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1α,25-DIHYDROXY-VITAMIN D-3 REGULATES ATP-DEPENDENT CALCIUM TRANSPORT IN BASOLATERAL PLASMA MEMBRANES OF RAT ENTEROCYTES

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Basolateral plasma membrane vesicles of rat small intestinal epithelium accumulate calcium through an ATP-dependent pumping system. The activity of this sytem is highest in duodenum and decreases towards the ileum. This distribution along the intestinal tract is similar as the active calcium absorption capacity of intact intestinal epithelial segments. ATP-dependent calcium uptake in basolateral membrane vesicles from duodenum and ileum increased significantly after repletion of young vitamin D-3-deficient rats with 1α,25-dihydroxy-vitamin D-3. Ca^{2+}-ATPase activity in duodenal basolateral membranes increased to the same extend as ATP-dependent calcium transport, but ($Na^+ + K^+$)-ATPase activity remained unaltered.

Active calcium absorption in young rats occurs primarily in the duodenum and it is generally accepted that this process is regulated by the seco-steroid hormone $1\alpha,25(OH)_2D_3$, a metabolite of vitamin D-3 [1,2]. At least three steps can be distinguished in the transport of Ca^{2+} through epithelial cells: transport of Ca^{2+} across the brush border membrane, transport of Ca^{2+} through the cytosol and transport across the basolateral plasma membrane. Calcium influx across the brush border is down the electrochemical gradient for Ca^{2+} and $1\alpha,25(OH)_2D_3$ is involved in the regulation of the Ca^{2+} permeability of this membrane [3,4]. A cytosolic calcium binding protein, induced by $1\alpha,25(OH)_2D_3$, may play a role in intracellular Ca^{2+} buffering and transport through the cytosol [5,6,7]. The final step, efflux of Ca^{2+}, is against the electrochemical gradient and requires an active transport system. Recently it has been shown that purified basolateral plasma membranes of rat duodenum contain Ca^{2+}-ATPase activity with high affinity for Ca^{2+} [8,9], and that basolateral membrane vesicles accumulate calcium when incubated in the presence of ATP [10,11,12]. We like to report here effects of the vitamin D status of young rats on Ca^{2+}-ATPase activities and on ATP-dependent calcium transport in small intestinal basolateral membranes.

Basolateral plasma membranes were isolated from 10-cm pieces of proximal duodenum, mid jejunum or terminal ileum of male Wistar rats (160–200 g) according to a slightly modified procedure of Mircheff et al. [13] and described in detail elsewhere [12]. Of the initial ($Na^+ + K^+$)-ATPase activity, 15% was routinely recovered in a final pellet enriched 10–15-fold. Contamination with fragments of brush border, mitochondrial and endoplasmic reticulum was minimal since sucrase and succinate dehydrogenase activity in the final preparation was less than 1% and NADPH-dependent cytochrome *c*-reductase was about 2% of the initial activity. After purification, basolateral membranes were washed free of sorbitol and resuspended in 100 mM KCl, 5 mM $MgCl_2$

Abbreviations: Hepes, 4-(2-hydroxyethyl)-1-piperazineethane sulphonic acid; EGTA, ethyleneglycol bis(β-aminoethyl ether)-*N*,*N*'-tetraacetic acid.

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and 20 mM Hepes-Tris (pH 7.4). Enzyme assays have been described previously [8,9]. Ca^{2+}-ATPase activity was assayed in a leaky vesicle preparation to avoid enzyme latency due to inaccessible sites [8]. Male rats were raised from weaning under vitamin D-deficient conditions for six weeks [2,14]. Repleted rats received 160 ng $1\alpha,25(OH)_2D_3$ intraperitoneally 48 and 24 h before sacrifice. Plasma calcium was determined fluorimetrically [15] and plasma calcium levels verified the vitamin D-deficient (−D) and repleted (+D) state of the animals. Plasma calcium increased from 1.65 ± 0.06 mM (−D) to 2.58 ± 0.06 mM (+D) ($n = 24$). Calcium uptake into basolateral vesicles was measured by means of a rapid filtration technique using ^{45}Ca as radioactive marker [12].

In Fig. 1 results are summarized of ATP-dependent calcium uptake activities in basolateral membrane vesicles from three intestinal segments and the effect of the vitamin D status on this calcium transport. In duodenal membranes the rate of ATP-dependent calcium uptake is highest. A similar distribution along the small intestinal tract has been reported for net calcium fluxes across intact intestinal epithelium [2,14] and for vitamin D-dependent calcium-binding protein [16]. This close correlation between ATP-dependent calcium uptake in basolateral membranes and net calcium fluxes across intact epithelium strongly suggests a primary role for the calcium pump in intestinal calcium absorption. Addition of calcium ionophore A23187 prevents calcium accumulation, while oligomycin (10 μg/ml) has no effect on ATP-dependent calcium transport [12].

In duodenum as well as ileum a significant increase of 70% ($P < 0.001$, paired Student's t-test) is observed in the rate of ATP-dependent calcium transport after repletion of vitamin D-deficient rats with $1\alpha,25(OH)_2D_3$. These results agree with calcium transport studies in vitro [17] and in vivo [18] and support the idea that only the most proximal and distal segments of the small intestine respond to $1\alpha,25(OH)_2D_3$ with respect to calcium absorption [19].

In Fig. 2 the kinetics of ATP-dependent calcium transport in duodenal basolateral membranes is shown. The data indicate that the effect of $1,25(OH)_2D_3$ is especially on the V_{max} of the calcium pump and not so much on the affinity of

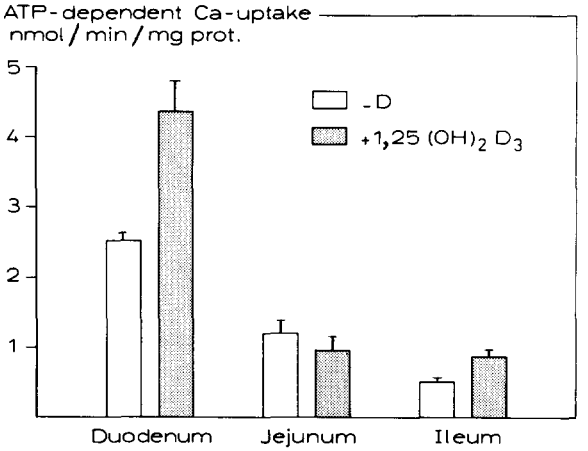

Fig. 1. ATP-dependent Ca^{2+}-uptake in basolateral plasma membranes from different segments of rat small intestine and the effects of vitamin D deficiency (−D) and repletion with $1\alpha,25(OH)_2D_3$. Ca^{2+}-uptake was measured at 25°C in a medium containing 100 mM KCl, 5 mM $MgCl_2$, 3 mM Tris-ATP, 0.5 mM EGTA, 1 μM free Ca^{2+} (1.5 μCi ^{45}Ca/ml) and 20 mM Tris-Hepes buffer (pH 7.4). Control Ca^{2+}-uptake experiments in the absence of ATP were done separately and ATP-dependent Ca^{2+}-uptake was corrected for ATP-independent uptake. The rate of ATP-independent Ca^{2+}-uptake in duodenal basolateral membranes was: 1.21 ± 0.13 (−D) and 1.22 ± 0.15 (+$1,25(OH)_2D_3$) nmol Ca^{2+}/min·mg protein. In jejunal and ileal basolateral membranes the ATP-independent Ca^{2+}-uptake rates were not significantly different from duodenal values. The data given represent mean values with S.E. values. Number of observations: duodenum ($n = 8$), jejunum ($n = 4$) and ileum ($n = 4$). Each observation is made on pooled intestinal segments of at least three rats.

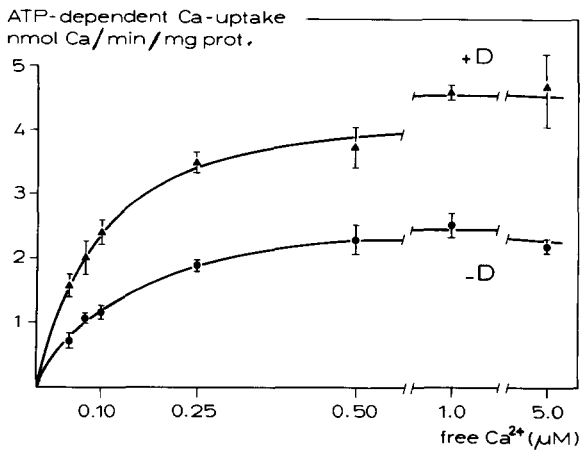

Fig. 2. Kinetics of ATP-dependent calcium uptake in basolateral plasma membranes of duodenal epithelium: Effect of $1,25(OH)_2D_3$. ●——●, vitamin-D-deficient rats (−D); ▲——▲, $1\alpha,25(OH)_2D_3$-repleted rats (+D).

this system for calcium. The affinity for calcium in Fig. 2 is between 0.10 and 0.15 μM Ca^{2+} and this value is in good agreement with the affinity for Ca^{2+} of the Ca^{2+}-ATPase activity [12].

In Table I the activities are given of $(Na^+ + K^+)$-ATPase and Ca^{2+}-ATPase in leaky basolateral membrane fragments of duodenal epithelium. Ca^{2+}-ATPase activity increased 75% ($P < 0.05$) after repletion with $1\alpha,25(OH)_2D_3$, while no significant change in $(Na^+ + K^+)$-ATPase activity is observed. This result indicate that the effect of $1\alpha,25(OH)_2D_3$ is rather specific and not of a general trophic nature as to increase all enzymatic activities. The results of Fig. 1 and Table I clearly demonstrate that $1\alpha,25(OH)_2D_3$ is also involved in the regulation of Ca^{2+} transport across the basolateral membrane. The secosteroid hormone increases the activity of an ATP-dependent Ca^{2+}-transport system with a concomittant increase in high affinity Ca^{2+}-ATPase activity. However, the relative importance of the basolateral membrane in regulating transcellular calcium transport must be assessed in the intact epithelium since the effects of $1\alpha,25(OH)_2D_3$ on the brush border membrane, on cytosolic Ca^{2+}-buffering systems and on the basolateral membrane must be perfectly balanced in order to increase the transcellular flux of calcium, while the free calcium concentration in the cytosol has to remain fairly constant.

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TABLE I

Ca^{2+}-ATPase AND $(Na^+ + K^+)$-ATPase ACTIVITIES IN LEAKY BASOLATERAL MEMBRANE FRAGMENTS OF RAT DUODENAL EPITHELIUM

Enzyme activities are assayed at 37°C and given as μmol P_i per h per mg protein (mean values ± S.E., number of observations in parentheses). Ca^{2+}-ATPase activity is measured at 1 μM free calcium in the presence of 2.5 mM theophylline to inhibit non-specific Ca^{2+}-induced ATP hydrolysis due to alkaline phosphatase which is present in basolateral membranes [8].

	Ca^{2+}-ATPase	$(Na^+ + K^+)$-ATPase
− D rats	2.08 ± 0.38 (4)	23.5 ± 3.7 (4)
+ $1\alpha,25(OH)_2D_3$ rats	3.66 ± 0.23 (4)	25.9 ± 4.3 (4)

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