium concentration, which is the focal point for the control of calcium (Ca) metabolism in mammals [1]. Each hormone releases Ca from bone into the extracellular fluid, and there is a synergism between them in this action [2]. PTH increases Ca reabsorption from the tubular fluid in specific segments of the nephron [3, 4], whereas calcitriol increases Ca absorption from the luminal contents of specific segments of the intestine [5]. A controversial issue is whether a vitamin D metabolite, either independently or in concert with PTH, is important in augmenting renal tubular Ca reabsorption [3, 4].

Evidence supporting a physiologic role of vitamin D to increase renal tubular Ca reabsorption has been derived primarily from clearance studies in rats [6, 7]. Costanzo, Sheehe and Weiner [6] observed that thyroparathyroidectomized (TPTX) vitamin D-deficient rats had significantly higher fractional excretions of Ca than TPTX vitamin D_3-repleted rats when Ca clearance was normalized for sodium clearance. More recently, Yamamoto et al [7] reported that the "apparent serum Ca threshold"[1] concentration for urinary Ca excretion was significantly lower in vitamin D-deficient than in vitamin D_3-replete TPTX rats. In addition, more exogenously infused PTH was required to incrementally augment the apparent serum threshold-concentration of Ca in the vitamin D-deficient rats than in the vitamin D_3-replete controls. These investigators concluded that vitamin D deficiency decreases both renal tubular Ca reabsorption (either in the absence or the presence of PTH) and the effect of PTH to stimulate tubular reabsorption of Ca [7]. Hugi, Bonjour and Fleisch [8] studied the effects of sustained variations in the endogenous production of—or physiologic supplementation with—calcitriol on the tubular handling of Ca in vitamin D-replete rats. In contrast to the conclusions reached by Costanzo, Sheehe and Weiner [6] and Yamamoto et al [7] in vitamin D-deficient rats, they found no influence of calcitriol on the renal tubular transport of Ca.

The purpose of this study was to investigate the potential role of vitamin D in the regulation of epithelial Ca transport in a Ca-reabsorbing segment of the rabbit nephron. We measured absorptive Ca fluxes across isolated perfused cortical thick ascending limbs of Henle's loop from chronically vitamin D-deficient adult rabbits and vitamin D-replete controls. We found that both the baseline and the PTH-stimulated Ca efflux were indistinguishable between tubules from the two groups, providing no evidence for an essential role of endogenous vitamin D in the regulation of Ca transport in this portion of the rabbit nephron.

Methods

Animals

Albino rabbits were purchased commercially and maintained in a vivarium. Vitamin D deficiency was induced as described previously [9]. Briefly, rabbits were housed in a room illuminated solely with incandescent light and fed a synthetic diet (TD

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[1] Yamamoto et al [7] defined the "apparent serum Ca threshold" as "the serum Ca concentration at which Ca appeared in the urine." This is a virtual value derived from the x-intercept of a linear regression of urinary Ca excretion (expressed as μmol Ca per 100 ml of glomerular filtrate) versus serum Ca concentration.

82321, Teklad, Madison, Wisconsin, USA) that contained (by weight) 1% Ca and 0.5% P but was devoid of vitamin D. Vitamin D-replete control animals consisted of two rabbits fed an identical diet except that it contained 2.2 IU of vitamin D_3/g feed (TD 82322, Teklad) and four rabbits fed a vitamin D-supplemented standard laboratory feed (Wayne Rabbit Ration, Allied Mills, Inc., Chicago, Illinois, USA) that contained 1.0% Ca, 0.5% P, and 2.6 IU of vitamin D_3/g feed.

Serum vitamin D metabolites

Blood was obtained from each rabbit immediately prior to sacrifice. The central auricular artery was punctured with a siliconized hypodermic needle that was attached to a short length of Teflon tubing, and blood was collected in a siliconized glass tube. After a clot had formed, serum was separated by centrifugation. Aliquots were frozen immediately and stored at −20°C until analyzed. Serum concentrations of vitamin D metabolites were measured as described previously [9].

Serum parathyroid hormone (PTH)

Aliquots of serum were frozen immediately and stored at −70°C in Chicago. They were shipped on dry ice to Leuven, where concentrations of PTH were measured by radioimmunoassay using synthetic human [53-84] PTH as the standard [10].

Isolated tubule perfusion

Segments of cortical thick ascending limbs of Henle's loop were dissected and perfused in vitro using techniques identical to those described previously [11], with the following exceptions. The rabbits were killed by infusing approximately 6 ml of pentobarbital sodium injection, USP, 50 mg/ml (Nembutal Sodium Solution, Abbott Laboratories, North Chicago, Illinois, USA), into a marginal ear vein. Tubule segments, 0.60 to 1.45 mm in length, were dissected in a saline solution that was maintained at 17°C and that contained 0.1 g/dl defatted bovine albumin. The composition of this solution was (in mM): NaCl, 150; K_2HPO_4, 2.5; $CaCl_2$, 2; $MgSO_4$, 1.2; L-alanine, 6; and D-glucose, 5.6. Prior to the addition of albumin, the dissection solution was bubbled with 100% O_2, and its pH was titrated to a value between 7.35 and 7.45 with 1 M HCl. Tubules were bathed in and perfused with protein-free solutions. The composition of the bathing solution was (in mM): NaCl, 120; $NaHCO_3$, 25; Na lactate, 4; NaH_2PO_4, 1; KCl, 5; $CaCl_2$, 2; $MgSO_4$, 1.2; L-alanine, 6; and D-glucose, 5.6. The composition of the perfusion solution was (in mM): NaCl, 59; NaH_2PO_4, 1; KCl, 5; $CaCl_2$, 2; and $MgSO_4$, 1.2. (These solutions are identical to bath 150 Na and perfusate 60 Na (Table 1 of [11]) that were used previously to study the effects of PTH on Ca transport across cortical thick ascending limbs of Henle's loop from normal laboratory rabbits.)

Transepithelial voltages were measured between calomel cells connected to the solutions in the bath and the perfusion pipette with bridges containing 0.154 M NaCl-3% agarose. The electrical potential differences were displayed on a digital multimeter (Model 8000A, John Fluke Manufacturing Co, Inc, Mountlake Terrace, Washington, USA) connected through a high input-impedance electrometer-amplifier (Model 750 Dual Micro-Probe, World Precision Instruments, Inc, New Haven, Connecticut, USA). Corrections for liquid junction voltages were identical to those reported previously [11, 12].

Table 1. Characteristics of albino rabbits when sacrificed for isolated tubule perfusion experiments

Vitamin D status	Sex	Body weight, *g*	Consumption of diet prior to decapitation, *days*	Serum concentration, mM	
				Calcium	Phosphorus
Replete	M	4000	303[a]	3.42	0.95
	F	5629	358[a]	3.39	0.93
	F	4829	262[b]	3.03	1.27
	F	5201	269[b]	3.15	0.81
	F	4513	276[b]	3.07	0.77
	F	3889	290[b]	3.01	1.21
Mean		4677	293	3.18	0.99
SE		278	14	0.07	0.08
Deficient[c]	F	3694	951	3.38	1.43
	F	4456	623	2.81	0.45
	F	3268	387	2.93	0.17
	F	4570	365	3.36	0.86
	F	3670	428	3.24	0.24
Mean		3932	551[d]	3.14	0.63
SE		250	110	0.11	0.23

[a] Teklad TD 82322
[b] Wayne Rabbit Ration
[c] Teklad TD 82321
[d] $P < 0.05$ for comparison with vitamin D-replete rabbits

Unidirectional, lumen-to-bath transepithelial Ca fluxes were measured as described previously [11]. The mean recovery of perfused ^{45}Ca activity from the collectate plus the bath was 101.1 ± 1.2%.

Synthetic bovine PTH (amino-terminal 1-34 tetratriacontapeptide; Peninsula Laboratories, Inc, Belmont, California, USA) was dissolved in 50 mM acetic acid, and 30 μl aliquots of this solution were pipetted into siliconized glass vials. Each vial was gassed with 100% N_2, and its contents were frozen immediately by immersion of the vial in a slurry of solid CO_2-acetone. These vials were stored at −20°C until used on the day of an experiment. The final concentration of synthetic bovine PTH in the bathing solutions was 7.3×10^{-8} M.

Because there was no appreciable difference between Ca fluxes in tubules taken from rabbits fed the two types of vitamin D-replete diets, these results were combined and analyzed as a single group.

Statistics

Results are reported as means ± SEM. Statistical analyses were performed using either paired or group *t*-tests. *P* values less than 0.05 were considered to be significant.

Results

General characteristics of the rabbits (Table 1)

When sacrificed, the vitamin D-deficient rabbits had been consuming a synthetic diet devoid of vitamin D for 1 to 2.6 years. Mean serum Ca concentrations were not significantly different between the groups. Although mean serum *P* concentration tended to be lower in the vitamin D-deficient animals,

Table 2. Serum concentrations of vitamin D metabolites in rabbits

Vitamin D status	Calcidiol[a] *ng/ml*	Calcitriol[b] *pg/ml*
Replete		
$N = 6$	33 ± 3	60 ± 7
Deficient		
$N = 5$	<1.6	<10

[a] 25-hydroxycholecalciferol
[b] 1,25-dihydroxycholecalciferol

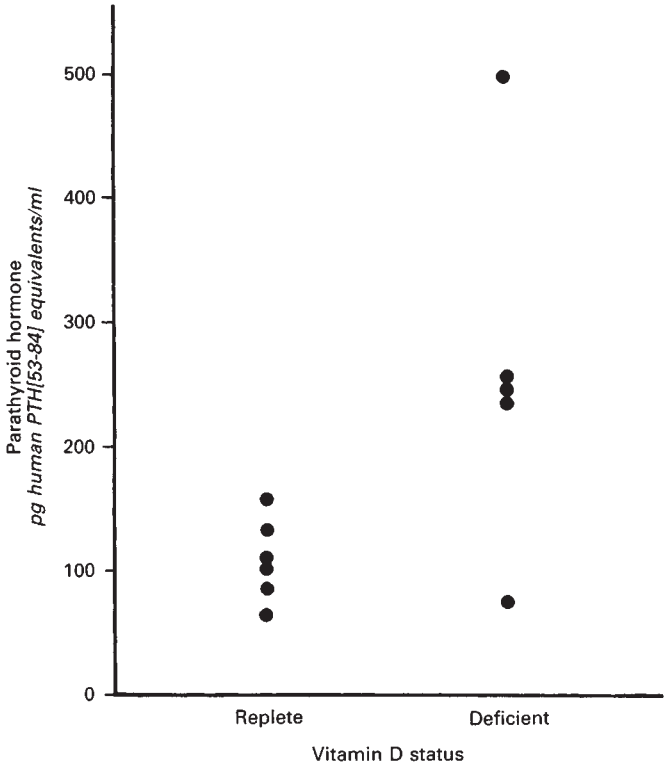

Fig. 1. *Immunoreactive PTH concentrations in sera collected from rabbits immediately prior to sacrifice for isolated tubule perfusion experiments.* Each point represents the measurement for a single rabbit.

Table 3. Effect of synthetic bovine parathyroid hormone on unidirectional lumen–to–bath transepithelial calcium fluxes

Period	Transepithelial voltage *mV*		Calcium flux *pmol · sec⁻¹ · cm⁻¹*	
	+D $N = 6$	−D $N = 5$	+D $N = 6$	−D $N = 5$
First control	+22.0 ± 1.0	+23.7 ± 1.1	0.255 ±0.044 *	0.206 ±0.043 *
Last control	+21.1 ± 0.7	+23.2 ± 1.2	0.147 ±0.023 *	0.132 ±0.026 *
Parathyroid hormone	+17.9 ± 1.5	+20.2 ± 1.1	0.536 ±0.062	0.511 ±0.081

Abbreviations are: +D, vitamin D-replete rabbits; −D, vitamin D-deficient rabbits. In tubules from +D and −D rabbits, the mean tubular fluid collection rates were 3.51 ± 0.38 and 3.55 ± 0.40 nl · min^{-1} · mm^{-1}, respectively. * Significant difference for the paired comparison between the mean values above and below the asterisk.

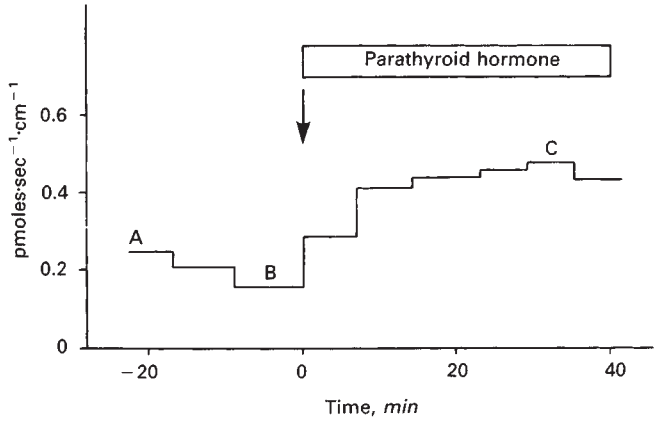

Fig. 2. *Effect of synthetic bovine PTH on the unidirectional lumen-to-bath transepithelial Ca flux across an isolated perfused cortical thick ascending limb of Henle's loop obtained from a vitamin D-deficient rabbit.* The letters on the flux versus time curve indicate the first control period (A), the last control period (B), and the PTH period (C) referred to in Table 3.

the greater variability in this group precluded a statistically significant difference.

Vitamin D status (Table 2)

Calcidiol and calcitriol were measurable in the sera from vitamin D-replete rabbits, but were undetectable in the sera from vitamin D-deficient rabbits.

Serum parathyroid hormone (Fig. 1)

With one exception, serum PTH concentrations were higher in vitamin D-deficient than -replete rabbits. Accordingly, mean serum [PTH] in the former was significantly greater than in the latter, 258 ± 67 versus 106 ± 14 pg human[53-84]PTH equivalents/ml, respectively.

Calcium efflux (Table 3)

Mean time-averaged transepithelial voltage was +20.3 mV in tubules from vitamin D-replete rabbits and +22.4 mV in tubules from vitamin D-deficient rabbits. The transepithelial voltages were not significantly different between the two groups of tubules during any study period. These voltages are similar to values reported previously under these experimental conditions [11, 12]. As observed previously [11], the unidirectional lumen-to-bath Ca fluxes declined with time during the control periods, despite a constant transepithelial voltage (Fig. 2 and Table 3). After PTH was added to the bathing solution, there was an immediate increase in the lumen-to-bath Ca flux. Thereafter, the mean flux increased to a maximal value that was fourfold greater than the mean of the control fluxes immediately preceding addition of the hormone. The unidirectional lumen-to-bath Ca fluxes were not significantly different between the two groups of tubules during any study period (Table 3).

Discussion

Ca is absorbed from the luminal fluid of the cortical portion of the thick ascending limb of Henle's loop when this nephron segment is studied in vitro under simulated physiologic conditions [12–14]. Furthermore, under these conditions (or other conditions producing a lumen positive voltage) synthetic bovine PTH in the bathing solution increases Ca absorption by augmenting the unidirectional lumen-to-bath Ca flux [11, 15, 16]. Apparently, this latter effect is mediated via cyclic AMP [11, 14, 16, 17].

In the present study we investigated whether chronic vitamin D-deficiency alters Ca transport across the cortical thick ascending limb of Henle's loop from the rabbit. We found that the baseline and the PTH-stimulated lumen-to-bath Ca fluxes were the same between tubules from the vitamin D-replete and the vitamin D-deficient rabbits (Table 3), providing no evidence for an essential role of endogenous vitamin D in regulating Ca absorption in this nephron segment. These results are the same as those reported previously for smaller, growing normal rabbits (Table 3 of [11]). Although we are unaware of any direct evidence for calcitriol binding in cells of the thick ascending limb of Henle's loop in the rabbit, the absence of calbindin-D_{28K} in this segment of the nephron [18] implies that calcitriol receptors are absent [19], which is consistent with the lack of an effect of vitamin D on Ca transport.

In this study we employed experimental solutions that simulate physiologic conditions in the terminal portion of the diluting segment [20, 21], which is the cortical thick ascending limb of Henle's loop. These solutions cause a large lumen-positive voltage because of the sodium permselectivity of this epithelium [20, 21], which in turn may drive diffusional Ca^{2+} absorption via the paracellular pathway [21, 22]. These conditions, though physiologic, might obscure a relatively small effect of vitamin D on a PTH-stimulated cellular Ca absorptive process. We cannot exclude this possibility, which might be tested by using perfusion and bathing solutions that result in a smaller—or absent—electrochemical driving force for epithelial Ca^{2+} transport. Likewise, although we believe it to be unlikely, we cannot exclude the possibility that chronic vitamin D deficiency may increase the unidirectional flux of Ca from bath to lumen, thus reducing net Ca absorption. We were unable to investigate these two issues because of insufficient numbers of vitamin D-deficient rabbits.

The results of the serum Ca, P, and PTH measurements in our middle-aged rabbits (Table 1, Fig. 1) are qualitatively similar to observations in middle-aged rats [23]. In that study, rats were fed a vitamin D-deficient diet for 1.5 to six months. Serum Ca or P concentrations were similar in vitamin D-deficient and -replete rats at 1.5, 3.0, 4.5, and 6.0 months. Serum PTH concentrations were increased one- to twofold in the vitamin D-deficient, compared to -replete, rats at each of these times. Previous studies have shown that chronic vitamin D deficiency is associated with elevated serum PTH concentrations in middle-aged [9] and young [10] rabbits, although in both of those studies vitamin D-deficiency was accompanied by modest hypocalcemia relative to the vitamin D-replete state.

Because the vitamin D-deficient rabbits in the present study demonstrated increased serum PTH concentrations at the time of sacrifice (Fig. 1), it is reasonable to consider why baseline Ca fluxes (study period A in Fig. 2) across cortical thick ascending limbs from this group were not greater than in tubules from vitamin D-replete controls. We do not have experimental evidence to answer this question, but we suggest the following possibilities. First, variability of endogenous serum PTH concentrations at the time of sacrifice coupled with variability in the duration of dissection and mounting of individual tubule segments prior to measurement of Ca fluxes, during which time the effects of endogenous PTH would be dissipating, might preclude detection of a relationship between the Ca flux during the first study period and the ante-mortem serum PTH concentration. Second, the increases in endogenous serum PTH concentrations associated with vitamin D deficiency may have been too small to increase detectably the absorptive Ca fluxes to values above those of the controls. In this regard, Chabardes et al [24] have shown that the cortical thick ascending limb of Henle's loop is less sensitive to PTH stimulation of adenylate cyclase, through which the hormone presumably acts to increase Ca absorption, than the more distally located connecting tubule. These two possibilities are not mutually exclusive.

In conclusion, both baseline and PTH-stimulated Ca absorptive fluxes were indistinguishable between cortical thick ascending limbs dissected from chronically vitamin D-deficient or -replete rabbits and studied in vitro. Because calcidiol and calcitriol were undetectable in the serum of the vitamin D-deficient rabbits, we find no evidence for an essential role of endogenous vitamin D metabolites in the regulation of Ca transport in this portion of the rabbit nephron.

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Reprint requests to Dr. James E. Bourdeau, Division of Renal Medicine, Michael Reese Hospital and Medical Center, Lake Shore Drive at 31st Street, Chicago, Illinois 60616, USA.

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