Modulation of Phosphate Absorption by Calcium in the Rabbit Proximal Convoluted Tubule

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Abstract

Proximal convoluted (S_2) and straight (S_3) renal tubule segments were studied to determine the effect of Ca on lumen-to-bath phosphate flux ($J^{lb}_{PO_4}$). Increasing bath and perfusate Ca from 1.8 to 3.6 mM enhanced $J^{lb}_{PO_4}$ from 3.3±0.7 to 6.6±0.6 pmol/mm per min in S_2 segments ($P < 0.001$) but had no effect in S_3 segments. Decreasing bath and perfusate Ca from 1.8 to 0.2 mM reduced $J^{lb}_{PO_4}$ from 3.7±0.6 to 2.2±0.6 in S_2 segments. These effects were unrelated to changes in fluid absorption and transepithelial potential difference. Increasing cytosolic Ca with a Ca ionophore, inhibiting the Ca-calmodulin complex with trifluoperazine, or applying the Ca channel blocker nifedipine had no effect on $J^{lb}_{PO_4}$ in S_2 segments. Increasing only bath Ca from 1.8 to 3.6 mM did not significantly affect $J^{lb}_{PO_4}$. However, increasing only perfusate Ca enhanced $J^{lb}_{PO_4}$ from 3.4±0.7 to 6.1±0.7 pmol/mm per min ($P < 0.005$). Inhibition of hydrogen ion secretion, by using a low bicarbonate, low pH perfusate, both depressed base-line $J^{lb}_{PO_4}$ and abolished the stimulatory effect of raising perfusate Ca. Net phosphate efflux ($J^{net}_{PO_4}$) also increased after ambient calcium levels were raised, ruling out a significant increase in PO_4 backflux. When net sodium transport was abolished by reducing the bath temperature to 24°C, $J^{net}_{PO_4}$ at normal ambient calcium was reduced and increasing ambient calcium failed to increase it, ruling out a simple physicochemical reaction wherein phosphate precipitates out of solution with calcium.

The present studies provide direct evidence for a stimulatory effect of Ca on sodium-dependent PO_4 absorption in the proximal convoluted tubule, exerted at the luminal membrane. It is postulated that Ca modulates the affinity of the PO_4 transporter for the anion.

Introduction

The renal handling of phosphate may be influenced by a variety of factors, one of which is serum calcium concentration. Changes in serum calcium levels may alter renal phosphate absorption indirectly by altering the amount of phosphate filtered at the glomerulus. Infusion of calcium may increase the filtered load of phosphate by raising serum phosphate concentration (1–8). Opposing this effect is a decrease in the filterability of phosphate and in glomerular filtration rate (GFR).[1] Filterable phosphate levels may decline as a result of the formation of calcium-phosphate-proteinate complexes (9, 10). The fall in GFR is due to not only a decline in renal plasma flow (3, 8, 11, 12) but also to a decline in the ultrafiltration coefficient (13). However, while the reduction in GFR reduces the filtered load of phosphate, it actually may enhance tubular absorption (14).

Changes in serum calcium also may indirectly alter tubular handling of phosphate. As serum calcium levels are raised, parathyroid hormone (PTH) secretion is inhibited, and the tubular absorption of phosphate increases (2, 4–7). Conversely, as serum calcium levels decline, PTH secretion increases and tubular absorption of phosphate declines (15, 16).

In addition to these indirect effects, clearance and micropuncture studies have suggested a direct effect of calcium on renal tubular absorption of phosphate. Clearance studies performed in hypoparathyroid humans have generally shown a decrease in phosphate absorption as serum calcium levels are raised acutely (4, 5, 7) and an increase as serum calcium levels are lowered by EDTA infusion (17). Conversely, clearance studies in animals have shown an increase in phosphate absorption as serum calcium levels are increased (11, 18) and a decrease (19, 20) or no change (21) as serum calcium levels are lowered, independently of PTH.

Micropuncture studies have not resolved the conflicting results of human and animal clearance studies. Amiel and colleagues (22) have shown that raising serum calcium levels from low towards normal in parathyroidectomized rats stimulates phosphate absorption in the proximal convoluted (S_2) tubule, loop of Henle, and the distal nephron. In the thyroparathyroidectomized dog, Goldfarb and colleagues (23) also found distal absorption of phosphate to be enhanced by mild hypercalcemia. In contrast to the study of Amiel et al., however, this group of investigators found that fractional phosphate absorption in the S_2 tubule was reduced. Using the standing droplet technique with simultaneous perfusion of peritubular capillaries in parathyroidectomized rats, Ullrich and colleagues (24) found that raising luminal calcium levels had no effect on phosphate transport in S_2 tubules in the absence of fluid absorption. However, these investigators did find a reduction in phosphate transport when calcium was eliminated from the perfusate. From these studies, it appears that as calcium delivery to the proximal tubule is increased

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1. *Abbreviations used in this paper:* DMSO, dimethylsulfoxide; GFR, glomerular filtration rate; $J^{lb}_{PO_4}$, lumen-to-bath phosphate flux; JM, juxtamedullary; $J^{net}_{PO_4}$, net phosphate efflux; J_v, fluid absorption; PD, potential difference; PTH, parathyroid hormone; S_2, proximal convoluted; S_3, proximal straight; SF, superficial; TFP, trifluoperazine.

from low to normal values, phosphate absorption increases. However, above normal values calcium may depress or have no effect on proximal phosphate absorption.

Thus, while there exists considerable evidence for a direct effect of calcium on the renal tubular absorption of phosphate, the direction of this effect remains uncertain. Since all of the previous studies have been performed in vivo, the indirect effects of calcium on phosphate delivery and absorption may have played a role in their outcome. For this reason, an in vitro study using the isolated tubule microperfusion technique was planned in an attempt to better understand the effects of calcium on renal phosphate handling. The first aim of the present study was to determine if phosphate absorption in the proximal tubule, the major site of phosphate absorption, was altered by changes in calcium concentration. Once the effect was clearly defined, the mechanism was then investigated.

Methods

General procedure

The general procedure used in the present studies is similar to that described previously (25) and is summarized below.

Female New Zealand White rabbits, fed a normal phosphate diet and allowed tap water ad lib., were killed by guillotine. Within 30 min of sacrifice, the left kidney was removed, transverse slices were made, and S_2 or proximal straight (S_3) tubule segments dissected with careful attention to anatomical origin (superficial [SF] or juxtamedullary [JM]). The segments were then transferred to a lucite perfusion chamber and perfused according to the method of Burg et al. (26). All groups of tubules were studied at 38°C except for group XIV which was studied at 24°C.

An artificial solution resembling plasma ultrafiltrate ("A" solution, Table I) was used to perfuse and bathe the tubules. Solutions were gassed with 5% CO_2/95% O_2 to maintain a normal pH and P_{CO_2}. The perfusate and bath differed in the following ways.

Perfusate. To each perfusate was added exhaustively dialyzed [*methoxy*-^{3}H]inulin (Amersham Corp., Arlington Heights, IL) for the measurement of fluid absorption (J_v) and ^{32}P (ICN Pharmaceuticals, Inc., Irvine, CA), for the measurement of lumen-to-bath phosphate flux ($J^{lb}_{PO_4}$) or net phosphate efflux ($J^{net}_{PO_4}$). For two protocols, the calcium concentration was increased above that in the bath, and in one of these the bicarbonate was reduced to 8.0 mM and the pH reduced to 6.8.

*Table I. Composition of A Solution**

Component	Millimoles per liter
Sodium	145.0
Potassium	5.0
Chloride	112.0
Bicarbonate	25.0
Phosphate	2.3
Magnesium	1.0
Acetate	10.0
Sulfate	1.0
Glucose	8.0
d-Alanine	5.0
Calcium	1.8
Ionized calcium (pH 7.40)	1.3

* An artificial solution resembling plasma ultrafiltrate.

Bath. To each 100 ml of bath, 5 ml of fetal calf serum (Gibco Laboratories, Grand Island, NY) was added. This small concentration of protein (0.3 g/dl) did not alter the concentration of any component of the bath. For one protocol, the calcium concentration was increased above that in the perfusate. When $J^{net}_{PO_4}$ was measured, both bath and perfusate contained ^{32}P in identical concentration and specific activity.

Oils used to minimize evaporation were equilibrated with H_2O saturated with CO_2. To prevent evaporative water loss, the bath was exchanged continuously at 0.5 ml/min. The reservoir of bath was continuously gassed with 5% CO_2/95% O_2 at 4°C so that the pH of the aliquot in the perfusion chamber was maintained at 7.4.

Transepithelial potential difference (PD) was measured as described previously by Rocha and Kokko (27) and corrected for the calculated liquid junction potential (28) when appropriate. The voltage response to an imposed 50 meq/liter NaCl gradient (lumen greater than bath) was used to verify the origin of the tubule segments (29).

Timed fluid collections were made with a constant-volume constriction pipette, and each expelled into a counting vial containing 1 ml of water and 10 ml of Biofluor (New England Nuclear, Boston, MA). The isotopic concentrations were determined in a liquid-scintillation spectrometer (Packard Instrument Co., Inc., United Technologies, Downers Grove, IL).

Calculations

J_v in nanoliters per millimeter per minute was calculated using the following formula:

$$J_v = \frac{V_i - V_0}{L}, \quad (1)$$

where V_i and V_0 are the rates of perfusion and collection in nanoliters per minute, respectively, and L is the length of the tubule segment in millimeters, as measured by a reticle in the eyepiece of the microscope used for perfusion.

$J^{lb}_{PO_4}$ and $J^{net}_{PO_4}$ in picoequivalents per millimeter per minute were calculated using the following balance formula:

$$J_{PO_4} = \left(\frac{V_iC_i - V_0C_0}{L}\right)\left(\frac{[PO_4]_i}{C_i}\right) \quad (2)$$

where C_i and C_0 are the disintegrations per minute per nanoliter in the perfusate and collected sample, respectively, and $[PO_4]_i$ is the chemical concentration of phosphate in the perfusate in millimoles per liter.

The means of three to four collections from each of the control and experimental periods were compared statistically using the t test for paired samples.

Experimental protocols

After a 30–40-min equilibration period, one of the following protocols was performed.

Group I. Five SF and six JM S_3 segments (mean length, 1.7±0.1 mm) were studied using first normal calcium concentration (1.8 mM) in the bath and perfusate and then high calcium concentration (3.6 mM) in the bath and perfusate. The order in which the tubules were exposed to normal and high calcium concentrations was alternated among experiments.

Group II. The protocol outlined in group I was repeated in five SF and five JM S_2 segments (mean length, 1.0±0.1 mm).

Group III. Three SF and four JM S_2 segments (mean length, 0.8±0.1 mm) were studied using first normal calcium concentration in the bath and perfusate and then low calcium concentration (0.2 mM) in the bath and perfusate.

Group IV. As a time control, four SF and four JM S_2 segments (mean length, 0.8±0.1 mm) were studied using only normal calcium bath and perfusate over the same time intervals as groups I, II, and III.

Group V. Using normal calcium bath and perfusate, three SF and four JM S_2 segments (mean length, 0.9±0.1 mm) were studied before

and after the addition of the calcium ionophore A23187 (Sigma Chemical Co., St. Louis, MO) to both solutions to the final concentration of 5×10^{-6} M. Control bath and perfusate contained the vehicle for the ionophore, dimethylsulfoxide (DMSO), in the same concentration as in the experimental solutions.

Group VI. The protocol outlined in group V was repeated in five SF and two JM S_2 segments (mean length, 0.9±0.0 mm) except that low calcium (0.2 mM) perfusate and bath were used.

Group VII. The protocol outlined in group IV was repeated in four SF and two JM S_2 segments (mean length, 0.8±0.1 mm) except that DMSO was added to the bath and perfusate in the same concentration as in groups V and VI.

Group VIII. Four SF and three JM S_2 segments (mean length, 0.8±0.1 mm) were studied before and after the addition of trifluoperazine (Sigma Chemical Co.), the Ca-calmodulin complex inhibitor, to a normal calcium bath and perfusate to the final concentration of 10^{-4} or 10^{-6} M.

Group IX. Using normal calcium bath and perfusate, four SF and four JM S_2 segments (mean length, 0.9±0.1 mm) were studied before and after the addition of the calcium channel blocker nifedipine to the perfusate to the concentration of 10^{-6} M.

Group X. Three SF and two JM S_2 segments (mean length, 0.8±0.1 mm) were studied before and after raising only the bath calcium concentration from 1.8 to 3.6 mM. Perfusate calcium was 1.8 mM throughout.

Group XI. The protocol outlined in group X was repeated in one SF and four JM S_2 segments (mean length, 0.6±0.1 mm) except that perfusate calcium concentration was raised to 3.6 mM. Bath calcium was 1.8 mM throughout.

Group XII. The effect of raising only perfusate calcium was again studied in two SF and four JM S_2 segments (mean length, 0.8±0.1 mm) except a perfusate resembling late proximal tubule luminal fluid was used. The bicarbonate of this perfusate was 8.0 mM and the pH 6.8.

Group XIII. In two SF and three JM S_2 segments (mean length, 0.98±0.08 mm), $J^{net}_{PO_4}$ was measured using normal and high calcium concentrations in the bath and perfusate.

Group XIV. The protocol used in group XIII was repeated in three SF and three JM S_2 segments (mean length, 0.8±0.1 mm) except the temperature of the bath was reduced to 24°C.

Results

The responses of SF and JM tubule segments were identical in all 14 groups studied and thus were combined for statistical analysis.

Effect of Increasing or Decreasing Ambient Calcium

In S_3 tubule segments (group I), increasing bath and perfusate Ca from 1.8 to 3.6 mM had no significant effect on J_v, PD, or $J^{lb}_{PO_4}$. J_v was 0.3±0.1 nl/mm per min using 1.8 mM Ca and 0.2±0.1 using 3.6 mM Ca; PD was −0.7±0.1 mV using either Ca concentration; and $J^{lb}_{PO_4}$ was 1.2±0.2 pmol/mm per min for both concentrations. However, identical experiments performed in S_2 tubule segments (group II) revealed an increase of $J^{lb}_{PO_4}$ from 3.3±0.7 to 6.6±0.6 ($P < 0.001$) as ambient Ca was increased (Table II). In these experiments, J_v and PD were stable after raising ambient Ca. When bath and perfusate Ca were lowered from 1.8 to 0.2 mM in S_2 segments (group III), $J^{lb}_{PO_4}$ fell from 3.7±0.6 to 2.2±0.6 pmol/mm per min ($P < 0.05$) (Table II). PD was unchanged; however, J_v increased from 0.6±0.1 to 0.8±0.1 nl/mm per min ($P < 0.05$) as ambient Ca was decreased, an effect also observed by Friedman and colleagues (30).

Time control studies in S_2 segments, using only normal ambient Ca (group IV), revealed no changes in J_v, PD, or $J^{lb}_{PO_4}$.

Mechanism by Which Calcium Modulates PO_4 Reabsorption

Cytosolic changes. With the use of normal ambient Ca in seven S_2 segments (group V), the Ca ionophore A21387 was added to the perfusate and bath to increase cytosolic Ca (31) and mimic the effect of increasing ambient Ca. PD declined from −0.9±0.1 mV in control to −0.4±0.1 mV ($P < 0.01$), and J_v increased from 0.8±0.1 nl/mm per min to 1.3±0.1 ($P < 0.005$) with the addition of ionophore (Table III). Despite these changes, $J^{lb}_{PO_4}$ was not significantly altered, being 3.7±0.6

Table II. Relationship of Bath and Perfusate Calcium Concentrations and $J^{lb}_{PO_4}$ in S_2 Segments

	V_i		Jv		$J^{lb}_{PO_4}$		PD	
n	C	E	C	E	C	E	C	E
	nl/min	*nl/min*	*nl/mm per min*	*nl/mm per min*	*pmol/mm per min*	*pmol/mm per min*	*mV*	*mV*
Bath and perfusate Ca increased from 1.8 (C) to 3.6 (E) mM								
10	25.0±1.5	25.8±1.4	0.6±0.1	0.5±0.1	3.3±0.7	6.6±0.6*	−1.0±0.2	−1.3±0.3
Bath and perfusate Ca decreased from 1.8 (C) to 0.2 (E) mM								
7	19.2±1.2	19.5±1.4	0.6±0.1	0.8±0.1*	3.7±0.6	2.2±0.6*	−1.2±0.3	−0.9±0.3
Bath Ca increased from 1.8 (C) to 3.6 (E) mM								
5	19.7±1.3	18.6±0.5	0.8±0.1	0.7±0.1	3.9±0.8	3.3±0.7	−1.1±0.2	−1.4±0.3
Perfusate Ca increased from 1.8 (C) to 3.6 (E) mM								
5	19.3±2.0	19.7±2.3	1.2±0.2	0.9±0.2	3.4±0.7	6.1±0.7*	−1.7±0.4	−1.5±0.4

V_i is perfusion rate. * Denotes a significant difference between control (C) and experimental (E) periods.

Table III. Mechanism by Which Calcium Stimulates Phosphate Efflux in S_2 Segments

	V_i		J_v		J_{PO_4}		PD	
n	C	E	C	E	C	E	C	E
	nl/min	*nl/min*	*nl/mm per min*	*nl/mm per min*	*pmol/mm per min*	*pmol/mm per min*	*mV*	*mV*
$J^{lb}_{PO_4}$ after addition of ionophore (E), group V								
7	25.5±1.3	26.3±0.9	0.8±0.1	1.3±0.1*	3.7±0.6	4.7±1.0	−0.9±0.1	−0.4±0.1*
$J^{lb}_{PO_4}$ after addition of TFP (E), group VIII								
7	21.5±1.9	20.0±2.0	0.7±0.1	0.4±0.0*	4.5±0.4	3.8±0.5	−1.5±0.5	−0.2±0.2*
$J^{lb}_{PO_4}$ after addition of nifedipine (E), group IX								
8	16.5±0.8	17.4±0.5	1.0±0.1	0.9±0.3	5.1±0.6	4.5±0.3	−1.2±0.1	−1.0±0.2
$J^{lb}_{PO_4}$ after increasing perfusate calcium (E), absence of H^+ secretion, group XII								
6	16.4±1.0	16.7±1.4	0.8±0.2	0.7±0.1	2.4±0.3	2.1±0.3	−0.7±0.5	−0.5±0.6
$J^{net}_{PO_4}$ using 1.8 (C) and 3.6 (E) mM ambient calcium, group XIII								
5	17.9±1.3	17.9±1.0	1.2±0.3	1.0±0.2	3.9±0.8	6.5±1.3*	−2.1±0.5	−1.5±0.3
$J^{net}_{PO_4}$ in the absence of a sodium gradient, using 1.8 (C) and 3.6 (E) mm ambient calcium, group XIV								
6	18.2±0.6	16.9±0.8	0.11±0.07	0.17±0.06	1.2±0.3	1.2±0.4	−0.3±0.1	−0.3±0.8

Definition of terms same as in Table II. J_{PO_4}, phosphate flux, both net and lumen-to-bath. * Denotes a significant difference between C and E periods.

pmol/mm per min before and 4.7±1.0 after the addition of ionophore. The effect of ionophore was studied in an additional seven S_2 segments maintained in low ambient Ca (0.2 mM) (group VI). Again, PD fell from −1.3±0.3 to −0.7±0.2 mV with the addition of ionophore ($P < 0.05$); however, J_v was unchanged, being 0.9±0.1 nl/mm per min before and after the addition of ionophore. Although basal $J^{lb}_{PO_4}$ was lower due to the lower ambient calcium, the addition of ionophore failed to stimulate the $J^{lb}_{PO_4}$, being 2.8±0.6 pmol/mm per min before and 2.7±0.5 after ionophore addition.

Six S_2 segments were studied as time controls (group VII), as in group IV, except that the vehicle for the ionophore, DMSO, was added to the bath and perfusate. J_v, PD, and $J^{lb}_{PO_4}$ were not significantly altered.

Changes in ambient Ca may alter cytosolic processes that require Ca. To test the possibility that the activation of Ca-calmodulin–dependent reactions stimulates PO_4 absorption S_2 segments were studied before and after the addition of trifluoperazine (TFP) to a normal Ca bath and perfusate. With the addition of TFP, J_v declined from 0.7±0.1 to 0.4±0.0 nl/min per min ($P < 0.025$), as did PD from −1.5±0.4 mV to −0.2±0.2 after TFP addition ($P < 0.05$) (Table III). Despite these changes, $J^{lb}_{PO_4}$ was unchanged, being 4.5±0.4 pmol/mm per min in control and 3.8±0.5 pmol/mm per min after TFP was added.

To block Ca entry and lower cytosolic Ca, nifedipine, a calcium channel blocker in smooth and cardiac muscle, was added to the perfusate in eight S_2 segments (group IX). J_v, PD, and $J^{lb}_{PO_4}$ were not affected by the calcium channel blocker (Table III). The lack of effect of nifedipine addition may only indicate that the calcium channels of this epithelium are not blocked by this compound. Calcium fluxes were not determined to verify this effect of nifedipine.

Membrane alterations. To determine the sidedness of the effect of calcium on phosphate absorption, the calcium concentration was raised in the bath (group X) and perfusate (group XI) separately. When bath calcium was increased from 1.8 to 3.6 mM, neither J_v, PD, nor $J^{lb}_{PO_4}$ was significantly altered (Table II). By contrast, increasing perfusate Ca significantly enhanced $J^{lb}_{PO_4}$ from 3.4±0.7 to 6.1±0.7 pmol/mm per min ($P < 0.005$) (Table II), an effect comparable to that seen when both perfusate and bath calcium concentrations were raised (group II, Table II) simultaneously. Neither J_v nor PD was altered by increasing perfusate Ca.

Changes in pH. To study the possibility that Ca stimulates PO_4 absorption by stimulating H^+ secretion in S_2 segments, perfusate Ca concentrations were again increased, except a low bicarbonate (8.0 mM) low pH (6.8) perfusate-simulating late proximal tubule luminal fluid was employed (group XII). Using this perfusate, H^+ secretion should have been abolished. Increasing perfusate Ca from 1.8 to 3.6 mM in this setting failed to stimulate $J^{lb}_{PO_4}$, which was 2.4±0.3 before and 2.1±0.2 pmol/mm per min after perfusate Ca concentrations were raised. Neither J_v nor PD was significantly altered by increasing perfusate Ca concentrations (Table III). However, basal $J^{lb}_{PO_4}$ was depressed by the low bicarbonate low pH perfusate, the value observed being lower than those observed in the other study groups.

Net efflux studies. An increase in medium calcium may be expected to increase the concentration of the complex $CaHPO_4$, which may permeate both the luminal and basolateral membrane more easily than a charged PO_4 species. Measuring only

unidirectional PO_4 efflux ignores the possibility of enhanced influx; thus, net efflux studies were performed. At bath temperature of 38°C, $J^{net}_{PO_4}$ was 3.9±0.8 pmol/mm per min with the use of normal ambient calcium and 6.5±1.3 pmol/mm per min with the use of high ambient calcium ($P < 0.05$, Table III). Again, no significant changes in PD or J_v were observed. These results are similar to those of the unidirectional efflux studies (group II), which used normal and high ambient calcium.

The final group of tubules was studied at 24°C to determine if calcium stimulates sodium-dependent PO_4 transport, and if the apparent stimulation is due to precipitation and/or binding of Ca-PO_4 complexes at the brush border villi. The absence of net sodium transport was confirmed by the low J_v and PD (Table III). $J^{net}_{PO_4}$ was also reduced to 1.2±0.3 pmol/mm per min with the use of normal ambient Ca and was unchanged when ambient Ca was increased to 3.6 mM being 1.2±0.4 pmol/mm per min.

Discussion

While a direct relationship between the concentration of calcium in the serum and phosphate absorption in the proximal tubule has been described, its nature is controversial. Previous studies were performed in vivo and their results may have reflected not only changes in serum calcium concentrations but also changes in other variables induced by calcium infusion. The present study was designed to examine the effect of calcium on phosphate handling in the proximal tubule in vitro, in the absence of any influence of alterations in renal hemodynamics or serum phosphate.

The results of this study demonstrate that the magnitude of sodium-dependent phosphate absorption in the proximal convoluted tubule is directly related to the calcium concentration of the luminal fluid. Alterations of cytosolic calcium by the addition of a calcium ionophore or inhibition of the calcium-calmodulin complex did not mimic the effects seen when ambient calcium was varied.

These results are consistent with those of Amiel and colleagues (22) and of Frick and Durasin (32) in the rat, but not with those of Goldfarb et al. (23) in the dog, and Ullrich et al. (24) in the rat. However, in the study of Goldfarb et al., as hypercalcemia was induced, serum phosphate rose and fractional phosphate absorption declined. While ultrafilterable calcium and phosphate increased as well, it is possible that this pair may have been in some form of ion association that is not available for transport. Species differences also may account for the discrepancy between the results of Amiel et al. and of the present studies, and those of Goldfarb et al. The apparent discrepancy with the results of Ullrich et al. may be explained by the lack of net sodium absorption in their study. Our results indicate that calcium stimulates the sodium-dependent but not passive phosphate absorption.

Popovtzer et al. (18), in the rat, and Glorieux and Scriver (33), in familial hypophosphatemic patients, have suggested two mechanisms for phosphate absorption in the nephron. One mechanism appears to be stimulated by calcium and inhibited by saline expansion; the other inhibited by PTH. The distribution of these mechanisms along the nephron may vary among the species. In the proximal tubule of the rabbit, unlike that of the rat and dog, PTH does not inhibit phosphate absorption in the early convolutions and has a moderate or no effect in later convolutions (34, 35). Perhaps in the rabbit, the absorption of phosphate in the S_2 tubule is controlled primarily by the calcium-stimulated mechanism. In the S_3 tubule segment of the rabbit, PTH does inhibit phosphate absorption (34). Our studies indicate that calcium does not modulate phosphate absorption in this segment, thus, PTH may be the primary control in this portion of the rabbit nephron.

The lack of effect of the calcium ionophore is not surprising. McKeown (36) presented similar results in their studies using the calcium ionophore and normal ambient calcium. Popovtzer et al. (37) and Ullrich et al. (24) also found no effect of the ionophore on phosphate absorption in vivo without the influence of PTH. The effect of the ionophore on J_v is not consistent among these studies, however. McKeown (36) found an enhancement of J_v very similar to ours, and Ullrich et al. (24) and Friedman et al. (30) found an inhibition with ionophore addition. Information to date offers no explanation for these discrepancies. Friedman et al.'s and Ullrich et al.'s results support the sodium-calcium antiport theory, whereas McKeown's and the present studies do not.

The results obtained with the calcium-calmodulin complex inhibitor were not expected. The addition of TFP produced a significant decline in PD and J_v but no depression of phosphate absorption. The decline in PD and J_v is indicative of reduced sodium absorption. One possible explanation for these results is that the calcium-calmodulin–stimulated phosphodiesterase (38) activity was reduced resulting in an increase in intracellular cAMP, which is known to inhibit sodium and fluid absorption (39). Not consistent with this interpretation, however, is the lack of effect on phosphate absorption. Agus and colleagues (39) found an inhibition of phosphate as well as sodium absorption with cAMP infusion in the dog. Also, Brazy et al. (35) have shown that PTH, which presumably acts by stimulating cAMP production, does moderately inhibit fluid and phosphate absorption in late S_2 tubule segments from the rabbit. The lack of effect of TFP on phosphate absorption in our studies suggests that calcium acts independently of cAMP to modulate this process.

Kessler et al. (40) have isolated a 3,000-mol-wt proteolipid from brush border membrane vesicles from rabbit renal cortex which exhibits sodium-dependent phosphate binding. The Michaelis constant for binding is 8 μM phosphate, while saturation occurs at 20 μM. A further report by this group has shown that phosphate binding by this proteolipid is dependent on divalent metal ions, manganese and calcium, sharing the highest order of effectiveness (41). If this proteolipid is, indeed, the phosphate transporter, calcium may stimulate phosphate transport by activating this transporter. Alternatively, calcium may act by recruiting additional proteolipid units.

The level of serum calcium has been shown to affect hydrogen ion secretion in the kidney. Lowering serum calcium levels inhibits hydrogen ion secretion in thyroparathyroidectomized dogs (20) and isolated rabbit proximal tubules perfused in vitro (42), while raising serum calcium levels stimulates secretion in thyroparathyroidectomized dogs (43). One possible mechanism by which increases in calcium enhance phosphate absorption is by this stimulation of hydrogen ion secretion. Increasing hydrogen ion concentration may titrate the dibasic ion to its monobasic form, reduce its polarity, and promote its entry into the cell. Indeed, in our studies, when calcium concentration was raised in an acid perfusate which limits H^+

secretion, $J^{lb}_{PO_4}$ did not increase, and PD and J_v did not change. It is not likely, however, that calcium augments phosphate absorption by acidifying the luminal contents, since basal flux with the low bicarbonate perfusate was lower than that with the normal bicarbonate perfusate. This observation is of interest and has been shown in other studies where pH and bicarbonate were varied (44, 45). The decline in phosphate absorption when the luminal contents are acid, along with studies on phosphate uptake by brush border (luminal) membrane vesicles (46, 47), has been taken as evidence that the divalent phosphate species is preferentially transported. However, phosphate absorption also declines as luminal pH is increased above 7.5 (44). The similar effects of the two extremes in pH suggest that there is a modification of a membrane component rather than promotion of the divalent or monovalent species. In contrast to our study and those of others (44, 45), Hamm and colleagues (48) have shown an increase in phosphate absorption when the perfusate pH was lowered from 7.4 to 6.2 in the presence or absence of bicarbonate. The different results of Hamm et al. may be related to the concentration of phosphate used in their study, which was 10 mM vs. the 2.3 mM used in our study and those of Dennis et al. (44). In the study of Hamm et al. lowering pH from 7.4 to 6.2 reduces the concentration of the divalent phosphate species from 8 to 2 mM, whereas in our study the concentration fell from 1.8 to 1.2 mM and in the study of Dennis et al. (44) from 1.8 to 0.7 mM. The lower concentrations of the divalent species in the latter studies may account for the reduction in phosphate absorption.

In conclusion, the present study shows that increasing ambient calcium stimulates sodium-dependent phosphate absorption in the S_2 tubule of the rabbit, whereas lowering ambient calcium inhibits phosphate absorption. There is no effect when calcium levels are raised in S_3 tubule segments. The effect in the convoluted tubule segments does not appear to be mediated by changes in cytosolic ionized calcium but instead appears to occur at the luminal membrane. Calcium translocation and stimulation of hydrogen ion secretion by calcium do not appear to modulate phosphate absorption, although the inhibition of calcium translocation was not verified. The present results are consistent with a role for calcium in modulating the affinity of the luminal phosphate transporter for the anion.

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