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Na^+/Ca^{2+} exchange in coated microvesicles

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Coated microvesicles isolated from bovine neurohypophyses could be loaded with Ca^{2+} in two different ways, either by incubation in the presence of ATP or by imposition of an outwardly directed Na^+ gradient. Na^+, but not K^+, was able to release Ca^{2+} accumulated by the coated microvesicles. These results suggest the existence of an ATP-dependent Ca^{2+}-transport system as well as of a Na^+/Ca^{2+} carrier in the membrane of coated microvesicles similar to that present in the membranes of secretory vesicles from the neurohypophysis. A kinetic analysis of transport indicates that the apparent K_m for free Ca^{2+} of the ATP-dependent uptake was 0.8 μM. The average $V_{max.}$ was 2 nmol of Ca^{2+}/5 min per mg of protein. The total capacity of microvesicles for Ca^{2+} uptake was 3.7 nmol/mg of protein. Both nifedipine (10 μM) and NH_4Cl (50 mM) inhibited Ca^{2+} uptake. The ATPase activity in purified coated-microvesicle fractions from brain and neurohypophysis was characterized. Micromolar concentrations of Ca^{2+} in the presence of millimolar concentrations of Mg^{2+} did not change enzyme activity. Ionophores increasing the proton permeability across membranes activated the ATPase activity in preparations of coated microvesicles from brain as well as from the neurohypophysis. Thus the enzyme exhibits properties of a proton-transporting ATPase. This enzyme seems to be linked to the ion accumulation by coated microvesicles, although the precise coupling of the proton transport to Ca^{2+} and Na^+ fluxes remains to be determined.

INTRODUCTION

Clathrin-coated microvesicles (Pearse, 1975) are involved in endocytosis and receptor retrieval in many tissues (Steinman *et al.*, 1983). On the basis of morphological studies, coated microvesicles in the neurohypophysis have been suggested to be involved in membrane retrieval after exocytosis. However, quantitative morphological investigations have shown that coated microvesicles do not take part in this process (Nordmann *et al.*, 1979; Morris & Nordmann, 1980, 1981), and biochemical data supported these observations (Swann & Pickering, 1976; Saermark *et al.*, 1984).

Some reports have suggested that coated microvesicles contain a Ca^{2+}-activated ATPase and are involved in the regulation of intracellular free [Ca^{2+}] in nerve cells, including the neurohypophysis (Blitz *et al.*, 1977; Torp-Pedersen *et al.*, 1980; Nordmann & Chevallier, 1981). However, the presence of a Ca^{2+}-activated ATPase may be due to contamination of the preparations by cell membrane components (Saermark & Thorn, 1982). Other work has demonstrated that the ATPase activity in isolated brain coated microvesicles (Saermark & Thorn, 1982) may be a Mg^{2+}-dependent H^+-transporting ATPase (Forgac *et al.*, 1983; Stone *et al.*, 1983). A similar enzyme in liver endosomes has been characterized (Saermark *et al.*, 1984) and may be involved in acidification of the endosomal compartment, known to take place soon after endocytosis (Marsh, 1984). These findings have made necessary a re-investigation of the properties of Ca^{2+} accumulation by neurohypophysial coated microvesicles.

The secretory vesicles of the nerve endings in the neurohypophysis containing vasopressin and oxytocin have been demonstrated to accumulate Ca^{2+} (Saermark *et al.*, 1982). This process is almost completely inhibited by Na^+ and is presumably due to the presence of a Na^+/Ca^{2+}-exchange protein in the secretory-vesicle membrane (Saermark *et al.*, 1983). A similar system has also been described in chromaffin vesicles from the adrenal medulla (Phillips, 1981; Krieger-Brauer & Gratzl, 1981, 1982, 1983).

The results presented here suggest that coated microvesicles are able to transport Ca^{2+} in a Na^+-dependent manner, which exhibits the same properties as that found in secretory vesicles. The presence of this activity in coated microvesicles indicates their involvement in a specific retrieval of the Na^+/Ca^{2+} carrier from the cell membrane after exocytosis.

EXPERIMENTAL

Materials

BAPTA was obtained from BDH. EGTA, Mes, Tes and adenosine 5'-[β,γ-imido]triphosphate were from Sigma. Nitrilotriacetic acid and EDTA were from Merck. All other reagents used were of analytical grade. ATP was vanadate-free (Sigma). The ionophores monensin and FCCP and nifedipine were from Sigma.

Isolation of subcellular fractions

Coated microvesicles were prepared essentially as described by Pearse (1975). Bovine neurohypophyses (eight to ten) were minced and homogenized in a Teflon/glass homogenizer in a medium containing 20 mM-Mes, pH 6.5, 100 mM-KCl, 0.5 mM-EGTA and 1 mM-$MgCl_2$. The material was centrifuged at 3000 $g_{av.}$ for 5 min, and the supernatant from this at 35000 $g_{av.}$ for 30 min. The supernatant was centrifuged at 80000 $g_{av.}$

Abbreviations used: BAPTA, bis(aminophenoxy)ethane-*NNN'N'*-tetra-acetic acid; FCCP, carbonyl cyanide *p*-fluoromethoxyphenylhydrazone.

for 30 min, and the resulting pellet was resuspended in the homogenization medium. It was placed on top of a sucrose step gradient (0.58 M, 0.88 M, 1.17 M, 1.46 M and 1.75 M) in the homogenization medium. The gradient was centrifuged in a SW 36 rotor (Beckman) for 18 h at 50000 $g_{av.}$. The material at the interface between 1.46 M- and 1.75 M-sucrose medium was recovered, diluted 1:10 in the homogenization buffer and centrifuged at 100000 $g_{av.}$ for 60 min. The resulting pellet was resuspended in the buffer and centrifuged for 30 min at 50000 $g_{av.}$ (SW 36 rotor) on a sucrose step gradient (0.29 M, 0.88 M and 1.75 M) in the homogenization buffer. Material at the 0.29 M-/0.88 M-sucrose interface was recovered and diluted 1:10 in the buffer, followed by centrifugation at 100000 $g_{av.}$ for 30 min. In a series of experiments, rat brain coated microvesicles were prepared in the same way. The initial homogenization in these experiments was carried out as described by Pearse (1975).

Measurement of Ca^{2+} uptake and release

The accumulation of Ca^{2+} was measured by using ^{45}Ca and a Millipore-filtration technique (Saermark & Vilhardt, 1979; Saermark *et al.*, 1983). The incubation volume was 200 μl, and uptake was stopped by adding 5 ml of 25 mM-EGTA/20 mM-Tes (pH 7.0)/190 mM-sucrose, followed by filtration.

^{45}Ca efflux was measured by addition of 2 ml of 130 mM-KCl/0.5 mM-EGTA/20 mM-Tes, pH 7.3, including bivalent cations, with replacement of KCl by NaCl as indicated. The addition of 10 μM-BAPTA revealed that the free Ca^{2+} concentration was lowered to less than 50 nM when compared with a standard curve based on calculated free Ca^{2+} concentrations (see below). KOH was used for pH adjustments, and all solutions contained 0.5 mM-ouabain. Na^+ in the microvesicles was measured by atomic-emission spectroscopy by using a Perkin–Elmer inductively coupled plasma spectrometer (type 5500).

Preparation of Ca^{2+}-buffered media

The Ca^{2+}-uptake measurements and ATPase assays were based on a medium containing 0.5 mM-EGTA, 5 mM-nitrilotriacetic acid and 0.5 mM-EDTA for buffering of the free Ca^{2+} concentration; 20 mM-Tes was used for buffering the pH, and Ca^{2+} binding to this substance was without importance below 0.1 mM free Ca^{2+}. The medium included 130 mM-KCl, 130 mM-NaCl, or 260 mM-sucrose, as indicated. Other additions are described in the text.

The free Ca^{2+} and Mg^{2+} concentrations were calculated as described by Flodgaard & Fleron (1974) by using the binding constants stated by Sillen & Martell (1971). For Ca^{2+} the calculations were verified by using a Ca^{2+}-specific electrode to prepare Scatchard plots for the individual buffer substances. Inclusion of BAPTA (10 μM) was used to verify the calculated Ca^{2+} concentrations when Mg^{2+} was present (Tsien, 1980). The Ca^{2+}-dependent absorption by BAPTA was read at 254 nm.

Enzyme assays

ATPase activity (EC 3.6.1.3) was measured with Malachite Green (Itaya & Ui, 1966) as described by Saermark & Thorn (1982). Succinate dehydrogenase (EC 1.3.99.1) was measured as described by Saermark *et al.* (1985). Acid phosphatase (EC 3.1.3.2) was measured as described by Saermark *et al.* (1983), by using Malachite Green to estimate P_i liberation from α-glycerophosphate. Protein was determined by using *o*-phthaldehyde (Benson & Hare, 1975).

Other procedures

Electron microscopy was carried out on Epon-embedded pellets (0.5 mm thickness) fixed in 4% (v/v) glutaraldehyde in 0.1 M-sodium phosphate buffer, pH 7.4, for 4 h and stained with OsO_4 (1%). SDS/polyacrylamide-gel electrophoresis was carried out as described by Laemmli (1970).

The uptake of [^{14}C]methylamine was measured in a buffer containing 10 mM-Tes, 10 mM-Mes, 1 mM-$MgCl_2$, 1 mM-ATP and 260 mM-sucrose at different pH values and with the substitutions indicated in the text. The amount of methylamine trapped was determined as described by Forgac *et al.* (1983), by using a 1 ml column of Sephadex G-50.

RESULTS

Purity of the fractions of coated microvesicles

The purity of the preparation of coated microvesicles from the neurohypophysis was evaluated by electron microscopy. The fraction appeared homogeneous, and in particular it did not contain mitochondrial profiles or lysosomes. The purity was further confirmed by measurement of marker enzymes for mitochondria and lysosomes (Table 1). Succinate dehydrogenase and

Table 1. Marker enzymes present in the coated-microvesicle fraction from the neurohypophysis

The results are expressed as percentage recovered in the fraction relative to the homogenate (means ± S.D.; $n = 5$) and as relative specific activity (% of enzyme activity divided by % of protein in the fractions; homogenate = 100%). Succinate dehydrogenase and $Na^+ + K^+$-dependent ATPase activities were not measurable in the fractions, and instead the detection limits of the assays were used to calculate the highest possible degree of contamination.

Assay	Recovery in the coated vesicles (%)	Relative specific activity
Protein	0.2 ± 0.03	1.00
Succinate dehydrogenase	< 0.01	< 0.05
Acid phosphatase	0.01 ± 0.003	0.05
Mg^{2+}-dependent ATPase	0.3 ± 0.05	1.50
$Na^+ + K^+$-dependent ATPase	< 0.015	< 0.08

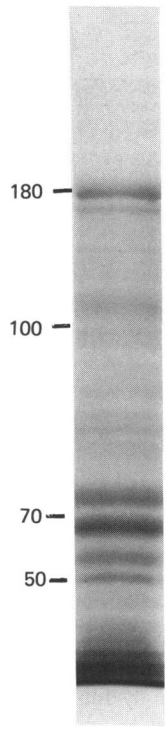

Fig. 1. SDS/polyacrylamide-gel electrophoresis of a preparation of coated microvesicles from the neurohypophysis

Acrylamide (7%) gels were prepared as described by Laemmli (1970). The sample (70 μg) was dissolved in boiling SDS-sample buffer (Laemmli, 1970) including dithiothreitol. The indicated M_r values ($\times 10^{-3}$) were determined by running standards in parallel. The gel was stained in Coomassie Blue.

lysosomal acid phosphatase were not co-purified with the coated microvesicles. Also shown in Table 1 is the enrichment in Mg^{2+}-dependent ATPase activity and the low $Na^+ + K^+$-dependent ATPase activity.

The SDS/polyacrylamide-gel-electrophoresis pattern (Fig. 1) shows a protein composition very similar to that seen in other preparations of coated microvesicles (Pearse, 1978; Simion *et al.*, 1983).

Clathrin, of M_r 180000, and a component of M_r 120000 could be identified (Fig. 1). Proteins of Mr 85000, 70000 and 50000 were also present. The major peak in front of the gel chromatogram could be resolved into the clathrin-associated proteins when 10%-acrylamide gels were used [see Daiss & Roth (1983) and Simion *et al.* (1983) for comparison].

Ca^{2+} uptake and release from coated microvesicles

Coated microvesicles isolated from bovine neurohypophyses were able to accumulate Ca^{2+} in two different ways. As shown in Fig. 2, ATP-dependent Ca^{2+} accumulation could be demonstrated by incubation of the vesicles in the presence of 130 mM-KCl, 2 mM-ATP and 1 mM-Mg^{2+} (free). The amount of calcium accumulated was decreased when Mg^{2+} or ATP was omitted from the medium (Fig. 2). When the coated microvesicles were incubated under these conditions, they accumulated 3.7 ± 1 nmol of Ca^{2+}/mg of protein within 30 min. The kinetic parameters of Ca^{2+} uptake were derived from

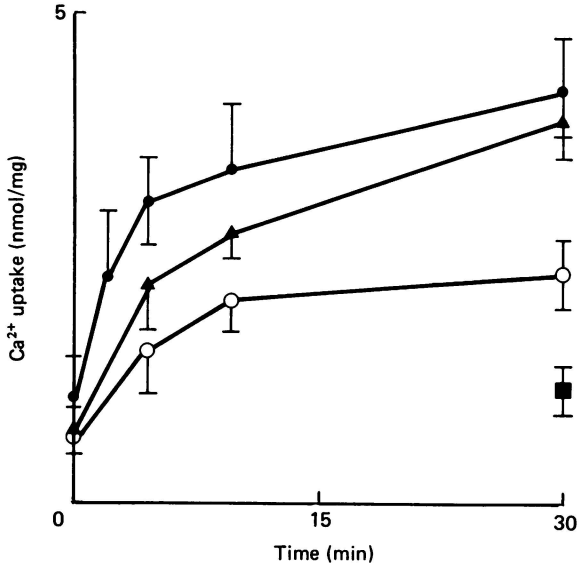

Fig. 2. Ca^{2+} uptake by coated microvesicles from the neurohypophysis

Coated microvesicles from bovine neurohypophyses were incubated in a buffer containing 130 mM-KCl, 20 mM-Tes (pH 7.3) and nitrilotriacetic acid, EGTA and EDTA as described in the Experimental section. The buffer also contained 0.1 mM free Ca^{2+}, 1 mM free Mg^{2+} and 2 mM-ATP (●). The Ca^{2+} uptake, at 1 μM free Ca^{2+}, 1 mM free Mg^{2+} and 2 mM-ATP (▲), under the same conditions, but in the absence of Mg^{2+} ions (○), or in the absence of ATP (■), is also shown. Results are means ± S.D. ($n = 3$). Ouabain (0.5 mM) was included in the media.

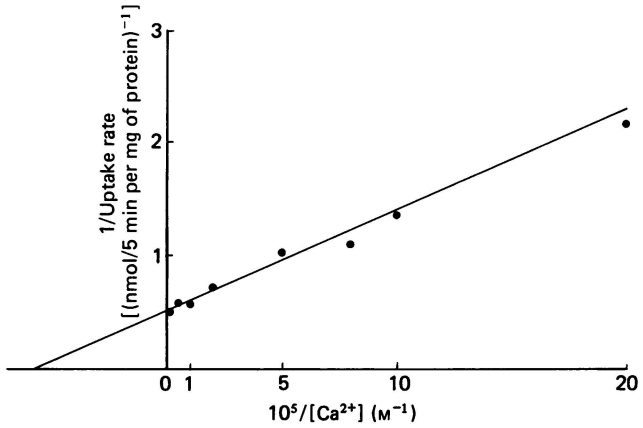

Fig. 3. Lineweaver–Burk plot of the Ca^{2+} uptake in coated microvesicles.

Coated microvesicles (50 μg) were incubated at various free Ca^{2+} contentrations, and the Ca^{2+} uptake was measured after 5 min as described in Fig. 2. The medium contained 2 mM-ATP and 1 mM-Mg^{2+} as described in Fig. 2. The K_m value determined from this experiment was 0.8×10^{-6} M and the $V_{max.}$ was 2 nmol/5 min per mg of protein.

Lineweaver–Burk plots (Fig. 3). The K_m value for Ca^{2+} from four such experiments was 0.9 (± 0.3; S.D.) $\times 10^{-6}$.

An alternative procedure to demonstrate Ca^{2+} uptake was to incubate the vesicles for 20 h at 0 °C in the presence of Na^+ before incubation in the presence of Ca^{2+}. When such vesicles were added to a medium

Table 2. Effect of Na^+ load on Ca^{2+} uptake by coated microvesicles

Coated microvesicles isolated from bovine neurohypophyses were incubated for 20 h (at 0 °C) in a medium containing 130 mM-KCl (without Na^+ load) or 130 mM-NaCl (Na^+ load) as well as 0.5 mM-EGTA, 1 mM-$MgCl_2$ and 20 mM-Mes, pH 6.5. The vesicles were diluted with 130 mM-KCl/0.5 mM-EGTA/1 mM-$MgCl_2$/20 mM-Mes, pH 6.5, and centrifuged for 20 min at 160000 $g_{av.}$ (4 °C). The pellet was resuspended in the Ca^{2+}-uptake medium (see the Experimental section), and the Ca^{2+} uptake was measured at 0.1 mM free Ca^{2+} with and without ATP (2 mM) present. The results are given as means ± S.D. ($n = 3$).

Experimental details	Ca^{2+} uptake (nmol/5 min per mg of protein)
Without Na^+ load	0.6 ± 0.1
Na^+ load	2.7 ± 0.2
Na^+ load, +2 mM-ATP	4.2 ± 0.1
Without Na^+ load, +2 mM-ATP	4.4 ± 0.3

containing 130 mM-KCl and 1 mM-Mg^{2+} (free) they accumulated Ca^{2+} (Table 2). This uptake was greater (by a factor of 4.5) than the uptake by vesicles not loaded with Na^+ and led to a decrease in their Na^+ content as measured directly by atomic-emission spectroscopy. The vesicles contained 22.8 nmol of Na^+/mg of protein after the Na^+-loading procedure. This was decreased to 13.4 nmol/mg after 30 min of incubation in the presence of Ca^{2+} (0.1 mM). Addition of ATP to the vesicles loaded with Na^+ resulted in a further increase in Ca^{2+} uptake. Vesicles not loaded with Na^+ in the presence of ATP accumulated about the same amount of Ca^{2+} (Table 2).

The Ca^{2+} uptake was inhibited by both NH_4Cl and nifedipine. Addition of 50 mM-NH_4Cl decreased the Ca^{2+} uptake to 35 ± 7% ($n = 3$) relative to controls after 5 min at 1 μM free Ca^{2+}. Only a minor effect was seen at 0.1 mM-Ca^{2+}. Nifedipine (10 μM) dissolved in dimethyl sulphoxide (0.5% final concn.) decreased the Ca^{2+} uptake in 5 min to 17 ± 8% relative to controls containing dimethyl sulphoxide alone.

The influence of Na^+ on Ca^{2+} transport could also be demonstrated as a Na^+-induced Ca^{2+} efflux from vesicles loaded with $^{45}Ca^{2+}$. In these experiments coated microvesicles were allowed to take up Ca^{2+} for 15 min as described in Fig. 2 at 0.1 mM free external Ca^{2+}. Then the samples were diluted in a medium containing Na^+ or K^+ as well as 0.5 mM-EGTA (Fig. 4). This procedure lowered the external free Ca^{2+} concentration to less than 50 nM, as revealed by the addition of BAPTA (see the Experimental section). An efflux of Ca^{2+} could only be demonstrated when the medium contained Na^+ (Fig. 4).

A similar rapid release of Ca^{2+} by Na^+ was found when rat brain coated microvesicles were used for the experiment. Their content of $^{45}Ca^{2+}$ was decreased to 39 ± 8% (S.D.; $n = 3$) after 2 min and further decreased to 19 ± 9% (S.D.; $n = 3$) 10 min after incubation in the presence of NaCl. No significant release of Ca^{2+} from the brain coated microvesicles was observed when they were incubated in the presence of KCl.

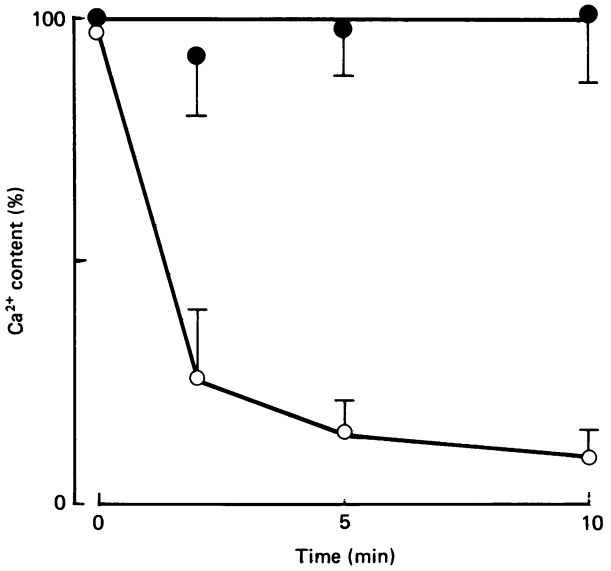

Fig. 4. Na^+-induced Ca^{2+} efflux from coated microvesicles

Coated microvesicles from the neurohypophysis were incubated for 15 min in the presence of 0.1 mM free Ca^{2+} and 2 mM-ATP, followed by dilution in a buffer containing 130 mM-KCl (●) or -NaCl (○) as described in the Experimental section. The efflux of Ca^{2+} was determined at the indicated time points by filtration (means ± S.D.; $n = 4$); 100% on the ordinate is equal to 4.1 ± 0.3 nmol/mg of protein.

Characterization of the ATPase activity present in preparations of coated microvesicles

The ATPase activity present in coated-microvesicle fractions isolated from the neurohypophysis was activated by Mg^{2+} over a narrow range of concentrations. The activity was half-maximal at 0.4 mM free Mg^{2+} (2 mM-ATP). The specific activity of the ATPase determined at 1 mM-Mg^{2+} (free) and 2 mM-ATP (pH 7.0) was 11 ± 3 μmol/h per mg of protein (S.D.; $n = 4$). Changes in pH had only a minor influence in the range pH 5.5–8.0, with an optimum at pH 7.0.

The ATPase activity was insensitive to Ca^{2+} at concentrations of 0.1–10 μM free Ca^{2+} when Mg^{2+} (1 mM free) and ATP (2 mM) were included in the medium (results not shown). The presence of neither calmodulin (10 μg/100 μg of vesicle protein) nor Triton X-100 (0.01%) caused a Ca^{2+}-dependent activation of the ATPase activity, although the addition of Triton X-100 increased the enzyme activity by a factor of 2 (results not shown). The ATPase was activated by Ca^{2+} in the absence of Mg^{2+}. However, this activity was lower than that in the presence of Mg^{2+} alone. Assay of the ATPase activity in the presence of 0.1 mM-Ca^{2+} (free) yielded 70 ± 8% (S.D.; $n = 3$) of the activity observed with 1 mM-Mg^{2+} (free).

Addition of the ionophores monensin and FCCP activated the Mg^{2+}-dependent ATPase in coated microvesicles from the neurohypophysis (Table 3). The activation by FCCP was maximal at 1 μg/ml and decreased at higher concentrations. Monensin activated the ATPase maximally at 0.1 μg/ml. FCCP and monensin also activated the ATPase activity in rat brain coated microvesicles (Table 3).

Table 3. Effect of FCCP and monensin on the Mg^{2+}-dependent ATPase activity in coated-microvesicle fractions

Coated microvesicles were purified on sucrose gradients. The ATPase activity in the preparations was measured in the presence of the ionophores FCCP (1 μg/ml) and monensin (1 μg/ml). The medium contained 1 mM-Mg^{2+} (free), 0.5 mM-EGTA, 2 mM-ATP and 20 mM-Tes, pH 7.3. The membranes incubated in the presence of methanol (1 μl/ml) used for solubilization of the ionophores were used as controls. Results are expressed as % of controls (S.D.; $n = 4$).

Coated microvesicles from:	Control	+FCCP	+Monensin
Neurohypophysis	100	175±15	183±18
Brain	100	172±10	178±12

Coated microvesicles accumulated [^{14}C]methylamine when incubated in the presence of ATP. The ratio of the amount accumulated in the presence of ATP to that with the ATP analogue adenosine 5'-[β,γ-imido]triphosphate was 4.1±0.8 (S.D.; $n = 3$) at pH 7.0. This ATP-dependent accumulation was decreased when NH_4Cl (50 mM) was added to the medium, and under these conditions no effect of ATP was found. Using the effect of NH_4 Cl as an indicator of the pH gradient, we determined the methylamine accumulation at pH 7.5, 7.0, 6.5 and 6.0 in the presence of ATP. The difference in the amount of methylamine accumulated in the presence and in the absence of NH_4Cl was decreased at low pH. At pH 6.0 no effect of NH_4Cl was found, indicating that the internal pH of the vesicles in the presence of ATP is close to this value. These findings confirm the results of Forgac *et al.* (1983) and Stone *et al.* (1983).

DISCUSSION

The coated microvesicles isolated from the neurohypophysis were able to transport Ca^{2+} in an ATP-dependent manner as well as in a Na^+-dependent manner. These vesicles also contained ATPase activity. However, the nature of the coupling between the ATPase activity and the Ca^{2+} uptake is not obvious. No activation by Ca^{2+} of the ATPase activity was found in the presence of 1 mM-Mg^{2+}, and this was not changed when calmodulin was added. The same properties were found for the ATPase activity in rat brain coated microvesicles. Blitz *et al.* (1977) reported that a Ca^{2+}-dependent ATPase activity was present in coated microvesicles incubated in the presence of Triton X-100. This was not found for the preparation of coated microvesicles used in the present study. We have previously reported the presence of a Mg^{2+}-dependent ATPase in brain microsomal fractions which is very similar to the enzyme described here (Saermark & Vilhardt, 1979).

The Ca^{2+} accumulation by coated microvesicles isolated from the neurohypophysis seems to differ from the accumulation by plasma-membrane fractions from this tissue. These pituitary membranes appear to have a calmodulin-activated $Ca^{2+}+Mg^{2+}$-dependent ATPase which is responsible for their Ca^{2+} accumulation (Conigrave *et al.*, 1981). Also the endoplasmic-reticulum type of Ca^{2+} accumulation described in brain synaptosomes (Blaustein *et al.*, 1978) and in pituitary microsomal fractions (Conigrave *et al.*, 1981) seems to be driven by a Ca^{2+}-activated Mg^{2+}-dependent ATPase. The demonstration by Forgac *et al.* (1983) and Stone *et al.* (1983) of a Mg^{2+}-dependent proton-ionophore-activated ATPase in coated microvesicles is supported by our results, and this enzyme is present also in coated microvesicles isolated from the neurohypophysis. On the basis of our results it seems reasonable to suggest that the proton gradient created by this enzyme may act as the primary driving force for the Ca^{2+} accumulation by the coated microvesicles, although this remains to be shown. However, the finding that NH_4Cl affects the Ca^{2+} uptake at low Ca^{2+} concentrations supports this idea, since it is known to affect the internal pH in coated microvesicles (Marsh, 1984; Forgac *et al.*, 1983; Stone *et al.*, 1983). This is similar to the effect of NH_4Cl on the intravesicular pH of the coated vesicles used in the present study, as indicated by the distribution of methylamine (see the Results section).

The ATPase activity in coated microvesicles is very high compared with that in other subcellular fractions isolated from the neurohypophysis. It is at least 3 times that in preparations of plasma membranes (Conigrave *et al.*, 1981), and more than 50 times the residual ATPase activity in preparations of secretory vesicles (Saermark *et al.*, 1983). These results, and the absence of smooth-microsomal-type membranes, mitochondria and lysosomes (see the Results section), make it unlikely that the Mg^{2+}-dependent ATPase activity in the coated-microvesicle fraction is due to contamination. This is also supported by the previous demonstration that further fractionation of brain coated microvesicles on immunoabsorbents removes trace amounts of $Na^+ + K^+$-dependent ATPase, but does not remove the ouabain-insensitive ATPase activity (Saermark & Thorn, 1982).

Secretory vesicles isolated from the neurohypophysis are able to accumulate Ca^{2+} independently of the addition of ATP (Saermark *et al.*, 1982) but as a consequence of a Na^+ gradient. The presence of a proton pump in these vesicles, as has been found in certain studies, is probably a contamination, since it has been demonstrated that the ATPase activity in secretory-vesicle fractions from the neurohypophysis prepared as described by Russell (1981, 1984) can be removed by further purification of the vesicles on sucrose gradients (Saermark *et al.*, 1983), confirming previous results obtained by Vilhardt & Hope (1974). This indicates that the driving forces for the carrier-mediated Ca^{2+}/Na^+ exchange in secretory vesicles and coated microvesicles may not be the same.

The results presented here on the Ca^{2+} accumulation by coated microvesicles from the neurohypophysis have certain features in common with the Ca^{2+} accumulation by secretory vesicles from the neurohypophysis described previously (Saermark *et al.*, 1982, 1983). None of these systems accumulate Ca^{2+}, owing to the presence of a Ca^{2+}-dependent ATPase, and both of the systems release Ca^{2+} when incubated in the presence of Na^+ (see the Results section, and Saermark *et al.*, 1982, 1983). Although the driving forces for the Ca^{2+} accumulation may be different, it is apparent that both systems can exchange Ca^{2+} for Na^+ across their membrane mediated by a carrier mechanism. Both processes can be saturated and have an affinity below 1 μM free Ca^{2+} in common as well as their sensitivity to Na^+.

The coated microvesicles in the neurohypophysis are probably not involved in membrane retrieval after exocytosis (Morris & Nordmann, 1980; Saermark *et al.*, 1984). The data presented here suggest that they are involved in a specific retrieval of certain cell membrane components, excluding the Ca^{2+}-dependent ATPase and the $Na^+ + K^+$-dependent ATPase, but including a Ca^{2+}/Na^+ carrier.

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