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Ca^{2+} Control of Electrolyte Permeability in Plasma Membrane Vesicles from Cat Pancreas

Irene Schulz and Klaus Heil

Max-Planck-Institut für Biophysik, Kennedyallee 70, 6000 Frankfurt (Main) 70, Germany

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Summary. The influence of Ca^{2+} and other cations on electrolyte permeability has been studied in isolated membrane vesicles from cat pancreas.

Ca^{2+} in the micromolar to millimolar concentration range, as well as Mg^{2+}, Sr^{2+}, Mn^{2+} and La^{3+} at a tested concentration of 10^{-4} M, increased Na^+ permeability when applied at the vesicle inside. When added to the vesicle outside, however, they decreased Na^+ permeability. Ba^{2+} was effective from the outside but not from the vesicle inside.

When Ca^{2+} was present at both sides of the membrane, Na^+ efflux was not affected as compared to that in the absence of Ca^{2+}. Monovalent cations such as Rb^+, Cs^+, K^+, $Tris^+$ and $choline^+$ decreased Na^+ permeability when present at the vesicle outside at a concentration range of 10 to 100 mM. Increasing Na^+ concentrations from 10 to 100 mM at the vesicle inside increased Na^+ permeability.

The temperature dependence of Na^+ efflux revealed that the activation energy increased in the lower temperature range (0 to 10 °C) when Ca^{2+} was present at the outside or at both sides, but not when present at the vesicle inside only or in the absence of Ca^{2+}.

The results suggest that the Ca^{2+} outside effect is due to binding of calcium to negatively charged phospholipids with a consequent reduction of both fluidity and Na^+ permeability of the membrane. The Ca^{2+}-inside effect most likely involves interaction with proteins with consequent increase in Na^+ permeability.

The data are consistent with current hypotheses on secretagogue-induced fluid secretion in acinar cells of the pancreas according to which secretagogues elicit NaCl and fluid secretion by liberating Ca^{2+} from cellular membranes and by stimulating Ca^{2+} influx into the cell. The increased intracellular Ca^{2+} concentration in turn increases the contraluminal Na^+ permeability which leads to NaCl influx. The luminal sodium pump finally transports Na^+ ions into the lumen.

The role of Ca^{2+} and Na^+ in the mechanism of both enzyme and NaCl secretion from the exocrine pancreas has become one of the main points of interest within the last years. Although the importance of Ca^{2+} in secretagogue-induced enzyme release has been generally accepted [1, 16, 18, 19, 49, 55, 58, 59, 71], the role of Na^+ in stimulus-secretion events leading to enzyme, NaCl, and fluid secretion is still a matter of controversy [4, 49, 69].

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Electrophysiological studies have shown that during stimulation of pancreatic enzyme secretion the conductance of the plasma membrane to ions is increased with a consequent Na^+ influx into the cell [34, 42]. Evidence that secretagogues increase Na^+ permeability of the cell membrane not directly but by an increase in intracellular free Ca^{2+} concentration came from the observation that Ca^{2+}-dependent depolarization could be induced by the Ca^{2+} ionophore A23187. In the absence of Na^+, this depolarization was abolished, suggesting that it is due to an increased influx in Na^+ resulting from a Ca^{2+}-mediated increase in Na^+ permeability [53]. Furthermore, the finding that intracellular Ca^{2+} application results in membrane depolarization similar to that evoked by acetylcholine suggests that the acetylcholine effect is caused by a rise of cytosol intracellular free Ca^{2+} which in turn influences the Na^+ permeability [18]. In this paper we present results showing that an asymmetric distribution of Ca^{2+} or other di- and trivalent cations such as Mg^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, and La^{3+} influence the Na^+ permeability. Ca^{2+} in the micromolar and millimolar concentration range or other divalent cations at a tested concentration of 10^{-4} M applied to the outside of isolated membrane vesicles decrease, but applied from the inside they increase the Na^+ permeability.

Materials and Methods

All reagents used were of the highest purity available. Ficoll (mol wt approx. 400,000), ethyleneglycol-bis (β-aminoethyl ether) N,N′-tetraacetic acid (EGTA) and phenylmethyl sulfonyl-fluoride (PMSF) were obtained from Sigma, St. Louis, Mo. Radiochemicals were obtained from NEN, Dreieich, Germany (^{22}NaCl, carrier free; ^{86}RbCl, 0.5–10 Ci/g Rb; ethylenediaminetetraacetic-2-^{14}C-acid, tetrasodium salt (^{14}C-EDTA) 0.05 mCi/0.4 mg) and from the Radiochemical Centre, Amersham, England (^{134}CsCl, 1–10 Ci/g Cs; L-(2,3-^{3}H)-alanine, 30–50 Ci/mmol). D(-)mannitol, sucrose, Tris (hydroxymethyl) aminomethane, NaCl, KCl, CsCl, RbCl, $CaCl_2$, $BaCl_2$, and $MnCl_2$ were obtained from Merck, Darmstadt, Germany; $SrCl_2$ and $MgCl_2$ from BDH Chemicals, Poole, England, $LaCl_3$ from Roth, Karlsruhe, Germany, LiCl and HCl from Alfa Prod. (Ventron) Danvers, Mass., N-2-hydroxyethylpiperazine-N′-2-ethane-sulfonic acid (Hepes) and L-alanine were bought from Serva, Heidelberg, Germany, and carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (CFCCP) from Boehringer, Mannheim, Germany. Millipore filters (HAWP 02500, pore size 0.45 μm) were purchased from Millipore Corporation, Bedford, Mass. The calcium ionophore A23187 was a gift from the Lilly Research Laboratories, Indianapolis. Ind.

Isolation of Plasma Membranes

The procedure used for the fractionation of tissue is described in a previous paper [38], and it will be only outlined here. Cat pancreas was homogenized by hand, using a loose Teflon-glass homogenizer in 5 volumes of 10% (w/w) sucrose containing 10 mM

Tris-Cl, pH 7.4, 0.1 mM ethyleneglycol-bis (β-aminoethylether), N,N'-tetraacetic acid (EGTA), and 0.1 mM phenylmethyl sulfonylfluoride (PMSF). To remove nuclei and intact cells, the homogenate was centrifuged by accelerating the SS34 rotor on a Sorvall RC 2B centrifuge to 2,000 rpm and then shutting off the motor. After the pellet had been removed, the supernatant was centrifuged through an exponential gradient between 5% ficoll (w/w) in 0.3 M sucrose and 45% sucrose (w/w) with a 60% sucrose (w/w) cushion in a Beckman zonal rotor (Ti 14) for 3.5 hr at $137{,}000 \times g$. The supernatant distributed among four protein bands, of which the first one at lowest density (1.07 g/ml) representing the plasma membranes was collected, diluted with the same buffer but without sucrose, and centrifuged for 1 hr at $123{,}000 \times g$. The pellet was taken up in 0.3 M mannitol containing 7.5 mM Hepes-Tris, pH 7.0, or the respective pH desired, homogenized 5 times by hand in a glass homogenizer, and centrifuged again for 1 hr at $300{,}000 \times g$. The resulting pellet was resuspended in the same mannitol buffer. This procedure was repeated to obtain the final pellet of membranes, which were used for the Na^+-flux studies.

Efflux Studies

Two volume parts of the membrane suspension were combined with one part of a solution which resulted in a final concentration of 2–3 mg of membrane protein/ml in 200 mM mannitol, 5 mM Hepes-Tris, pH 7.0, 10 mM NaCl containing 400 μCi $^{22}Na^+$/ml, 1 mM EGTA without or with Ca^{2+} adjusted to free Ca^{2+} concentrations between 10^{-8} M and 5×10^{-3} M, according to Portzehl *et al.* [51]. Since radioactive EGTA is not available, we have measured uptake of the similar compound ^{14}C-EDTA into membrane vesicles. After 5 hr incubation, equilibrium uptake for ^{14}C-EDTA was obtained. These results provide evidence that EGTA enters the vesicles and that a concentration of the ionized calcium inside the vesicles according to the calculated value should be expected after 20 hr preincubation. If the effect of Ca^{2+} at the inside or at both sides of the membrane vesicles was to be tested, the preincubation medium contained calcium (Fig. 1). If the effect of calcium

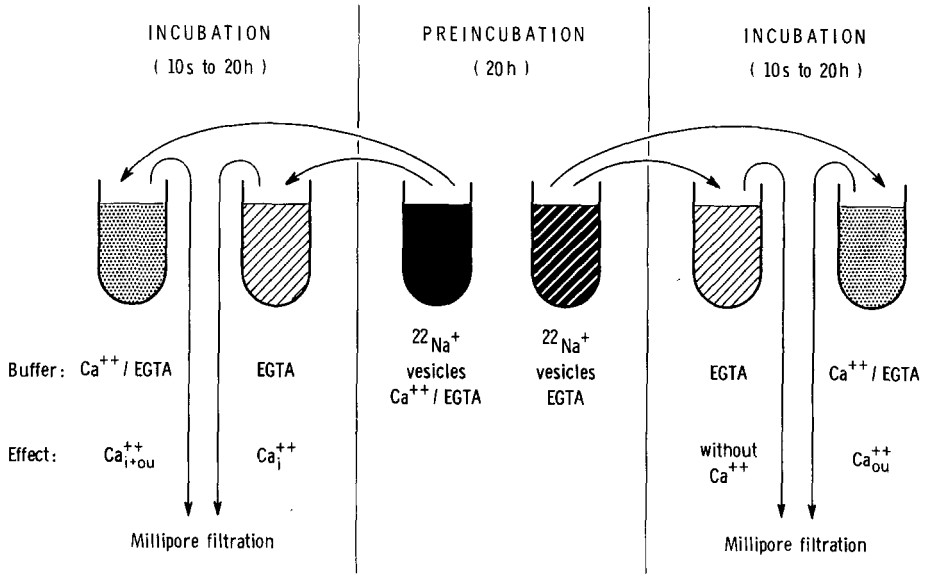

Fig. 1. Procedure to perform $^{22}Na^+$-efflux experiments. For explanation, *see* text

at the vesicle outside or the $^{22}Na^+$ efflux in the absence of calcium should be tested, the preincubation medium did not contain calcium. Other additions are indicated in the legends to the figures and tables. The membranes were incubated in this solution for 20 hr (overnight) at 4 °C to equilibrate with the preincubation medium. Equilibration time was considered to be reached when no further uptake of $^{22}Na^+$, $^{45}Ca^{2+}$, ^{14}C-mannitol, ^{3}H-L-alanine or ^{14}C-EDTA was measured with time.

For efflux studies 30 µl of the preloaded vesicles were transferred to 1.47 ml of a nonradioactive incubation solution at 4 °C containing 200 mM mannitol, 5 mM Hepes-Tris, pH 7.0. If the effect of calcium at both sides of the vesicles or at the vesicle outside was to be tested, this incubation medium also contained calcium as indicated (Fig. 1). Other additions are indicated in the text. After different incubation times at 4, 10, 20, or 30 °C, aliquots of 120 µl were poured on Millipore filters and washed with 5 ml of the same solution. Washing time took 15 sec. The filters were dissolved in 10 ml of Bray's solution and counted in a Packard liquid scintillation counter.

Determination of the Intravesicular Space

The $^{22}Na^+$ radioactivity remaining on the Millipore filter in the absence of membranes was less than 0.05% of total counts transferred to the filter and was subtracted from the total number of counts remaining on the filter for each incubation condition applied. Since the dilution of the concentrated membrane suspension was 50-fold, the equilibrium concentration of $^{22}Na^+$ in the membrane vesicles after dilution should be the same as in the diluted suspension, i.e., 2% of the $^{22}Na^+$ concentration in the starting medium. From the counts remaining in the vesicles at equilibrium conditions (20 hr after the start of the efflux experiment) the intravesicular space was calculated. Alternatively, the uptake of ^{3}H-L-alanine, ^{14}C-mannitol or ^{14}C-sucrose of which appreciable binding could not be detected was measured at equilibrium and used to calculate the intravesicular space. All values agreed very well with the data derived from the equilibrium $^{22}Na^+$ uptake and were between 6–8 µl/mg protein. The uptake of ^{3}H-L-alanine was routinely used in each experiment for measurement of the intravesicular space at each condition applied. The 100% value of $^{22}Na^+$ present in the vesicles at time 0 was derived from the intravesicular space and the standard counts present in the preincubation medium.

ATPase Assay

To estimate the orientation of the membrane vesicles, the Mg^{2+}-dependent, Na^+-K^+-activated ATPase was determined at 2 different incubation conditions, (i) membranes were preincubated for 30 min on ice in a 300 mM mannitol medium (hypertonic as compared to the preparation medium (200 mM mannitol) in order to preserve vesicles) plus 3 mM EDTA with or without desoxycholate (DOC) at indicated concentrations. Incubation was performed for 15 min at room temperature in 300 mM mannitol with 50 mM NaCl, 10 mM KCl, 3 mM $MgCl_2$, 10 mM Tris-Cl, pH 7.0 and 3.3 mM ATP with or without 2 mM ouabain (ii). Membranes were freeze-thawed for 1 time in liquid nitrogen in a hypotonic medium (100 mM mannitol plus 3 mM EDTA) and kept for 30 min on ice. Incubation was performed as described under *i* except that 100 mM mannitol and no DOC were used. After incubation the samples were boiled for 2 min, chilled, and centrifuged. The amount of inorganic phosphate liberated was determined in aliquots of the supernatant by the method of Fiske and Subbarow [6].

Results

I. Uptake and Binding of $^{22}Na^+$

To differentiate between $^{22}NaCl$ fluxes into the intravesicular space and $^{22}Na^+$ binding to the plasma membranes, we have measured $^{22}Na^+$ uptake into membrane vesicles at different osmotic pressure and at different $^{22}Na^+$ concentrations. Fig. 2 shows that varying the osmolarity of the incubation medium by the addition of mannitol[1] influenced $^{22}Na^+$ uptake measured after 10 min incubation. With increasing osmolarity plotted as reciprocal of osmolarity of the incubation medium, the $^{22}Na^+$ uptake into vesicles was reduced. Extrapolation to infinite osmolarity shows zero uptake, indicating that uptake is a function of an osmotically sensitive intravesicular space and that binding to membranes is negligible since otherwise an intercept at the ordinate would be present.

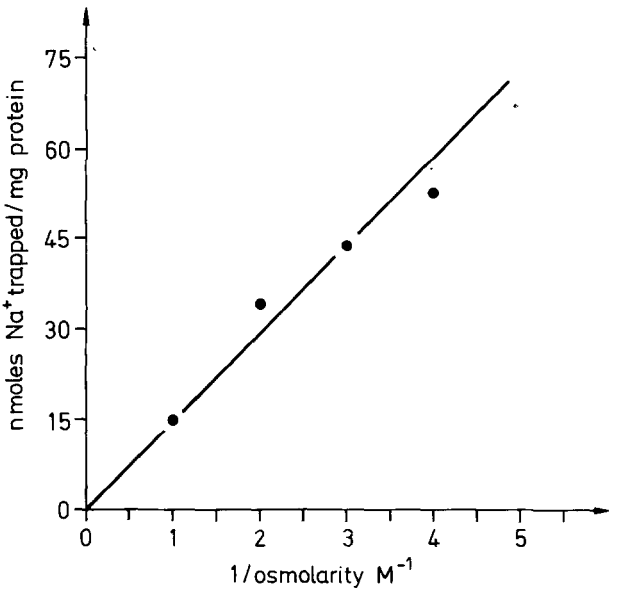

Fig. 2. Variation of medium osmolarity on $^{22}Na^+$ uptake. Membrane vesicles in 200 mM mannitol were added to a medium containing 10 mM NaCl, 5 mM Hepes-Tris, pH 7, and different concentrations of mannitol to vary the medium osmolarity. The samples were taken 10 min after mixing. The uptake was performed at 4 °C, and the final protein concentration was 2–3 mg/ml. Each point represents the mean of 5 determinations (2 experiments)

[1] Since mannitol used as a nonpermeant substance slowly penetrates the vesicles (equilibrium was reached after several hours), only 10 min exposure to the mannitol medium was used.

A quantitative estimate of the contribution of $^{22}Na^+$ binding to $^{22}Na^+$ uptake was derived from $^{22}Na^+$ uptake experiments at equilibrium and at different $^{22}Na^+$ concentrations in the incubation medium. A Scatchard plot of the Na^+ taken up at varying Na^+ concentrations between 0.5 and 10 mM showed that above 2.5 mM Na^+ a zero slope was observed as would be expected of uptake into a nonsaturating intravesicular space (Fig. 3). Since the measured $^{22}Na^+$ uptake at varying concentrations of Na^+ higher than 2.5 mM corresponds exactly to the $^{22}Na^+$ uptake as calculated by the L-alanine space, it can be assumed that the zero slope line represents a reliable estimation of the uptake of unbound Na^+.

Below 2.5 mM Na^+ concentration the slope of the line suggests that some binding occurs, which at a medium concentration of 2.5 mM Na^+ makes up 3% of the amount taken up by vesicles. At 10 mM Na^+, the concentration used for efflux studies, 0.8% is bound. The value corresponds also to the amount of bound $^{22}Na^+$ estimated by treatment of membrane vesicles with Triton X 100 or by 50-fold dilution in water instead of mannitol. Both methods were expected to rupture vesicles

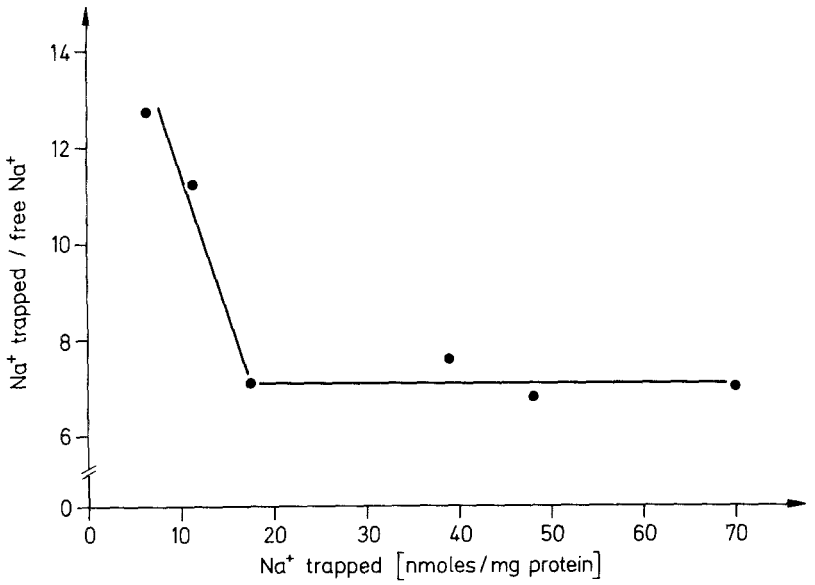

Fig. 3. Scatchard plot for equilibrium uptake of Na^+. Uptake was performed at 4 °C for 20 hr with varying Na^+ concentrations with 5 mM Hepes-Tris, pH 7. Points show Na^+ uptake (abscissa) at 0.5, 1.0, 2.5, 5.0, 7.0, and 10 mM Na^+ concentration of the incubation medium. The ordinate shows nmol Na^+ trapped/mM Na^+ in the incubation medium. Sampling was performed in duplicates at 4 °C. The final protein concentration was 1.63 mg/ml. Data show 1 experiment which is representative for 3 similar experiments

and only $^{22}Na^+$ bound to cell membranes should be retrieved on the Millipore filter. In the case of Triton X-100 treatment no $^{22}Na^+$ uptake was seen, whereas when hypotonic shock was applied uptake was still 10%, probably due to revesiculation since the intravesicular space as measured by ^{3}H-L-alanine uptake was also still 10% of the original space.

II. Efflux of $^{22}NaCl$

Efflux of $^{22}Na^+$ from vesicles which had been preloaded by equilibration with 10 mM $^{22}NaCl$ was influenced by temperature and the cation composition of the intravesicular and the external medium.

Temperature Dependence

Na^+ efflux increased with increasing temperature. The Arrhenius plots of the initial 10-sec efflux rates show straight lines resulting in

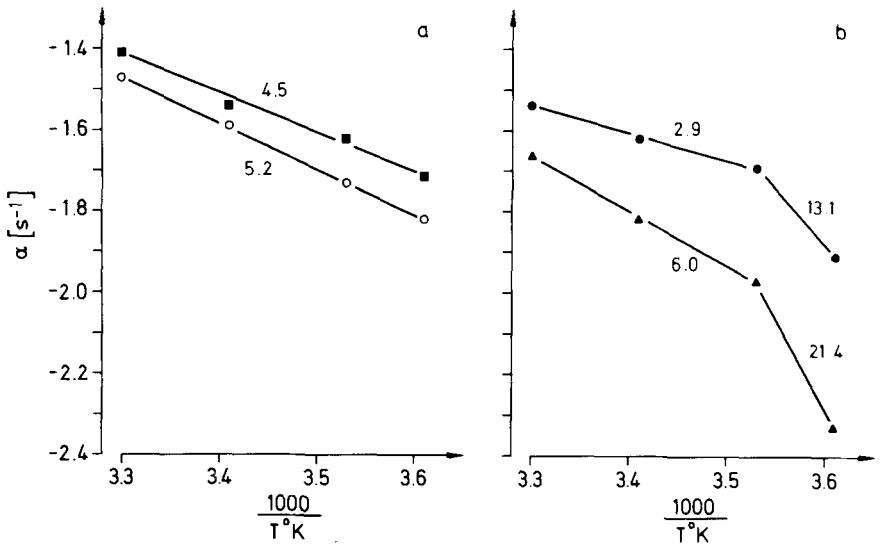

Fig. 4. Effect of temperature on Na^+ efflux in the absence or presence of calcium. (*a*): The Arrhenius plot of the initial Na^+ efflux rate in the presence of 10^{-4}M calcium at the inside only and in the absence of calcium (% Na^+ loss·sec^{-1}); ■—■, $Ca^{2+}{}_i$; ○—○, without calcium. (*b*): This plot at 10^{-4}M calcium at the outside only and at both sides; ●—●, Ca_{i+ou}; ▲—▲, Ca_{ou}. The data taken in duplicate are representative for 1 out of 2 similar experiments

activation energies of 5.2 kcal/mol when no Ca^{2+} was present and 4.5 kcal/mol when 0.1 mM Ca^{2+} was present at the vesicle inside only (Fig. 4*a*). When Ca^{2+} was present at the outside only or at both sides of the membrane at a temperature above 10 °C, the activation energy was in the same range (2.9 and 6.0 kcal/mol, respectively); however, below 10 °C an increase to 13.0 and 21.4 kcal/mol, respectively, was observed (Fig. 4b).

Since in vesicular systems the rate constants for Na^+ fluxes are quite high [63] and in our system even increased in the presence of some test substances, the experiments have been performed at 4 °C to minimize efflux rates and to handle samples within a sequence of 10 sec incubation time. It was also necessary to avoid activation of proteases and lipases present in pancreatic tissue, which could damage the membranes at higher temperatures.

Effect of Asymmetric Ca^{2+} Distribution on $^{22}Na^+$ Efflux from Membrane Vesicles

Figure 5 shows Na^+ efflux in the presence of Ca^{2+} on either the outside or the inside or on both sides of the membrane vesicles and in the absence of Ca^{2+}. Data are expressed as percent deviation from the value obtained in the presence of calcium at both sides of the vesicle membrane. Na^+ efflux was increased when 10^{-4} M Ca^{2+} was present at the vesicle inside only as compared to Ca^{2+} being present at both sides. Ca^{2+} at a 10^{-4} M concentration at the vesicle outside only, however, decreased Na^+ efflux. A plot of the natural logarithm of the fraction of $^{22}Na^+$ remaining within the vesicles *vs.* time (Fig. 6*a*) shows that within the first 40 sec $^{22}Na^+$ efflux was a linear function of time only in the presence of Ca^{2+} at the vesicle outside. Presence of Ca^{2+} at the vesicle inside or at both sides increased the initial rate of $^{22}Na^+$ efflux as indicated by the increased initial slope of the curves. After 40 sec, however, the fractional efflux curves became nearly parallel, showing that after that time $^{22}Na^+$ efflux was of the same rate for each condition initially present. A plot of the efflux rate coefficients *vs.* time (Fig. 6*b*) demonstrates this behavior even better.

Equilibrium at which the $^{22}Na^+$ concentration inside the vesicles was that of the outside medium, i.e., 2% of the value originally present within the vesicles was reached within 20 hr for all efflux curves. Since only the initial efflux was influenced by the composition of the intra-

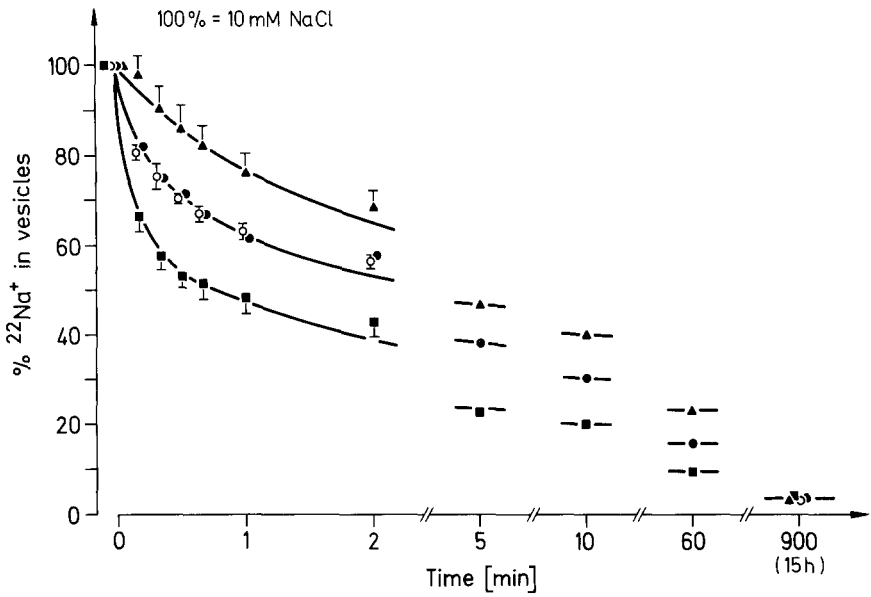

Fig. 5. Effect of Ca^{2+} on $^{22}NaCl$ efflux: ▲—▲, in the presence of Ca^{2+} (10^{-4}M) at the outside of membrane vesicles; ●—●, in the presence of Ca^{2+} (10^{-4}M) at both sides of the vesicle membrane; ○—○, in the absence of Ca^{2+}; ■—■, in the presence of Ca^{2+} (10^{-4}M) at the vesicle inside. The points at 10 sec to 2 min were obtained from 7 experiments and 13 determinations. The points at 5 min to 15 hr are the mean values from 3 experiments and 4 determinations. The points obtained at calcium present at both sides of the vesicles are the mean values in % of $^{22}Na^+$ remaining in the vesicles after different periods of incubation. All other points are expressed in % deviation from these values ± SEM

and extravesicular medium, all further experiments were performed for a time course of 2 min.

A stimulating effect of intravesicular Ca^{2+} on $^{22}Na^+$ efflux similar to that shown in Fig. 5, but at lower[2] $Ca^{2+}{}_i$ concentrations, was obtained when the vesicle outside Ca^{2+} concentration was fixed at a relatively high value (10^{-4}M) and the Ca^{2+} concentration at the vesicle inside was varied between 10^{-6} and 10^{-3}M. As Fig. 7 shows, an intravesicular Ca^{2+} concentration of only 10^{-6}M increased the initial $^{22}Na^+$-efflux to a value twice that obtained in the absence of intravesicular Ca^{2+}. At 10^{-3}M, the highest inside Ca^{2+} concentration tested, the initial $^{22}Na^+$ efflux was six times higher than without Ca^{2+} at the vesicle inside. 10^{-7}M Ca^{2+} at the inside did not change Na^+ efflux as compared to

[2] Throughout this communication $Ca^{2+}{}_i$, $Ca^{2+}{}_{ou}$ and $Ca^{2+}{}_{i+ou}$ will represent Ca^{2+} at the inside, at the outside, and at both sides of membrane vesicles, respectively.

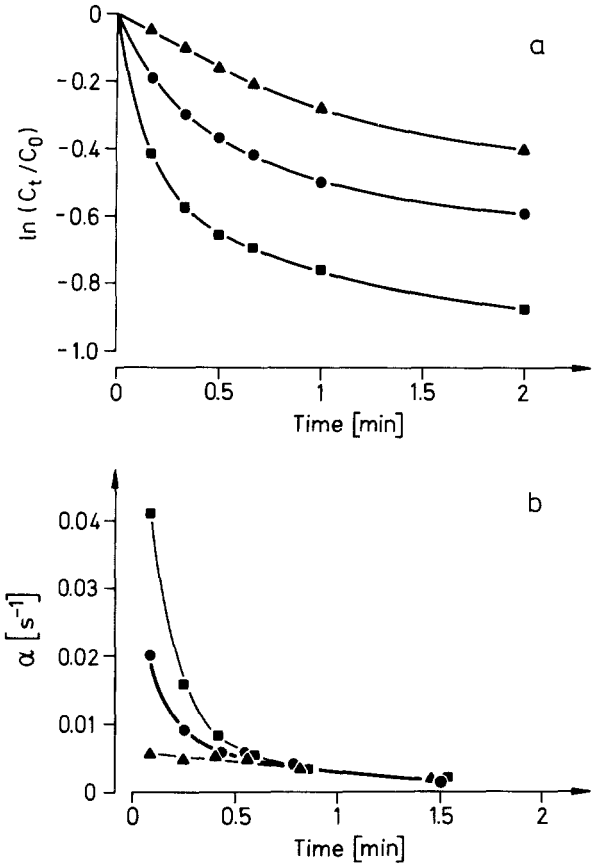

Fig. 6. (*a*): Effect of calcium (10^{-4}M) on ^{22}NaCl efflux: ▲—▲, with Ca^{2+}; ●—●, with $Ca^{2+}{}_{i+ou}$; ■—■, with $Ca^{2+}{}_{i}$. The natural logarithm of the values in fractional $^{22}Na^{+}$ efflux as shown in Fig. 5 is plotted as a function of time. (*b*): Na^{+} efflux rate coefficients as calculated according to

$$\alpha = \frac{1}{t_{i+1} - t_i} \cdot \ln \frac{N(t_i)}{N(t_{i+1})}$$

from the values shown in Fig. 5 plotted *vs.* time. N=% of $^{22}Na^{+}$ counts remaining in vesicles after time t_i and t_{i+1}, respectively

the data obtained in the absence of intravesicular calcium (curve not shown). A plot of the natural logarithm of the fractional efflux of these curves showed, similar to those in Fig. 6*a*, a change from a single exponential—at Ca^{2+} outside only—to curves with more exponentials and increasing rates for $^{22}Na^{+}$ efflux at increasing intravesicular Ca^{2+} concentrations within the first minute. Later the flux rates decreased, becoming similar for each condition (plot not shown).

When vesicles were preincubated with the Ca^{2+} ionophore A23187 (10^{-6}M) both the effect of Ca^{2+} at the outside only and Ca^{2+} at the

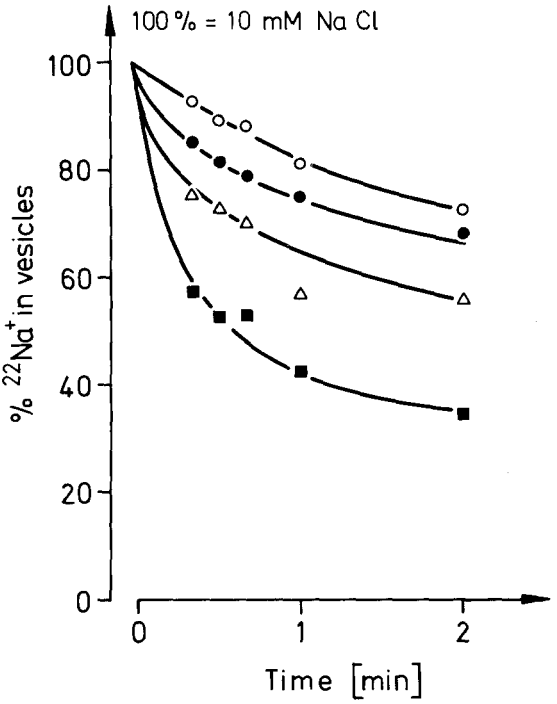

Fig. 7. $^{22}NaCl$ efflux from vesicles at different calcium concentrations at the inside but at constant calcium concentration (10^{-4}M) at the vesicle outside. ○—○, without $Ca^{2+}{}_i$; ●—●, with 10^{-6}M $Ca^{2+}{}_i$; △—△, with 10^{-4}M $Ca^{2+}{}_i$; ■—■, with 10^{-3}M $Ca^{2+}{}_i$. One experiment; each point is taken in duplicates

vesicle inside only was abolished (Fig. 8*b*). A comparison of Fig. 8*a* and *b* shows that in the presence of A23187 all $^{22}Na^{+}$-efflux curves became similar to that obtained in the absence of A23187 when no or the same Ca^{2+} concentration at both sides of the vesicle membrane was present, indicating that the effect of asymmetric Ca^{2+} distribution was abolished.

Since only the initial efflux rates occur at satisfactorily defined experimental conditions, the values obtained for the first 10 sec of $^{22}Na^{+}$ efflux were used to demonstrate the Ca^{2+} concentration-sodium efflux relationship. A plot of the relative amounts of NaCl remaining in plasma membrane vesicles after 10 sec of Na^{+} efflux is shown in Fig. 9. With increasing Ca^{2+} concentrations from 10^{-8} to 2×10^{-3}M an increase in the Ca^{2+} effect on $^{22}Na^{+}$ efflux could be observed. Between 10^{-7} to 10^{-6}M, similar to the free Ca^{2+} concentration in intact cells [35], the effect of Ca^{2+} at the vesicle inside showed an increase in $^{22}Na^{+}$ efflux, whereas at a free Ca^{2+} concentration between 10^{-8} and 10^{-7} and between 10^{-4}

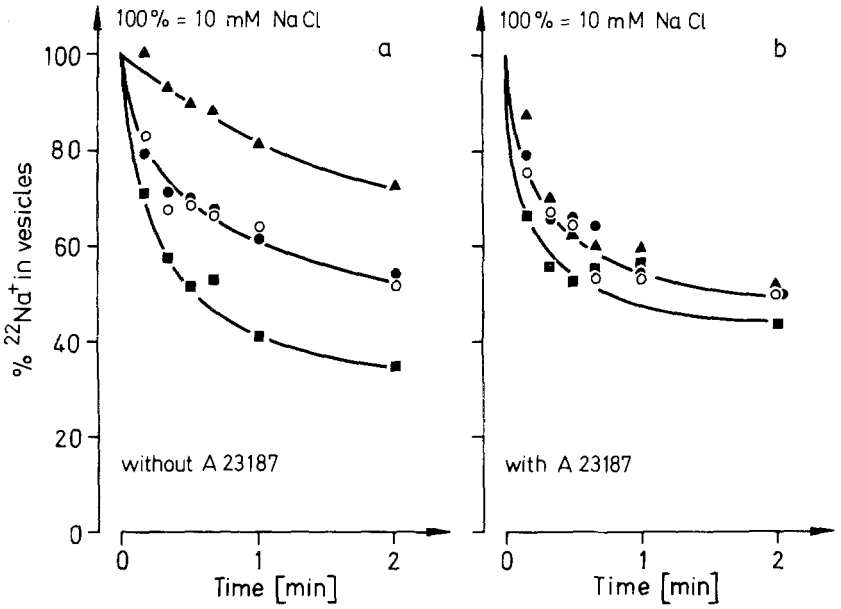

Fig. 8. Effect of Ca^{2+} on $^{22}NaCl$ efflux from vesicles. Data are shown out of 2 similar experiments, and each point is the mean of 2 determinations. The membrane vesicles were preincubated for 20 hr without (*a*) or with (*b*) A23187 (10^{-6}M) and processed as usual. ○—○, without Ca^{2+}; ●—●, with $Ca^{2+}{}_{i+ou}$, 10^{-4}M; ▲—▲, with $Ca^{2+}{}_{ou}$, 10^{-4}M; ■—■, with $Ca^{2+}{}_{i}$, 10^{-4}M

and 10^{-3}M at the vesicle outside two steps of Na^+ efflux change were observed. At the higher Ca^{2+} concentrations similar to that found in blood plasma the membrane vesicles became relatively tight to Na^+, showing a drastic decrease in $^{22}Na^+$ efflux.

In the presence of an electrical potential difference across the membrane, unequal distribution of calcium could influence the potential profile and thereby also ion fluxes. Furthermore, it could be possible that calcium might influence an electroneutral Na^+/H^+ exchange similar to that found in the brush border of kidney and intestine [39]. Both possibilities could be ruled out by the simultaneous application of KCl plus the K^+-selective ionophore valinomycin and of the H^+ ionophore CFCCP in the presence of a buffer. As shown by Murer *et al.* [39] this combination not only abolished an existing electrical potential difference but also "short-circuited" a Na^+/H^+ ion exchange mechanism. With our membrane preparation in the presence of 10 mM KCl,valinomycin (1 μg/mg protein) and CFCCP (10 μg/mg protein), respectively, in 2 experiments the Ca^{2+} effect on $^{22}Na^+$ efflux was not changed (data not shown). When the membranes had been preincubated in the presence of the Na^+ ionophore monensin for 15 hr before the efflux experiment was started, the half-time of Na^+ efflux within the first 10 sec was

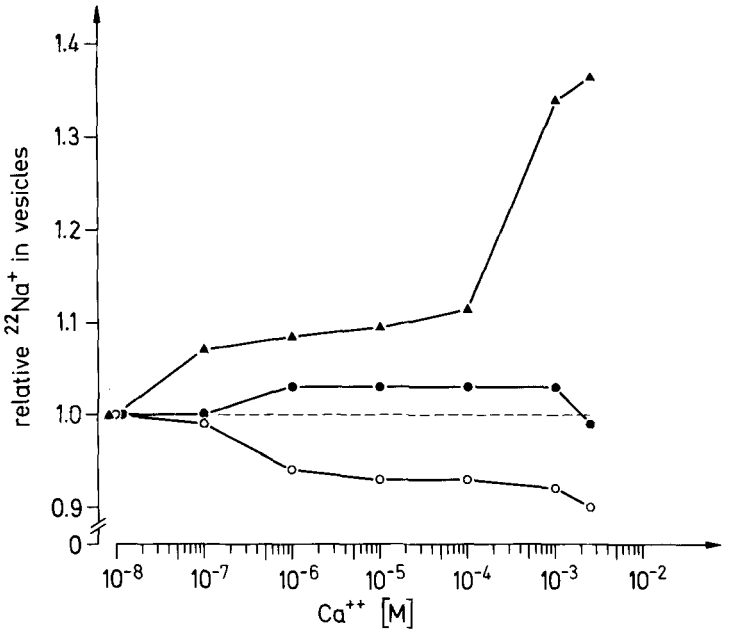

Fig. 9. Relative amount of $^{22}NaCl$ trapped in membrane vesicles after 10 sec at different calcium concentrations as compared to values in the absence of Ca^{2+} (---). ▲—▲, Ca^{2+} present at the vesicle outside only; ●—●, Ca^{2+} present on both sides; ○—○, Ca^{2+} present at the vesicle inside. Each point shows the mean value of 2 experiments

increased to a value 5.3 times higher than without monensin. Thereby the effect of Ca^{2+} at the vesicle outside on Na^+ efflux was only 10% of the effect without monensin, whereas the Ca^{2+}-inside effect was abolished (data not shown).

To compare the effect of calcium on a pancreatic membrane fraction other than plasma membrane, endoplasmic reticulum was prepared from the zonal fraction *II* as has been described elsewhere [38]. Although these membrane vesicles had an intravesicular space comparable to that of the plasma membranes, no effect of calcium on $^{22}Na^+$ efflux could be observed. Plasma membranes from liver, which had been isolated after the method of Neville *et al.* [40], did not show a Ca^{2+} effect on $^{22}Na^+$ efflux either.

Effect of Other Di- and Trivalent Cations on $^{22}Na^+$ Efflux

When other di- and trivalent cations were tested on their effects on Na^+ efflux and compared to the effectiveness of Ca^{2+} the following

sequence was found for the inside effect[3]: Ca^{2+} (100%) > Mn^{2+} (89%) > La^{3+} (78%) > Sr^{2+} (74%) > Mg^{2+} (48%) > Ba^{2+} (0%), and for the outside effect[4]: La^{3+} (120%) > Sr^{2+} (106%) > Ca^{2+} (100%) > Mn^{2+} (81%) > Mg^{2+} (66%) > Ba^{2+} (29%).

Figure 10 shows the effect of Ba^{2+} on Na^+ efflux. It can be seen that Ba^{2+} did not increase Na^+ efflux when applied to the vesicle inside.

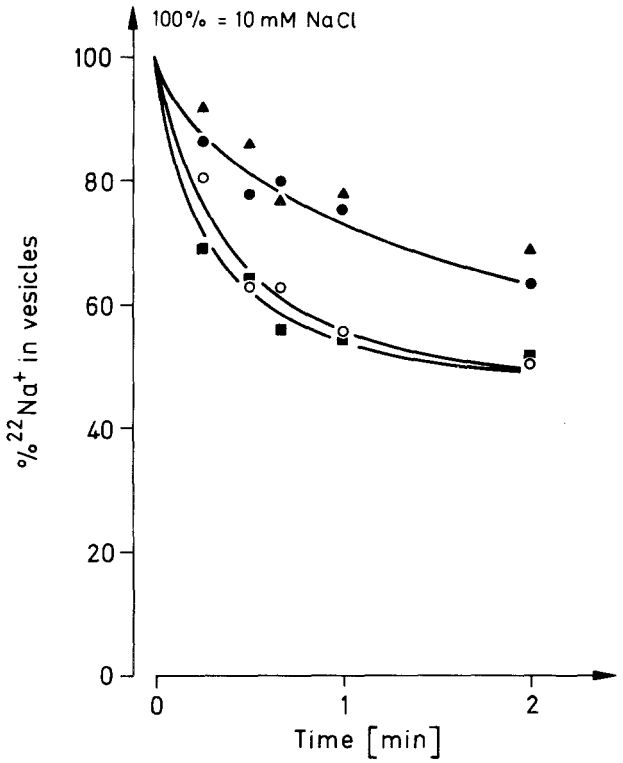

Fig. 10. Effect of Ba^{2+} (10^{-4}M) on Na^+ efflux. Each point represents the mean of 4 determinations (3 experiments). ▲—▲, with $Ba^{2+}{}_{ou}$; ○—○, without Ba^{2+}; ●—●, with $Ba^{2+}{}_{i+ou}$; ■—■, with $Ba^{2+}{}_{i}$

[3] Since in the presence of other divalent cations at both sides of the membrane the Na^+ efflux curves were not always identical to those in the absence of divalent cations, the following comparisons have been made: The inside effect was estimated by comparing the percent deviation of the initial (20 sec) value for trapped Na^+ in the presence of the respective cation at both sides of the membrane as to the initial value obtained when the cation was at the outside only (i.e., the percent decrease by $Me^{2+}{}_{i}$). Each value is the mean of 2–3 experiments for each divalent cation tested.

[4] The outside effect was obtained by comparing trapped Na^+ in the presence of the divalent cation at the outside only to the amount of Na^+ remaining in the vesicles in the absence of cations. Each value is the mean of 2–3 experiments for each divalent cation tested.

However, Ba^{2+} at the vesicle outside or at both sides decreased Na^+ efflux as compared to Na^+ efflux in the absence of Ba^{2+}.

Effect of pH on $^{22}Na^+$ Efflux

The effect of calcium on $^{22}Na^+$ efflux was qualitatively not influenced by the pH in the range of 5 to 8: As Fig. 11 shows, $^{22}Na^+$ efflux was not changed considerably in a Hepes-Tris buffered preincubation and incubation medium of pH 5, 6, 7, and 8 within the first 20 sec since 70 to 80% of the $^{22}Na^+$ counts initially present remained in vesicles after that time. The highest effect of Ca^{2+} was obtained at pH 7. How-

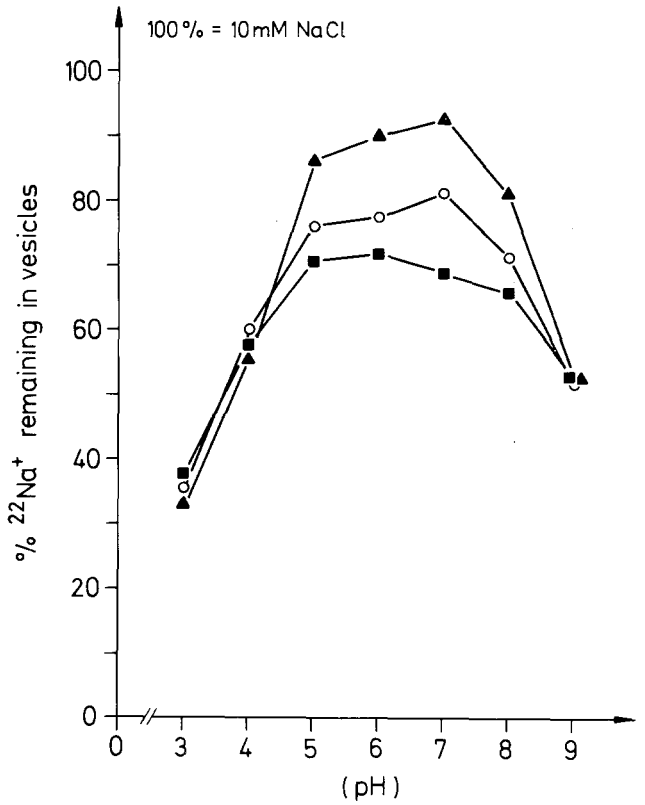

Fig. 11. Effect of calcium on $^{22}NaCl$ at different H^+ ion concentrations. Membranes were preincubated for 20 hr in the presence of 5 mM Hepes-Tris adjusted to the desired pH with NaOH or HCl. The effect of 10^{-4}M calcium was tested as described in *Materials and Methods*. Each point shows % $^{22}NaCl$ remaining in vesicles after 20 sec of efflux and is the mean value from 2 experiments. ▲—▲, with $Ca^{2+}{}_{ou}$; ○—○, without Ca^{2+}; ■—■, with $Ca^{2+}{}_i$

ever, at pH 3 and 9 a considerable increase in $^{22}Na^+$ efflux was observed and the effect of calcium was abolished.

$^{22}Na^+$-Isotope Exchange as Compared to $^{22}Na^+$ Net Flux

Under conditions of Na^+ net efflux the effect of Ca^{2+} on $^{22}Na^+$ efflux could be secondary to an increase in Cl^- permeability rather than to a direct effect on Na^+ permeability. In this case Ca^{2+} should affect Cl^- but not Na^+ exchange at equilibrium. In order to investigate such a possibility, experiments had been performed at equilibrium condition for NaCl, at which 10 mM NaCl was present at both sides of the vesicle and the Na^+ tracer only at the vesicle inside. Under these conditions the effect of 10^{-4} M Ca^{2+} concentration at both sides or at the inside or outside only on $^{22}Na^+$ exchange was qualitatively the same as on Na^+ net efflux although reduced by 50% (3 experiments, data not shown). This could indicate an effect of $Ca^{2+}{}_i$ on Cl^- exchange or, as outlined below, an inhibitory effect of $Na^+{}_{ou}$ itself on Na^+ permeability.

$^{22}Na^+$ Efflux and Na^+ Concentration at the Inside, at the Outside or at Both Sides of the Vesicles

When $Na^+{}_i$ was increased to 20, 50, or 100 mM, osmolarity at the vesicle outside in the incubation medium and washing solution being adjusted with mannitol, Na^+ efflux was increased (series I_1 in Table 1). A similar dependence on Na^+ concentration was seen at equilibrium, Na^+ being present at 10, 20, 50, and 100 mM at both sides of the membrane (series I_2). The Na^+ exchange rates, however, were lower for each Na^+ concentration between 10 and 50 mM when Na^+ was present at both sides of the membrane as compared to Na^+ net efflux rates (series I_1 compared to series I_2). Similar to the effect of varying Na^+ concentrations on tracer exchange, Rb^+ and Cs^+ affected $^{86}Rb^+$ and $^{134}Cs^+$ fluxes, respectively (series II_1 and II_2 in Table 1).

Since at 10, 20, 50, or 100 mM Na^+ efflux was slower at conditions of tracer exchange as compared to Na^+ net efflux, it might be suggested that Na^+ similar to Ca^{2+} but at higher concentrations has an inhibitory influence on $^{22}Na^+$ efflux. To substantiate this possibility, experiments

Table 1. Effect of increasing cation concentration at the vesicle inside only (*i*) at both sides (*i*+*ou*) and at the vesicle outside (*ou*) on half times ($t_{1/2}$) of cation efflux (1 experiment for each series)

I_1	Na^+_i (mM) (Na^+_{ou}=2% of each concentration)	$^{22}Na^+$ efflux $t_{1/2}$ (sec)	$t_{1/2}$ (%)
	10	34.2	100.0
	20	12.8	37.4
	50	5.9	17.3
	100	4.0	11.7
I_2	Na^+_{i+ou}	$^{22}Na^+$efflux	
	10	64.4	100.0
	20	24.7	38.4
	50	9.2	14.3
	100	4.0	6.2
II_1	Rb^+_{i+ou}	$^{86}Rb^+$efflux	
	10	64.4	100.0
	50	15.7	24.4
	100	11.3	17.5
II_2	Cs^+_{i+ou}	$^{134}Cs^+$efflux	
	10	81.4	100.0
	50	19.0	23.3
	100	13.7	16.8
III_1	Na^+_{ou} (Na^+_i=10 mM)	$^{22}Na^+$efflux	
	0.2	31.2	100.0
	2.0	34.3	109.0
	5.0	34.3	109.0
	10.0	81.4	261.0
	20.0	81.4	261.0
	50.0	53.1	170.2

The half-times are calculated from the initial slope of efflux curves which were composed of 12 experimental values each. Only the values within one series (*I, II, III*) can be compared with each other since they are from the same vesicle preparation.

were performed in which 10 mM $^{22}Na^+$ was present at the vesicle inside, whereas the external Na^+ concentration series was varied between 0.2 and 50 mM Na^+ (series *III* in Table 1), Na^+ efflux was not affected by 2 and 5 mM Na^+ but was slower in the presence of 10, 20, and 50 mM Na^+ at the vesicle outside as compared to our standard condition when no additional Na^+ was added to the outside medium.

Effect of Different Monovalent Cations at the Vesicle Outside on Na^+ Efflux

When the effectiveness of the outside concentrations of different cations was tested it was found that Rb^+, Cs^+, Li^+, and K^+ had the same inhibitory potency on $^{22}Na^+$ efflux as Na^+, all being in the same way effective as Ca^{2+} but in higher concentrations (Table 2). Increase in the H^+ ion concentration by 100-fold to pH 5 at the vesicle outside, however, did not affect $^{22}Na^+$ efflux (data not shown).

Table 2. Effectiveness of different cations at the vesicle outside on $^{22}Na^+$ efflux within the initial 10 sec[a]

Cations present at the outside (20 mM)	$t_{1/2}$ (%)
No addition	100.0
NaCl (or 10^{-4} M $CaCl_2$)	116.4
RbCl, CsCl, LiCl or KCl	122.4
Tris-Cl or choline-Cl	194.8

[a] Relative values of 20 mM cation concentration at the vesicle outside as compared to the $^{22}Na^+$-efflux rate in the absence of added cations are shown. Initial $^{22}Na^+$ concentration at the vesicle inside was 10 mM (1 experiment).

Effect of Ca^{2+} on $^{86}Rb^+$ and $^{134}Cs^+$ Efflux

The effect of Ca^{2+} on either $^{86}Rb^+$ or $^{134}Cs^+$ efflux was not comparable to that on NaCl efflux. In both cases 10^{-4} M Ca^{2+} at the vesicle outside had only a small inhibitory effect on both $^{86}Rb^+$ efflux (Fig. 12) and $^{134}Cs^+$ efflux (data not shown). The effect of Ca^{2+} at the inside was even absent. The $^{86}Rb^+$ efflux at equilibrium condition (tracer exchange) was not affected by Ca^{2+} at all (data not shown).

Orientation of Membrane Vesicles

A comparison of the effects of Ca^{2+} on $^{22}Na^+$ permeability in isolated membrane vesicles to the situation in intact cells would only make sense if the membranes are orientated outside out. One way of determining the orientation of the vesicles is to measure the Na^+-K^+-activated, Mg^{2+}-dependent ATPase before and after rupture of the vesicles. Since penetra-

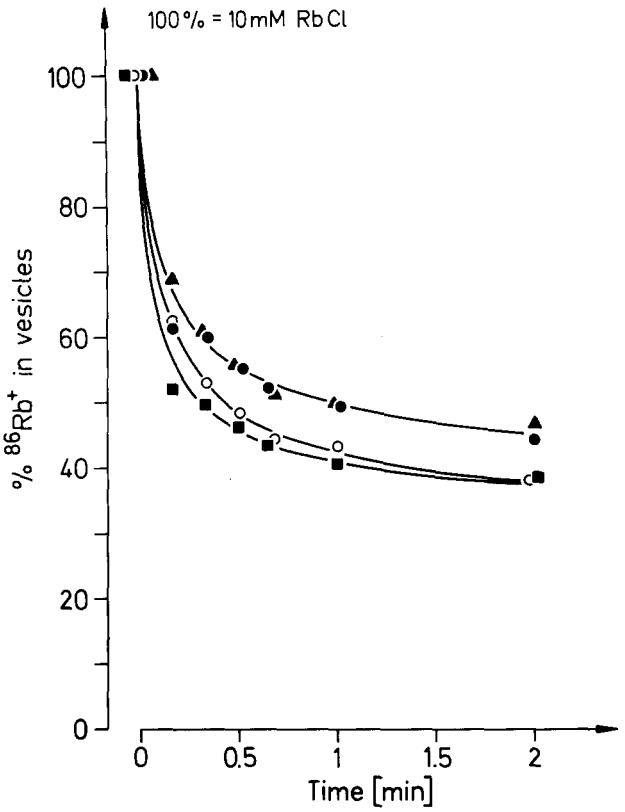

Fig. 12. Effect of calcium (10^{-4}M) on ^{86}RbCl efflux. ▲—▲, with $Ca^{2+}{}_{ou}$; ●—●, with $Ca^{2+}{}_{i+ou}$; ○—○, without calcium; ■—■, with $Ca^{2+}{}_{i}$. Each point is taken in duplicate and the data are representative for 1 out of 3 similar experiments

tion of ATP into closed membrane vesicles is not likely, a Na^{+}-K^{+}-ATPase activity should be expected only if ATP had access to the respective ATP binding sites at the inner face of the cell membrane. Thus an increase in Na^{+}-K^{+}-ATPase activity should be expected after rupture of closed outside-out oriented vesicles. As shown in Fig. 13 in the absence of detergents and taking care that vesicles were not destroyed during the incubation period of the ATPase test, the Na^{+}-K^{+} ATPase showed a small activity. With increasing concentration of desoxycholate (DOC) Na^{+}-K^{+}-ATPase activity increased by 4 times at 1.9 mM desoxycholate, whereas at higher DOC concentrations inhibition of ATPase activity was observed. Freeze-thawing for one time increased the activity only twofold, probably due to the fact that not all vesicles had been ruptured or revesiculation had occurred. It had been mentioned above that also ^{3}H-alanine remained trapped after this procedure.

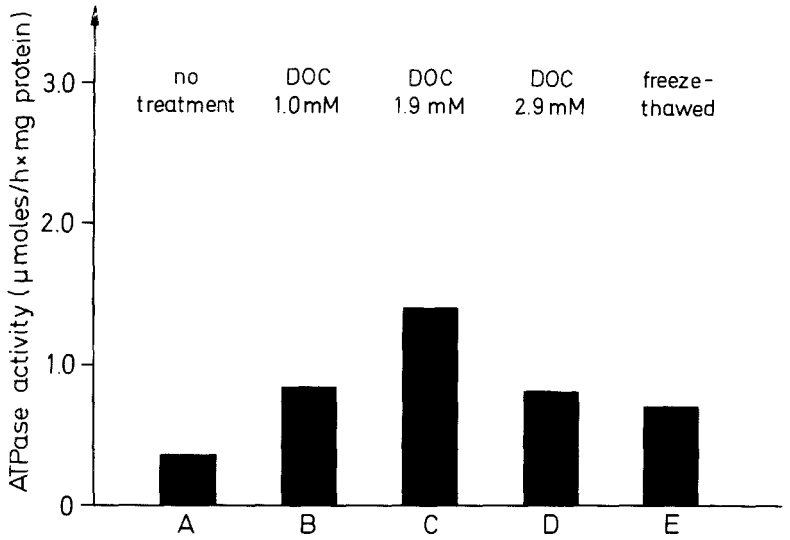

Fig. 13. Effect of different desoxycholate (DOC) concentrations and of 1-time freeze-thawing on Na^+-K^+ ATPase activity in membrane vesicles. One out of 2 similar experiments

Assuming that the Na^+-K^+ ATPase is located at the baso-lateral cell side and taking into account that in the absence of detergents no damage to vesicles had occurred during the Na^+-K^+-ATPase test, the increase in ATPase activity would indicate that at least 75% of Na^+-K^+ ATPase-containing membrane vesicles are orientated outside out.

Could the Calcium Effect be Due to Aggregation or Fusion of Vesicles?

If the effect of calcium present at the vesicle outside were due to fusion or aggregation of vesicles [5, 12, 37], vesicles with higher volume/surface ratio would be formed which should result in decreased $^{22}Na^+$-efflux rates as compared to smaller vesicles. We have measured the efflux of ^{3}H-L-alanine at the conditions used for $^{22}Na^+$ efflux and found no difference in the efflux curves whether calcium was present at the outside, at both sides, at the inside, or in the absence of calcium, suggesting that the calcium effect observed on Na^+ efflux could not be explained by an unspecific effect due to aggregation or fusion of vesicles. The same conclusion can be drawn from the Rb^+ efflux curves (Fig. 12). Furthermore, the total intravesicular space was also not altered by Ca^{2+}_{ou}

since neither the $^{22}Na^+$ uptake at equilibrium nor the ^{3}H-L-alanine uptake showed an increase in the presence of calcium.

Discussion

I. The Use of Cell Membrane Vesicles as a Tool for Na^+-Flux Studies

The method to use isolated membrane vesicles for flux studies has been successfully applied in a variety of tissues to localize and to characterize specific transport systems [13, 14, 17, 24–28, 39, 57, 66]. The advantage of this approach is due to the fact that membrane transport properties can be investigated without participation or interference of cellular metabolism. There are, however, several problems attached to the use of such preparations, which have to be considered. So it is necessary to show that the membranes form closed vesicles of which the orientation is known and to distinguish whether changes in uptake or release are caused by changes in fluxes or changes of binding. An appropriate judgement of the calcium effect on Na^+ release from our membrane vesicle preparation seems to be possible by the following considerations.

1) Evidence for closed vesicles and their orientation: The decreasing ^{22}NaCl uptake with increasing medium osmolarities as depicted in Fig. 2 suggests that Na^+ uptake occurred into an osmotic active space. In a previous study [66] the same effect was seen with ^{3}H-L-alanine uptake using the same membrane preparation. The demonstration of a Na^+-L-alanine cotransport system in these membrane vesicles, into which uptake of L-alanine against its concentration gradient was observed [66], further documents the presence of closed membrane vesicles. The increase in Na^+-K^+-ATPase activity with treatment of desoxycholate at 1.0 and 1.9 mM and once freeze-thawing (Fig. 13) again not only shows the presence of closed cell membrane vesicles but also of right-side out orientation. Rupture of vesicles exposes ATP-binding sites, located at the inside of the cell membrane, and therefore would cause an increase in Na^+-K^+-ATPase activity. Higher concentrations of detergent inhibit the enzyme activity itself, as has also been shown in another system [22].

2) Does calcium affect Na^+ permeability, Na^+ binding, or the vesicle size? The negligible contribution of Na^+ binding to the overall Na^+

uptake by the vesicles was not only assessed by extrapolation of Na^+ uptake to infinite osmolarity (Fig. 2) but also by measuring the Na^+ taken up at varying Na^+ concentrations in the medium (Fig. 3): At 10 mM Na^+, the concentration used for efflux studies, binding was only 0.8% of the total Na^+ present within the vesicles and could be neglected at least for the initial 2 min, the time at which the intravesicular Na^+ concentration was still 5 mM. Appreciable binding, however, occurred at a Na^+ concentration lower than 2.5 mM as could be judged from the Scatchard plot shown in Fig. 3. This binding could be the reason for the relatively long time of 20 hr necessary to reach equilibrium for Na^+ distribution. Another possible effect of Ca^{2+} concerns its ability to aggregate and to fuse membrane vesicles [3, 5, 12, 37]. Since our method of measuring $^{22}Na^+$ efflux was by diluting membranes in a medium which either contained or did not contain Ca^{2+}, the presence of Ca^{2+} in the efflux medium could cause aggregation or fusion of vesicles, thereby diminishing the number of small and increasing the number of large vesicles. Na^+ efflux from larger vesicles should be slower because of an increase in the volume/surface ratio. By the same reason, the total available space for $^{22}Na^+$ distribution would also have to become larger. Several points make such an effect of Ca^{2+} unlikely. Since the intravesicular space was measured by uptake of ^{3}H-L-alanine at equilibrium at each condition applied, ^{3}H-L-alanine and $^{22}Na^+$-equilibrium uptake should be increased in the presence of extravesicular Ca^{2+} which was not the case. Furthermore, calcium did not influence ^{3}H-alanine efflux. The Rb^+ efflux (Fig. 12) was affected only to a small extent, which suggests some specificity for the Ca^{2+} effect on Na^+ efflux. In addition, if a decrease in $^{22}Na^+$ efflux was due to fusion or aggregation of vesicles, the same efflux curve as in the presence of extravesicular Ca^{2+} should be observed in the presence of Ca^{2+} at both sides of the vesicles. In analogy, vesicles submitted to the same preincubation conditions as for testing the calcium effect from the inside or at both sides of the membrane should show the same Na^+ efflux curves. Both was clearly not the case, arguing against fusion or aggregation. A fusogenic effect of Ca^{2+} also should not be influenced by a small concentration of ionophores. However, in the presence of either monensin or A23187 which increases the Na^+ or Ca^{2+} permeability, respectively, the effect of Ca^{2+} at the vesicle outside as well as at the vesicle inside was nearly abolished (Fig. 8*a–b*). These data show that the Ca^{2+} control of Na^+ efflux was compensated by the increase in Na^+ permeability in the presence of a Na^+-specific ionophore on the one hand and by a rapid equili-

bration of different Ca^{2+} concentrations at both sides of the membrane in the presence of the Ca^{2+} ionophore A23187 on the other hand. All these observations indicate that Ca^{2+} changes the Na^+ permeability of our membrane vesicles.

3) Membrane specificity of the calcium effect. In membranes enriched in the endoplasmic reticulum marker ribonucleic acid prepared from the same tissue [38] as well as in liver plasma membranes, a similar effect of Ca^{2+} as in pancreatic cell membranes on $^{22}Na^+$ efflux was absent. These observations further argue for the calcium effect being specific for a certain type of membranes. Since our vesicles probably originate from different cell membrane areas, the possibility exists that only a part of the vesicles present respond to an increased $Ca^{2+}{}_i$ with an increase in Na^+ permeability. Thus, it could be that the $Ca^{2+}{}_i$ effect is much larger when compared to the responding vesicles than when compared to all vesicles present.

II. Control of the Membrane Permeability for Na^+ by Calcium

1) Time course of the calcium effect. After an initial rapid phase within the first 40 sec, all Na^+-efflux curves changed to the same slower efflux rate (Figs. 5 and 6*a–b*). A similar decrease in the $^{22}Na^+$-efflux rate coefficient was also observed in experiments in which no Ca^{2+} but the same Na^+ concentration (10 mM) was present at both sides of the vesicle membrane (curve not shown). In this case the change of an initial Na^+-efflux rate of 0.01 sec^{-1} to the same value for all efflux curves at 0.003 sec^{-1} between 40 and 60 sec could be attributed to different size and/or different permeabilities of vesicles from a heterogeneous vesicle population. The initial Na^+-efflux rates, however, could be influenced by an asymmetric distribution of Ca^{2+} ions and to a smaller extent also of Na^+ ions. Thus, Ca^{2+} at the inside only increased Na^+ efflux. When added to the outside, however, it decreased Na^+ efflux.

The finding that unequal Ca^{2+} distribution affects Na^+ efflux only within the first 40 sec could be interpreted to mean that the Ca^{2+} gradient initially present decreases during this time interval by an equilibrating Ca^{2+} flux over the membrane. Similarly a change of flux rates could result by reduction of the Na^+ gradient itself. Experiments with increasing NaCl concentrations at the inside or at both sides of the vesicle membranes (Table 1) suggest a cation effect of Na^+ similar to that of Ca^{2+}

at the vesicle inside. Thus a decrease in the Na^+ concentration at the vesicle inside due to Na^+ efflux would decrease the membrane permeability to Na^+ continuously. Calcium being present at the outside, however, would diminish this effect by inhibiting Na^+ efflux. If both the outside inhibitory as well as the inside accelerating effect of Na^+ and Ca^{2+} are additive, a smaller effect of Ca^{2+} on $^{22}Na^+$ isotope exchange would be expected as compared to $^{22}Na^+$ net efflux. Indeed, all Ca^{2+} effects on $^{22}Na^+$-tracer exchange were only half as large as on Na^+ efflux.

Curves similar to those in Fig. 7 showing a transition from a rapid to a slow phase were obtained by Riordan and Passow who studied the effect of different Ca^{2+} concentrations on K^+ efflux from erythrocyte ghosts [54]. This change of efflux rates had been interpreted to mean that either all ghosts of the population were similarly affected, responding to increasing Ca^{2+} concentrations with increasing K^+-efflux rates, or that an all or none effect was involved. In the latter case the initial rate constants would reflect the response of those ghosts whose threshold was exceeded, the amount of K^+ loss during the rapid phase being proportional to the number of ghosts whose threshold was surpassed [54]. Although such a possibility has to be considered for our case, the fact that after 40 sec the same slower efflux rates were observed even in the absence of Ca^{2+} or when present at the outside only (Fig. 5) would rather support the interpretation that under our conditions the Ca^{2+} gradient initially present tends to break down and that at equilibrated Ca^{2+} concentrations Na^+-efflux rates became the same. If Ca^{2+} equilibration was accelerated artificially by the use of the Ca^{2+} ionophore, even the initial Na^+-efflux rates became similar for all Ca^{2+} conditions tested (Fig. 8).

2) Nature of the Ca^{2+} effect. Having no direct information about the nature of Ca^{2+}-binding sites involved in the control of Na^+ permeability in our system, we can only speculate about the mode of Ca^{2+}-action to increase or to decrease Na^+ permeability of the membrane. From our data it becomes more likely that Ca^{2+} interacts with two independent membrane sites at both sides of the membrane: Thus the fact that Ca^{2+} and Ba^{2+} have similar effects on the Na^+ efflux from the vesicle outside but different effects on the increase in Na^+ efflux when applied at the vesicle inside suggests differences in the nature of extracellular and intracellular sites of Ca^{2+} action. Further support for this is provided by findings which demonstrate differences of intra- and extracellular Ca^{2+} action in Ca^{2+}-concentration dependencies (Fig. 9),

pH dependence (Fig. 11), and activation energies (Fig. 4). The temperature dependence of the Ca^{2+} effect indicates a phase transition in the presence of Ca^{2+} at the vesicle outside. Since it was shown that divalent cations increase the transition temperature in phospholipid bilayers [64] and phospholipid vesicles [21, 68], our data could indicate that membrane lipids are fluid in the whole temperature range tested when no Ca^{2+} or Ca^{2+} at the inside only was present. However, when Ca^{2+} was added to the outside, phospholipids might have been turned into the solid state in the temperature range between 4 and 10 °C with a consequent highly decreased Na^+ permeability in this temperature range. It cannot be excluded, however, that temperature changes exert critical conformational changes of membrane proteins which lead to a break in the Arrhenius plot.

III. Action of Calcium on Electrolyte Permeability in Lipid and Biological Membranes

We have no direct information concerning the molecular mechanism by which calcium influences the Na^+ permeability in our system.

A plausible interpretation can nevertheless be suggested on the basis of available information obtained by experiments with artificial membranes. So far most model experiments have been performed with defined phospholipids. Therefore, primary interactions of calcium with phospholipids and resulting secondary effects on proteins embedded in the lipid phase are better understood than direct Ca^{2+} effects on membrane proteins. In general one can say that specific adsorption of Ca^{2+} and other bivalent cations to negatively charged bilayers reduce the surface charge, condense or "stabilize" the membrane [7], reduce its fluidity, and decrease its permeability [64, 65, 67].

In membranes of mixed phospholipids, however, Ca^{2+} can induce phase separation and formation of solid phospholipid islets within the fluid moiety [41]. Thereby the permeability is increased at the border of solid to fluid domains [43, 44, 46]. In these systems the presence of membrane proteins can facilitate the Ca^{2+}-induced formation of solid domains with consecutive increase in permeability [23]. Because of its high affinity, it is likely that in biological membranes Ca^{2+} control of permeability involves specific interaction of Ca^{2+} with proteins. Conformational changes in the protein—alone or in concert with the surrounding lipids—could be responsible for the observed permeability changes [23, 47].

The effect of Ca^{2+} to increase membrane permeability has been described for a variety of tissues. Thus the effect of intracellular Ca^{2+} to increase K^+ conductance is not only present in red cells [9, 10, 11, 15, 32, 33, 48, 52, 54, 56, 61] but also in excitable tissues [20, 31, 35, 36]. In another secretory tissue, the insect salivary gland, intracellular calcium increases the chloride permeability [2]. Similar to our observations, an effect of internal free calcium in the micromolar concentration range on Na^+ conductance in the tunicate egg has been reported [62]. In these systems the view of calcium-activated protein channels or carriers seem to be preferred to that of calcium-induced phase separation. It can be suggested that our observations of Ca^{2+}-controlled Na^+ permeability might have some molecular events in common with other systems; however, we do not know detailed processes. Although we have no indication whether the described Ca^{2+} effects on pancreatic membranes have some relevance for Ca^{2+}-dependent secretion in the exocrine pancreas [50], our observations are compatible with the interpretation that a rise in free Ca^{2+} in pancreatic acinar cells by Ca^{2+} influx and/or displacement of Ca^{2+} from intracellular stores [4, 8, 29, 30, 49, 60, 70] would increase the NaCl permeability and therefore NaCl influx into the cell. Electrophysiological data of Petersen and his colleagues suggest that Ca^{2+} increases both Na^+ and Cl^- permeability of the acinar cell membrane and to a smaller extent that of K^+, too [19].

Our data on NaCl efflux agree with these observations, and comparing $^{86}Rb^+$ with K^+ also suggests a difference in membrane permeability for both Na^+ and K^+.

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