VITAMIN D-INDUCED TRANSEPITHELIAL PHOSPHATE AND CALCIUM TRANSPORT BY CHICK JEJUNUM

Effect of microfilamentous and microtubular inhibitors

Renate FUCHS and Meinrad PETERLIK
Department of General and Experimental Pathology, University of Vienna, Währingerstrasse 13, A-1090 Vienna, Austria

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1. Introduction

Microfilaments and microtubuli form a cytoskeletal system in many types of eukaryotic cells [1,2]. This system has been implicated not only in cellular motion, intracellular transport and maintenance of cell form but also in the control of certain intrinsic membrane properties, e.g., distribution of cell surface receptors [3]. Intestinal cells have a specific transport system for inorganic phosphate (P_i) to effectively transfer this ion from the lumen to the interstitial space [4–6]. Vitamin D has been shown to enhance transmural phosphate transport [7–9] by increasing the maximal velocity of this active 'phosphate pump' which is located at the mucosal surface of the epithelial cell layer [9]. Thus, a normal absorptive surface together with the correct orientation of transport complexes within the mucosal plasma membrane may be vital for an effective uptake of P_i from the lumen and, hence, for the expression of the vitamin D effect on intestinal P_i absorption. Of importance in this regard is the fact, that intestinal cells display a highly distinct cytoskeletal structure at their mucosal pole [10,11] which might be a key factor in maintaining the characteristic ultrastructure of the absorptive surface. The core of the microvilli consists of actin filaments which terminate in a 'terminal web' [12]. This microfilamentous system is susceptible to the action of the inhibitor cytochalasin B [13]. The latter and anti-microtubular agents like colchicine and vinblastine [2] have been shown to inhibit transport of ions [14] and solutes [15,16] in various types of cells. The subject of this study is, whether these anti-microfilamentous and anti-microtubular inhibitors influence the mucosal-to-serosal (M–S) P_i pathways. It was hoped that the results would furnish some evidence for the involvement of cytoskeletal structures in basal and vitamin D-induced P_i absorption by the small intestine. Also reported are observations on the effect of these inhibitors on vitamin D-dependent and -independent intestinal calcium transport.

Address correspondence to: Dr Meinrad Peterlik

2. Materials and methods

Day 1 White Leghorn cockerels were housed in a windowless room under constant lighting and fed a vitamin D-free diet [17]. After 4 weeks the chicks were divided into 2 groups. One group was given 1000 I.U. vitamin D_3 (per chick) dissolved in propylene glycol by intramuscular injection (+D group). The control group (–D group) received the vehicle only. The animals were killed 48 h later. They were fasted 16 h prior to sacrifice.

Unidirectional P_i and Ca^{2+} fluxes in the M–S direction were assessed by incubation of everted gut sacs [18] prepared from the jejunum. $^{32}P_i$ ($H_3{}^{32}PO_4$ in 0.02 N HCl) and $^{45}CaCl_2$ were added as radioactive tracers to the mucosal bathing solution only. Details of the experimental technique and calculation of flux data have been reported elsewhere [9].

Colchicine and cytochalasin B were purchased from Sigma, USA. Vinbalstine sulfate (Velbe®) was a gift of Kwizda, Vienna.

3. Results

Administration of vitamin D_3 to vitamin D-deficient chicks stimulates active entry of P_i [9] from the intestinal lumen into the epithelium ~2-fold above basal (–D) levels (table 1, P_i influx). This results in increased net absorption of P_i (data not shown) and in enhanced transfer into the serosal compartment (table 1, P_i M–S transfer). This increase in M–S transport is entirely due to the action of vitamin D_3 on the entry step and the thereby induced migration of P_i on a transcellular route [9].

In contrast to our expectations, none of the inhibitors tested had any significant influence on P_i influx into the epithelial cell layer (table 1). Thus, the P_i translocator [9] is not under direct control of the cytoskeletal system and, in addition, is not subject to changes in the plasma membrane induced by disintegration of cytoskeletal structures.

However, cytochalasin B inhibits M–S P_i transfer. While not significantly effective at 10 μM, the drug completely abolished vitamin D_3-induced P_i transfer into the serosal compartment at higher concentrations (50–100 μM in the mucosal bathing solution, table 1). Colchicine and vinblastine, at 100 μM mucosal concentration caused only a slight but statistically insignificant reduction of transmural P_i transfer (table 1).

Cytochalasin B caused ~30% reduction in vitamin D-induced transmural Ca^{2+} transport (table 1, Ca^{2+} M–S transfer). This might be the consequence of a decreased tissue uptake from the lumen observed in the +D group (table 1).

Colchicine at 100 μM lowered Ca^{2+} uptake marginally in both the –D and +D group. This did not result, however, in a significant depression of M–S transfer (table 1).

4. Discussion

The routes of intracellular P_i and Ca^{2+} migration have not yet been elucidated. There is some evidence

Table 1
Effect of cytochalasin B, colchicine and vinblastine on intestinal P_i and Ca transport[a]

Inhibitor concentration in mucosal solution	P_i influx from lumen[b]		Ca tissue uptake from lumen[c]		P_i M–S transfer[c]		Ca M–S transfer[c]	
	–D	+D	–D	+D	–D	+D	–D	+D
None	96 ±21	199[d] ±13	115 ±10	278[d] ±17	153 ± 6	334[d] ±40	61 ±8	122[d] ±12
10 μM cytochalasin B	137 ±25	209[d] ±25	n.d.	n.d.	166 ±20	240[d] ±40	60 ±6	100[d] ±10
50 μM cytochalasin B	74 ±13	167[d] ±11	n.d.	n.d.	144 ±20	123[e] ±11	54 ±8	78[d, e] ±8
100 μM cytochalasin B	124 ±29	241[d] ±28	115 ±12	220[d, e] ±10	160 ±20	120[e] ±12	64 ±7	86[d, e] ±7
100 μM colchicine	80 ±19	174[d] ±24	85 ±6	231[d, e] ±11	143 ±14	279[d] ±40	48 ±6	105[d] ±12
100 μM vinblastine	86 ±11	179[d] ±24	n.d.	n.d.	142 ±10	214[d] ±40	69 ±5	112[d] ±13

[a] Data are expressed as mean ± SE of 12–14 determinations; –D, vitamin D-deficient group; +D, vitamin D-replete group; n.d., not determined

[b] Since unidirectional P_i influx from lumen into epithelium is linear during the 20 min incubation period [9], data are given as flux rate (nmol/min.g tissue^{-1})

[c] nmol/g tissue transferred into respective compartment during 20 min incubation period; M–S indicates mucosal-to-serosal direction of transport

[d] Significantly different (Student's t-test) from –D group at least at $P < 0.05$ level

[e] Significantly different from respective control group (no inhibitor present) at least at $P < 0.05$ level

that the P_i transport pool does not readily exchange with other intracellular pools during M–S transfer ('channelized transport') [5,9]. This would, however, implicate the participation of intracellular macromolecules or organelles in the intracellular migration of P_i. Our results suggest that the latter can be blocked by high concentrations of cytochalasin B since the drug inhibits vitamin D-induced M–S transfer without affecting the preceeding entry step. The effective concentration range (50–100 μM) is appropriate to cause disturbance of cellular functions normally associated with an intact microfilamentous system [19]. The lack of any significant effect of 10 μM cytochalasin B speaks against a specific interaction of the drug with membrane transport systems [19].

For the first time, the combined evidence allows the implication of a particular cellular structure – the microfilamentous system of the intestinal brush border – in the transfer of inorganic phosphate from the microvillous to the baso-lateral surface of the enterocyte. In contrast to P_i transport, the small inhibitory effect of cytochalasin B on Ca^{2+} pathways does not speak in favor of a major role of microfilaments in transmural Ca^{2+} transport.

Of particular importance is the observation [20] that the active metabolite of vitamin D_3, 1,25-dihydroxyvitamin D_3, increases the amount of actin associated with the intestinal brush border. Since this effect is one of the earliest actions of the sterol which might coincide with or even preceed the rapid induction of P_i transport [21] this finding would strengthen our suggestion for a special role of microfilaments in the mediation of cellular responses to vitamin D.

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