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Ca^{++}-Transport across Basal-Lateral Plasma Membranes from Rat Small Intestinal Epithelial Cells

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Summary. Basal-lateral plasma membrane vesicles were isolated from rat duodenum and jejunum by a Percoll gradient centrifugation technique. Ca-uptake into and Ca-release from the vesicles was studied by a rapid filtration method. In the absence of Na (K-medium) at a Ca concentration of 0.05 mmol/liter and pH 7.4, addition of 5 mM MgATP stimulated Ca-uptake up to 10-fold as compared to a control without ATP. Since the Ca-ionophore A23187 (2 μg/ml) prevented the accumulation of Ca above the equilibrium uptake and rapidly released Ca accumulated by the vesicles in the presence of ATP, it is concluded that the ATP-dependent uptake of Ca involves accumulation of Ca inside the vesicles. The ATP-driven Ca-transport comigrates with the (Na +K)-ATPase and dissociates from the marker enzymes for mitochondrial inner membrane, endoplasmic reticulum and brush border membrane. It is not inhibited by 1 μg/ml oligomicin or 0.1 mmol/liter ruthenium red. Replacing K by Na inhibits ATP-dependent Ca-uptake by 60%. Efflux of Ca from passively preloaded vesicles is strongly temperature sensitive and enhanced by A23187. An inwardly directed Na-gradient stimulates Ca-efflux as compared to a K-gradient. Addition of gramicidin reduces the Na-stimulation of Ca-efflux, indicating direct coupling of Na and Ca fluxes across basal-lateral membranes. The results suggest that basal-lateral membranes possess two distinct mechanisms for Ca-transport: *a)* ATP-driven Ca-transport and *b)* Na/Ca-exchange.

Key words calcium-transport · small intestine · vesicles Ca^{++}-ATPase · Na^+/Ca^{++}-exchange · basal-lateral membranes

Introduction

Net absorbtion as well as secretion of calcium ions (Ca) may occur in the small and large intestine (Nellans & Kimberg, 1979; Lee, Walling, Gafter, Silis & Coburn 1980). Active transport occurs transcellularly in the absorptive direction. Ca absorption is regulated according to the demands of the body. In the intestine, 1,25-dihydroxy-cholecalciferol is the most important effector of the endocrine system involved in the regulation of calcium metabolism (Norman, 1979). In the rat small intestine, *in vitro*, the absorptive flux of Ca is largest in the duodenum and decreases over the jejunum to the ileum (Walling, 1977). Because of the electrical potential profile (cell interior negative to both the mucosal and serosal compartment) across the epithelial cell layer, and the Ca-activities prevailing in the intracellular and extracellular fluid – estimated to be <1 μmol/-liter and >1 mmol/liter, respectively – active steps of Ca-transport have to be expected in the basal-lateral plasma membrane of the enterocyte.

Suggestions for the molecular mechanisms involved in the active extrusion of Ca from the cell have been a Na/Ca-exchange system (Martin & De Luca, 1969) – on the basis of the Na-dependence of serosal Ca-efflux from the epithelium – and a calcium-dependent ATPase (Birge & Gilbert, 1974; Mircheff & Wright, 1976). These concepts are in agreement with Ca-transport mechanisms which have been characterized in a variety of other plasma membranes (Carafoli & Crompton, 1978). The Na-dependence of transepithelial Ca-transport has also been explained by the suggestion of a Ca-ATPase additionally stimulated by Na in the basal-lateral membrane (Birge & Gilbert, 1974). The evidence for the Na/Ca-exchange system (Martin & De Luca, 1969) had been subject to criticism because of possible interference from subepithelial tissue (De Luca, 1979). The evidence for the Ca-ATPase was criticized because of the high Ca-concentrations used in the studies, which were far from the Ca-concentration in the micromolar range (Ghijsen & van Os, 1979).

The development of a rapid isolation method yielding a relatively tight fraction of basal-lateral plasma membranes containing in part inside-out oriented vesicles (Scalera, Storelli, Storelli-Joss, Haase & Murer, 1980), allowed us to study ATP-driven Ca-transport across these membranes. Transport studies using tracer calcium suggest the exis-

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tence of two different mechanisms in isolated basal-lateral membranes: *a)* ATP-driven Ca-transport and *b)* Na/Ca-exchange.

Materials and Methods

Isolation of Basal-Lateral Plasma Membranes

Basal-lateral plasma membranes were isolated from rat (Sprague Dawley, 190–210 g) duodenum and jejunum according to Scalera et al. (1980) with some modifications. For each batch of Percoll (Pharmacia, Uppsala, Sweden), a crude plasma membrane fraction was centrifuged in concentrations of Percoll ranging from 10 to 12% for 30 to 60 min. In such preliminary experiments gradients were selected by eye for fractionation with the criterion that two bands had clearly separated: one in the upper third of the tube and one in the lower fourth of the tube. These gradients were fractionated into 50 to 60 fractions and assayed for the distribution of protein and (Na+K)-ATPase, and in some cases of leucine-amino-peptidase, succinate: cytochrome *c*-oxidoreductase and KCN-resistant NADH-oxidoreductase. The conditions leading to migration of the (Na+K)-ATPase to the middle third of the gradient and to clear separation from the upper and lower protein bands containing contaminating enzyme activites, were chosen for preparative purposes. The basal-lateral membrane fraction was defined by the volume which had to be pumped out of the tubes from above the band and containing the band. These fractions from 3 to 4 gradients were each filled up to 35 ml with the buffer appropriate for the uptake experiment and centrifuged at 48,000 × g for 90 min. The membranes floating on the glassy Percoll pellet were collected with a syringe and suspended in the appropriate amount of buffer needed for the uptake experiment. The purity of the basal-lateral membrane fractions was controlled by enzyme assays with frozen samples. The average enrichment of (Na+K)-ATPase was 12. Experiments with an enrichment below 9 were disregarded.

Enzyme Assays

(Na+K)-ATPase, KCN-resistant NADH: (acceptor)-oxidoreductase, succinate: cytochrome *c*-oxidoreductase and leucine-aminopeptidase were measured as by Scalera et al. (1980). Protein was measured by a modified Lowry procedure (Scalera et al., 1980), except for the assay of protein distribution in the Percoll gradient fractions; in this case, the Bio-Rad protein assay, No. 500-0006 was used (Bio-Rad-Laboratories, Munich, Germany).

Transport Studies

The final basal-lateral membrane pellet was resuspended in a buffer solution containing 300 mmol/liter mannitol, 100 mmol/liter KCl, 5 mmol/liter $MgCl_2$ and 20 mmol/liter HEPES, adjusted to pH 7.4 by Tris. For some experiments KCl was replaced by choline chloride. Incubations were carried out at 25 °C, except where otherwise stated. For the assay of uptake, Ca concentrations of 50 to 100 µmol/liter were used. 20 to 40 µl of membrane suspension were added to 100 to 240 µl of incubation medium, containing the same buffer as the suspension medium and the additions mentioned in the legends of the Figures. Ionophores were added to the membrane suspension at the concentrations given with the individual experiments. At given times, 20 µl of the incubation medium were pipetted into 1 ml of ice-cold stop solution containing 1 mmol/1liter EGTA and otherwise the same solutes as the suspension solution. The stop solution was then filtered through a cellulose-nitrate membrane filter (Sartorius, Göttingen, Germany; 0.65 µm pore size) which was then immediately rinsed with additional 6 ml of stop solution. In efflux experiments membrane vesicles were equilibrated with 100 µmol/liter $^{45}CaCl_2$ by incubation for 1 hr at 25 °C either in the usual suspension buffer or the buffer with choline chloride substituting for KCl. Efflux was started by adding 10 µl of 20 mmol/liter EGTA to 130 µl of preincubated membrane. More details are given in the legends of Figs. 6 and 7. The efflux was terminated in the same way as in the uptake experiments. The protein concentration during incubation was 0.8–1.7 mg/ml. Radioactivity retained on the filters was measured by liquid scintillation.

Single experiments will be presented throughout the paper. All experiments were repeated at least 3 times with qualitatively identical results. Within the same experiments all uptake measurements are carried out in duplicate. The experimental scatter among the individual values of the duplicates was around 5%. However, the absolute equilibrium uptake values varied by a factor of 3 from membrane preparation to membrane preparation. A possible explanation for this variation could be a difference in the sealing and/or orientation of the vesicle preparation. As the qualitative conclusions of the present paper are always based on findings obtained within the same experiment, the experimental scatter does not represent a major problem in evaluating the data obtained in this study. However, a statistical evaluation of our data based on the averaging of several experiments was not possible, for such a procedure necessarily implies normalization of all the data, e.g. by referring the different values to the control curve obtained in the same experiment.

Materials

$^{45}CaCl_2$ was obtained from NEN (Dreieich, Germany, NEZ 0-13) at a specific activity from 10 to 42 mCi/mg. ATP (Mg-salt, No. A-0770), CTP (Tris-salt, No. C-1756), ADP (Tris-salt, A-9021), EGTA (No. E-4378), oligomycin (No. 04876) and ruthenium red (No. R-2751) were from Sigma (Munich, Germany). Adenylyl (β-γ-methylen)-diphosphate (AMP–PCP, tetralithium-salt No. 102555), Gramicidin D (No. 106160), valinomycin (No. 161608) and ouabain (No. 109363) were from Boehringer (Mannheim, Germany). A-23187 was from Calibiochem (Giessen, Germany). HEPES was from SERVA (Heidelberg, Germany). Tris and other chemicals were from Merck (Darmstadt, Germany).

Results and Discussion

ATP-Driven Ca-Transport across Basal-Lateral Membranes of Rat Small Intestine

Basic observations. ATP-driven Ca-transport was established by experiments, in which basal-lateral plasma membrane vesicles were incubated with and without ATP, and with ATP after addition of the Ca-ionophore A23187 to the membrane suspension (Fig. 1). In the presence of ATP the initial Ca-uptake rate (15 sec time point) at 50 µmol/liter Ca was two- to fivefold that observed without ATP. Without ATP the Ca-uptake reached its equilibrium between 4 and 10 min, which amounted to 300 to 800 pmol/mg of protein in different preparations. With ATP added, Ca-uptake increased up to 10-fold

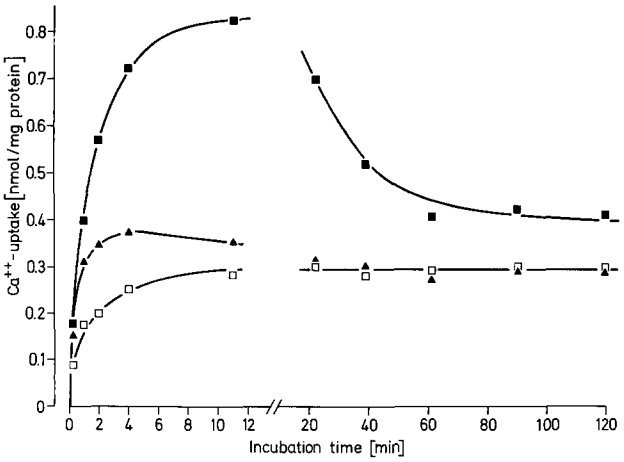

Fig. 1. ATP-driven transport of Ca^{++} into rat small intestinal basal-lateral membrane vesicles. The membranes were suspended in 300 mmol/liter mannitol, 20 mmol/liter HEPES, adjusted to pH 7.4 by Tris, 100 mmol/liter KCl, 5 mmol/liter $MgCl_2$. The incubation medium contained the same solutes and, in addition, 1 µg/ml oligomycin, 2 mmol/liter ouabain, 0.05 mmol/liter $CaCl_2$ and 5 mmol/liter Mg-ATP (■, ▲) or no ATP (□). In one condition A23187 was added to a concentration of 2 µg/ml to the membrane suspension (▲)

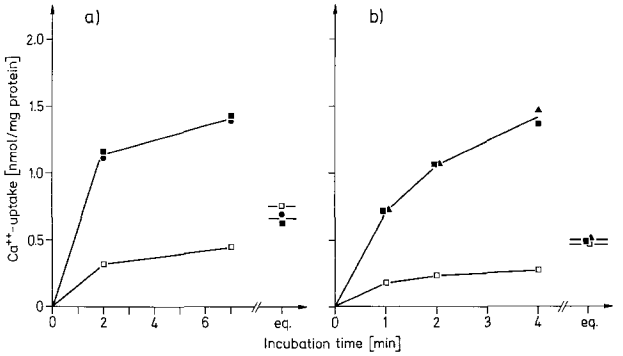

Fig. 2. Lack of inhibition of ATP-driven Ca^{++}-uptake into rat small intestinal basal-lateral plasma membrane vesicles by inhibitors of mitochondrial Ca^{++}-transport. *a*) Oligomycin (1 µg/ml (●); *b*) ruthenium red (0.1 mmol/liter) (▲). 20 µl of membrane suspension in 300 mmol/liter mannitol, 20 mmol/liter HEPES/Tris, pH 7.4, 100 mmol/liter KCl, 5 mmol/liter $MgCl_2$ were added to 80 µl (*a*) or 100 µl (*b*) of incubation medium containing in addition 100 µmol/liter $CaCl_2$ (*a*) or 70 µmol/liter $CaCl_2$ (*b*) and 5 mmol/liter Mg-ATP (■), 5 mmol/liter Mg-ATP + 1 µg/ml oligomycin (●), 5 mmol/liter Mg-ATP + 0.1 mmol/liter ruthenium red (▲) or no nucleotide (□). 20 µl samples were taken at the time points indicated. For the assay of equilibrium uptake (*eq.*) 2 µg/ml A23187 was added to the media and samples taken after a minimum of 20 min

above the equilibrium level, reaching a plateau after 10 to 15 min of incubation. When the protein concentration during incubation was high, the vesicles released Ca during the first hour of incubation. This release started during the period, in which complete hydrolysis of ATP could be expected from the values for specific activity of Mg-ATPase and (Ca + Mg)-ATPase given by Ghijsen and van Os (1979) and Ghijsen, De Jong and van Os (1980). In the experiment of Fig. 1, this was after 10 to 15 min. When 2 µg/ml of ionophore A23187 had been added to the membrane suspension before incubation, the initial uptake rate of Ca was also increased as compared to the condition without ATP. A slight accumulation above equilibrium uptake was still observed, which decreased to equilibrium uptake within 20 min. Also, when A23187 was added to a suspension of membrane vesicles during the plateau phase which had taken up Ca in the presence of ATP, Ca was released to a Ca-content near the equilibrium uptake (*data not shown*).

Insensitivity of ATP-Driven-Ca-Transport of Basal-Lateral Plasma Membranes to Mitochondrial Inhibitors. Mitochondrial ATP-dependent uptake of Ca is known to be inhibited by oligomycin (1 µg/ml) and ruthenium red (0.1 mmol/liter). These inhibitors have been tested in our system. The results of Fig. 2 show that these inhibitors have no influence on ATP-driven Ca-transport in basal-lateral membranes. Thus, the properties of this plasma membrane Ca-transport system are different from the mitochondrial ones, as it has been shown to the level of reaction mechanism in red blood cell plasma membranes (Sarkadi, 1980). Together with the observation that in fractions of the Percoll gradient below the basal-lateral membrane fraction, which contain more mitochondrial marker enzyme activity, oligomycin at the same concentration does inhibit ATP-dependent Ca-uptake (*data not shown*), these results provide evidence that the ATP-effect observed is not attributable to mitochondrial contamination. In order to be on the safe side with respect to mitochondrial contamination, 1 µg/ml oligomycin was included into the incubation media of the other experiments on ATP-dependent Ca^{++}-transport.

Distribution of ATP-Driven Ca-Uptake in Fractions of the Percoll Gradient. ATP-driven Ca-transport has been observed in three organelles of different cell types: mitochondria, endoplasmic (sarcoplasmic) reticulum, plasma membrane (e.g. Carafoli & Crompton, 1978). The same has also been suggested for the cell membranes of enterocytes, because of the presence of Ca-stimulated ATPase activity (Melancon & De Luca, 1970; Ghijsen & van Os, 1979). It is tempting to attribute the ATP-driven Ca-transport to contaminating membrane fractions present in our basal-lateral membrane fraction. Mitochondrial ATP-dependent Ca-transport has already been excluded

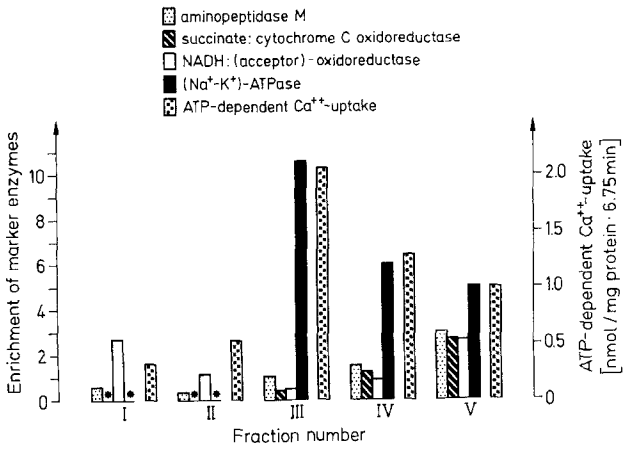

Fig. 3. Distribution of marker enzymes and ATP-dependent Ca-uptake in subfractions of a heavy microsomal fraction separated by a Percoll gradient. A crude plasma membrane fraction was separated in a Percoll gradient as described in Materials and Methods and divided into five fractions, designated *I–V* from the top of 7.5 ml (*I, II, IV, V*) and 5 ml (*III*). The marker enzymes of brush border membranes (aminopeptidase M), mitochondrial inner membrane (succinate: cytochrome *c* oxidoreductase), endoplasmic reticulum (NADH: acceptor-oxidoreductase) and basal-lateral plasma membrane ((Na+K)-ATPase) as well as ATP-dependent Ca^{++}-transport were assayed in each fraction. *=no detectable activity in membrane fraction. Incubation media containing: 300 mmol/liter mannitol, 20 mmol/liter HEPES/Tris, 100 mmol/liter KCl, 5 mmol/liter ATP or ADP, 5 mmol/liter $MgCl_2$, 1 μg/ml oligomycin and 50 μmol/liter $CaCl_2$

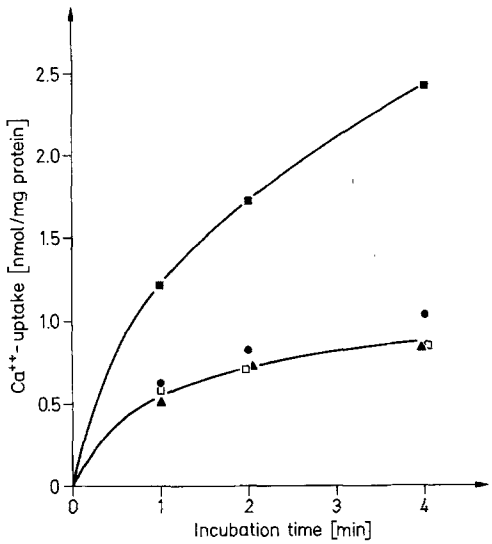

Fig. 4. Lack of stimulation of Ca^{++}-uptake into small intestinal basal-lateral plasma membrane vesicles by CTP and AMP-PCP. 20 μl of membrane suspension in 300 mmol/liter mannitol, 20 mmol/liter HEPES/Tris, pH 7.4, 100 mmol/liter KCl, 5 mmol/liter $MgCl_2$ were added to 100 μl of incubation medium which contained the same solutes and in addition 1 μg/ml oligomycin, 100 μmol/liter $CaCl_2$ and 5 mmol/liter Mg-ATP (■), 5 mmol/liter CTP (Tris-salt) + 5 mmol/liter $MgCl_2$ (●), 5 mmol/liter AMP-PCP (Li-salt) + 5 mmol/liter $MgCl_2$ (▲) or no nucleotide, but 5 mmol/liter $MgCl_2$ (□)

by the previous experiments. To exclude also endoplasmic reticulum and brush border membrane, we followed the ATP-stimulation of Ca-uptake in five fractions of the Percoll gradient and estimated, in the same fractions, the enrichment of marker enzymes of the basal-lateral membrane, brush border membrane, mitochondrial inner membrane and endoplasmic reticulum. The diagram of Fig. 3 gives the result of such an experiment. Leucine-aminopeptidase (aminpeptidase M) and succinate: cytochrome *c* oxidoreductase migrate to the bottom of the gradient. NADH-oxidoreductase is relatively enriched in the top as well as in the bottom of the gradient and is minimal in the midfraction. The upper small peak of endoplasmic reticulum marker has been observed in fractionation experiments of enterocyte membranes with different separation techniques and might be due, in part, to Golgi apparatus membranes (e.g., van Os et al., 1980 and Weiser, Neumeier, Quaroni & Kirsch, 1978). The (Na+K)-ATPase has its peak in the midfraction of the gradient where none of the other specific activities is maximal. The ATP-dependent Ca-uptake is also maximal in the midraction and should thus clearly be attributed to the basal-lateral membrane.

Inefficiency of Other Nucleotides. The requirement of the adenine-base and the hydrolysis of the β,γ-phosphodiester bond for the stimulatory effect of ATP have been tested by the replacement of ATP in the incubation medium for CTP and the nonhydrolyzable ATP analogous adenylyl-(β,γ-methylen-diphosphonate (AMP-PCP). Both were not able to stimulate Ca-uptake into basal-lateral plasma membrane vesicles indicating that the hydrolysis of the β,γ-phosphodiester bond is essential for the accumulation of Ca and that the adenine base is required for efficient binding of a trinucleotide to the specific site on the transport system (Fig. 4).

Na/Ca-Exchange

Na-Inhibition of ATP-Driven Ca-Transport. A Na/Ca-exchange system in the basal-lateral membrane should be activated by Na, opening a pathway for Ca through the membrane not present in the absence of Na. Thus, the availability of Na should antagonize the ATP-dependent Ca-accumulation, since the gradient of Ca across the membranes favors the efflux of Ca from the vesicles, once the

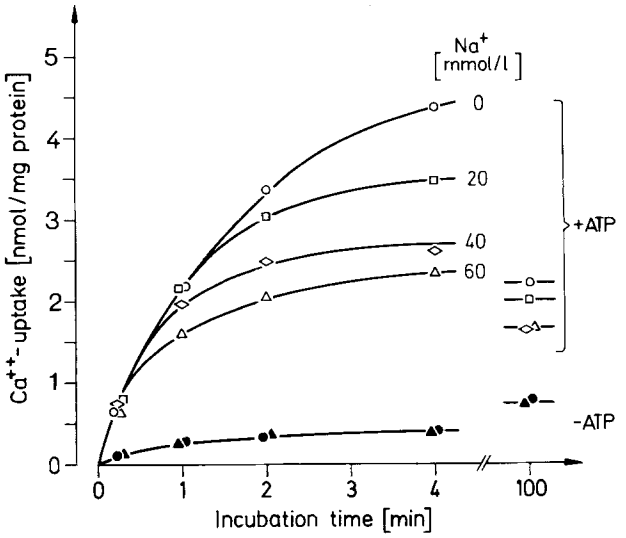

Fig. 5. Influence of Na^+ in the incubation medium on ATP-driven Ca^{++}-uptake into basal-lateral plasma membrane vesicles of rat small intestine. 20 µl of membranes suspended in 300 mmol/liter mannitol, 20 mmol/liter HEPES/Tris, pH 7.4, 100 mmol/liter KCl, 5 mmol/liter $MgCl_2$ and 1 µg/ml oligomycin were added to 120 µl of incubation medium either containing the same concentrations of solutes, namely with KCl (●, ○) or with KCl stepwise replaced by increasing amounts of NaCl (20 mmol/liter (□); 40 mmol/liter (◇); 60 mmol/liter (△, ▲)). The incubation media contained in addition 2 mmol/liter ouabain, 50 µmol/liter $CaCl_2$ and 5 mmol/liter Mg-ATP (○, □, ◇, △) or no ATP (●, ▲). 20 µl samples were taken at the times indicated

intravesicular concentration exceeds the extravesicular one. This could be observed when K in the incubation medium was stepwise replaced by increasing concentrations of Na (Fig. 5). At each Na concentration a characteristic plateau was approached after 4 min, being 50% of the ATP-dependent uptake (control = 0 mmol/liter Na^+) at 60 mmol/liter Na. Also in experiments with complete replacement of K by 100 mmol/liter Na the plateau was never reduced below 40% (*data not shown*). The Na-effect cannot be attributed to an activation of the (Na+K)-ATPase and increased hydrolysis of ATP, since the experiment was carried out with 2 mmol/liter ouabain, and other experiments showed that the Na-effect during 7 min of incubation was independent of the presence or absence of ouabain. In the absence of ATP, no difference of Ca-uptake can be seen during 4 min of incubation whether 60 mmol/liter Na is present or not. After 100 min of incubation Ca has reached its equilibrium distribution without ATP and has decreased from the plateau to different extents in the medium containing 5 mmol/liter ATP in the beginning, depending on the concentration of Na.

These observations are compatible with the existence of a Na/Ca-exchange system in the same membranes which contain the ATP-driven Ca-transport system. A direct inhibitory effect of Na on the ATP-dependent system is not entirely excluded by this experiment. It cannot be of major importance, however, since the effect of Na is not apparent at the initial time point; it rather develops when the actual vesicular Ca-content exceeds the equilibrium content. Furthermore, the high affinity Ca-ATPase activity described by Ghijsen et al. (1980) is not inhibited by Na (Ghijsen & van Os, *personal communication*, April 1980). An alternative interpretation of the data presented in Fig. 5 is that Na−Ca competition for intravesicular binding sites would lead to Ca displacement as Na concentration is raised. Consequently, net Ca-uptake would be reduced. This seems to be unlikely, as in the absence of ATP the Ca-content of the vesicles is not altered by the addition of sodium (Fig. 5). Furthermore, such an interpretation is also countered by the data presented in Fig. 7. If Na−Ca competition for intravesicular membrane binding explained the Na-effect, then increased Na-influx would be expected to increase the rate of Ca loss in the presence of gramicidin.

Acceleration of Ca-Efflux by Na. For these experiments basal-lateral membrane vesicles were equilibrated with 100 µmol/liter $CaCl_2$. Then a small volume of EGTA was added to obtain an EGTA concentration of 1.5 mmol/liter outside the vesicles instantaneously creating a Ca-concentration gradient from the inside of the vesicles to the outside. First, an experiment showing the effect of temperature and A23187 on the release of Ca from the vesicles (Fig. 6) is presented. There is a pronounced effect of temperature reduction from (25 °C (○) to 0 °C (●), more than 50% of the vesicular Ca being released during 3 min at 25 °C and less than 20% at 0 °C. Furthermore, the addition of 2 µg/ml A23187 stimulates Ca-efflux at both temperatures. Altogether this indicates that the release process, which we are studying at 25 °C, represents a transmembrane movement of Ca and not a dischargement of a minor fraction of Ca from the external membrane surface, which we were possibly not able to eliminate by our EGTA wash procedure with the stop solution.

In order to assess an effect of external Na on Ca-efflux from Ca-preloaded vesicles, vesicles were prepared in 100 mmol/liter choline chloride in the usual buffer instead of KCl. Efflux of Ca was then initiated, adding a small volume of EGTA to obtain 1.5 mmol/liter EGTA outside the vesicles, and with the same volume 71 mmol/liter Na or K were introduced to the incubation medium, creating a Na-

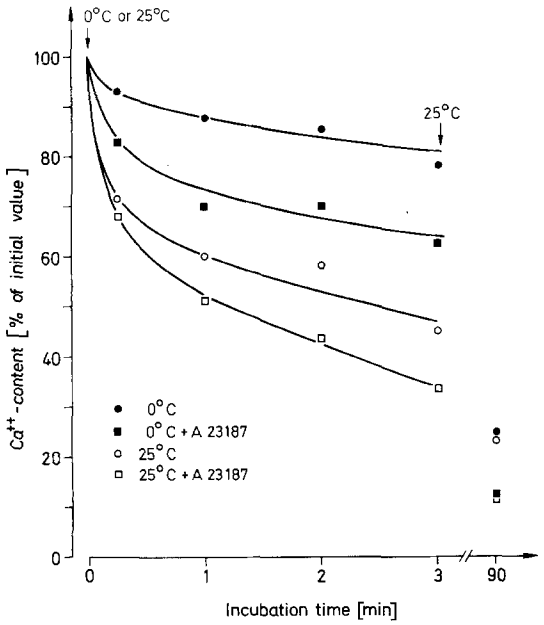

Fig. 6. EGTA-induced Ca-loss from Ca-loaded basal-lateral plasma membrane vesicles. Temperature dependence and stimulation by A23187. Membranes were suspended in 300 mmol/liter mannitol, 20 mmol/liter HEPES/Tris, pH 7.4, 100 mmol/liter KCl and 5 mmol/liter $MgCl_2$. They were allowed to equilibrate with 0.1 mmol/liter $^{45}CaCl_2$ for 1 h at 25 °C and then kept on ice. For each incubation 170 µl of membrane suspension were taken, 20-µl samples were withdrawn (2×), to estimate the initial Ca-content. In order to start Ca-efflux 10 µl of a 20-mmol/liter EGTA-solution in the same buffer as the calcium-containing membrane suspension were added and 20-µl samples taken at the times indicated. Incubation was carried out at 0 °C (●, ■) or 25 °C (○, □) with (■, □) or without (●, ○) 2 µg/ml A23187. After the withdrawal of the 3-min sample, all incubation media were kept at 25 °C

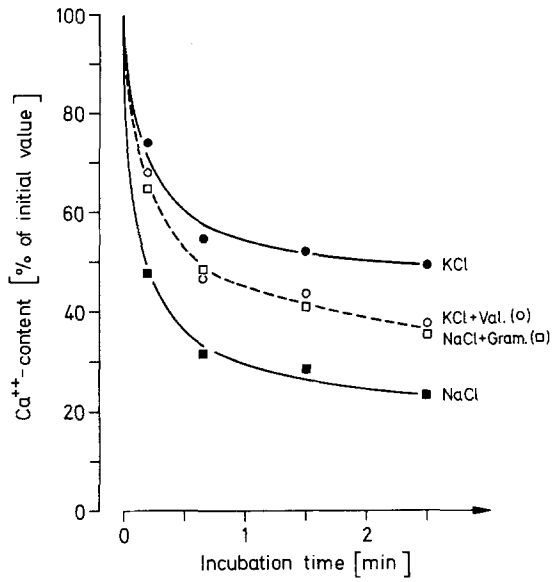

Fig. 7. Efflux of Ca^{++} from basal-lateral plasma membrane vesicles of rat small intestine; effect of Na^+. Membranes were preincubated for 1 h at 25 °C with 0.1 mmol/liter $^{45}CaCl_2$ in 300 mmol/liter mannitol, 20 mmol/liter HEPES/Tris, pH 7.4, 100 mmol/liter choline chloride, 5 mmol/liter $MgCl_2$. Efflux of Ca was initiated by the addition of 10 µl of 20 mmol/liter EGTA in 300 mmol/liter mannitol, 20 mmol/liter HEPES/Tris, pH 7.4, 5 mmol/liter $MgCl_2$ and 1 mol/liter NaCl (■, □) or KCl (●, ○) to 130 µl of membranes suspension. Valinomycin (○) or gramicidin (□), dissolved in ethanol were added to a concentration of 20 µg/ml to the membrane suspension 5 min before the start of efflux; the respective controls (●, ■) received the same amount of ethanol (2%)

or a K-gradient directed to the interior of the vesicles. Under these conditions a considerably faster efflux of Ca was observed with Na (>50% during 15 sec) than with K (~25% during 15 sec). When ionophores were added to the membranes before the addition of EGTA, valinomycin for K and gramicidin for Na, the efflux of Ca with a Na-gradient was reduced, whereas it was accelerated with a K-gradient (Fig. 7). The coincidence of the efflux curves with the two ionophores is accidental in this experiment; however, the change relative to the condition with the respective salt gradient without ionophore has been observed consistently.

These results are well compatible with the existence of a Na/Ca-exchange system, too. Na does increase the efflux of Ca from the vesicles, as compared to K. When an increased Na permeability is introduced by an ionophore preventing part of the Na from entering the vesicles via the Na/Ca-exchanger and thus, Ca from being exchanged for Na, Ca-efflux from the vesicles is reduced. On the other hand, with a potassium gradient the addition of an ionophore elicits the opposite effect, which might be explained by a diffusion potential inside more positive as compared to the absence of ionophore during dissipation of the K-gradient acting as a driving force for efflux of calcium ions by conductive pathways (leaks).

Conclusion

Because of the criticism on previous evidence for ATP-dependent Ca-transport and Na/Ca-exchange across basal-lateral plasma membrane of small intestine mentioned in the beginning, the availability of a rapid preparation method for this membrane seemed to offer a chance to provide additional evidence for these mechanisms. For the former, the demonstration of an ATP-dependent Ca-transport function appeared to be the most direct way, and for the latter it could be hoped that circumstantial evidence

would be provided without interference from other tissues or even other organelles. By the experiments presented in Fig. 1 to 4, clearcut evidence was obtained for Ca-transport against a concentration gradient dependent on the hydrolysis of ATP, localized in the basal-lateral plasma membrane. While this work was in progress, information became available on a Mg-ATPase stimulated by micromolar concentrations of Ca (Ghijsen & van Os, 1979; Ghijsen et al., 1980) which most probably is the enzymatic expression of the transport system described by us, although the demonstration of ATP-driven Ca-transport was not possible in that preparation because of the leakiness of the vesicles (Ghijsen & van Os, 1979).

The work on Ca-Mg-ATPase also provided substantial support to our interpretation of the Na-inhibition of ATP-dependent Ca-accumulation as a consequence of the operation of a Na/Ca-exchange system by finding that the high affinity Ca-ATPase is not inhibited by Na (Ghijsen & van Os, *personal communication*). This and the demonstration of Na-stimulation of Ca-efflux from basal-lateral membrane vesicles in the absence of ATP or ADP indicate that the two systems operate independently.

The question of interest is, which might be the relative contribution of the two transport systems for transport of Ca out of the small intestinal epithelial cell across the serosal surface? This question cannot be answered from the experiments presented. It was not possible to quantify Ca-fluxes experimentally at initial time-points; during which the Na-gradient could be regarded as constant, due to the high Na-permeability of basal-lateral membranes; i.e. the application of a constant and known driving force to this system was not possible. Furthermore, the question of charge transfer by these systems has not been assessed in our study, so that it cannot be decided if and to which extent the membrane potential contributes to the physiologic driving force. To obtain answers instead of speculation, experimental conditions have to be aspired to, under which meaningful driving force and kinetic analysis of the transport phenomena are possible as e.g. in heart sarcolemmal preparation (Pitts, 1979; Caroni & Carafoli, 1980; Caroni, Reinlib & Carafoli, 1980). This could be achieved by finding methods for the preparation of tighter vesicles and the application of transport assays with higher time resolution. A uniformly oriented basal-lateral membrane preparation is also a prerequisite for such a quantitative study. Unfortunately, the isolation methods used today for epithelial plasma membrane preparations do not allow such experiments in the near future.

In summary, the presented experiments suggest that an ATP-driven mechanism and a Na/Ca-exchange are involved in the extrusion of Ca^{++} from the cytosol of the enterocyte into the interstitium. Together with a passive entry mechanism located in the brush border membrane and regulated by 1.25 $(OH)_2$ Vit D_3 (Rasmussen et al., 1979; Hildmann, Storelli, Schmidt & Murer, 1980), these mechanisms might represent the plasma membrane-bound mechanisms involved in transcellular transport of calcium in the small intestine. Similar mechanisms were described for rat renal proximal tubular basal-lateral membranes (Gmaj, Murer & Kinne, 1978).

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Note Added in Proof

Since our first report on the experiments presented in this contribution (Hildmann, Schmidt & Murer, 1979), other research groups have reported on similar experiments (Ghijsen, de Jonge & van Os, 1981; Nellans & Popovitch, 1981). Furthermore, it has been documented in both reports that ATP-dependent Ca-uptake in basolateral membranes from rat small intestinal epithelial cells is stimulated by calmodulin.