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KINETIC PROPERTIES OF Na^+/Ca^{2+} EXCHANGE IN BASOLATERAL PLASMA MEMBRANES OF RAT SMALL INTESTINE

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The presence of an Na^+/Ca^{2+} exchange system in basolateral plasma membranes from rat small intestinal epithelium has been demonstrated by studying Na^+ gradient-dependent Ca^{2+} uptake and the inhibition of ATP-dependent Ca^{2+} accumulation by Na^+. The presence of 75 mM Na^+ in the uptake solution reduces ATP-dependent Ca^{2+} transport by 45%, despite the fact that Na^+ does not affect Ca^{2+}-ATPase activity. Preincubation of the membrane vesicles with ouabain or monensin reduces the Na^+ inhibition of ATP-dependent Ca^{2+} uptake to 20%, apparently by preventing accumulation of Na^+ in the vesicles realized by the Na^+-pump. It was concluded that high intravesicular Na^+ competes with Ca^{2+} for intravesicular Ca^{2+} binding sites. In the presence of ouabain, the inhibition of ATP-dependent Ca^{2+} transport shows a sigmoidal dependence on the Na^+ concentration, suggesting cooperative interaction between counter transport of at least two sodium ions for one calcium ion. The apparent affinity for Na^+ is between 15 and 20 mM. Uptake of Ca^{2+} in the absence of ATP can be enhanced by an Na^+ gradient (Na^+ inside > Na^+ outside). This Na^+ gradient-dependent Ca^{2+} uptake is further stimulated by an inside positive membrane potential but abolished by monensin. The apparent affinity for Ca^{2+} of this system is below 1 μM. In contrast to the ATP-dependent Ca^{2+} transport, there is no significant difference in Na^+ gradient-dependent Ca^{2+} uptake between basolateral vesicles from duodenum, midjejunum and terminal ileum. In duodenum the activity of ATP-driven Ca^{2+} uptake is 5-times greater than the Na^+/Ca^{2+} exchange capacity but in the ileum both systems are of equal potency. Furthermore, the Na^+/Ca^{2+} exchange mechanism is not subject to regulation by 1α,25-dihydroxy vitamin D-3, since repletion of vitamin D-deficient rats with this seco-steroid hormone does not influence the Na^+/Ca^{2+} exchange system while it doubles the ATP-driven Ca^{2+} pump activity.

Introduction

The extrusion of Ca^{2+} out of intestinal cells is against the electrochemical gradient and requires energy. In general, two types of Ca^{2+} transport systems can perform Ca^{2+} extrusion across mammalian plasma membranes: a high affinity Ca^{2+}-ATPase and Na^+/Ca^{2+} exchange in which the electrochemical gradient for Na^+ provides the energy for Ca^{2+} extrusion [1]. Recent work indicates that intestinal basolateral plasma membranes contain ATP-dependent Ca^{2+} transport and high affinity Ca^{2+}-ATPase activity [2–5]. In squid axon [6] and cardiac sarcolemma [7–9] the Na^+/Ca^{2+} exchange system has been studied in detail. In these tissues the exchange of Na^+ for Ca^{2+} has been shown to be electrogenic and the capacity exceeds greatly that of the ATP-dependent Ca^{2+} pump [10,11]. The presence of Na^+/Ca^{2+} ex-

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

change has also been suggested in basolateral membranes of rat kidney cortex [12] and toad bladder epithelium [13]. In intestine, the extrusion of Ca^{2+} by way of an Na^+/Ca^{2+}-exchange mechanism is a possibility suggested by some authors who report a dependency of Ca^{2+} absorption on Na^+ [14,15]. However, Na^+ dependency of Ca^{2+} absorption has also been questioned by others [16,17]. In isolated basolateral membranes of rat small intestine, the presence of Na^+/Ca^{2+} exchange has been claimed by Hildmann et al. [5]. These authors showed that ATP-dependent Ca^{2+} accumulation was inhibited by Na^+ in the uptake medium, apparently by inducing a Ca^{2+} leak via the Na^+/Ca^{2+} exchanger.

In this study we demonstrate the presence of Na^+/Ca^{2+} exchange in rat small intestine by studying Ca^{2+} uptake into basolateral plasma membranes driven by an electrochemical Na^+ gradient. Since ATP-dependent Ca^{2+} transport is most active in the duodenum and influenced by the vitamin D-3-status of the rat [18], we also studied the distribution of Na^+/Ca^{2+} exchange along the small intestinal tract and the effect of vitamin D deficiency on the capacity of this exchanger.

Methods and Materials

Membrane preparation. Male wistar rats (170–200 g) are killed by a blow on the head. Immediately, 15 cm pieces of proximal duodenum, mid jejunum and terminal ileum are removed and rinsed with ice-cold isotonic saline solution. Epithelial cells are isolated according to Stern [19]. Isolation and purification of basolateral plasma membranes has been done exactly as described recently [4]. Vitamin D-deficient rats are raised as described in detail by Ghijsen [20]. In short, male rats are raised from weaning under vitamin D-deficient conditions for 6 weeks. Repleted animals receive 160 ng 1α,25-dihydroxy vitamin D-3 intraperitoneally 48 and 24 h before killing. Low plasma calcium levels verify the vitamin D-deficient state of the animals [18,20]. Plasma calcium increased from 1.37 ± 0.03 (54) (D-deficient) to 2.45 ± 0.04 (51) after repletion with 1α,25$(OH)_2D_3$.

ATP-dependent Ca^{2+}-uptake measurements. Isolated basolateral membrane vesicles are suspended in a meduim containing 150 mM KCl, 5 mM $MgCl_2$ and 20 mM Hepes-Tris (pH 7.4). The vesicles are used for experiments within 6 h after the start of the isolation, or within 5 days after freezing in liquid N_2 and storage at $-80°C$. The ATP-dependent Ca^{2+} uptake is measured in a medium containing 150 mM KCl, 7.9 mM MgCl, 6 mM Tris-ATP, 1 μM free Ca^{2+} (1.5 μCi ^{45}Ca/ml) and 20 mM Hepes-Tris (pH 7.4) at 25°C. The free Ca^{2+} concentration is buffered with 0.5 mM EGTA and 0.5 mM nitrilotriacetic acid [20,21]. Equimolar amounts of KCl are replaced by NaCl in order to get the desired Na^+ concentrations. The membrane protein content in the uptake assay is maximally 0.2 mg/ml to avoid ATP depletion in the presence of Na^+, due to activation of $(Na^+ + K^+)$-ATPase. In some experiments the basolateral membrane vesicles are pre-incubated with 1–2 mg/ml ouabain on ice for 45 min or more prior to the Ca^{2+}-uptake studies. The Ca^{2+}-uptake reaction is started by adding basolateral membrane vesicles, preincubated for 5 min at 25°C, to the ATP-containing uptake medium. At appropriate times, 50 μl aliquots are removed and the membranes are collected by rapid filtration [4]. The Na^+ dependence of the inhibition of Ca^{2+} accumulation is studied by measuring the initial uptake rates by sampling after 0.5 and 1.0 min of incubation, where the Ca^{2+} uptake is linear with time, both in the absence and presence of Na^+.

Na^+ gradient-dependent Ca^{2+}-uptake. Freshly prepared basolateral membrane vesicles are suspended in the isolation medium described above with 150 mM NaCl instead of KCl. The suspension is kept on ice for 90 min prior to the experiments. Ca^{2+} uptake is started by 25-fold dilution of the Na^+-loaded vesicles (5 mg/ml protein) in a medium containing 0.1–50 μM free Ca^{2+} (40 μCi ^{45}Ca/ml), 2.1 mM $MgCl_2$, 150 mM NaCl or KCl, 0.5 mM EGTA, 0.5 mM nitrilotriacetic acid and 20 mM Hepes-Tris (pH 7.4) at 25°C. Nitrilotriacetic acid is present in the medium for improved buffering of free Ca^{2+} concentrations above 1 μM [21]. Short incubations are carried out by starting the Ca^{2+} uptake reaction by mixing 5 μl membrane suspension with 125 μl incubation medium. Rapid quenching is accomplished by addition of 1 ml ice-cold medium containing 150 mM KCl, 1 mM $LaCl_3$, 5 mM $MgCl_2$ and 20 mM

Hepes-Tris (pH 7.4) at appropriate times (5–90 s). The membranes are collected by rapid filtration of 1 ml of the quenched solution and analysed for ^{45}Ca [4]. When effects of ionophores are studied, basolateral membrane vesicles are preincubated for 5 min at 25°C in the presence of the ionophore. Ethanolic solutions of monensin and valinomycin are used and the controls are assayed in the presence of the same amount of ethanol (less than 0.1%, v/v).

Enzyme assays. Ca^{2+}-ATPase activity is assayed with 1 μM free Ca^{2+} in the Ca^{2+}-uptake medium as described previously [22], with 2.5 mM theophylline and 2 mg/ml ouabain present to suppress ATP hydrolysis by alkaline phosphatase and ($Na^+ + K^+$)ATPase.

The orientation of the vesicle membranes has been studied by measuring the effect of Triton X-100 and of alamethacin on ($Na^+ + K^+$)-ATPase and Ca^{2+}-ATPase activity. Membranes are preincubated with Triton X-100 (0.5–0.75 mg/mg protein) or alamethacin (0.25–0.50 mg/mg protein) for 10–15 min at 25°C. These pretreated membranes are diluted 20–25-fold in the assay mixture for ($Na^+ + K^+$)-ATPase or Ca^{2+}-ATPase, and incubated for 15 min at 25°C. ($Na^+ + K^+$)-ATPase is assayed as before [22]. Protein is assayed by means of a Coomassie blue assay (Bio-Rad).

Materials. Tris-ATP, EGTA, nitrilotriacetic acid and valinomycin were obtained from Sigma (St. Louis). Monensin was from Calbiochemicals (La Jolla). $^{45}CaCl_2$ (20 mCi/mg) was purchased from New England Nuclear (Dreieich, F.R.G.).

Results

Effects of Na^+ on ATP-dependent Ca^{2+}-uptake

For these experiments basolateral membrane vesicles from duodenum are used since these membranes contain the highest ATP-dependent Ca^{2+} transport capacity [18]. In Fig. 1 it is shown that ATP-dependent Ca^{2+} uptake is inhibited by 45% by the presence of 75 mM Na^+ in the uptake solution. This inhibition may indicate the presence of an Na^+/Ca^{2+} exchange mechanism, causing exchange of accumulated Ca^{2+} against Na^+. Other possible explanations are inhibition of the ATP-dependent Ca^{2+}-pump by Na^+ or involvement of ($Na^+ + K^+$)-ATPase activity. However, the absence of an effect of Na^+ on high affinity Ca^{2+}-ATPase activity (0.94 ± 0.10 compared to 1.00 ± 0.13 μmol P_i per h per mg protein ($n = 5$) in the absence of Na^+) makes the first of these alternative explanations unlikely. Inhibition of Ca^{2+} uptake due to ATP depletion as a result of ($Na^+ + K^+$)-ATPase activation, is not very likely for two reasons. Firstly, the Ca^{2+} uptake is assayed in the presence of 6 mM ATP and the inhibition by Na^+ is already observed at incubation times of 1 and 2 min, when the ATP supply is not yet exhausted (Fig. 1). Secondly, the presence of 2 mg/ml ouabain has no effect on the ATP-dependent Ca^{2+} uptake. However, preincubation of the basolateral membrane vesicles with ouabain for 45 min or longer causes a significant decrease in the Na^+-induced loss of Ca^{2+} from the vesicles (Fig. 1 and Table I). This effect of ouabain points to a role of the Na^+-pump in the observed Na^+ inhibition of ATP-dependent Ca^{2+} uptake. The presence of Na^+, K^+, ATP and Mg^{2+} in the Ca^{2+} uptake assay provides optimal conditions for activating ($Na^+ + K^+$)-ATPase in the basolateral membrane. Since prolonged preincubation with ouabain is needed, it is clear that ouabain exerts its effect inside the vesicles. Therefore, it is likely

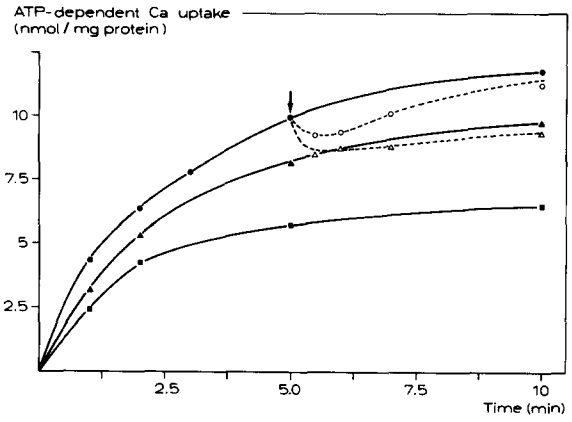

Fig. 1. Effects of Na^+ on the ATP-dependent Ca^{2+} accumulation in basolateral membrane vesicles of rat duodenum. ATP-dependent Ca^{2+} uptake is assayed, as described previously [4] at 1 μM Ca^{2+}. The Na^+ concentration is 75 mM. Ouabain effects are determined after preincubation of the vesicles in 2 mg/ml ouabain for 45 min on ice. The arrow indicates that in some experiments after 5 min of Ca^{2+} accumulation, either 75 mM KCl (○) or 75 mM NaCl (△) is added to the uptake medium in the form of 2 M solutions. ●, K^+; ▲, Na^+ + ouabain; ■, Na^+.

TABLE I

EFFECTS OF OUABAIN, MONENSIN AND IONOPHORE A23187 ON Na^+-INDUCED INHIBITION OF ATP-DEPENDENT Ca^{2+} UPTAKE IN BASOLATERAL MEMBRANE VESICLES FROM DUODENUM

Effects are shown on the initial rate of Ca^{2+}-accumulation and are expressed as percentages of the control value (4.61 ± 0.42 nmol Ca^{2+}/min per mg protein, $n = 5$). When ouabain effects are determined, vesicles are preincubated with 2 mg/ml ouabain for 45 min on ice. Values are given as means $\pm$S.E., with the number of experiments in parentheses.

	No addition	+2 μM monensin	+10 μg/ml A23187
Control	100	98.8 ± 6.5 (4)	22.6 ± 1.1 (4)
+ 100 mM Na^+	62.4 ± 5.1 (5)	74.6 ± 4.3 (9)	14.8 ± 0.8 (4)
+ 100 mM Na^+ + 2 mg/ml ouabain	75.7 ± 4.3 (7)	79.7 ± 2.9 (6)	22.0 ± 2.2 (4)

that $(Na^+ + K^+)$-ATPase accumulates Na^+ inside the vesicles and that a high Na^+ concentration on the inside displaces Ca^{2+} from intravesicular binding sites. Ouabain, by inhibiting the Na^+ pump, prevents Na^+ accumulation and thereby Ca^{2+} displacement. A critical test for this explanation will be to make the vesicles leaky for Na^+ by means of an Na^+ ionophore. The results of such experiments are shown in Table I. This table shows clearly that the effect of Na^+ on ATP-dependent Ca^{2+} uptake is reduced by monensin to the same extent as preincubation with ouabain. That there is intravesicular binding of Ca^{2+} and displacement of bound Ca^{2+} by high inside Na^+ is also suggested by the effects of ionophore A23187 on ATP-dependent Ca^{2+} uptake. In Table I it is shown that ATP-dependent Ca^{2+} uptake is not completely abolished by ionophore A23187. In the presence of Na^+ there is a slight increase in the loss of Ca^{2+} induced by ionophore A23187. Also, this Na^+ effect is abolished by preincubation with ouabain, most likely by preventing Na^+ accumulation.

Addition of 75 mM Na^+ or K^+ to ouabain-preincubated membrane vesicles, actively loaded with Ca^{2+} for 5 min, causes an initial rapid efflux of Ca^{2+} from the vesicles (Fig. 1). The Ca^{2+} efflux induced by Na^+ is more rapid and the plateau phase is the same as observed when Na^+ is present from the beginning (Fig. 1). The Ca^{2+} leak induced by addition of K^+ appears to be restored within a few minutes. This KCL-induced leak is probably an osmotic effect.

The effects of various Na^+ concentrations (10–100 mM) on the rate of ATP-dependent Ca^{2+} accumulation in ouabain-pretreated vesicles are shown in Fig. 2. At 10 mM Na^+ no decrease in Ca^{2+} load is observed, while between 15 and 20 mM Na^+, a sharp rise in Ca^{2+} efflux out of the vesicles is observed. A plateau is reached between 50 and 100 mM Na^+, and about 15–20% of the accumulated Ca^{2+} is lost. The sigmoidal relationship between Na^+ concentration and inhibition of ATP-induced Ca^{2+} uptake suggests a cooperative interaction between transport of two or more sodium ions with one calcium ion. The affinity for Na^+ of the Na^+/Ca^{2+} exchanger is between 15 and 20 mM.

Na^+ gradient-dependent Ca^{2+} uptake

The best way to demonstrate the presence of Na^+/Ca^{2+} exchange, are experiments in which it

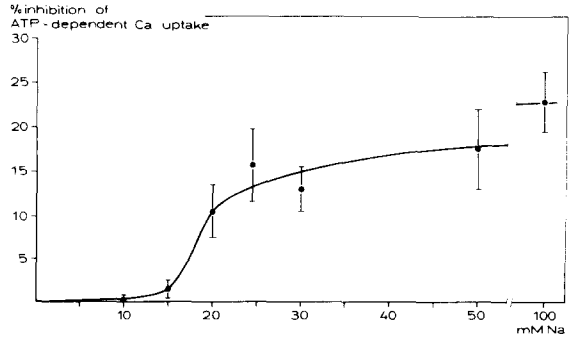

Fig. 2. Concentration dependence of Na^+ inhibition of ATP-induced Ca^{2+} uptake in duodenal basolateral membranes. Ca^{2+} uptake is determined in vesicles pretreated with ouabain (see Fig. 1). Results are expressed as percent inhibition of ATP-dependent Ca^{2+} uptake after 1 min incubation in the presence of Na^+. Mean values are given $\pm$S.E. for four experiments.

is shown directly that a Na^+ gradient across the vesicle membrane $[Na^+]_i > [Na^+]_o$, accumulates Ca^{2+} in the absence of ATP. For these experiments, vesicles are passively loaded with 150 mM NaCl and diluted 25-fold in an equimolar NaCl or KCl solution containing 50 μM free Ca^{2+}. The time course of Ca^{2+} uptake into duodenal basolateral membrane vesicles in the presence and absence of a Na^+ gradient is shown in Fig. 3. The difference in Ca^{2+} uptake between the two conditions is very small. After 15 s incubation this difference is both maximal and significant ($P < 0.01$). Upon longer incubation times the difference between the two curves is slowly decreasing. If the extra Ca^{2+} uptake in the presence of an Na^+ gradient is due to Na^+/Ca^{2+} exchange, it should decrease in the presence of an Na^+ ionophore and increase in the presence of an inside positive electrical potential. In Table II it is shown that Ca^{2+} uptake in the presence of an Na^+ gradient can be stimulated by an inwardly-directed K^+ diffusion potential, generated by addition of valinomycin and K^+ to Na^+-loaded vesicles. This valinomycin effect indicates that there is charge separation during Na^+/Ca^{2+} exchange as demonstrated in squid axon [23] and in cardiac sarcolemma [8]. In Table II it is also shown that the difference in Ca^{2+} uptake disappears when the vesicles are incubated with monensin. This effect of monensin strongly suggests that the extra Ca^{2+} uptake, albeit small, is driven by an Na^+ gradient.

In order to get information of the segmental

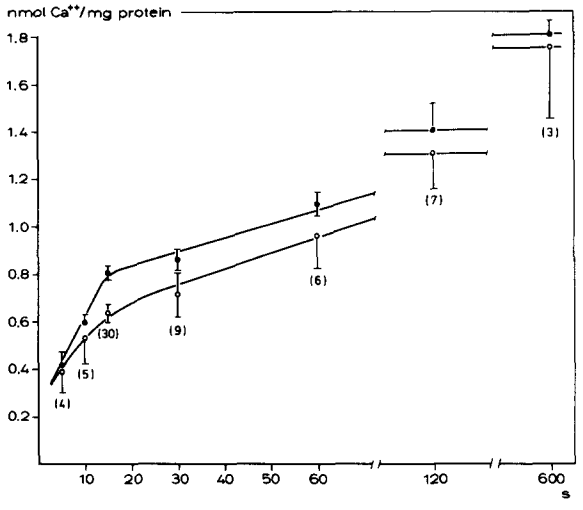

Fig. 3. Effect of a Na^+ gradient on Ca^{2+} uptake in basolateral membranes from rat duodenum in the absence of ATP. Membrane vesicles were passively loaded with 150 mM NaCl and diluted 25-fold into equimolar NaCl (Na_i^+/Na_o^+) (○) or KCl medium (Na_i^+/K_o^+) (●). In all experiments membrane vesicles were preincubated with 10 μg/ml valinomycin. The free Ca^{2+} concentration was 50 μM.

distribution of Na^+/Ca^{2+}-exchange capacity in the small intestine, we studied Na^+-induced Ca^{2+} uptake into basolateral membrane vesicles from duodenum, mid jejunum and terminal ileum, under conditions which create a favourable electrochemical driving force for Na^+ (25 × dilution into KCl solutions, presence of valinomycin, 15 s incubations and 50 μM free Ca^{2+}). The mean values

TABLE II

EFFECT OF MONENSIN AND VALINOMYCIN ON Ca^{2+} UPTAKE IN BASOLATERAL MEMBRANES OF RAT DUODENUM

Basolateral vesicles were passively loaded with Na^+ and diluted 25-fold in equimolar NaCl (Na_i^+/Na_o^+) or KCl medium (Na_i^+/K_o^+). Membranes were preincubated with 10 μl/ml valinomycin (Na_i^+/K_o^+ + val) or with valinomycin and 10 μM monensin (Na_i^+/K_o^+ + val + mon). The free Ca^{2+} concentration was 50 μM. Normalized relative increase means that the Ca^{2+} uptake in the Na_i^+/Na_o^+ condition is taken as 100% and the increase is expressed per experiment as percentage of the control value.

	Na_i^+/Na_o^+	Na_i^+/K_o^+	Na_i^+/K_o^+ + val	Na_i^+/K_o^+ + val + mon
Ca^{2+} uptake (nmol Ca^{2+}/mg protein per 15 s)	0.52 ± 0.05	0.59 ± 0.06	0.68 ± 0.07	0.48 ± 0.06 (n = 7)
Normalized relative increase (%)	100	113.2 ± 3.8 ($P < 0.05$)	128.8 ± 5.8 ($P < 0.01$)	103.8 ± 7.7 ($P > 0.2$)

TABLE III

EFFECTS OF $1\alpha,25(OH)_2D_3$ ON ATP-DEPENDENT Ca^{2+} UPTAKE AND Na^+/Ca^{2+} EXCHANGE

The data given represent mean values ± S.E., with number of observations in parentheses. Each observation is made on pooled intestinal segments of at least two deficient (−D) or repleted (+D) rats. ATP-dependent Ca^{2+} uptake has been corrected for ATP-independent Ca^{2+} uptake. Inhibition by Na^+ of ATP-dependent Ca^{2+} uptake was measured after 1 h preincubation with 2 mg/ml ouabain. Na^+-gradient induced Ca^{2+} uptake is the difference between Ca^{2+} uptake after 15 s incubation under gradient (Na_i^+/K_o^+ + val) and non-gradient (Na_i^+/Na_o^+ + val) conditions.

	Duodenum		Jejunum		Ileum	
	−D	+D	−D	+D	−D	+D
ATP-dependent Ca^{2+} uptake (nmol Ca^{2+}/min per mg prot)	2.45 ± 0.13	4.65 ± 0.15 ($n = 15$)	1.09 ± 0.16	0.84 ± 0.10 ($n = 6$)	0.28 ± 0.08	0.48 ± 0.10 ($n = 6$)
% inhibition of ATP-dependent Ca^{2+} uptake by 75 mM Na^+	22 ± 3	17 ± 3 ($n = 4$)	48 ± 4	37 ± 6 ($n = 4$)	45 ± 5	35 ± 4 ($n = 3$)
Na^+ gradient-induced Ca^{2+} uptake (nmol Ca^{2+}/15 s per mg protein)	0.18 ± 0.03	0.22 ± 0.04 ($n = 4$)	0.21 ± 0.03	0.17 ± 0.04 ($n = 4$)	0.18 ± 0.03	0.15 ± 0.03 ($n = 3$)

of four experiments in triplicates were 0.19 ± 0.03, 0.22 ± 0.05 and 0.17 ± 0.03 nmol Ca^{2+}/15 s per mg protein, respectively. Since these values are not significantly different ($P > 0.3$), this experiment implies that the capacity of the Na^+/Ca^{2+} exchanger is constant along the small intestine. This is in sharp contrast with the segmental distribution of ATP-dependent Ca^{2+} transport, the activity of which is highest in duodenum and decreases towards the ileum (Tables III and V).

So far, the Na^+-driven Ca^{2+} uptake was measured at 50 μM free Ca^{2+}, but a physiological role for this carrier requires an affinity for Ca^{2+} around 1 μM or lower. Therefore, we tried to determine the affinity of the Na^+/Ca^{2+} exchange system for Ca^{2+}. Since the differences between the uptakes under gradient and non-gradient conditions are small, we have concentrated our efforts on four Ca^{2+} concentrations: 0.1, 1, 10 and 50 μM. The Na^+ induced Ca^{2+} uptake at these concentrations are 0.03 ± 0.01, 0.08 ± 0.01, 0.14 ± 0.02 and 0.13 ± 0.02 nmol Ca^{2+}/15 s per mg protein (mean values of seven experiments run in triplicate in the presence of valinomycin). At 1.0 μM Ca^{2+}, the Na^+ gradient-dependent uptake amounts to 65% of the uptake reached at 50 μM Ca^{2+}. Although this kinetic analysis is hardly quantitative, the data suggest an affinity for Ca^{2+} below 1 μM.

Effects of vitamin D deficiency

In an earlier report we demonstrated that the maximal activity of ATP-dependent Ca^{2+} transport and Ca^{2+}-ATPase is duodenal basolateral membranes was 50% reduced in vitamin D deficient rats. Repletion with $1\alpha,25(OH)_2D_3$ restored Ca^{2+}-ATPase activity to normal levels [18]. Therefore, it is of interest to study the effect of vitamin D deficiency on the Na^+/Ca^{2+}-exchange capacity of basolateral membranes. In Table III the results are summarized. Repletion with $1\alpha,25(OH)_2D_3$ of vitamin D-deficient rats resulted in a 90% increase in ATP-dependent Ca^{2+} transport in duodenal and ileal basolateral membrane vesicles, as reported previously [18]. The inhibitory effect of Na^+ on ATP-dependent Ca^{2+} transport and the Na^+ gradient-dependent Ca^{2+} transport were not influenced by the vitamin D status of the animals. These results suggest strongly that the Na^+/Ca^{2+}-exchange capacity of basolateral membranes of rat enterocytes is not regulated by $1\alpha,25(OH)_2D_3$.

Comparison of transport capacities of Na^+/Ca^{2+} exchange and ATP-dependent Ca^{2+} uptake along the small intestinal tract

In order to compare transport capacities of Na^+/Ca^{2+} exchange and ATP-dependent Ca^{2+} uptake in basolateral membrane vesicles, we need information on the orientation of the vesicle population. Only vesicles with an inside-out orientation (ATP-binding site on the outside) and sealed for Ca^{2+} will contribute to ATP-dependent uptake, whereas all vesicles sealed for Ca^{2+} (inside-out and outside-out orientation) will contribute to Na^+ gradient-dependent Ca^{2+} uptake, since the Na^+/Ca^{2+} exchanger has been shown to be a symmetrical carrier, at least in heart sarcolemma [24]. Previously, we have shown that Triton X-100, alamethacin and an osmotic shock appeared to be the most reliable tools to unmask latency in ($Na^+ + K^+$)-ATPase activity [4,20]. From the stimulation of ($Na^+ + K^+$)-ATPase activity, it was calculated that 40% of the vesicles were resealed [4]. However, these studies have been done at 37°C and it turned out that at 25°C, the temperature of the Ca^{2+}-uptake studies, more vesicles are resealed. In Table IV, the results are given of preincubation at 25°C with Triton X-100 and alamethacin on the latency of ($Na^+ + K^+$)-ATPase and Ca^{2+}-ATPase in freshly prepared basolateral

TABLE IV

EFFECTS OF TRITON X-100 AND ALAMETHACIN ON THE LATENCY OF ($Na^+ + K^+$)-ATPase AND Ca^{2+}-ATPase ACTIVITIES IN BASOLATERAL MEMBRANE VESICLES FROM DUODENUM

Mean values ±S.E., with the number of experiments in parentheses. Membrane vesicles are preincubated with Triton X-100 or alamethacin for 10–15 min at 25°C. Pretreated membranes are diluted 20–25-fold into the assay mixture and incubated for 15 min at 25°C.

Treatment	Enzyme activity (μmol P_i/h per mg protein)	
	($Na^+ + K^+$) ATPase	Ca^{2+}-ATPase
Control	8.5 ± 0.7 (10)	1.07 ± 0.05 (6)
Triton X-100 (0.5 mg/mg protein)	23.9 ± 1.3 (10)	1.37 ± 0.30 (3)
Alamethacin (0.5 mg/mg protein)	23.6 ± 6.7 (3)	1.57 ± 0.04 (3)

TABLE V

SPECIFIC ACTIVITY OF ATP-DEPENDENT Ca^{2+} TRANSPORT AND OF Na^+/Ca^{2+} EXCHANGE IN THREE SMALL INTESTINAL SEGMENTS

The specific activity of ATP-dependent Ca^{2+} uptake is measured at 1 μM free Ca^{2+} (Table III), while Na^+/Ca^{2+} exchange is measured at 50 μM free Ca^{2+} (Fig. 4, Table III). Both activities have been corrected for the orientation of the vesicle population as explained in the text. Na^+/Ca^{2+}-exchange capacity is measured directly with Na^+ gradient-dependent uptake studies, and indirectly from the inhibition of ATP-dependent Ca^{2+} uptake by 75 mM Na^+ (Table III). Specific activities in nmol Ca^{2+}/min per mg protein.

	ATP-dependent Ca^{2+} transport	Na^+/Ca^{2+} exchange	
		Direct	Indirect
Duodenum	14.0 ± 0.5 (15)	1.2 ± 0.2 (8)	2.4 ± 0.1 (4)
Jejunum	2.5 ± 0.3 (6)	1.2 ± 0.2 (8)	1.2 ± 0.1 (4)
Ileum	1.4 ± 0.2 (6)	1.0 ± 0.2 (8)	0.6 ± 0.1 (3)

membrane vesicles from rat duodenum. Both alamethacin and Triton X-100 increase ($Na^+ + K^+$)-ATPase activity to the same extent, indicating that 65% of the vesicles are sealed for ATP or ouabain. Triton X-100 does not increase significantly Ca^{2+}-ATPase activity ($P > 0.4$), whereas alamethacin stimulates Ca^{2+}-ATPase activity by 50% ($P < 0.05$). The absence of an effect of Triton X-100 may be due to solubilization of the enzyme, as described recently by Scharff [25] for erythrocyte Ca^{2+}-ATPase. The stimulation of Ca^{2+}-ATPase by alamethacin suggests that 32% of the vesicles have an outside-out orientation, when 35% of the vesicles are leaky for ATP. It seems reasonable to conclude that one-third of the vesicles are leaky, one-third is inside-out and one-third is outside-out or right-side-out oriented. Therefore, the ATP-dependent Ca^{2+}-uptake represents one third of the total activity, while the Na^+/Ca^{2+} exchange represents two-thirds of the total. In Table V we have summarized the specific activity of ATP-dependent Ca^{2+} uptake and of Na^+/Ca^{2+} exchange in the three intestinal segments, after correction for the orientation of the vesicles. It is clear that ATP-dependent Ca^{2+}-transport greatly exceeds Na^+/Ca^{2+} exchange in duodenum but that in ileum both activities are of equal potency.

Discussion

The Na^+ gradient-dependent Ca^{2+} uptake together with the inhibition of ATP-dependent Ca^{2+} accumulation by sodium ions provides evidence for the presence of an Na^+/Ca^{2+} exchange system in basolateral plasma membranes of rat small intestinal epithelium.

Hildmann et al. [5] reported 45% inhibition of ATP-dependent Ca^{2+} transport by sodium ions in basolateral membrane vesicles from rat duodenum and jejunum. These authors did not observe an effect of ouabain, and ascribed the Na^+ inhibition to Ca^{2+} leakage via the Na^+/Ca^{2+} exchanger. We observed the effect of ouabain only after prolonged incubation, which means that ouabain has to be present on the inside of the vesicles in order to inhibit Na^+ accumulation in inside-out oriented vesicles. The similarity between the ouabain and monensin effects on Na^+ inhibition of ATP-dependent Ca^{2+} uptake, indicate that when Na^+ is accumulated by the Na^+ pump there will be competition between high Na^+ and Ca^{2+} for intravesicular binding sites. Therefore, it is obvious that no firm conclusions can be drawn from Na^+ effects on ATP-dependent Ca^{2+} uptake with regard to the capacity of the Na^+/Ca^{2+} exchange mechanism. A similar conclusion was reached for Na^+ effects on Ca^{2+} accumulation in rat myometrium membrane vesicles [26]. An explanation for the higher inhibition of ATP-dependent Ca^{2+} uptake by Na^+, reported by Hildmann et al. [5], and the absence of an effect of ouabain, could be that these authors used duodenum and jejunum as a source for membrane vesicles. In view of the steep fall in ATP-dependent Ca^{2+} pumping capacity further down in the intestinal tract, Na^+/Ca^{2+} exchange becomes relatively more important, and

therefore Na^+ effects on ATP-dependent Ca^{2+} uptake differ with the location in the intestine (see Tables III and V). Also, the competition between Na^+ and Ca^{2+} on the inside of duodenal vesicles may be more pronounced proximally than further down in the small intestine.

More direct evidence for the presence of Na^+/Ca^+ exchange came from Ca^{2+}-uptake studies in the absence of ATP but in the presence of an Na^+ gradient. The initial Na^+ gradient, present upon dilution of the Na^+-loaded vesicles into a KCl solution, will dissipate rather quickly, but the large initial Na^+ gradient ($Na_i^+ = 25\ Na_o^+$) and the presence of a favourable membrane potential should guarantee a physiologically relevant Na^+ gradient at least the first 15 s after starting the experiment. This is what we can conclude from Fig. 3. The Na^+ gradient-dependent Ca^{2+} uptake is maximal around 15 s and does not further increase upon longer incubations probably due to dissipation of the Na^+ gradient. With heart sarcolemma vesicles, accurate values for initial rates of the Na/Ca^{2+} exchanger could be obtained by several investigators with a continuous monitoring assay using Arsenazo III [8,27]. Applying this technique to basolateral membrane vesicles did not work, which is not suprising when we realize that the Na^+/Ca^{2+}-exchange capacity in heart sarcolemma is three orders of magnitude greater than the one in small intestine [1,8,27]. In spite of the much lower activity we find comparable Na^+ and Ca^{2+} affinities for the intestinal Na^+/Ca^{2+} exchanger as reported for heart sarcolemma. From Na^+ inhibition of ATP-dependent Ca^{2+} transport, we arrived at an affinity for Na^+ between 15 and 20 mM, which is close to the value of 22 mM, reported by Reeves et al. [28] from similar experiments with heart sarcolemma. We find an affinity for Ca^{2+} below 1 μM, while in heart sarcolemma this value is in the order of 1.5 μM [1,8]. Whether this difference is real cannot be concluded from our rather qualitative approach, but during excitation of heart muscle the free cytosolic Ca^{2+} concentration is undoubtedly higher than in small intestinal cells, hence the affinity for Ca^{2+} of Na^+/Ca^{2+} exchange in heart plasmalemma may be less.

We have compared the activities of ATP-dependent Ca^{2+} uptake and Na^+/Ca^{2+} exchange in different intestinal segments after a correction for the fact that different vesicle populations are involved in these activities (Table V). With one-third of the vesicles inside-out and one-third outside-out oriented and one-third leaky we can calculate, from the Na^+ effect on ATP-dependent Ca^{2+} uptake, that the Na^+/Ca^{2+}-exchange capacity is about 2 nmol Ca^{2+}/min per mg protein in duodenal membranes. This value represents an upper limit in view of the possible influence of Na^+ upon displacing Ca^{2+} from intravesicular binding sites. From the Na^+ gradient-dependent Ca^{2+} uptake we calculate for this exchanger an activity of about 1 nmol Ca^{2+}/min per mg protein, which may be an underestimation due to the rapidly dissipating Na^+ gradient. Since both methods give more or less equal activities for the Na^+/Ca^{2+} exchanger, we can be sure that the total activity in duodenum is between 1 and 2 nmol Ca^{2+}/min per mg protein, which is about 10% of the activity of the ATP-driven Ca^{2+} pump. In view of the finding that the Na^+/Ca^{2+}-exchange activity is constant along the intestinal tract, this exchange mechanism becomes important in the extrusion of Ca^{2+} out of enterocytes in more distal intestinal segments. This view is supported by studies on intact intestinal epithelium in which it was shown that sodium ions in the serosal solution are required for net Ca^{2+} fluxes across the ileum [15,29], but that Na^+ is not required for net Ca^2 transport across the duodenum [30].

Our observation that the vitamin D status of the animal has no influence on the activity of Na^+/Ca^{2+} exchange, makes the effect of $1\alpha,25(OH)_2D_3$ on Ca^{2+}-ATPase rather specific, since previously we reported that this hormone did not change the $(NA^+ + K^+)$-ATPase activity either [18].

In conclusion, the presence of an Na^+/Ca^{2+}-exchange system in basolateral membranes of rat small intestine is demonstrated, and the segmental distribution of this system suggests an increasing importance in Ca^{2+} extrusion across basolateral membranes from duodenum to ileum.

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