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EFFECT OF 1,25-DIHYDROXYVITAMIN D-3 ON PHOSPHATE UPTAKE INTO CHICK INTESTINAL BRUSH BORDER MEMBRANE VESICLES *

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Summary

Brush border membrane vesicles prepared from the vitamin D-deficient chick duodenum take up phosphate and show an overshoot phenomenon in the presence of NaCl. Substitution of choline chloride for NaCl reduces phosphate uptake. Prior treatment of vitamin D-deficient chicks with 1,25-dihydroxyvitamin D-3 increases the initial rate of Na^+-dependent phosphate uptake into the brush border vesicles. This Na^+-dependent phosphate uptake is a saturable process, exhibiting an apparent K_m of 0.31 mM and a V of 385 pmol/mg per 15 s. Pretreatment of chicks with 1,25-dihydroxyvitamin D-3 leads to an increase in V (750 pmol/mg per 15 s) without significantly altering the apparent K_m (0.33 mM). Addition of Ca^{2+}, either in the presence or absence of the polyene antibiotic, filipin, or of calmodulin, has no effect on Na^+-dependent phosphate uptake. Pretreatment of the vitamin D-deficient chick with a dose of cycloheximide sufficient to inhibit membrane protein synthesis blocks the 1,25-dihydroxyvitamin D-3-induced increase in alkaline phosphatase activity, but does not affect the stimulation of Na^+-dependent phosphate uptake. From these data, it is concluded that 1,25-dihydroxyvitamin D-3 stimulates Na^+-dependent phosphate transport at the brush border membrane of the enterocyte, that alkaline phosphatase is not directly involved in this process, and that this effect of 1,25-dihydroxyvitamin D-3 is independent of new protein synthesis.

Abbreviations: EGTA, ethyleneglycol bis(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid; Hepes, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid.

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Introduction

Intestinal phosphate transport is known to be stimulated by vitamin D-3 and by its active metabolite, 1,25-dihydroxyvitamin D-3. Administration of vitamin D-3 or 1,25-dihydroxyvitamin D-3 to vitamin D-deficient chicks or rats causes an increase in intestinal phosphate transport measured either in situ with a ligated loop technique [1], or in vitro employing an everted gut sac method [2—6]. Studies using either an Ussing-type chamber [7,8] or organ culture of intestinal explants [9—11] have yielded similar results. Some of these studies have also shown that the presence or absence of Ca^{2+} does not affect the action of vitamin D-3 or its metabolite on phosphate transport, suggesting that vitamin D-3 or its metabolite stimulates phosphate transport independently of its action on Ca^{2+} transport [1,3—6].

From the accumulated evidence it appears that phosphate transport across the intestinal epithelial cell involves at least three steps: (1) phosphate entry across the brush border membrane into the epithelial cell; (2) phosphate transfer to the serosal side of the cell; and (3) phosphate transport from the enterocyte into the extracellular space across the basolateral membrane. It has been shown that mucosal phosphate transport requires the presence of mucosal Na^+ [5,8] and that ouabain, metabolic inhibitors and anaerobic conditions block transcellular phosphate transport [5,6,9]. It is, at present, thought that the energy-dependent step in transcellular phosphate transport is the Na^+-dependent entry into the cell across the brush border membrane [6], in analogy to the situation in the proximal renal tubule [12]. However, controversy remains concerning the specific cellular site of action of vitamin D.

The introduction by Hopfer et al. [13,14] of the use of isolated membrane vesicles from both the brush border and basolateral membranes of intestinal epithelial cells has helped to define the steps in transcellular glucose and amino acid transport [15]. Phosphate transport has also been extensively studied by Hoffman et al. [12] employing membrane vesicles from renal tubular epithelial cells. However, less work has been carried out with regard to intestinal phosphate transport. The only detailed study of phosphate uptake into brush border membrane vesicles from the small intestine is that of Berner et al. [16] using vesicles prepared from rats on a normal chow diet. They showed that phosphate uptake into the vesicles was a saturable process, that it was competitively inhibited by arsenate, and that Na^+ and phosphate shared an electroneutral cotransport at pH 7.4. From these data they concluded that a 1 : 1 Na^+ and univalent phosphate cotransport system existed in the intestinal brush border membrane.

Recently we developed a method for isolating highly-purified brush border membrane vesicles from the chick duodenal mucosa, and have employed these vesicles to study the mechanism of action of vitamin D [17]. Ca^{2+} uptake into these brush border membrane vesicles was stimulated by prior treatment of vitamin D-deficient animals with either 1-α-hydroxyvitamin D-3 or 1,25-dihydroxyvitamin D-3 [18]. The present study was undertaken to examine the effect of 1,25-dihydroxyvitamin D-3 on phosphate transport into these vesicles. The results show that 1,25-dihydroxyvitamin D-3 stimulates Na^+-dependent phosphate entry at the brush border membrane.

Materials and Methods

Animals

1-day old White Leghorn cockerels (Moyer Chick, Quakertown, PA) were maintained on a vitamin D-deficient diet (Teklad Test Diet TD 75007, Madison, WI) in a darkened room for 3 weeks. All chicks were fasted 16 h prior to killing, with free access to deionized water. The 1,25-dihydroxyvitamin D-3-treated chicks received, by intraperitoneal injection, 1 μg of 1,25-dihydroxyvitamin D-3 dissolved in 100 μl of propylene glycol usually 16 h before killing. For the experiments in which response time to 1,25-dihydroxyvitamin D-3 was studied, the animals were given 1,25-dihydroxyvitamin D-3 at the indicated time before killing.

The effect of cycloheximide treatment was examined by the procedure of Bikle et al. [19]. An initial dose of 20 μg cycloheximide in 100 μl propyleneglycol was administered to vitamin D-deficient chicks intraperitoneally. 1 h later, the treated group of chicks were given 1,25-dihydroxyvitamin D-3 (1 μg/100 μl) in propyleneglycol. Cycloheximide (20 μg) was given every 4 h after the initial dose, then 1 h after the third injection of cycloheximide the chicks were killed.

Preparation of brush border membranes

Chicks were killed by decapitation and the duodena quickly removed, chilled in ice-cold 0.15 M NaCl, flushed, slit open and the mucosa gently scraped with a glass slide. Membrane vesicles were prepared by the method previously described [17]. In short, the scrapings were homogenized with a glass-Teflon homogenizer in ice-cold hypotonic buffer (2.5 mM Na^+-EGTA/2 mM Na^+-Hepes, pH 7.4) and the pellet of the low-speed centrifugation was collected. Intact brush borders were isolated by sucrose gradient centrifugation and then homogenized in a Waring Blender in 0.5 M Tris-HCl, pH 7.2. Purified brush border membranes were collected by discontinuous glycerol gradient centrifugation and resuspended in an appropriate buffer for each experiment indicated below.

Phosphate uptake

Membrane vesicles were usually resuspended in a buffer containing 100 mM mannitol, 20 mM Hepes-Tris, 0.5 mM $MgCl_2$ and 0.3 mM KH_2PO_4, pH 7.4. Uptake of phosphate was determined by a method modified from that of Berner et al. [16].

Purified membranes were preincubated for 20 min at 25°C. Phosphate uptake was initiated by addition of membrane suspensions at a final concentration of 0.5 mg/ml membrane protein to the same buffer as used for resuspension of the membranes except that the incubation buffer contained labelled substrate ([^{32}P]phosphoric acid, $1 \cdot 10^7$ cpm/ml) and 100 mM NaCl or 100 mM choline chloride. For kinetic experiments, the concentrations of KH_2PO_4 in the resuspension and incubation buffer were changed from 0.1 to 1 mM. At indicated times, 100-μl aliquots of the incubation were removed and directly pipetted onto Millipore filters (HAWP 02500, Millipore Corp., Bedford, MA). The filters were immediately washed with 5 ml of ice-cold buffer containing

100 mM mannitol, 20 mM Hepes-Tris, 1 mM $MgCl_2$, 90 mM choline chloride and 15 mM Na_2HAsO_4, pH 7.4. The amount of ^{32}P retained on the filter was determined by liquid scintillation counting.

For the experiments in which the effect of filipin was examined, the vesicles were preincubated for 15 min at 25°C in 100 mM mannitol, 20 mM Hepes-Tris, 0.5 mM $MgCl_2$ and 0.3 mM KH_2PO_4 containing [^{32}P]phosphoric acid, pH 7.4. Then 5 μg filipin in 5 μl of 95% ethanol, or 5 μl ethanol alone per ml were added and incubated for 10 min. After base-line phosphate uptake was obtained, Na^+-dependent phosphate uptake was examined by adding 4 M NaCl to attain the final concentration of 100 mM NaCl.

Ca^{2+} uptake

Ca^{2+} uptake was carried out as described previously [18] except that the brush border vesicles were resuspended in the same buffer as used for phosphate uptake. From the previous observation [18], Ca^{2+} uptake into the vesicles shows two components: a rapid-binding component and a slower accumulation into an osmotically-active space. Ca^{2+} uptake presented in this paper represents subtraction of the rapid-binding component from total uptake.

Alkaline phosphatase activity

Alkaline phosphatase was assayed using *p*-nitrophenyl phosphate (Sigma 104, Sigma Chemical Co., St. Louis, MO) as a substrate. The brush border membranes were incubated with 9 mM *p*-nitrophenyl phosphate in 0.75 M 2-amino-2-methyl-1-propanol solution (Sigma 221, Alkaline Buffer Solution, pH 10.3) containing 4 mM $MgCl_2$ and 1 mM $ZnCl_2$ at 37°C for 15 min in a final volume of 1 ml, and the reaction was terminated by the addition of 5 ml of 0.05 N NaOH. Free *p*-nitrophenol was determined spectrophotometrically at 410 nm.

Materials

$^{45}CaCl_2$ and $H_3^{32}PO_4$ were obtained from New England Nuclear, Boston, MA. All the reagents used were analytical grade. The 1,25-dihydroxyvitamin D-3 was obtained from the Upjohn Company, Kalamazoo, MI, filipin from the Upjohn Co. and cycloheximide from Sigma Chemical Co. All the solutions and buffers used for phosphate uptake were filtered before use.

Results are expressed as the mean ± S.E. Statistical analysis of the difference between these means was carried out using the Student's *t*-test [20].

Results

Time course of phosphate uptake

Phosphate uptake into the brush border membrane vesicles prepared from vitamin D-deficient chicks incubated at 25°C in the presence of an NaCl gradient showed an overshoot phenomenon. This overshoot reached its peak between 1–2 min after Na^+ addition (Fig. 1). The amount of phosphate taken up by the vesicles then gradually decreased and reached an equilibrium value after approx. 60 min. When the membrane vesicles were incubated with a choline chloride gradient instead of an NaCl gradient, phosphate uptake was

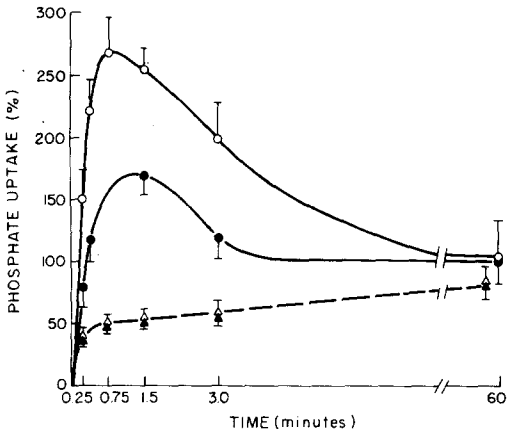

Fig. 1. Time course of phosphate uptake into intestinal brush border membrane vesicles prepared from vitamin D-deficient (•) and 1,25-dihydroxyvitamin D-3-treated (○) chicks in the presence of 100 mM NaCl, or in the presence of 100 mM choline chloride (▲, from vitamin D-deficient; △, from 1,25-dihydroxyvitamin-treated chicks). Phosphate uptake was initiated by the addition of the brush border vesicles to the incubation buffer (0.5 mg protein/ml) containing 100 mM mannitol, 20 mM Hepes-Tris, 0.5 mM $MgCl_2$, 0.3 mM phosphate with $1 \cdot 10^7$ cpm/ml $H_3^{32}PO_4$ and 100 mM NaCl or 100 mM choline chloride, pH 7.4, at 25°C. The data are expressed as the percentage of the 60-min uptake of the vesicles from vitamin D-deficient animals in the presence of NaCl, which amount to 415 ± 38 pmol/mg protein. The vertical bars represent the standard error of the means of eight experiments.

reduced and no overshoot was observed. Subtraction of the uptake with the choline chloride gradient from that with the NaCl gradient was taken to represent Na^+-dependent phosphate uptake. As shown in Fig. 1, the vesicles prepared from 1,25-dihydroxyvitamin D-3-treated chicks consistently showed a higher initial and peak Na^+-dependent phosphate uptake than those from vitamin D-deficient chicks, whereas the equilibrium values were almost the same for the two preparations. There was no difference in phosphate uptake in the presence of choline chloride (Na^+-independent phosphate uptake) between the vesicles from 1,25-dihydroxyvitamin D-3-treated and vitamin D-deficient chicks. The vesicular space calculated from the equilibrium value in the presence of NaCl was 1.38 μl/mg protein in vesicles from the vitamin D-deficient chicks and 1.43 μl/mg protein in vesicles from 1,25-dihydroxyvitamin D-3-treated animals. These values are very close to the vesicular space calculated from glucose uptake (1.35 in vitamin D-deficient and 1.39 μl/mg protein in 1,25-dihydroxyvitamin D-3-treated group, see also Ref. 18). As shown by Hopfer et al. [13], glucose uptake into the brush border is known to represent the uptake into osmotically-active space and does not contain binding component to the membrane vesicles.

From these data, it was concluded that most of the phosphate taken up was transported into the vesicular space, and that 1,25-dihydroxyvitamin D-3 specifically stimulated Na^+-dependent phosphate uptake into the brush border membrane vesicles without changing the vesicular size or Na^+-independent phosphate uptake. As Na^+-dependent glucose uptake was not affected by vitamin D-treatment [18], the stimulation of Na^+-dependent phosphate uptake by 1,25-dihydroxyvitamin D-3 was not due to a change in Na^+-permeability of the brush border membrane but to a change in the phosphate transport system.

Kinetics of phosphate uptake

Phosphate uptake into the vesicles increased as the concentration of phosphate in the incubation buffer was increased. The initial 15-s uptake value was taken to represent an approximation of initial velocity. The data in Fig. 2A show the 15-s uptake into vesicles prepared from vitamin D-deficient and 1,25-dihydroxyvitamin D-3-treated chicks as a function of phosphate concentration. As indicated by Berner et al. [16], this increase in initial uptake consists of two components: a saturable Na^+-dependent uptake and a linear Na^+-independent uptake seen with a choline chloride gradient. At all phosphate concentrations employed (0.1—1 mM) the Na^+-dependent uptake into the vesicles prepared from 1,25-dihydroxyvitamin D-3-treated chicks was higher than that observed in vesicles from vitamin D-deficient controls. An Edie-Hofstee plot of the Na^+-dependent uptake calculated from Fig. 2A is shown in Fig. 2B. The Na^+-dependent phosphate uptake gave an apparent Michaelis constant (K_m) of 0.31 mM in vesicles from vitamin D-deficient animals and 0.33 mM in those from 1,25-dihydroxyvitamin D-3-treated ones. The maximum velocity (V) of the initial uptake was increased from 385 pmol/mg protein in vesicles from vitamin D-deficient animals to 750 pmol/mg protein in those from 1,25-dihydroxyvitamin D-3-treated ones. Hence, 1,25-dihydroxyvitamin D-3 stimulated phosphate transport at the brush border membrane by increasing V without changing the apparent K_m of the transport system.

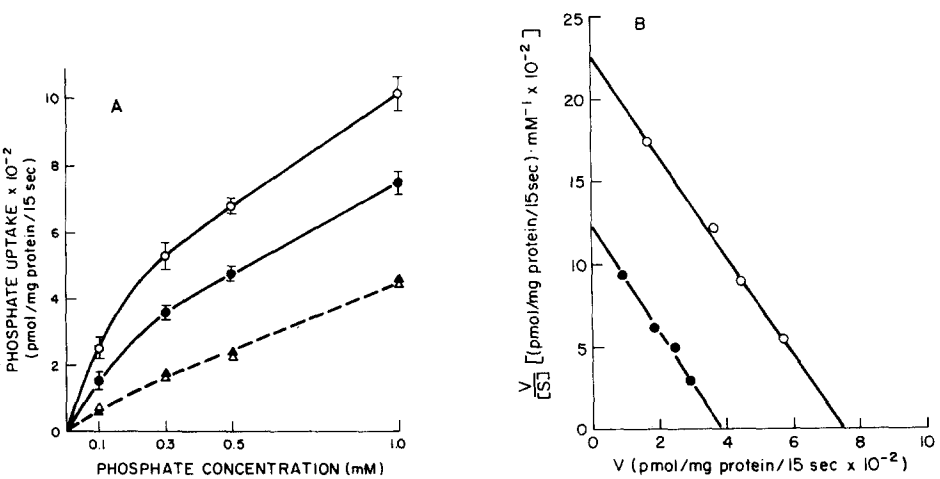

Fig. 2. (A) The kinetics of phosphate uptake into intestinal brush border membrane vesicles prepared from vitamin D-deficient (•) and 1,25-dihydroxyvitamin D-3-treated (○) chicks in the presence of 100 mM choline chloride (▲, from vitamin D-deficient; △, from 1,25-dihydroxyvitamin D-3-treated chicks). The buffers used for resuspension of the vesicles and those for incubation contained the same concentration of phosphate varying from 0.1 to 1 mM in order to eliminate phosphate gradient across the membrane. Phosphate uptake was studied 15 s after the addition of the brush border vesicles to the incubation buffer containing $1 \cdot 10^7$ cpm/ml $H_3^{32}PO_4$ and 100 mM NaCl or 100 mM choline chloride. The data are the means ± S.E. of four experiments. (B) Edie-Hofstee plot of Na^+-dependent phosphate uptake into intestinal brush border membrane vesicles from vitamin D-deficient (•) and 1,25-dihydroxyvitamin D-3-treated (○) chicks. The data are the subtraction of the mean values in the presence of 100 mM choline chloride from those in the presence of 100 mM NaCl.

Effect of filipin on phosphate uptake

The influence of the polyene antibiotic, filipin, on phosphate uptake was examined because previous works had shown that this antibiotic, when added directly to vesicles in vitro, induces an increase in the Ca^{2+} accumulation into brush border membrane vesicles from vitamin D-deficient but not in those from 1,25-dihydroxyvitamin D-3-treated chicks [18]. As shown in Table I, addition of 5 μg filipin/ml led to no change in phosphate uptake into the vesicles from either vitamin D-deficient or 1,25-dihydroxyvitamin D-3-treated chicks. In the same preparation, 5 μg filipin/ml caused a 2.5-fold increase in the rate of Ca^{2+} uptake into the vesicles from vitamin D-deficient animals without changing the uptake into those from 1,25-dihydroxyvitamin D-3-treated ones (Table I).

To test the possibility that filipin might influence phosphate transport in the intestinal mucosal cell of the vitamin D-deficient chick secondary to an increase in Ca^{2+} entry at the brush border membrane, the effect of Ca^{2+} and Ca^{2+}-dependent regulator protein [21], or calmodulin [22], on phosphate uptake in the presence of filipin was examined. The results are listed in Table II. Addition of 100 μM Ca^{2+} or 5 μg/ml calmodulin with 100 μM Ca^{2+} in the presence of filipin did not cause any significant increase in Na^+-dependent phosphate uptake into the vesicles from vitamin D-deficient chicks.

Time course of the response to 1,25-dihydroxyvitamin D-3

The rate of phosphate uptake into the brush border membrane vesicles and the alkaline phosphatase activity of the brush border membrane were measured as a function of time after administration of 1,25-dihydroxyvitamin D-3. Phosphate uptake into the vesicles significantly increased 4 h after the treatment, when it was stimulated to approx. 60% of the maximum response (Fig. 3). It is noteworthy that alkaline phosphatase activity in the brush border membrane was still at a basal level 4 h after administration of 1,25-dihydroxyvitamin D-3 and increased only after 8 h (Fig. 3). Therefore, phosphate uptake into the vesicles and alkaline phosphatase activity in the brush border mem-

TABLE I

EFFECT OF FILIPIN ON PHOSPHATE AND Ca^{2+} UPTAKE INTO CHICK INTESTINAL BRUSH BORDER MEMBRANE VESICLES

Phosphate uptake was examined by the addition of 4 M NaCl to attain the final concentration of 100 mM NaCl, and the samples were taken before and 15 s after the addition of NaCl. Na^+-dependent phosphate uptake was calculated by subtracting the values before the addition of NaCl from those after the addition. Ca^{2+} uptake was calculated as described in Materials and Methods. The data are the means ± S.E. of four experiments.

	Vitamin D-deficient		1,25-Dihydroxyvitamin D-3-treated	
	None	Filipin (5 μg/ml)	None	Filipin (5 μg/ml)
Na^+-dependent phosphate uptake (pmol/mg protein per 15 s)	193 ± 18	234 ± 23	478 ± 20	466 ± 28
Ca^{2+} uptake (pmol/mg protein per 10 min)	1.1 ± 0.1	2.6 ± 0.3	2.3 ± 0.2	2.4 ± 0.2

TABLE II

EFFECT OF Ca^{2+} AND CALMODULIN IN THE PRESENCE OF Na^+-DEPENDENT PHOSPHATE UPTAKE INTO CHICK INTESTINAL BRUSH BORDER MEMBRANE VESICLES

The vesicles were resuspended and preincubated in 100 mM mannitol, 20 mM Hepes-Tris, pH 7.4, with or without 5 μg/ml calmodulin, at 25°C for 5 min. Then $MgCl_2$ was added to the final concentration of 0.5 mM. 15 min later, 5 μg/ml filipin or 5 μl ethanol and 100 μM Ca^{2+} in Ca^{2+} + group were added and incubated for 40 min. Na^+-dependent phosphate uptake was examined and calculated as described in Table I. The data are the means ± S.E. of three experiments. Results are expressed as pmol/mg per 15 s.

Chemical in incubation buffer			Conditions	
Filipin	Ca^{2+}	Calmodulin	Vitamin D-deficient	1,25-Dihydroxyvitamin D-3-treated
—	—	—	270 ± 36	439 ± 46
+	—	—	259 ± 23	—
+	+	—	266 ± 32	—
+	+	+	277 ± 16	469 ± 53

brane responded with a different time course after administration of 1,25-dihydroxyvitamin D-3.

Effect of cycloheximide pretreatment

In order to determine whether the stimulation of phosphate uptake into the brush border vesicles caused by 1,25-dihydroxyvitamin D-3 is mediated through the synthesis of a new protein, the effect of cycloheximide pretreatment on phosphate uptake into the vesicles from vitamin D-deficient and 1,25-dihydroxyvitamin D-3-treated animals was examined. As shown in Table III, cycloheximide pretreatment did not block the stimulation of phosphate uptake by 1,25-dihydroxyvitamin D-3, although in the same brush border membrane, alkaline phosphatase activity was completely suppressed to values below those found in vesicles from vitamin D-deficient animals. This dose schedule of cyclo-

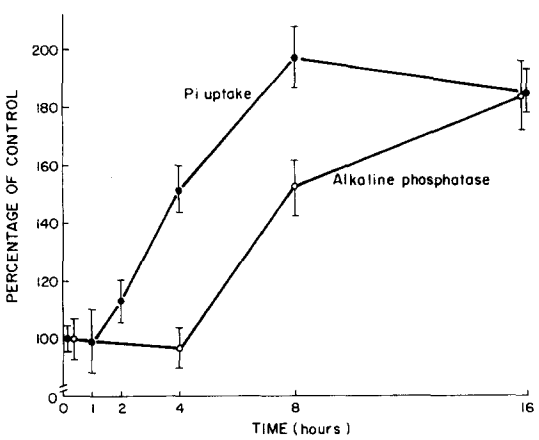

Fig. 3. Time course of effect of 1,25-dihydroxyvitamin D-3 on Na^+-dependent phosphate uptake (●) and alkaline phosphatase activity (○) of the chick intestinal brush border membrane vesicles. The data are the means ± S.E. of four experiments and expressed as the percentage of the vitamin D-deficient control.

TABLE III

EFFECT OF CYCLOHEXIMIDE ON Na^+-DEPENDENT PHOSPHATE UPTAKE AND ALKALINE PHOSPHATASE ACTIVITY IN CHICK INTESTINAL BRUSH BORDER MEMBRANE VESICLES

Chicks were given 20 μg cycloheximide in 100 μl of propylene glycol, intraperitoneally every 4 h, three times. 1 h after the first injection of cycloheximide, 1 μg 1,25-dihydroxyvitamin D-3 in propylene glycol was injected intraperitoneally. 1 h after the third injection of cycloheximide, chicks were decapitated and the brush border membranes were prepared. The control groups were given vehicles only. The data are the means ± S.E. of three experiments.

	Vitamin D-deficient		1,25-Dihydroxyvitamin D-3 treated	
	None	Cycloheximide	None	Cycloheximide
Na^+-dependent phosphate uptake (pmol/mg protein per 15 s)	116 ± 21	126 ± 7	293 ± 13	275 ± 22
Alkaline phosphatase activity (% of vitamin D-deficient control)	100 ± 7	71 ± 9	151 ± 11	88 ± 7

heximide also blocks nearly completely the incorporation of labeled amino acids into brush border membrane proteins [23].

Discussion

The present results indicate that phosphate entry into the chick intestinal mucosal cell is an Na^+-dependent process, which is saturable (Fig. 2), and altered by the vitamin D status of the animal (Figs. 1 and 2). They also demonstrate that the action of 1,25-dihydroxyvitamin D-3 upon Na^+-dependent phosphate entry does not depend upon the synthesis of new protein (Table III), and does not involve obligatory changes in the alkaline phosphatase activity of the brush border membrane (Fig. 3 and Table III).

In previous studies employing either the everted intestinal sac technique [6] or organ culture of embryonic chick intestine [11], the reported apparent K_m for phosphate uptake has been in the range 0.2—0.5 mM, values similar to the value found in the present study (Fig. 2). The present study also shows that the major effect of 1,25-dihydroxyvitamin D-3 is to increase the maximum transport capacity or V without altering the apparent K_m of this process. Hence, the simplest model of how 1,25-dihydroxyvitamin D-3 alters transcellular phosphate transport is to consider that the rate limiting step in this process is the Na^+-dependent entry of phosphate into the cell across the luminal membrane. In such a model, the previous data showing that the process requires metabolic energy and is inhibited by ouabain [1,5,6,9] can all be explained by the fact that the energy-dependent maintenance of the Na^+ gradient across the mucosa cell face is necessary for transcellular phosphate transport, and that the activity of the $(Na^+ + K^+)$-ATPase of the basolateral cell membrane is the means by which this gradient is formed and maintained.

Wong et al. [24] showed that mucosal addition of filipin stimulated transcellular phosphate transport if 0.1 mM Ca^{2+} was present in the mucosal solution but not when Ca^{2+} was absent. Their data raise the possibility that an increase in mucosal Ca^{2+} entry is the stimulus for the subsequent increase in the

rate of Na^+-dependent phosphate entry into the enterocyte. However, in the present experiments, addition of Ca^{2+} and calmodulin in the presence of filipin did not cause any significant increase in Na^+-dependent phosphate uptake into the brush border vesicles from vitamin D-deficient chicks (Table II).

A question of some interest has centered around the possible role of alkaline phosphatase in mediating either phosphate or Ca^{2+} transport [10,18,19,25]. It is known that the administration of vitamin D-3 or 1,25-dihydroxyvitamin D-3 to vitamin D-deficient animals leads to an increase in total and brush border alkaline phosphatase activity [10,18,25—27]. Moog and Glazier [28] reported that the rate of phosphate transport, both organic and inorganic, in the intestine changed in parallel with the alkaline phosphatase activity. They proposed that alkaline phosphatase acted in some way as a phosphate permease. However, the present results show: (1) that the time course in the change of Na^+-dependent phosphate uptake differs from the change in alkaline phosphatase activity of the brush border membrane (Fig. 3); and (2) that even though prior treatment of vitamin D-deficient animals with cycloheximide blocks the subsequent effect of 1,25-dihydroxyvitamin D-3 upon brush border alkaline phosphatase activity, it does not block the effect of 1,25-dihydroxyvitamin D-3 treatment upon Na^+-dependent mucosal phosphate entry (Table III). These data indicate that alkaline phosphatase does not serve as a phosphate permease in the intestinal brush border membrane.

The present results indicate that 1,25-dihydroxyvitamin D-3 administration alters the rate of Na^+-dependent mucosal entry of phosphate into the enterocyte by a process that is independent of protein synthesis (Table III). This is similar to its effect upon mucosal Ca^{2+} entry which is also expressed in the absence of new protein synthesis [18]. In both cases, there is substantial evidence that these entry steps are the rate-limiting steps in the transcellular transport of phosphate and Ca^{2+}, respectively. Our data do not rule out the possibility that effects of 1,25-dihydroxyvitamin D-3 at other sites within the cell are equally important in the regulation of the transcellular transport of these ions.

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References

1 Wasserman, R.H. and Taylor, A.N. (1973) J. Nutr. 103, 586—599
2 Harrison, H.E. and Harrison, H.C. (1961) Am. J. Physiol. 201, 1007—1012
3 Kowarski, S. and Schacter, D. (1969) J. Biol. Chem. 244, 211—217
4 Chen, T.C., Castillo, L., Korycka-Dahl, M. and DeLuca, H.F. (1974) J. Nutr. 104, 1056—1060
5 Taylor, A.N. (1974) J. Nutr. 104, 489—494
6 Peterlik, M. and Wasserman, R.H. (1978) Am. J. Physiol. 234, E379—E388
7 Walling, M.W. (1977) Am. J. Physiol. 233, E488—E494
8 Walling, M.W. (1978) in Homeostasis of Phosphate and Other Minerals, Advances in Experimental Medicine and Biology (Massry, S.G., Ritz, E. and Rapado, A., eds.), Vol. 103, pp. 131—148, Plenum Press, New York

9 Birge, S.J. and Miller, R. (1977) J. Clin. Invest. 60, 980—988
10 Morrissey, R.L., Zolock, D.T., Bikle, D.D., Empson, R.N., Jr. and Bucci, T.J. (1978) Biochim. Biophys. Acta 538, 23—33
11 Peterlik, M. (1978) Biochim. Biophys. Acta 514, 164—171
12 Hoffman, N., Thees, M. and Kinne, R. (1976) Pflügers Arch. 362, 147—156
13 Hopfer, U., Nelson, K., Perrotto, J. and Isselbacher, K.J. (1973) J. Biol. Chem. 248, 25—32
14 Hopfer, U., Sigrist-Nelson, K., Ammann, E. and Murer, H. (1976) J. Cell. Physiol. 89, 805—810
15 Hopfer, U. (1977) Am. J. Physiol. 233, E445—E449
16 Berner, W., Kinne, R. and Murer, H. (1976) Biochem. J. 160, 467—474
17 Max, E.E., Goodman, D.B.P. and Rasmussen, H. (1978) Biochim. Biophys. Acta 511, 224—239
18 Rasmussen, H., Fontaine, O., Max, E.E. and Goodman, D.B.P. (1979) J. Biol. Chem. 254, 2993—2999
19 Bikle, D.D., Zolock, D.T., Morrissey, R.L. and Herman, R.H. (1978) J. Biol. Chem. 253, 484—488
20 Brownlee, K.A. (1965) Statistical Theory and Methodology in Science and Engineering, John Wiley and Sons, Inc., New York
21 Wolff, D.J. and Brostrom, C.O. (1974) Arch. Biochem. Biophys. 163, 349—358
22 Cheung, W.Y., Lynch, T.J. and Wallace, R.W. (1978) Adv. Cyclic Nucl. Res. 9, 233—251
23 Fontaine, O., Matsumoto, T., Simoniescu, M., Goodman, D.B.P. and Rasmussen, H. (1979) in Vitamin D Basic Research and Its Clinical Application (Norman, A.W., Schaefer, K., Herrath, D.V., Grigoleit, H.G., Coburn, J.W., DeLuca, H.F., Mawer, E.B. and Suda, T., eds.), pp. 693—701, Walter de Gruyter, Berlin
24 Wong, R.G., Adams, T.H., Roberts, P.A. and Norman, A.W. (1970) Biochim. Biophys. Acta 219, 61—72
25 Haussler, M.R., Nagode, L.A. and Rasmussen, H. (1970) Nature 228, 1199—1201
26 Norman, A.W., Mircheff, A.K., Adams, T.M. and Spielvogel, A. (1970) Biochim. Biophys. Acta 215, 348—359
27 Holdsworth, E.S. (1970) J. Membrane Biol. 3, 43—53
28 Moog, F. and Glazier, H.S. (1972) Comp. Biochem. Physiol. 42A, 321—336