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VITAMIN D-INDUCED PHOSPHATE TRANSPORT IN INTESTINAL BRUSH BORDER MEMBRANE VESICLES

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SUMMARY

Vitamin D-independent and vitamin D-induced uptake of inorganic phosphate (P_i) by brush border vesicles is mediated by a sodium-dependent transport mechanism. Vitamin D_3 stimulates initial uptake rates by causing a twofold increase in the maximal velocity of vesicular P_i transport without any effect on its carrier affinity. Diffusional uptake of P_i, observed in the absence of sodium, is not affected by vitamin D_3. Thus, the first step in vitamin D_3 stimulation of intestinal P_i absorption is an effect of the sterol on a "secondary active" P_i transport mechanism located at the brush border membrane.

Vitamin D plays a role in phosphate homeostasis by regulating the intestinal absorption of inorganic phosphate (P_i) (1, 2). From previous in vitro experiments with everted sacs from chick intestine, it has been inferred that the increase in mucosal-to-serosal transepithelial P_i transport observed after vitamin D repletion is due to the vitamin D-induced stimulation of a Na^+-dependent active P_i transport system located on the mucosal surface of the intestine (3, 4, 5). Consequently, basal and vitamin D-induced P_i transport in isolated brush border membrane vesicles was studied in an attempt to further characterize the mode of vitamin D action on intestinal P_i absorption.

MATERIALS AND METHODS

Animals: One day-old White Leghorn cockerels were raised on a vitamin D-free diet (6) for 4 weeks. Some animals were repleted with vitamin D_3 (1000 I.U. per chick), given by i.m. injection 48 hours before experimentation.

Isolation of brush border membrane vesicles: Mucosal scrapings from the jejunum of three chicks, either vitamin D-deficient

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(-D) or vitamin D-replete (+D), were processed as described by Max et al. (7). Briefly, homogenization in HEPES buffer (pH 7.4) containing 2.5 mM EGTA was followed by low speed centrifugation (400 x g, 20 min). The pelleted material was layered on a discontinous sucrose gradient (50/63 %) and centrifuged for 75 min at 90 000 x g. Material at the lower interface was further purified by centrifugation on a glycerol gradient (37/45/60 %) at 58 500 x g for 10 min. The milky bands within the gradient were collected.

Purification of membranes was routinely checked by assay of the marker enzyme sucrase. A 17 to 25fold increase in specific enzyme activity was achieved. An average membrane protein yield of 1.6 % of total homogenate protein (3 mg protein/3 g mucosal scrapings) was obtained.

Electron microscopy of pelleted membranes showed a highly homogenous population of elongated membrane vesicles closely resembling those described by Max et al. (7).

P_i uptake studies: P_i accumulation by isolated vesicles was measured by a "rapid filtration technique" as described by Berner et al. (8): Membrane vesicles were prepared in 0.1 M mannitol, 20 mM HEPES-Tris buffer (pH 7.4). 150 µg membrane protein in 20 µl were added to 100 µl incubation medium which was 0.1 mM $^{32}P_i$ (if not otherwise stated), 0.1 M mannitol, 20 mM HEPES-Tris (pH 7.4) in either 0.1 M NaCl or 0.1 M choline chloride. In some experiments, vesicles were pre-incubated in 0.1 M NaCl buffer at 4° C for 1 h.

Incubation was at 25° C. Aliquots of the vesicles were collected on Sartorius SM membrane filters (pore size 0.45 µm) at stated time intervals, and vesicular P_i uptake was determined by liquid scintillation counting of retained radiophosphate.

Analytical procedures and reagents: Protein was determined by the Lowry method. Sucrase was assayed according to Dahlqvist (9). $^{32}P_i$ was obtained from the Radiochemical Centre Amersham, England, as $H_3{}^{32}PO_4$ in 0.02 N HCl. Radioactivity was determined after dissolving the filters in a liquid scintillation mixture consisting of 2 parts of 0.8 % PPO, 0.01 % dimethyl-POPOP in toluene and 1 part of triton X-100.

RESULTS

P_i uptake in +D and -D vesicles (Fig. 1): Using a 0.1 M NaCl buffer to provide an outside>inside Na^+ gradient, phosphate accumulation in brush border vesicles obtained from vitamin D-deficient (-D) or vitamin D-replete chicks (+D) was determined. The early phase of P_i uptake (up to 2 min) was compared to equilibrium concentrations (determined at 60 min incubation) which are thought to reflect steady-state conditions after dissipation of the initial Na^+ gradient. Within 2 min, +D vesicles accumulate phosphate to 114 % of the equilibrium level, whereas the cor-

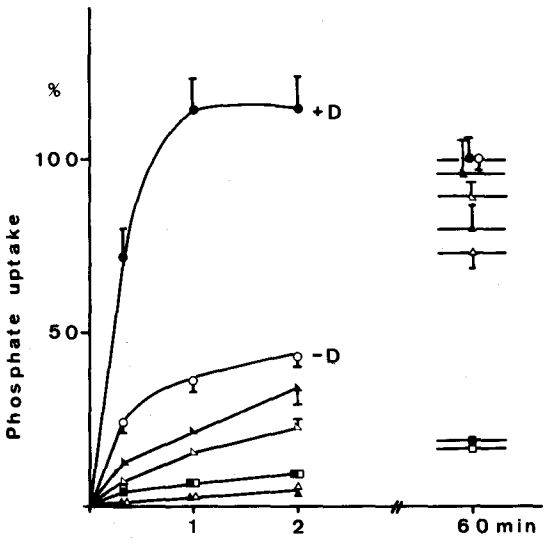

Figure 1: P_i uptake by isolated brush border membrane vesicles isolated from jejunum of either vitamin D-deficient (-D, open symbols) or vitamin D-replete chicks (+D, closed symbols). Extravesicular P_i concentration was 0.1 mM. ○,●: P_i uptake was measured in 0.1 M NaCl buffer. ◺,◣: Vesicles were equilibrated with Na^+ by pre-incubation in 0.1 M NaCl medium. □,■: 5 mM Na_3AsO_4 was included in 0.1 M NaCl incubation medium. △,▲: P_i uptake was determined in 0.1 M choline chloride medium. P_i uptake is expressed as "percentage of equilibrium concentration in 0.1 M NaCl". Data are means ± S.E. (vertical bars) from 6 to 8 determinations utilizing at least two vesicle preparations. P_i uptake (within 2 min incubation in 0.1 M NaCl) of -D and +D vesicles was significantly different at least at $P<0.05$ level at every point of time.

responding uptake by -D vesicles is only 45 %. Equilibrium concentrations are not significantly different in the +D and -D group (+D: 288±15, -D: 322±21 S.E. pmoles P_i/mg protein, n=10).

To verify that the Na^+ concentration gradient between the extra- and intravesicular space is the driving force for P_i uptake, vesicular P_i transport was measured in absence of a Na^+ gradient. Identical Na^+ concentrations on both sides of the vesicular membrane were established by pre-incubation of the vesicles in 0.1 M NaCl buffer. A substantial reduction of P_i uptake during the early phase of intravesicular P_i accumulation was observed in both +D and -D vesicles. In the complete absence of Na^+ (at an outside concentration of 0.1 M choline chloride), +D

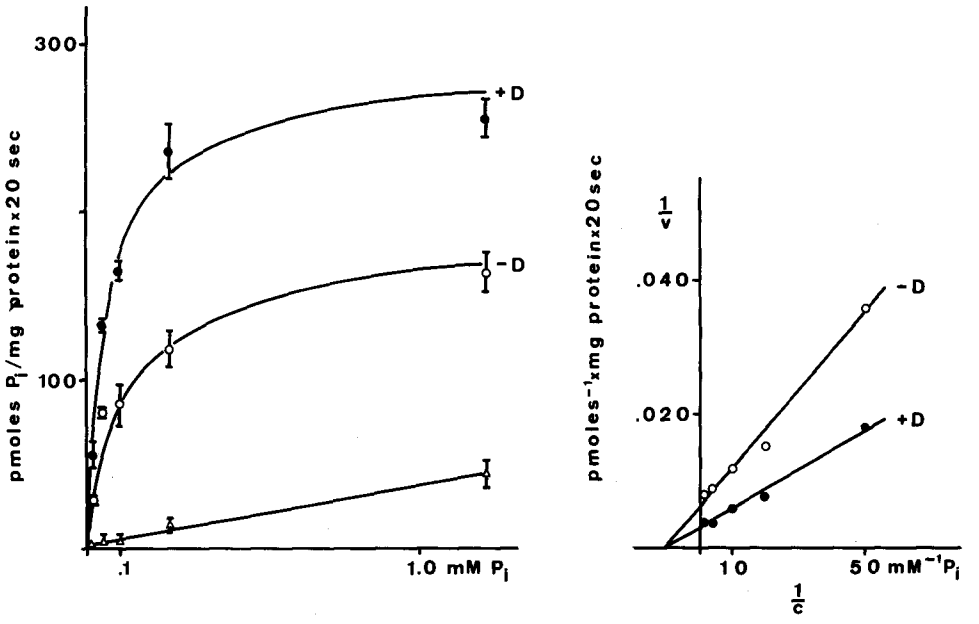

Figure 2: Kinetics of vitamin D-independent and vitamin D-induced P_i uptake by isolated brush border vesicles. Left part: Concentration depencence of initial uptake rates. Right part: Lineweaver-Burk plot of saturable fractions of P_i transport. Open circles refer to -D, closed ones to +D vesicles; Triangles: Measurements were done in 0.1 M choline chloride medium. All data are means ± S.E. from 6 determinations.

as well as -D uptake rates were further reduced to non-distinguishable levels. After 60 min, intravesicular P_i was close to the equilibrium concentration observed in 0.1 M NaCl. The combined data suggest that the transport mechanism mediating P_i uptake by +D as well as -D vesicles depends on the presence of Na^+ and its transmembrane gradient ("secondary active" transport).

When arsenate (5 mM) was included in the 0.1 M NaCl buffer, it depressed P_i uptake, possibly by acting as a substrate analog for the P_i transfer system.

Effect of vitamin D_3 on P_i transport kinetics (Fig. 2): Initial rates of vesicular P_i uptake were measured after 20 seconds incubation as a function of the extravesicular P_i concentration. Na^+-coupled P_i accumulation displays saturation,

while linear concentration dependence of P_i uptake is observed in the absence of any external driving force (at 0.1 M choline chloride). Subtraction of this linear term allowed calculation of the kinetic constants of saturable, vitamin D-independent and vitamin D-induced P_i transfer (Fig. 2, right part). As evidenced by the common abscissa intercept of linearized uptake curves, the phosphate transport systems of vitamin D-deficient and vitamin D-replete jejunum show the same affinity towards P_i (K_m 0.1 mM). The increase in the rate of P_i transfer due to vitamin D_3 is caused by a rise in the maximal velocity from 166 to 303 pmoles P_i/20 sec per mg protein.

DISCUSSION

Isolated brush border vesicles from rat proximal renal tubule or duodenum have been successfully used for characterization of the respective P_i transfer systems (8, 10). The current study is the first attempt to assess the effect of vitamin D repletion on P_i transport across the luminal plasma membrane of the intestinal epithelial cell by determination of P_i transport in brush border vesicles. Phosphate vesicular transport can be separated into two components which differ in their sensitivity towards vitamin D. A small fraction of P_i uptake, which does not respond to vitamin D_3, shows characteristics of diffusional transfer, while the major fraction of Na^+-linked saturable P_i uptake is clearly subject to vitamin D regulation. The maximal velocity of this carrier-mediated transport mechanism is increased after vitamin D_3 administration to vitamin D-deficient chicks. This probably reflects an increase in the number of available carrier sites. Whether this is by exposure of pre-existing latent carrier sites or by vitamin D-directed

synthesis of new carrier complexes cannot be determined from the current experiments. However the lack of any vitamin D effect on intestinal P_i transport in the presence of inhibitors of protein synthesis (11) favors the second assumption.

Previous investigations indicate that vitamin D stimulates only the transcellular - not the paracellular - route of transepithelial P_i transport (5). The current study with brush border vesicles demonstrates that it is the first step of the transcellular pathway which is subject to vitamin D regulation. The twofold increase in the rate of P_i uptake from the lumen might very well account for the elevated P_i absorption by the intestine observed after vitamin D repletion (4).

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REFERENCES

1. Harrison, H. E. and Harrison, H. C. (1960) Am. J. Physiol. 201, 1007-1012
2. Wasserman, R. H. and Taylor, A. N. (1973) J. Nutr. 103, 586-599
3. Taylor, A. N. (1974) J. Nutr. 104, 489-494
4. Peterlik, M. and Wasserman, R. H. (1978) Am. J. Physiol. 234, E379-388
5. Fuchs, R. and Peterlik, M. (1979) Pflügers Arch. 381, 217-222
6. Corradino, R. A. and Wasserman, R. H. (1970) Proc. Soc. Exp. Biol. Med. 133, 960-963
7. Max, E. E., Goodman, D. B. P. and Rasmussen, H. (1978) Biochim. Biophys. Acta 511, 224-239
8. Berner, W., Kinne, R. and Murer, H. (1976) Biochem. J. 160, 467-474
9. Dahlqvist, A. (1968) Anal. Biochem. 22, 99-107
10. Hoffmann, N., Thees, M. and Kinne, R. (1976) Pflügers Arch. 362, 147-156
11. Peterlik, M. (1978) Biochim. Biophys. Acta 514, 164-171