Vitamin D stimulates ($Na^+ + K^+$)-ATPase activity in chick small intestine

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Vitamin D_3 and 1,25-dihydroxyvitamin D_3 raise ($Na^+ + K^+$)-ATPase activity (ouabain-sensitive $^{86}Rb^+$ uptake) in cultured embryonic and 4-week-old chick small intestine. Vitamin D stimulation of the sodium pump, which requires genomic action of the sterol, may lead to enhanced Ca^{2+} extrusion via a basolateral Na^+/Ca^{2+} exchange mechanism, and, in addition, may provide a proliferative signal in undifferentiated enterocytes.

Vitamin D *($Na^+ + K^+$)-ATPase* *Embryonic intestine* *Proliferation* *Calcium transport* *Na^+-dependent transport*

1. INTRODUCTION

It is now well documented that administration of vitamin D, either vitamin D_3 or 1,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$), to vitamin D-deficient chicks results in increased Na^+ gradient-driven transport of inorganic phosphate [1,2], D-glucose [3,4], and amino acids (e.g., methionine and alanine [4]), across the brush-border membrane of chick small intestine epithelial cells. Similar effects of the steroid hormone have been observed in chick small intestine maintained in organ culture during the terminal period of embryonic development [3,5,6]. With respect to the mechanism by which vitamin D could stimulate Na^+-dependent transport systems in general, the possibility was considered that the sterol could act on transmembrane Na^+ fluxes in a way that would increase the electrochemical Na^+ gradient which is the driving force underlying the concentrative solute uptake. This led us to investigate possible effects of vitamin D on epithelial Na^+ transport: In fact, vitamin D was shown to reduce the rate of Na^+ entry into brush-border membrane vesicles derived from the jejuno-ileal portion of chick small intestine [3] (Fuchs and Peterlik, in preparation). In intact cells this vitamin D action would cause hyperpolarization which could at least partially explain the observed stimulatory effects of vitamin D on various Na^+-dependent transport systems [3,4]. By the same token, not only inhibition of Na^+ influx but also increased Na^+ extrusion across the basolateral plasma membrane could contribute to elevation of Na^+-dependent nutrient uptake from the intestinal lumen. In addition, it would be expected that any increase in the activity of the sodium pump would facilitate also the extrusion of calcium via the Na^+/Ca^{2+} exchange system.

We presented evidence that vitamin D, when added to organ cultures of embryonic chick small intestine or when administered to vitamin D-deficient chicks, actually increases the ($Na^+ + K^+$)-ATPase activity of enterocytes as reflected by elevated rates of ouabain-sensitive $^{86}Rb^+$ uptake. Vitamin D influences the sodium pump by increasing its efficiency through an event which requires prior nuclear action of the steroid hormone. Since elevation of ($Na^+ + K^+$)-ATPase activity due to vitamin D can be observed in cultured undifferentiated epithelium without any concomitant rise in Na^+-linked uptake of D-glucose or phosphate, the effect of the sterol on the extent of sodium pump-

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ing alone might be inadequate to raise Na^+-dependent transport. However, the vitamin D action on $(Na^+ + K^+)$-ATPase activity shows a striking parallelism to that on calcium absorption during embryonic development, lending support to the notion that vitamin D might facilitate the transcellular transport of calcium by coordinately regulating its entry and exit step. In addition, since stimulation of $(Na^+ + K^+)$-ATPase activity can be regarded as a signal for cell growth [7,8], the impressively high responsiveness of undifferentiated epithelium to the stimulatory action of vitamin D on the $(Na^+ + K^+)$-ATPase activity suggests a particular role of the sterol in promoting enterocyte proliferation.

2. MATERIALS AND METHODS

Methods used for organ culture of embryonic chick small intestine and for determination of Rb^+ and Ca^{2+} uptake by cultured guts have been detailed in [5,6,9]. Briefly, intestinal segments excised from chick embryos between day 15–20 were cultured (for 12–48 h) in serum-free McCoy's 5A mod. medium in the absence or presence of vitamin D_3 or 1,25-$(OH)_2D_3$, respectively. They were then incubated (for 30 min, if not otherwise indicated) in either Krebs–Henseleit bicarbonate buffer (P_i-free, pH 7.4) containing 0.1 mM RbCl (0.5 μCi $^{86}Rb^+$/ml), or in a low-sodium mannitol buffer with 0.25 mM Ca^{2+} (0.5 μCi $^{45}Ca^{2+}$/ml).

In one experiment 4-week-old vitamin D-deficient chicks [10] were repleted with vitamin D_3 (1000 IU/chick) 48 h before experiment. $^{86}Rb^+$ tissue uptake was measured in intestinal slices at ~0.5 cm length.

$(Na^+ + K^+)$-ATPase activity was determined according to [11]. Each determination, for which homogenates of mucosal scrapings from 6 cultured guts were combined, was done in triplicates.

3. RESULTS AND DISCUSSION

Ouabain-sensitive uptake of the K^+ tracer ^{86}Rb by intact cells is considered to reflect their $(Na^+ + K^+)$-ATPase and, thus, sodium pump activity [7]. Cumulative uptake of $^{86}Rb^+$ by chick small intestine (cultured on day 20 of embryonic life), which is a linear function of the incubation time up to 60 min (fig.1A), is found to be increas-

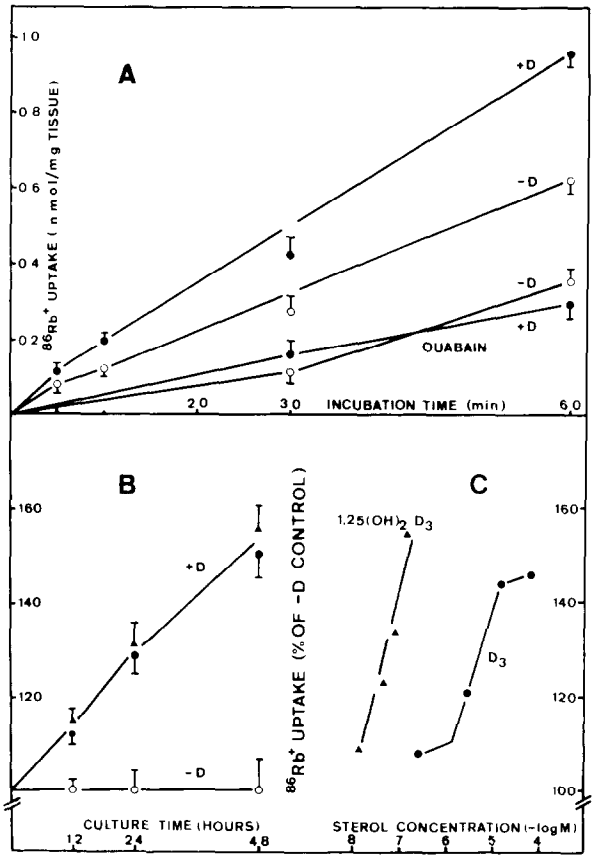

Fig.1. Effect of vitamin D on $^{86}Rb^+$ uptake in embryonic jejunum (day 20). Means ± SE (vertical bars), $n \geq 6$. Culture time 48 h, except when indicated otherwise. (○) Vitamin D-free controls (−D group); Vitamin D conc. in culture medium was either 26 μM vitamin D_3 (●, +D) or 70 nM 1,25-$(OH)_2D_3$ (▲, +D). (A) Stimulation of ouabain-sensitive $^{86}Rb^+$ uptake. Ouabain conc. in incubation buffer was 1.0 mM. (B) Time course of vitamin D induction of $^{86}Rb^+$ uptake. (C) Dose–response relationship of vitamin D_3 and 1,25-$(OH)_2D_3$.

ed at any time point due to the presence of vitamin D. The sterol was shown to act exclusively on the ouabain-sensitive fraction of $^{86}Rb^+$ uptake (fig.1A, table 1). Thus, a 2-fold rise in $(Na^+ + K^+)$-ATPase activity can be observed in the jejunum while duodenum and ileum exhibit much less responsiveness. The vitamin D effect on intestinal $(Na^+ + K^+)$-ATPase can be observed also in 4-week-old chicks where it is high in the jejuno-ileal portion of the small intestine, but becomes undetectable in the duodenum (table 1). This observation might explain why other laboratories

Table 1

Effect of vitamin D on $^{86}Rb^+$ uptake by chick small intestine

Segment	Vitamin D status	$^{86}Rb^+$ uptake (30 min incubation)			
		Ouabain-sensitive		Total	
		nmoles/mg	% of −D	nmoles/mg	% of +D
Embryonic day 20					
Duodenum	−D	0.25 ± 0.01	100 ± 4	0.46 ± 0.02	100 ± 4
	+D	0.34 ± 0.01[a]	136 ± 4[a]	0.52 ± 0.02[a]	113 ± 4[a]
Jejunum	−D	0.12 ± 0.01	100 ± 8	0.27 ± 0.01	100 ± 4
	+D	0.24 ± 0.01[a]	200 ± 8[a]	0.43 ± 0.01[a]	155 ± 4[a]
Ileum	−D	0.40 ± 0.01	100 ± 2	0.56 ± 0.02	100 ± 3
	+D	0.48 ± 0.02[a]	120 ± 5[a]	0.64 ± 0.03[a]	114 ± 5[a]
Day 28 posthatching					
Duodenum	−D	0.08 ± 0.01	100 ± 9	0.11 ± 0.01	100 ± 9
	+D	0.07 ± 0.01	94 ± 9	0.10 ± 0.01	96 ± 9
Jejunum	−D	0.21 ± 0.03	100 ± 11	0.26 ± 0.03	100 ± 11
	+D	0.29 ± 0.02[a]	138 ± 8[a]	0.36 ± 0.02[a]	138 ± 8[a]
Ileum	−D	0.11 ± 0.01	100 ± 9	0.13 ± 0.01	100 ± 7
	+D	0.15 ± 0.01[a]	175 ± 9[a]	0.19 ± 0.02[a]	146 ± 15[a]

[a] Statistically significant difference from −D controls at least at $P < 0.05$ level (Student's t-test)

Data are means ± SE ($n \geq 6$ per group). Embryonic intestines were cultured (48 h) in the absence (−D) or presence of 26 μM vitamin D_3 (+D). 28-day-old chicks: −D, vitamin D-deficient; +D, vitamin D-replete animals. $^{86}RbCl$ was added after guts were preincubated (20 min) in the absence or presence of 1.0 mM ouabain for determination of total or ouabain-insensitive uptake (not shown), respectively, which was subtracted from the former to yield ouabain-sensitive fraction

are unable to find an effect of vitamin D on the $(Na^+ + K^+)$-ATPase [12,13].

Because of its unique responsiveness, cultured embryonic jejunum was used for further investigations: Elevation of sodium pump activity, which becomes significant at 12 h and which was followed up to 48 h culture time (fig.1B), is brought about by vitamin D_3 and its biologically most active metabolite, 1,25-$(OH)_2D_3$, alike. The vitamin D sterols differ, however, in their ability to raise $^{86}Rb^+$ uptake by a factor > 200 (fig.1C). Their absolute biopotencies as shown by the dose-related responses to each sterol are in accordance with reported data on the effective concentration range for stimulation of other transport processes in the intestinal organ culture system [5,6,14].

Any effect of vitamin D on cells other than enterocytes is highly unlikely since only these but not smooth muscle or connective tissue cells were identified as target cells by demonstration of specific nuclear binding of vitamin D as a prerequisite for influencing the genome expression [15]. This mode of steroid hormone action implies transcriptional control of protein synthesis and is also underlying the elevation of $(Na^+ + K^+)$-ATPase activity by vitamin D since it can be completely blocked by addition of inhibitors of RNA and protein synthesis like actinomycin D and cycloheximide to the culture medium (table 2). Under the conditions employed, neither inhibitor has any apparent effect on $^{86}Rb^+$ uptake by guts which had been cultured in the absence of vitamin D.

When $(Na^+ + K^+)$-ATPase in mucosal homogenates of cultured jejunal segments was determined, no significant difference in hydrolytic enzyme activity could be detected between guts cultured in the absence or presence of vitamin D

Table 2

Effect of actinomycin D and cycloheximide on vitamin D-induced $^{86}Rb^+$ uptake by cultured embryonic jejunum

Vitamin D_3 in culture medium	Inhibitor in culture medium	Total $^{86}Rb^+$ uptake (30 min incubation) % of −D control
−	−	100 ± 4
+	−	155 ± 4
−	Actinomycin D	91 ± 3
−	Cycloheximide	94 ± 5
+	Actinomycin D	106 ± 6
+	Cycloheximide	100 ± 4

Data are means ± SD ($n \geq 6$ per group) and are expressed as percent of vitamin D-free control (−D). Culture time was 24 h. Actinomycin D (5.5 μg/ml) and cycloheximide (20 μg/ml) were present only during last 18 h of culture. Vitamin D_3 was 26 μM

(0.65 ± 0.07 *vs* 0.63 ± 0.03 μmol P_i liberated.mg protein^{-1}.h^{-1}; $n = 3$). If one assumes that the enzymatic assay of $(Na^+ + K^+)$-ATPase activity in a broken cell preparation is a measure of the total number of pump sites in contrast to the determination of $^{86}Rb^+$ uptake which reflects the actual rate of Na^+-pumping under various conditions in the intact cell, it might be concluded that vitamin D regulates the activity of the sodium pump in enterocytes rather indirectly by increasing its efficiency through an actinomycin D-sensitive step and not by a direct effect on the synthesis of $(Na^+ + K^+)$-ATPase complexes.

When the ability of vitamin D to raise enterocytic $(Na^+ + K^+)$-ATPase activity was determined in dependence of the stage of embryonic development (fig.2A), an increase of ouabain-sensitive $^{86}Rb^+$ uptake was observed already on day 16. This precludes that vitamin D affects Na^+-dependent transport systems via action on the sodium pump only, since no vitamin D-related increase in Na^+ gradient-driven transport of D-glucose or P_i was observed prior to day 18 or 20, respectively [6]. However, the extent of the vitamin D effect on the sodium pump strikingly resembles that on calcium accumulation (fig.2B). Increased Na^+ extrusion would certainly contribute to elevated calcium transport via a coupled Na^+/Ca^{2+} exchange mechanism. Its existence in embryonic

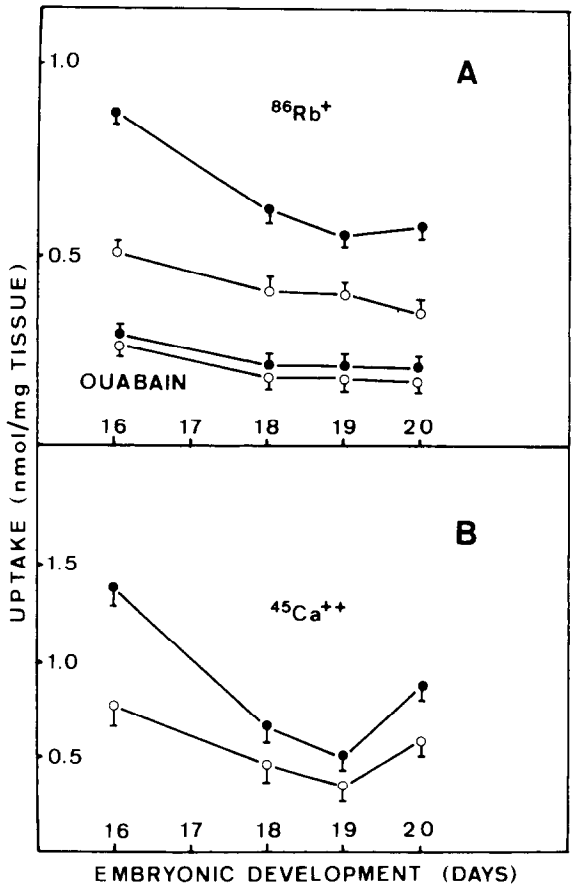

Fig.2. Vitamin D responses in dependence of embryonic development. Means ± SE (vertical bars), $n \geq 6$. Culture time 48 h. (○) Vitamin D-free controls (−D); (●) 26 μM vitamin D_3 in culture medium. (A) Stimulation of ouabain-sensitive $^{86}Rb^+$ uptake. Uptake was initiated by addition of 0.1 mM $^{86}RbCl$ to guts preincubated for 20 min in the absence or presence of 1.0 mM ouabain. (B) Stimulation of $^{45}Ca^{2+}$ uptake.

intestine can be inferred from the observation, that at low extracellular $[Na^+]$, when calcium extrusion via the Na^+/Ca^{2+} antiport is presumably blocked, calcium accumulation within the cell is markedly increased [5]. In addition, Na^+/Ca^{2+} exchange could be demonstrated in basolateral plasma membrane vesicles from rat small intestine [16]. Thus it seems possible that vitamin D stimulates transepithelial calcium transport by coordinately regulating the entry and also the exit steps. Vitamin D also activates the Ca^{2+}-stimulated ATPase [12], another calcium-transporting

mechanism located at the basolateral plasma membrane [17].

The finding that the response of the $(Na^+ + K^+)$-ATPase activity to vitamin D is highest on day 16, when the epithelium is made up exclusively of undifferentiated absorptive cells with high mitogenic potential [18], hints at another possible role of vitamin D in cell function: An increase in $(Na^+ + K^+)$-ATPase activity is widely regarded as the earliest signal for cell proliferation [7,19]. Our results support notions that vitamin D can promote the proliferation of absorptive intestinal cells [20,21]. However, it remains a subject of future investigations, whether enterocytes of cultured intestine can actually undergo proliferation as a response to the signal of elevated $(Na^+ + K^+)$-ATPase activity caused by vitamin D.

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