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STIMULATION OF D-GLUCOSE TRANSPORT

A NOVEL EFFECT OF VITAMIN D ON INTESTINAL MEMBRANE TRANSPORT

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Vitamin D stimulates absorption of D-glucose in chick jejunum and ileum by a specific action on the maximal velocity of Na^+-gradient driven D-glucose transport across the brush-border membrane of intestinal cells. Induction of D-glucose transport by either vitamin D-3 or 1,25-dihydroxyvitamin D-3 in embryonic intestine can be blocked by inhibitors of RNA and protein synthesis.

Vitamin D, by virtue of the biologically active metabolite 1α,25-dihydroxyvitamin D-3, stimulates calcium and inorganic phosphate absorption from the small intestine by independent action on the respective membrane transport system at the brush-border aspect of the intestinal absorptive cell (for review see Ref. 1). There is ample evidence that vitamin D induces calcium and phosphate transport by gene activation in a mode comparable to that of other steroid hormones [2,3], though a direct effect of the sterol on membrane transport has also been discussed [4,5]. In this report we wish to describe an additional effect of vitamin D on a well described brush-border absorptive mechanism which hitherto was not regarded as vitamin D-sensitive. Elevation of Na^+-dependent D-glucose transport [6] by vitamin D in certain segments of chick small intestine apparently is another specific effect of the sterol on intestinal membrane transport.

During our studies on vitamin D-3 induction of phosphate uptake by brush-border membrane vesicles from chick jejunum [7] we intended to use Na^+-gradient driven D-glucose accumulation as a non-vitamin D-related reference of vesicular transport activity. Although there was no difference in equilibrium values, higher initial uptake rates were consistently observed in vesicles from chicks which were given vitamin D-3, compared to those derived from vitamin D-3 deficient birds (cf. Fig. 1B). This lead us to further investigation of this unique effect of vitamin D-3 repletion.

Vitamin D-stimulated D-glucose uptake in different segments of small intestine. Cellular uptake of D-glucose in everted gut sacs [8] from four-week old chicks raised on a vitamin D-deficient [9] diet declines along the small intestine in the proximal-to-distal direction (Fig. 1A). Vitamin D-3 has no further influence on duodenal glucose transport but causes an approximately 2-fold increase in the jejunum and ileum (Fig. 1A). Likewise, transepithelial transfer in the lumen-to-blood direction (not shown) reflects this segmental pattern of vitamin D stimulation of D-glucose transport. Any vitamin D increment in either glucose uptake or transfer across the gut wall is abolished by incubation in Na^+-free buffer (Fig. 1A).

Vitamin D effect on Na^+-coupled D-glucose transport by isolated brush-border vesicles. Utilizing isolated brush-border membrane vesicles which were prepared according to Max et al. [10] from small intestinal segments of either vitamin D-3 deficient or vita-

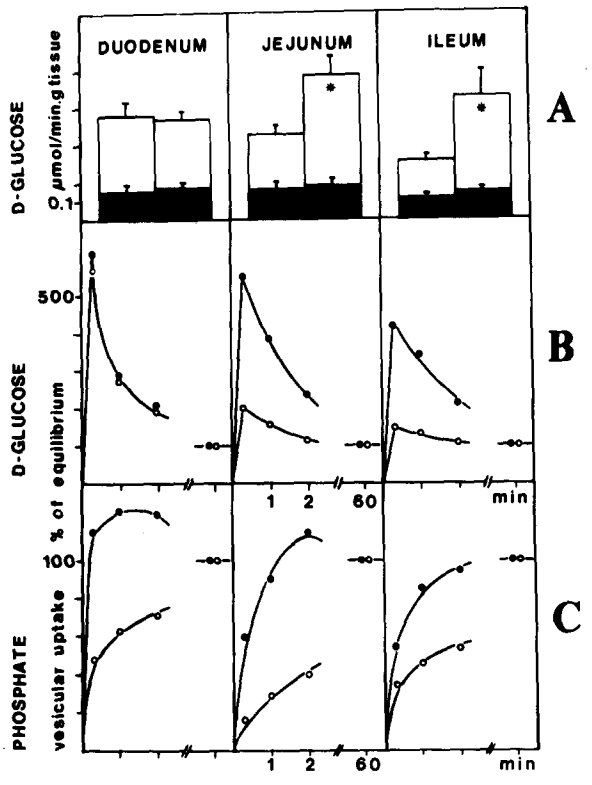

Fig. 1. Effect of vitamin D-3 on intestinal D-glucose (A, B) and P_i transport (C). (A) D-Glucose absorption measured by 5 min incubation of everted gut sacs [8] in phosphate-free Krebs-Henseleit bicarbonate buffer containing 1.0 mM D-[1-^{3}H]glucose. Open bars: accumulation of radiotracer in intestinal segments from vitamin D-deficient (left) and vitamin D-replete (right) chicks (1000 I.U. vitamin D-3 by intramuscular injection, 48 h before experimentation). Asterisk indicates significant stimulation by vitamin D-3 ($P < 0.001$). Black bars: D-glucose uptake in Na^+-free buffer. Mean ± S.E., $n \geq 8$. (B and C) D-Glucose and P_i uptake by isolated brush-border membrane vesicles [10] measured by rapid filtration technique [15]. Open circles: vitamin D-3 deficient chicks; intravesicular volume: 1.31 ± 0.10 µl/mg protein (calculated from D-glucose equilibrium values). Closed circles: vitamin D-3 replete chicks; intravesicular volume: 1.20 ± 0.08 µl/mg protein. Substrate concentration was 0.1 mM D-[1-^{3}H]glucose in phosphate-free, or 0.1 mM [^{32}P]P_i in glucose-free buffer, respectively. Each point represents the mean of triplicate measurements in one out of at least four independent preparations. Significant differences ($P < 0.005$) were obtained for rapid phase accumulation (0–2 min) of both solutes except for duodenal D-glucose uptake.

min D-3 replete chicks, vitamin D-3 was shown to enhance D-glucose absorption by an effect on mucosal Na^+-dependent transport [6]. Concentrative uptake of D-glucose by right side-out vesicles [10,11] energized by an extravesicular > intravesicular Na^+-gradient [6] is demonstrated by the characteristic 'overshoot' phenomenon (Fig. 1B). A pronounced effect on initial transport rates (measured at 20 s) is observed in jejunal and ileal vesicles, while duodenal uptake is not further enhanced. This is consistent with results obtained in everted gut segments (cf. Fig. 1A) and prior observation by others [12].

The effect of vitamin D-3 on transport kinetics was evaluated in jejunal brush-border vesicles. In the presence of an outside > inside Na^+-gradient, D-glucose uptake by vesicles derived from both vitamin D-3 deficient or vitamin D-3 replete chicks tends to saturate with rising D-glucose concentrations (Fig. 2). Subtraction of Na^+-independent glucose uptake, which is linear over the concentration range measured and not influenced by vitamin D (Fig. 2), yielded the Na^+-dependent fraction of D-glucose transfer (Fig. 2, dashed lines). Under the influence of the sterol there is a slight change in the apparent K_m from 0.8 to 0.5 mM, while the maximal uptake rate (V) is raised from 6.0 to 9.4 nmol/mg protein per 20 s. Therefore, the predominant effect of vitamin D is to increase the flux of the carrier or the number of carrier sites without significantly changing the affinity of the transport system for D-glucose.

Vitamin D-induction of Na^+-dependent D-glucose transport in embryonic intestine. In an organ culture system of embryonic small intestine [13] D-glucose transport could be induced by vitamin D-3 or 1,25-dihydroxyvitamin D-3 in the jejunum and ileum, but not in the duodenum. The extent of vitamin D-induced jejunal D-glucose accumulation increased proportionally with culture time. As demonstrated by the minimal amount of D-glucose accumulating upon incubation in Na^+-free buffer, the uptake as measured reflects an Na^+-sensitive process which is significantly altered by addition of vitamin D compounds to the culture medium (Table I).

When embryonic jejunum was cultured in the presence of actinomycin D or cycloheximide, Na^+-dependent D-glucose uptake was reduced and, in addition, any effect of vitamin D-3 or 1,25-dihydroxyvitamin D-3 was abolished (Table I). Apparently, induction of D-glucose transport by vitamin D can be blocked at the level of transcription as well as translation. We deduce therefore from these experiments

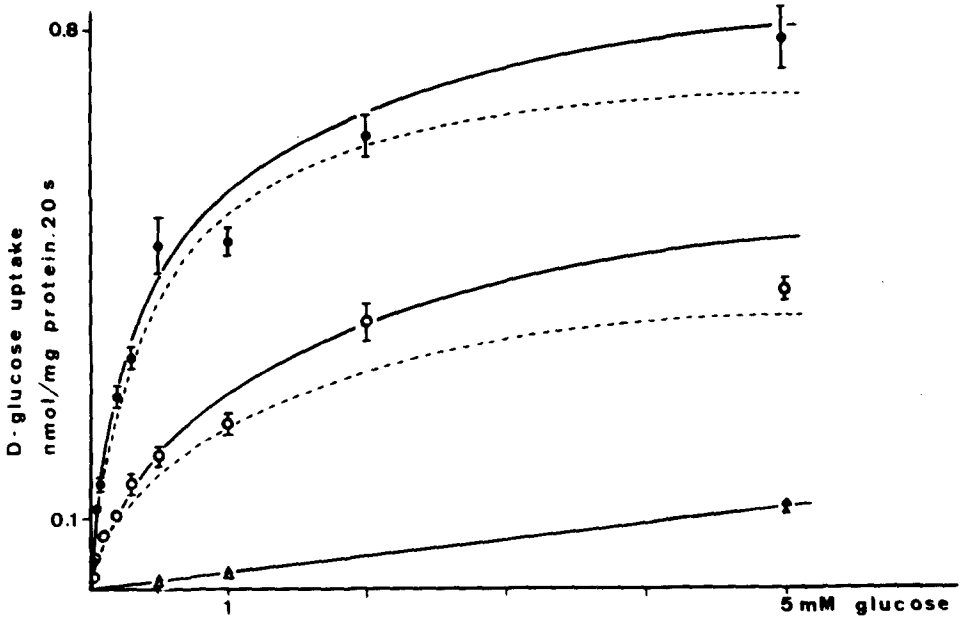

Fig. 2. Concentration dependence of D-glucose uptake by jejunal brush-border vesicles from vitamin D-deficient (open symbols) and vitamin D-replete chicks (closed symbols), measured in the presence of an Na^+-gradient (circles) or in the absence of Na^+ (triangles). Dashed lines: calculated curves of Na^+-dependent uptake (obtained by subtraction of the linear Na^+-independent term). Mean ± S.E. from at least four measurements per concentration.

that vitamin D action on intestinal D-glucose transport necessitates interaction of the sterol with the genome leading to elevated synthesis of protein(s) involved in stimulation of D-glucose translocation across the luminal plasma membrane of enterocytes.

Specificity of vitamin D action on Na^+-dependent D-glucose transport. The similar action of vitamin D on another Na^+-dependent transport system, such as phosphate transport [3,5,7], raises the question whether a common mechanism of action could be envisioned for stimulation of both D-glucose and phosphate uptake. According to Heinz and Geck [14] any effect of vitamin D on Na^+ permeability of the luminal plasma membrane would influence the efficiency of Na^+/solute cotransport in general. As shown in Fig. 3, vitamin D-3 repletion, in fact, significantly reduces Na^+ uptake by isolated brush-border vesicles. If this change in Na^+ permeability reflects solely a reduction of solute-independent Na^+-flow with no interference of vitamin D with Na^+/solute cotransport, one could expect that slower dissipation of the Na^+ gradient would lead to elevated rates of

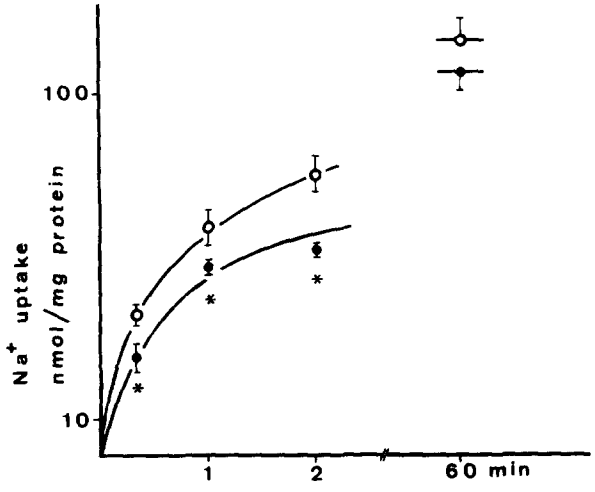

Fig. 3. Influence of vitamin D-3 on $^{22}Na^+$ uptake by jejunal brush-border vesicles. Extravesicular/intravesicular Na^+ concentration was 100/0 mM. Open circles: vitamin D-deficient chicks; intravesicular volume (calculated from equilibrium value): 1.17 ± 0.06 µl/mg protein. Closed circles: vitamin D-replete chicks; intravesicular volume: 1.06 ± 0.06 µl/mg protein. Each point represents the mean (±S.E.) from three independent preparations (n = 10). Asterisk: Significant difference at least at $P < 0.05$ level.

TABLE I

VITAMIN D INDUCTION OF D-GLUCOSE UPTAKE BY CULTURED EMBRYONIC CHICK INTESTINE

Concentration of vitamin D-3 or 1,25-dihydroxyvitamin D-3 (1,25-$(OH)_2D_3$) in medium (McCoy's 5A serum-free) was 26 μM or 120 nM, respectively. Actinomycin D (5.5 μg/ml) or cycloheximide (40 ng/ml) were added 24 h before end of culture period. For measurement of D-glucose uptake, cultured jejunum (day 20) was incubated for 10 min in Krebs-Henseleit bicarbonate buffer (143 mM Na^+ or Na^+ free) containing 1.0 mM D-[1-^{3}H]glucose. For experimental details cf. Refs. 3 and 13. Data (mean ± S.E., $n \geq 12$) are expressed as percent of the mean of vitamin D-free controls.

Segment	Sterol in culture medium	Inhibitor in culture medium	Culture period (h)	Incubation buffer	D-Glucose uptake (%)
Duodenum	None	None	48	Na^+	100 ± 3
	Vitamin D-3	None	48	Na^+	101 ± 3
Jejunum	None	None	24	Na^+	100 ± 4
	1,25-$(OH)_2D_3$	None	24	Na^+	113 ± 5 [a]
	None	None	48	Na^+	100 ± 4
	Vitamin D-3	None	48	Na^+	138 ± 6 [a]
	None	None	48	Na^+-free	11 ± 1 [b]
	Vitamin D-3	None	48	Na^+-free	11 ± 1 [b]
	None	Actinomycin D	24	Na^+	67 ± 7 [c]
	1,25-$(OH)_2D_3$	Actinomycin D	24	Na^+	70 ± 7 [c]
	None	Cycloheximide	24	Na^+	38 ± 5 [c]
	1,25-$(OH)_2D_3$	Cycloheximide	24	Na^+	40 ± 3 [c]
	None	Actinomycin D	48	Na^+	64 ± 8 [c]
	Vitamin D-3	Actinomycin D	48	Na^+	65 ± 13 [c]
	None	Cycloheximide	48	Na^+	58 ± 9 [c]
	Vitamin D-3	Cycloheximide	48	Na^+	47 ± 7 [c]
Ileum	None	None	48	Na^+	100 ± 7
	Vitamin D-3	None	48	Na^+	120 ± 12

[a–c] Superscripts indicate significant difference (at least at $P < 0.05$ level):
[a] from vitamin D-free controls,
[b] from respective controls incubated at 143 mM Na^+,
[c] from pertinent inhibitor-free control group.

Na^+/solute co-transfer. However, a stimulatory action of vitamin D on Na^+-dependent transport systems cannot be unequivocally established, since studies utilizing everted gut sacs as well as isolated brush-border vesicles yielded no conclusive results as yet on vitamin D dependence on Na^+-coupled transfer of other solutes, e.g. methionine (unpublished observations).

There is, however, some evidence against a common mechanism of action in vitamin D stimulation of D-glucose and phosphate transport. Phosphate transport can be induced by vitamin D without any concomitant increase in D-glucose uptake in embryonic duodenum (cf. Ref. 3 and Table I) as well as in the duodenum from four-week old chicks (cf. Fig. 1B and C). On the other hand, in immature (day 18) embryonic jejunum D-glucose transport but not phosphate uptake can be induced by vitamin D-3 (unpublished observations).

Although the physiological significance of this novel effect of vitamin D is not yet known and certainly warrants further investigation, the apparent specific influence on intestinal transport of an important nutrient other than calcium and phosphate points to a more general role of vitamin D in regulation of nutrient absorption.

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