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Calcium Ion Transport across Plasma Membranes Isolated from Rat Kidney Cortex

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Basal–lateral-plasma-membrane vesicles and brush-border-membrane vesicles were isolated from rat kidney cortex by differential centrifugation followed by free-flow electrophoresis. Ca^{2+} uptake into these vesicles was investigated by a rapid filtration method. Both membranes show a considerable binding of Ca^{2+} to the vesicle interior, making the analysis of passive fluxes in uptake experiments difficult. Only the basal–lateral-plasma-membrane vesicles exhibit an ATP-dependent pump activity which can be distinguished from the activity in mitochondrial and endoplasmic reticulum by virtue of the different distribution during free-flow electrophoresis and its lack of sensitivity to oligomycin. The basal–lateral plasma membranes contain in addition a Na^+/Ca^{2+}-exchange system which mediates a probably rheogenic counter-transport of Ca^{2+} and Na^+ across the basal cell border. The latter system is probably involved in the secondary active Na^+-dependent and ouabain-inhibitable Ca^{2+} reabsorption in the proximal tubule, the ATP-driven system is probably more important for the maintenance of a low concentration of intracellular Ca^{2+}.

In recent micropuncture studies using simultaneously tubular and capillary perfusion it could be demonstrated that in the rat kidney proximal tubule Ca^{2+} reabsorption is dependent on the presence of Na^+ ions and sensitive to ouabain (Ullrich *et al.*, 1976). On the other hand cell-fractionation studies on the distribution of plasma-membrane-bound enzymes in rat proximal tubular epithelial cells revealed a contraluminal localization of a Ca^{2+}-stimulated ATPase (Kinne-Saffran & Kinne, 1974). These results suggested that both Na^+-driven and ATP-driven Ca^{2+} transport systems might be involved in proximal tubular transepithelial Ca^{2+} transport. Considering the low concentration of intracellular Ca^{2+} one could expect that these active steps in Ca^{2+} reabsorption are located at the basal cell pole.

To our knowledge there have been two attempts to study the role of ATP in the Ca^{2+} transport of renal membranes. In one study increase in Ca^{2+} uptake by rabbit kidney membranes was observed, but this increase was attributed to a phosphorylation of the membranes and a concomitant binding of Ca^{2+} to the negative charges newly generated at the membrane surface. Moore *et al.* (1974) observed an ATP-dependent Ca^{2+} uptake distinct from that of the mitochondria in a crude fraction of renal plasma membranes as well as in rat renal microsomes. The two uptake systems differed in their capacity, their sensitivity to Na^+ and their apparent K_m values for Mg^{2+}–ATP. Their exact cellular location remained, however, uncertain.

In the present paper experiments are described on the Ca^{2+} transport into brush-border-membrane vesicles and basal–lateral plasma-membrane vesicles isolated from rat renal cortex. The results show that a primary active ATP-driven Ca^{2+} pump and an Na^+/Ca^{2+}-exchange system are present in the basal-lateral plasma membranes, but not in the brush-border membrane. These findings indicate that transepithelial Ca^{2+} transport in rat proximal tubule can be primarily active via the ATP-driven system as well as secondarily active if the Na^+/Ca^{2+} exchange system is involved. It is proposed that the Na^+/Ca^{2+} exchange system is responsible for the bulk flow of Ca^{2+} across the epithelium, whereas the ATP-driven system might be involved in the fine regulation of the concentration of intracellular Ca^{2+}.

A preliminary report of this work was given at the Spring Meeting of the German Physiological Society, Regensburg, 1977 (Gmaj *et al.*, 1977).

Abbreviations used: Ca^{2+}-ATPase: Ca^{2+}-stimulated adenosine triphosphatase; $Na^+ + K^+$-ATPase: $(Na^+ + K^+)$-stimulated adenosine triphosphatase; Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid.

Materials and Methods

Membrane purification

Male Wistar rats, 180g in weight, were killed by a blow on the neck and the kidneys were removed immediately. Partially purified renal plasma membranes were obtained from the renal cortex by

differential centrifugation after homogenization in iso-osmotic sucrose as previously described (Pockrandt-Hemstedt *et al.*, 1972). These membranes were then separated into brush-border and basal-lateral-membrane fractions by using the Desaga FF4 free-flow electrophoresis apparatus as detailed before (Heidrich *et al.*, 1972). Brush-border-membrane fractions and basal-lateral-membrane fractions respectively were suspended by homogenization with a Teflon/glass homogenizer (0.1 mm clearance, ten strokes, 1200 rev./min) in 40 ml of a solution containing 100 mM-KCl, 5 mM-$MgCl_2$ and 20 mM-Hepes/Tris (pH 7.0) and centrifuged for 20 min at 40000*g* and 4°C. The pellets were resuspended in 40 ml of the same buffer and the homogenization and centrifugation were repeated once. The final pellets were resuspended in approx. 400 μl of the same buffer by using a syringe fitted with a fine needle. Protein concentration of the final membrane suspension was about 10 mg/ml.

The fractions were routinely assayed for enzymes shown to be characteristic of brush-border microvilli, basal-lateral membranes, mitochondria and endoplasmic reticulum, and the purity of the membrane fractions was identical with that reported previously from our laboratory (Heidrich *et al.*, 1972; Kinne *et al.*, 1975; Berner & Kinne, 1976). Occasionally residual mitochondrial contamination was removed by an additional sucrose-density-gradient centrifugation. Basal-lateral-membrane fractions were suspended in 1.5 ml of 250 mM-sucrose/10 mM-triethanolamine/HCl (pH 7.0) and layered on 3 ml of 40% (w/v) sucrose/10 mM-triethanolamine/HCl (pH 7.0). After centrifugation for 90 min at 90000*g* the membranes at the interface were collected and suspended in the KCl-containing buffer as described above. The pellet, which contained almost all mitochondrial enzyme activity, was discarded.

Transport studies

Uptake of solutes (D-[^{3}H]glucose and $^{45}Ca^{2+}$) was assayed by a Millipore filtration technique as described earlier (Kinne *et al.*, 1975; Hoffmann *et al.*, 1976). The basic composition of the incubation media was 100 mM-KCl/5 mM-$MgCl_2$/20 mM-Hepes/Tris (pH 7.0). Changes in the composition of the incubation medium as well as the stop solution are indicated in the legends to the Figures and in the Tables. The stop solution contained usually 100 mM-KCl, 5 mM-$MgCl_2$, 20 mM-Hepes/Tris (pH 7.0) and 1 mM-EGTA. For rapid filtration cellulose acetate filters (0.6 μm pore size; Sartorius, Göttingen, Sweden) were used. Usually 20 μl of membrane suspension was added to 120–130 μl of incubation medium kept in a water bath at 25°C. At different time intervals a 20 μl sample was removed from the incubation suspension and diluted into 1 ml of ice-cold stop solution and immediately filtered. The filters were then washed with 3 ml of ice-cold stop solution to remove extravesicular radioactivity. The radioactivity remaining on the filters was counted by standard liquid-scintillation techniques.

All chemicals used in this study were of highest purity available. Ionophore A23187 was generously given by the Lilly Laboratories (Indianapolis, IN, U.S.A.). The radioactive compounds were obtained from New England Nuclear Corp. (Boston, MA, U.S.A.).

Results and Discussion

Uptake of Ca^{2+} in the absence of ATP

In Fig. 1 the uptake of Ca^{2+} by isolated renal brush-border-membrane vesicles and basal-lateral-plasma-membrane vesicles is presented. The apparent rate constants of Ca^{2+} uptake at 25°C calculated

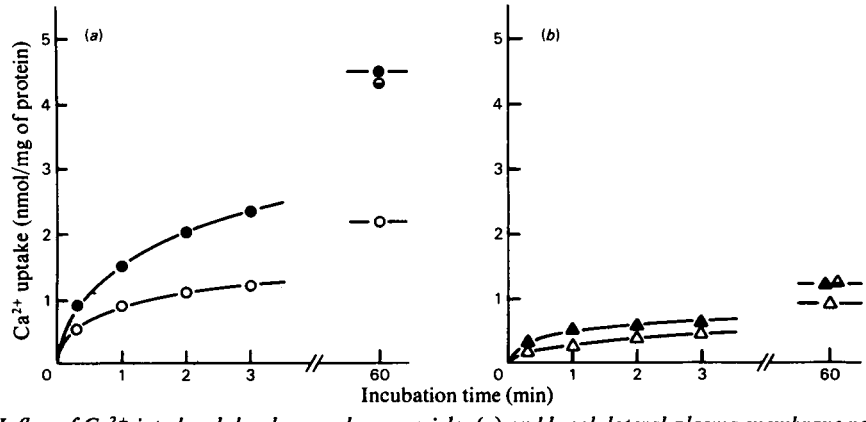

Fig. 1. *Influx of Ca^{2+} into brush-border-membrane vesicles (a) and basal-lateral-plasma-membrane vesicles (b)*
$^{45}CaCl_2$ concentration was 0.05 mM. ●, ▲, Ca^{2+} uptake at 25°C; ○, △, Ca^{2+} uptake at 0°C; ◒, ◭, stop solution contained in addition 1 mM-EGTA.

on the basis of several experiments are 0.13 min^{-1} for brush-border-membrane vesicles and 0.41 min^{-1} for basal-lateral-plasma-membrane vesicles, indicating a considerably faster uptake of Ca^{2+} by basal-lateral-plasma membranes. Since it is known that biological membranes show a high binding capacity for Ca^{2+}, it was possible that the uptake observed reflected, not only movement of Ca^{2+} into the vesicles, but also binding to the vesicle surfaces. From earlier studies with identical membrane preparations (Kinne *et al.*, 1975) it is known that glucose uptake by isolated renal proximal-tubular-plasma-membrane vesicles represents almost exclusively transmembrane movement into an intravesicular space and no binding to the membranes. Therefore the amount of glucose taken up at equilibrium represents a good estimate of the intravesicular space. At a concentration of 1 mM glucose uptake at equilibrium amounts to approx. 3 nmol/mg of protein in brush-border-membrane vesicles and to 1.3 nmol/mg of protein in basal-lateral-membrane vesicles, indicating an intravesicular volume of 3 μl/mg of protein and 1.3 μl/mg of protein respectively. Ca^{2+} uptake at a concentration of 0.1 mM was, however, much higher (5–10-fold both in the brush-border and in the basal-lateral-membrane vesicles) than expected from the intravesicular space calculated on the basis of the glucose distribution. These results indicate that Ca^{2+} uptake at equilibrium, as measured by our method, represents, to a large extent, binding of Ca^{2+} to the membrane of the vesicles (predominantly to the inside, see below).

As can be seen from Fig. 1, short exposure (approx. 10s) of the vesicles to EGTA in the ice-cold stop solution did not remove Ca^{2+} significantly from the membrane vesicles. The same was observed when La^{3+} (300 μM) was used instead of EGTA in the stop solution (Ca^{2+} concentration in the incubation medium 2–100 μM). These findings seem to indicate that the major part of Ca^{2+} bound to the membrane vesicles is not freely accessible to complexing by EGTA or to replacement by La^{3+}, and therefore suggest that Ca^{2+} binding measured under our conditions at equilibrium occurs at the inside of the vesicle. This notion is further supported by the experiments shown in Figs. 1 and 2. Ca^{2+} uptake

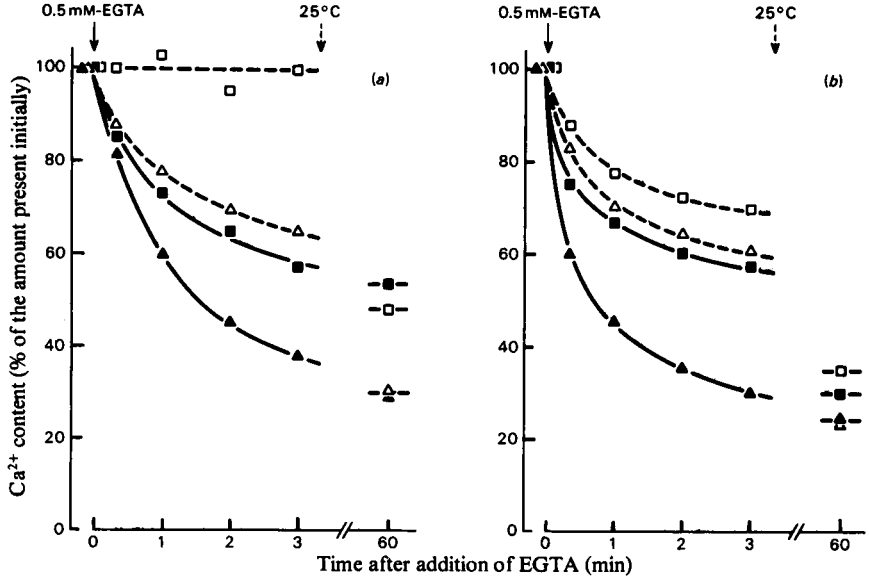

Fig. 2. *Efflux of Ca^{2+} from brush-border-membrane vesicles (a) and basal-lateral-plasma-membrane vesicles preloaded with $^{45}CaCl_2$ (b)*

The membrane vesicles suspended in the usual potassium containing buffer were pre-equilibrated for 2h with 0.1 mM-$^{45}CaCl_2$. Efflux of Ca^{2+} was started by the addition of 10 μl of incubation buffer containing 20 mM-EGTA to 130 μl of pre-equilibrated membranes. Samples were removed before addition of EGTA (100% value) and at the different time points after addition of EGTA as given in the Figure. The results are expressed in percentages of the amount of Ca^{2+} found in the vesicles immediately before starting efflux; 100% corresponded to 1.79 nmol/mg of protein in brush-border-membrane vesicles and to 1.19 nmol/mg of protein in basal-lateral-membrane preparations. □, Efflux at 0°C; △, efflux at 0°C in the presence of ionophore A23187 (1 μg/ml); ■, efflux at 25°C; ▲, efflux at 25°C in the presence of ionophore A23187 (1 μg/ml). After 4 min of incubation the samples previously incubated at 0°C were transferred to 25°C.

(Fig. 1) as well as Ca^{2+} efflux initiated by the addition of EGTA (Fig. 2) are strongly temperature-sensitive and accelerated by the addition of the ionophore for bivalent cation A23187. This indicates that both Ca^{2+} uptake into and Ca^{2+} release from the vesicles are preceded by a temperature-dependent rate-limiting step probably representing transfer of Ca^{2+} across the membrane. In the efflux experiments the vesicular space as measured by the vesicular content of pre-equilibrated [^{3}H]glucose was not altered as a consequence of the EGTA addition (results not shown).

The fact that under the experimental conditions used binding of Ca^{2+} occurs in the vesicle interior makes an analysis of the transport properties of the vesicle membrane very difficult. Thus it cannot be decided to date whether the equilibrating uptake or the EGTA-induced efflux as observed in the experiments given in Figs. 1 and 2 represents simple diffusion or facilitated diffusion of Ca^{2+} across the membranes.

Effect of ATP on transmembrane Ca^{2+} transport

ATP-driven transport of Ca^{2+} through biological membranes has been observed for plasma membranes of various bacterial and animal sources (for review see Vicenzi & Hinds, 1976). Similar to the situation with the Na^+/K^+ pump ($Na^+ + K^+$-ATPase) one has to assume that the active, ATP-hydrolysing site of a Ca^{2+}-ATPase, which acts as a Ca^{2+} pump, is present on the cytoplasmic face of the membrane. Furthermore, since the Ca^{2+}-ATPase located in the plasma membrane of epithelial cells should help to maintain a low intracellular Ca^{2+} concentration, the Ca^{2+} movement mediated by this pump should be directed from the cytoplasmic membrane face to the external membrane face. Considering in addition that ATP permeates plasma membranes slowly, if at all, it is evident that only the Ca^{2+}-ATPase pump sites of inside-out-oriented membrane vesicles would respond to ATP added to the extravesicular medium. Therefore, a system that extrudes Ca^{2+} from the intact cell would accumulate Ca^{2+} in inside-out-oriented vesicles. Such behaviour of a Ca^{2+} pump was indeed demonstrated by Cha *et al.* (1971) and Weiner & Lee (1972) in inside-out vesicles of erythrocyte membranes.

Since basal–lateral-membrane preparations contain inside-out oriented membrane vesicles in addition to outside oriented vesicles (Haase *et al.*, 1978; Kinne *et al.*, 1978) the study of ATP-driven Ca^{2+} transport in basal–lateral-membrane vesicles was possible. As can be seen in Fig. 3, the addition of ATP, in the presence of magnesium, to basal–lateral-membrane vesicles provoked a rapid Ca^{2+} uptake. Rapid Ca^{2+} uptake was not observed when ATP and the ionophore A23187 were added simultaneously, and under these conditions Ca^{2+} uptake was similar to the

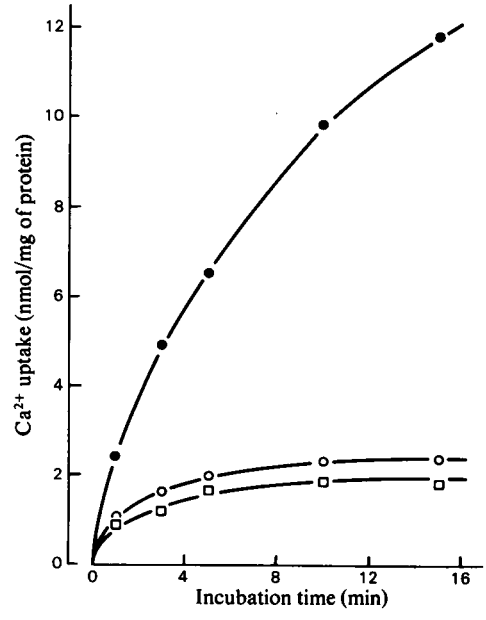

Fig. 3. *Influx of Ca^{2+} into basal–lateral-plasma-membrane vesicles: effect of ATP*

The incubation medium contained 100mM-KCl, 5mM-$MgCl_2$, 20mM-Hepes/Tris, pH7.0, and 0.05mM-$^{45}CaCl_2$ with (●) or without (□) 5mM-ATP (di-Tris salt). ○, Influx in the presence of ATP and ionophore A 23187 (1 μg/ml).

uptake in the absence of ATP. The increase of Ca^{2+} uptake by ATP can theoretically be brought about in several ways. Increased binding of Ca^{2+} to the external surface of the membrane might occur or the translocation of Ca^{2+} across the membrane might be increased either by generation of a vesicle inside-negative membrane potential or by ATP-driven active transport. The results obtained with A23187, an ionophore that increases the Ca^{2+} permeability of the membrane, support the latter explanation. Increased binding of Ca^{2+} to the membrane should not be affected by the ionophore and in the presence of an inside-negative membrane potential an increased uptake rather than a decreased uptake should be observed in the presence of the ionophore.

The ATP-driven uptake occurs most probably into the intravesicular space which in the presence of ionophore A23187 easily equilibrates with extravesicular space. Inside the vesicle the Ca^{2+} which entered the vesicle in an ATP-dependent manner can then associate with binding sites or storage sites of the membrane; therefore no information can be derived from our experiments on the actual concentration of free Ca^{2+} in the vesicles and of the concentration difference established by the ATP-driven Ca^{2+} pump.

Intracellular localization of ATP-dependent Ca^{2+} uptake

As demonstrated earlier, a Ca^{2+}-ATPase found in membrane fractions from renal cortical tissue co-purifies with the $Na^+ + K^+$-ATPase, suggesting an exclusive basal–lateral localization of this enzyme in the renal proximal tubular epithelial cell (Kinne-Saffran & Kinne, 1974). On the basis of this one might speculate that ATP-driven Ca^{2+} uptake is also exclusively located in the basal–lateral membrane. However, it is known that intracellular organelles, such as mitochondria and endoplasmic reticulum, show also ATP-dependent Ca^{2+} uptake (Bruns *et al.*, 1976). Therefore it had to be proved that the ATP-dependent Ca^{2+} uptake activity observed in the basal–lateral-membrane fraction was not due to contamination of the membrane fraction with other cellular organelles. As can be seen in Fig. 4 the distributions of the $Na^+ + K^+$-ATPase activity and ATP-dependent Ca^{2+}-uptake activity after free-flow electrophoresis of renal plasma membranes are quite similar. The ATP-dependent transport activity is highest in those membrane fractions with highest $Na^+ + K^+$-ATPase activity, whereas membrane fractions with a low content show very little ATP-dependent Ca^{2+}-transport activity. The membrane fractions (II+III) between the highly purified basal–lateral-membrane fraction (I) and brush-border-membrane fraction (IV) also have little activity in ATP-driven Ca^{2+} transport compared with the activity found in membrane fraction I. This is especially noteworthy because these fractions show the highest content in succinate dehydrogenase (mitochondria), glucose 6-phosphatase (endoplasmic reticulum) and acid phosphatase (lysosomes) (Heidrich *et al.*, 1972). The close correlation of ATP-driven Ca^{2+}-uptake activity with $Na^+ + K^+$-ATPase activity suggests very strongly that the ATP-dependent Ca^{2+} transport system is localized in basal–lateral plasma membranes.

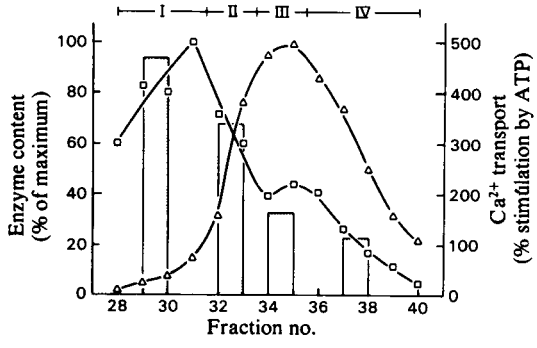

Fig. 4. *Distribution of marker enzymes for renal cortical plasma membranes and ATP-dependent Ca^{2+} transport activity during free-flow electrophoresis*

The crude plasma-membrane fraction was separated by free-flow electrophoresis as described by Heidrich *et al.* (1972). Enzyme content in the electrophoretic fractions is expressed in percentages of the fraction with the highest enzyme content. Ca^{2+} transport was measured in the pooled fractions (I, II, III, IV) in the presence or in the absence of ATP under the conditions presented in the legend to Fig. 3. The results on Ca^{2+} uptake are presented in the histogram as the percentages of the Ca^{2+} uptake in the absence of ATP and represent the Ca^{2+} uptake after 10min incubation. □, $Na^+ + K^+$-ATPase; △, alkaline phosphatase.

Properties of ATP-driven Ca^{2+} uptake by basal–lateral plasma membranes

Inhibition of ATP-driven Ca^{2+} uptake by different compounds was examined by measuring the uptake of Ca^{2+} in the presence of ATP after 10min (Table 1). The system was practically insensitive to oligomycin and ouabain. Occasionally observed sensitivity to oligomycin vanished if the basal–lateral membranes were further purified by an additional sucrose-density-gradient centrifugation, removing thereby mitochondrial contamination (results not shown). On the other hand, NaN_3, another inhibitor of mitochondrial Ca^{2+} uptake, inhibited the ATP-driven Ca^{2+} uptake by basal–lateral plasma membranes appreciably. The inhibition observed by NaN_3 reflects, however, probably an effect of sodium on the permeability of the vesicles for Ca^{2+} (see below) rather than a direct effect of azide on the pump system. Oligomycin and NaN_3 at the concentrations used in this experiment inhibit mitochondrial Ca^{2+} uptake completely. Among other inhibitors tested, La^{3+}, butacaine and compound D-600 have marked effects on ATP-driven Ca^{2+} uptake (inhibition approx. 40%). Qualitatively similar results were obtained by analysing the effects of the inhibitors on the ATP-driven Ca^{2+} uptake after 5 min of incubation.

Table 1. *Inhibition of ATP-dependent Ca^{2+} uptake by basal–lateral-plasma-membrane vesicles*

The incubation medium contained 100mM-KCl, 5mM-$MgCl_2$, 20mM-Hepes/Tris at pH7.0, 0.05mM-$^{45}CaCl_2$ and 5mM-ATP (di-Tris salt).

Addition to the incubation medium	Ca^{2+} uptake (nmol/10min per mg of protein)
Control	9.0
Oligomycin (1 μg/ml)	8.8
Ouabain (2mM)	8.2
NaN_3 (10mM)	5.4
Ruthenium Red (10 μM)	6.5
$La(NO_3)_3$ (20 μM)	5.4
Butacaine (0.5mM)	7.6
D-600 (0.5mM)	5.8

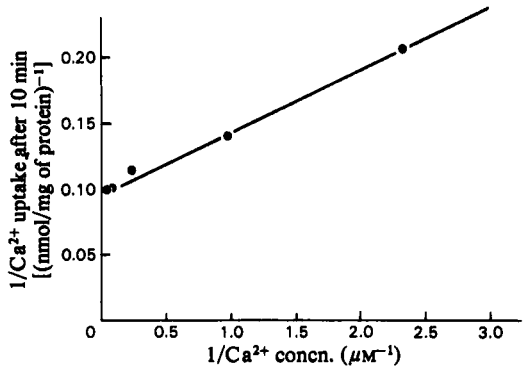

Fig. 5. *Kinetics of ATP-dependent Ca^{2+} uptake by basal-lateral plasma membranes*

The experiments were carried out as indicated in the legend to Fig. 3 in the presence of 5mM-ATP. $^{45}CaCl_2$ concentration was 100μM. The free Ca^{2+} concentration was varied by adding different concentrations of EGTA. The uptake after 10min of incubation at 25°C in the absence of ATP was subtracted from the uptake in the presence of ATP. The EGTA concentration in the incubation medium varied from 0.15mM (resulting in a free Ca^{2+} concentration of 0.5μM in the absence of ATP and 0.43μM in the presence of ATP) to 0.05mM (resulting in a free Ca^{2+} concentration of 50.2μM in the absence or in the presence of ATP). The plot gives the following results: $r = 0.973$; $K_m = 0.5\mu M$; $V_{max.} = 10.4$nmol/10min per mg of protein.

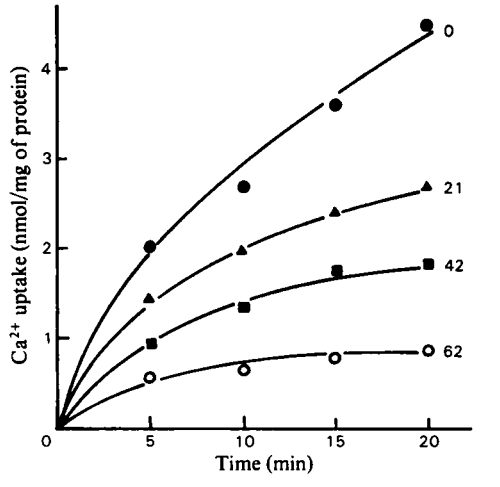

Fig. 6. *Effect of sodium on ATP-dependent Ca^{2+} uptake by basal-lateral plasma membranes*

The experiments were carried out as indicated in the legend to Fig. 3. Potassium was replaced in a stepwise manner by sodium as indicated in the Figure (concentration given in mM).

The ATP-driven Ca^{2+} uptake by basal-lateral-plasma-membrane vesicles was saturable by increasing Ca^{2+} concentrations. Double-reciprocal analysis of ATP-dependent Ca^{2+} uptake (Ca^{2+} uptake in the presence of ATP minus Ca^{2+} uptake in the absence of ATP) after 10min of incubation yielded an apparent K_m of the transport system for Ca^{2+} of 0.5μM and a $V_{max.}$ of 10.4nmol/10min per mg of protein (Fig. 5).

Influence of sodium on ATP-dependent Ca^{2+} uptake by basal-lateral plasma membranes

In the experiments on ATP-driven Ca^{2+} transport presented above, Ca^{2+} uptake was studied in the presence of high potassium concentrations. If potassium was replaced in a stepwise manner by sodium a

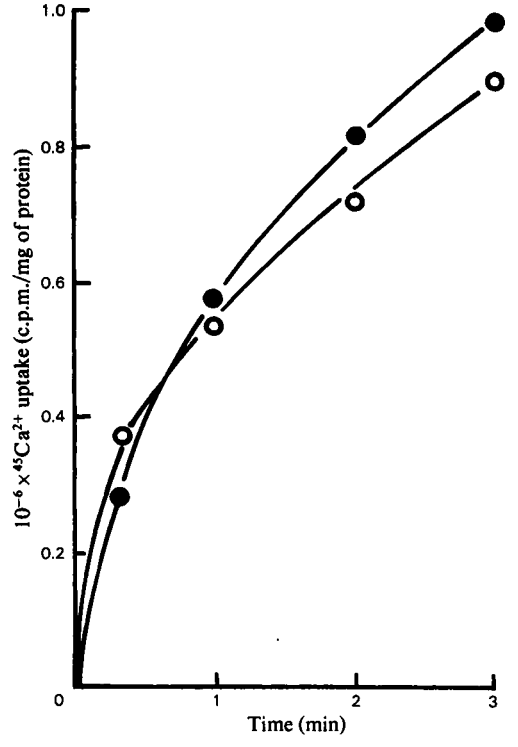

Fig. 7. *Effect of sodium on the unidirectional ATP-dependent influx of Ca^{2+} into basal-lateral-plasma-membrane vesicles*

Basal-lateral-plasma-membrane vesicles were first incubated for 20min in 120μl of a medium containing 6mM-$MgCl_2$, 20mM-Hepes/Tris, pH7.0, 1μg of oligomycin/ml, 5mM-ATP, 100μM-$CaCl_2$, 80μM-EGTA (20.8μM-free Ca^{2+}) and 100mM-KCl (●) or 100mM-NaCl (○). After preincubation 20μl of the respective medium containing in addition $^{45}CaCl_2$ was added and the uptake of $^{45}Ca^{2+}$ determined.

decrease in ATP-driven Ca^{2+} uptake by basal-lateral-membrane vesicles was observed (Fig. 6), a result which agrees with the observation of Moore *et al.* (1974). This decrease in the presence of sodium might be explained by two different mechanisms: firstly, the pump rate could be affected directly by sodium; secondly, sodium could increase the permeability of the membrane to Ca^{2+} and the decreased Ca^{2+} uptake would be explained by an increased back-leak of Ca^{2+}. A decreased pump rate of the ATPase system seems to be very unlikely. If membranes were first incubated with unlabelled Ca^{2+} in the presence of ATP in a potassium medium (absence of sodium) and influx of tracer $^{45}Ca^{2+}$ was studied subsequently during a second incubation period, no difference in the initial uptake rate of $^{45}Ca^{2+}$ in a potassium medium compared to a sodium medium was observed (Fig. 7). In this experimental situation the $^{45}Ca^{2+}$ entering the vesicles at initial time points is diluted into a large intravesicular Ca^{2+} pool, which has been accumulated during the first incubation period, and the conditions of unidirectional flux measurements are approximated since tracer efflux is minimal.

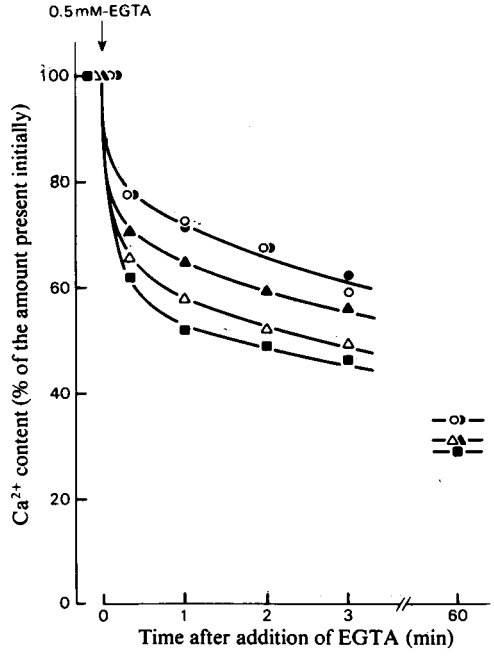

Fig. 8. *Effect of sodium on the efflux of Ca^{2+} from basal-lateral-membrane vesicles*

Basal-lateral-membrane vesicles were suspended in 100mM-choline cyclamate/5mM-$MgSO_4$/20mM-Hepes/Tris. In addition the membrane vesicles were pre-equilibrated with 100μM-$^{45}CaCl_2$ for 1h at 25°C. The efflux was started by the addition of 10μl of a solution containing 28mM-EGTA, 20mM-Hepes/Tris, 5mM-$MgSO_4$ and either 1M-potassium cyclamate or sodium cyclamate to 130μl of the pre-equilibrated membrane suspension: ○, efflux in the presence of potassium cyclamate gradient; ●, efflux in the presence of potassium cyclamate gradient plus valinomycin (10μg/ml); △, efflux in the presence of sodium cyclamate gradient; ▲, efflux in the presence of sodium cyclamate gradient plus Gramicidin D (10μg/ml); ■, efflux in the presence of sodium cyclamate gradient after valinomycin (10μg/ml) and 10mM-potassium cyclamate on both membrane sites. The results are expressed as percentages of the amount of Ca^{2+} found in the vesicles before starting efflux, which amounted to 1.34nmol/mg of protein.

Evidence for a Na^+/Ca^{2+}-exchange system in basal-lateral membranes

Since the pump rate of the ATP-driven Ca^{2+} uptake seems not to be affected by the presence of sodium, the decreased ATP-dependent Ca^{2+} uptake in the presence of sodium is probably caused by a sodium-dependent leak of Ca^{2+} out of the vesicles. This increased Ca^{2+} permeability of basal-lateral-membrane vesicles in the presence of sodium can be demonstrated directly in efflux experiments as shown in Fig. 8; where the effects of potassium and sodium on the efflux of Ca^{2+} from basal-lateral-membrane vesicles preloaded with choline was analysed in the presence of a relatively impermeant anion (cyclamate). The efflux of Ca^{2+} in the presence of an Na^+ gradient (extravesicular Na^+ concentration higher than intravesicular Na^+ concentration) was substantially stimulated over that observed in the presence of a K^+ gradient.

Such an observation does not necessarily imply a molecular coupling of Na^+ and Ca^{2+} movement via an Na^+/Ca^{2+} exchange system. A higher efflux rate in the presence of sodium could also occur if the Na^+ gradient creates a higher electrical diffusion potential across the membrane (vesicle inside-positive) than the K^+ gradient. However, since the K^+ gradient even in the presence of valinomycin is not able to mimic the effect of sodium, an electrical coupling seems unlikely. Further support for the existence of an Na^+/Ca^{2+}-exchange system was obtained from experiments with Gramicidin D, an ionophore for univalent cations. Gramicidin D inhibits the stimulatory effect of sodium on Ca^{2+} efflux; owing to the increased cation permeability of the membrane in the presence of the ionophore an Na^+-gradient-dependent diffusion potential should increase rather than decrease and accordingly electrically coupled Ca^{2+} fluxes should increase. Since on the other hand the Na^+ gradient might dissipate faster in the presence of the ionophore or the Na^+/Ca^{2+} exchange might be electrogenic the smaller effect of the sodium gradient on Ca^{2+} efflux can be explained by a lowering of the driving force.

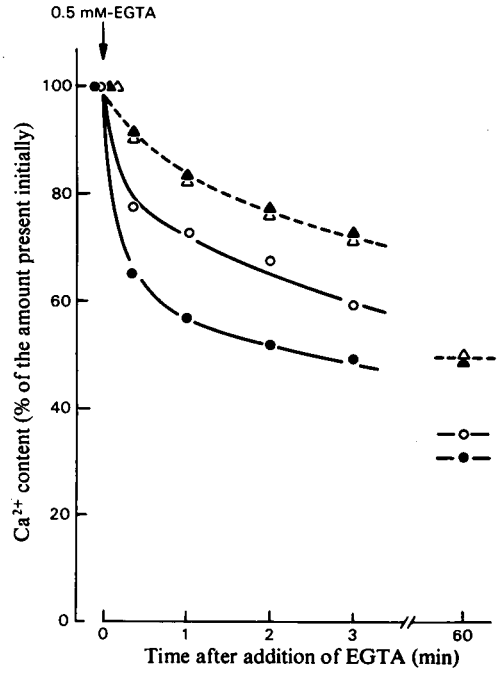

Fig. 9. *Effect of sodium on the efflux of Ca^{2+} from basal-lateral-plasma-membrane vesicles and brush-border-membrane vesicles*

The membrane vesicles are loaded with 100mM-choline chloride/5mM-$MgCl_2$/20mM-Hepes/Tris instead of the usual KCl buffer. In addition the membrane vesicles were pre-equilibrated with 100μM-$^{45}CaCl_2$ for 1h at 25°C. The efflux was started by the addition of 10μl of a solution containing 28mM-EGTA, 20mM-Hepes/Tris, 5mM-$MgCl_2$ and 1M-NaCl or 1M-KCl: ●, efflux from basal-lateral-plasma-membrane vesicles in the presence of a sodium gradient; ○, efflux from basal-lateral plasma membranes in the presence of a potassium gradient; ▲, efflux from brush-border membranes in the presence of a sodium gradient; △, efflux from brush-border membranes in the presence of a potassium gradient. The results are expressed as percentages of the amount of Ca^{2+} found in the vesicles before starting efflux, which amounted to 1.22nmol/mg of protein for basal-lateral-plasma-membrane vesicles and to 2.76nmol/mg of protein for brush-border membranes.

The most rapid Ca^{2+} efflux is observed when, in the presence of an Na^+ gradient and of K^+ on both membrane sides, the K^+ conductivity of the membrane is increased by the addition of valinomycin. This observation might indicate that a charge transfer is involved in the Ca^{2+} Na^+ exchange. In analogy to other tissues one might assume that three Na^+ ions are transported across the basal-lateral membrane in exchange for one Ca^{2+} ion. However, further experiments will be needed to prove this assumption.

The Na^+/Ca^{2+}-exchange system seems to be present only in the basal-lateral plasma membranes of the renal tubular cell. Sodium has no effect on the efflux of Ca^{2+} from brush-border-membrane vesicles (Fig. 9).

Conclusion

The experiments presented above provide evidence that at least two different transport systems for Ca^{2+} are present in basal-lateral plasma membranes of rat proximal-tubular epithelial cells: (*a*) an ATP-driven transport system for Ca^{2+} and (*b*) a Na^+-gradient driven Na^+/Ca^{2+}-exchange system. No evidence for the presence of these two systems in the brush-border membrane was obtained.

If we assume that the ATP-driven transport system is also operating in the intact cell and that owing to the action of the Na^+/Ca^{2+}-exchange system an Na^+ gradient can establish a concentration difference for Ca^{2+} across the membrane and does not only accelerate the dissipation of a Ca^{2+} gradient, as demonstrated experimentally, transepithelial transport of Ca^{2+} could proceed in the following way (Fig. 10). Ca^{2+} enters the cell at the luminal border driven by its electrochemical potential difference. The mechanism of this process could not be defined in our transport studies, because the high Ca^{2+}-binding capacity of the isolated brush-border-membrane vesicles made an interpretation of the results very difficult. At the basal-lateral cell side Ca^{2+} can be extruded from the cell via the ATP-driven pump or via the Na^+/Ca^{2+}-exchange system. In the former case Ca^{2+} reabsorption would be primarily active, in the latter secondarily active. In the secondarily active transport mode, Ca^{2+} extrusion from the cell would be driven by the electrochemical potential difference for sodium across the contra-

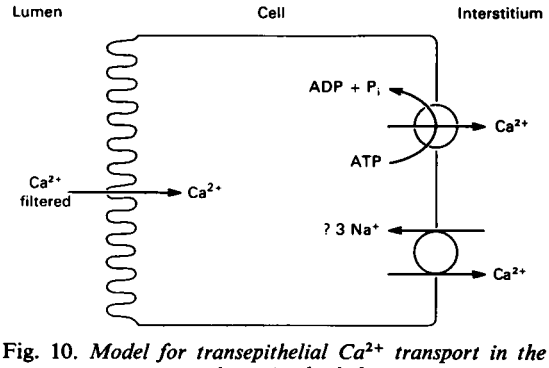

Fig. 10. *Model for transepithelial Ca^{2+} transport in the renal proximal tubule*

luminal membrane, which is maintained by the $Na^+ + K^+$-ATPase.

From microperfusion studies in the proximal convoluted tubule of rat and hamster it is known that removal of Na^+ from or addition of ouabain to the perfusion fluid inhibits active Ca^{2+} reabsorption (Ullrich *et al.*, 1976, 1977). According to these findings one could assume that the secondary active transport involving the Na^+/Ca^{2+}-exchange represents the predominant mechanism for Ca^{2+} reabsorption in the tubule. The ATP-driven Ca^{2+} pump on the other hand might be involved in the fine regulation of the intracellular Ca^{2+} concentration.

One could imagine that the Na^+/Ca^{2+}-exchange system and the ATP-driven Ca^{2+} pump are linked indirectly to each other via the $Na^+ + K^+$-ATPase. Since it is known that $Na^+ + K^+$-ATPase is inhibited by Ca^{2+}, removal of Ca^{2+} by the ATP-driven pump might be essential to maintain high $Na^+ + K^+$-ATPase activity, which in turn generates the electrochemical potential difference for Na^+ that drives the Na^+/Ca^{2+} exchange.

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