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ATP-Dependent Calcium Transport in Rat Parotid Basolateral Membrane Vesicles is Modulated by Membrane Potential

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Summary. The ATP-dependent Ca^{2+} transport activity (T. Takuma, B.L. Kuyatt and B.J. Baum, *Biochem. J.* **227**:239–245, 1985) exhibited by inverted basolateral membrane vesicles isolated from rat parotid gland was further characterized. The activity was dependent on Mg^{2+}. Phosphate (5 mM), but not oxalate (5 mM), increased maximum Ca^{2+} accumulation by 50%. Half-maximal Ca^{2+} transport was achieved at ~70 nM Ca^{2+} in EGTA-buffered medium while maximal activity required >1 μM Ca^{2+} (V_{max} = 54 nmol/mg protein/min). Optimal rates of Ca^{2+} transport were obtained in the presence of KCl, while in a KCl-free medium (mannitol or sucrose) ~40% of the total activity was achieved, which could not be stimulated by FCCP. The initial rate of Ca^{2+} transport could be significantly altered by preimposed membrane potentials generated by K^+ gradients in the presence of valinomycin. Compared to the transport rate in the absence of membrane potential, a negative (interior) potential stimulated uptake by ~30%, while a positive (interior) potential inhibited uptake. Initial rates of Ca^{2+} uptake could also be altered by imposing pH gradients, in the absence of KCl. When compared to the initial rate of Ca^{2+} transport in the absence of a pH gradient, pH_i = 7.5/pH_o = 7.5; the activity was ~60% higher in the presence of an outwardly directed pH gradient, pH_i = 7.5/pH_o = 8.5; while it was ~80% lower when an inwardly directed pH gradient was imposed, pH_i = 7.5/pH_o = 6.2. The data show that the ATP-dependent Ca^{2+} transport in BLMV can be modulated by the membrane potential, suggesting therefore that there is a transfer of charge into the vesicle during Ca^{2+} uptake, which could be compensated by other ion movements.

Key Words Ca^{2+} transport · plasma membrane · parotid acinar cells · membrane potential

Introduction

Ca^{2+} plays a key role in the process of secretion in parotid and other exocrine cells (Schulz, 1980; Putney, 1986). Stimulation of the rat parotid gland by α_1-adrenergic, muscarinic-cholinergic, and substance P-peptidergic agonists leads to the mobilization of cellular Ca^{2+} (Putney, 1986). Following receptor activation, there is an immediate increase both in cytosolic Ca^{2+} ($[Ca^{2+}]_i$) and Ca^{2+} efflux from the cell. It has been suggested that the rapid elevation in cytosolic Ca^{2+} follows the stimulation of phosphoinositide 4,5-bisphosphate hydrolysis by the secretagogues. This results in the generation of inositol trisphosphate (IP_3), which subsequently elicits release of a mobilizable Ca^{2+} pool from the endoplasmic reticulum. This elevated $[Ca^{2+}]_i$ could then contribute to the observed increase in Ca^{2+} efflux from the cell.

The mechanism involved in the efflux of Ca^{2+} from the cell is not well understood. Calcium, in the form of protein complexes sequestered inside the secretory granules (Feinstein & Schramm, 1970), is extruded from the cell via the luminal membrane during protein secretion, but because of temporal patterns, this process probably does not directly contribute towards $[Ca^{2+}]_i$ regulation. Moreover, α_1-adrenoreceptor and muscarinic receptor activation, which result in considerable efflux of Ca^{2+} and fluid secretion, elicit only small levels of protein secretion. It can therefore be suggested that cytosolic Ca^{2+} homeostasis in these receptor-stimulated conditions would be established by the concerted activities of ATP-dependent Ca^{2+} transport systems located in the endoplasmic reticulum (Kanagasuntheram & Teo, 1982; Immelmann & Soling, 1983) and in the basolateral plasma membrane (Takuma et al., 1985; Helman et al., 1986). The latter system is likely the key Ca^{2+}-transporting mechanism mediating Ca^{2+} efflux from this cell (Takuma et al., 1985).

Our studies have been directed towards understanding the mechanisms involved in acinar cell Ca^{2+} efflux, and their possible regulation, during neurotransmitter activation. A previous report from our laboratory (Takuma et al., 1985) showed that inverted basolateral plasma membrane vesicles accumulate Ca^{2+} in the presence of ATP. The Ca^{2+} accumulated in the vesicles could be discharged by imposing an inwardly directed Na^+ gradient, indi-

cating the presence of a Na^+/Ca^{2+} exchange mechanism in the same membrane vesicles and also confirming the plasma membrane origin of the ATP-dependent Ca^{2+} transporter. The presence of ATP-dependent Ca^{2+} transporters in plasma membranes from a wide variety of tissues has been described (for reviews *see* Carafoli & Zurini, 1982; Penniston, 1983; Rega, 1986). However, when the transport activities of membranes from various cell sources, both in situ and in reconstituted systems, are compared, many differences are observed in the actual mode of calcium flux, i.e. number of Ca^{2+} binding sites, Ca^{2+}/ATP stoichiometry, regulation by calmodulin and cAMP, and electrogenic nature of the transport. This suggests the presence of tissue-specific physiological determinants of the ATP-dependent Ca^{2+} transport system in plasma membranes.

In the present study, we have further characterized the ATP-dependent Ca^{2+} transport activity in rat parotid basolateral membrane vesicles (BLMV)[1] in terms of its kinetic properties and various membrane parameters associated with the Ca^{2+} flux. The kinetic properties measured in the presence of EGTA-buffered Ca^{2+} solutions, reveal that this transporter is a high-affinity ($K_m \sim 70$ nM) and a high-capacity ($V_{max} \sim 54$ nmol/mg protein/min) Ca^{2+}-transporting system. We also report here for the first time, that in BLMV from rat parotid gland, the initial rate of plasma membrane ATP-dependent Ca^{2+} transport can be modulated by preimposed membrane potentials induced by K^+ gradients in the presence of valinomycin and by pH gradients in the absence of K^+. The data suggest that during ATP-dependent Ca^{2+} transport in BLMV there is transfer of electric charge.

Materials and Methods

Materials

Tris-ATP, PMSF, valinomycin, FCCP and DTT were purchased from Sigma Chemical Company. Di-S-C2-(5) was obtained from Molecular Probes. $^{45}Ca^{2+}$ (2 mCi/ml) was routinely obtained from Amersham. All other reagents used were of the highest grade commercially available.

[1] Abbreviations used are: BLMV, basolateral membrane vesicles; DTT, dithiothreitol; PMSF, phenylmethyl sulfonyl fluoride; EGTA, ethyleneglycol bis (β-aminoethylether)-N,N tetracetic acid; ATP, adenosine triphosphate; TMA, tetramethylammonium; NMDG, N-methyl-D-glucamine; MES, (N-morpholino) ethanesulfonic acid; di-S-C2-(5), 3,3'-diethylthiadicarbocyanine iodide; FCCP, carbonylcyanide *p*-trifluromethoxy-phenylhydrazone.

Animals

Animals used in these studies were male Wistar strain rats, obtained from Harlan-Sprague Dawley. Rats were given ad libitum water and Purina Chow. The animals were anesthetized with ether, killed by cardiac puncture, and parotid tissue then excised.

Preparation of Basolateral Membrane Vesicles

BLMV were prepared as described previously (Takuma et al., 1985), with some minor modifications. The procedure, in brief, was as follows. Glands from at least 15 to 20 rats were pooled for each membrane preparation. The excised glands were immersed in ice-cold buffer containing 0.25 M sucrose, 0.1 mM PMSF, 1 mM DTT, 10 mM Tris-HEPES, pH 7.5, and homogenized (twice, 10 sec each) using a Polytron (Brinkman Instruments) at setting 5 to give a 10% (wt/vol) homogenate. The homogenate was further diluted to 7.5% (wt/vol) with the same buffer and then centrifuged at 2600 × *g* for 15 min. The supernatant was filtered through four layers of cheesecloth and recentrifuged at 23,500 × *g* for 20 min. The pellet obtained was rehomogenized (by hand) in the sucrose medium using a Teflon®-glass homogenizer, mixed with Percoll® (12% vol/vol) and centrifuged at 41,700 × *g* for 30 min. The basolateral membrane fraction was collected (Takuma et al., 1985) and washed three times with 100 mM mannitol, 0.1 mM PMSF, 1 mM DTT, 10 mM Tris-HEPES, pH 7.5, by centrifuging at 49,000 × *g* for 15 min. Membranes were finally washed in a medium containing 300 mM mannitol, 1 mM DTT, 10 mM Tris-HEPES, pH 7.5, and resuspended in a minimum volume of the same medium. All the procedures described above were carried out at 4°C. Aliquots of membrane vesicles were quick frozen in liquid nitrogen and stored at −70°C. Membranes were thawed in an ice-bath before each experiment and kept on ice until used. All membrane preparations were subjected to only one freeze-thaw. This freeze-thaw procedure did not alter calcium transport activity when compared to that of freshly prepared vesicles (*data not shown*).

Ca^{2+} Transport in Basolateral Membrane Vesicles

Calcium transport was measured by rapid filtration through Millipore filters (pore size 0.45 μm, HA). The assay medium typically contained (mM): 150 KCl, 10 Tris-HEPES, pH 7.5, 1 Mg-gluconate, and 1 Tris-ATP unless otherwise indicated. Ca^{2+} was added at the indicated concentrations and activity was maintained around 0.5 μCi $^{45}Ca^{2+}$/ml assay medium. The protein concentration was 50 μg/ml in all the experiments. Transport was initiated by the addition of membrane vesicles to the assay medium at 37°C.

The reaction was terminated by a "quench-filtration" method. Here, 1.0 ml of cold reaction medium, without ATP and Ca^{2+}, was added to 200-μl reaction medium at the indicated time. The sample was then vortexed and filtered. The filter was washed with 3 ml (three times) of the same medium. Inclusion of 0.1 mM $LaCl_3$ in the wash medium did not alter the values obtained and so $LaCl_3$ was not included in the wash medium. To obtain a "zero" time point, cold wash medium was added to the reaction mixture before the addition of the membrane vesicles. In kinetic experiments, Ca^{2+}-EGTA buffers (with 0.2 mM EGTA)

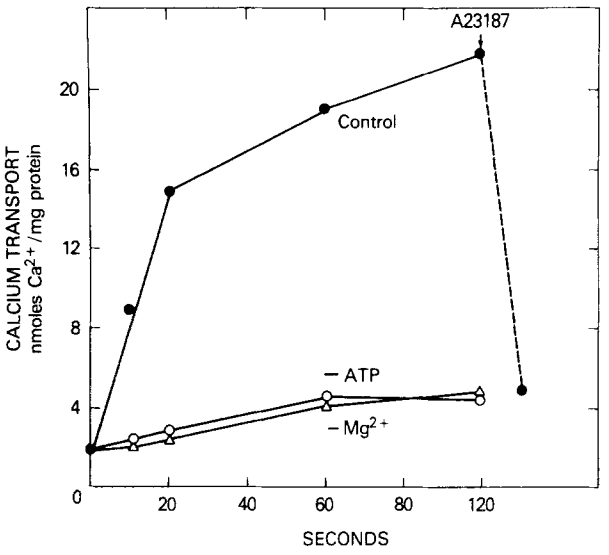

Fig. 1. ATP-dependent Ca^{2+} transport in rat parotid basolateral membrane vesicles. Ca^{2+} transport was assayed in the presence of 150 mM KCl, and 10 mM Tris-HEPES, pH 7.5, at 37°C. The control experiment contained 1 mM Mg-gluconate and 1 mM Tris-ATP in the assay medium. Ca^{2+} concentration in the assay was 12.3 μM (total). The intravesicular medium contained 300 mM mannitol, 10 mM Tris-HEPES, pH 7.5. Other details are as given in Materials and Methods. 5 μM A23187 as a 50% ethanolic : DMSO solution was added where indicated

were used to yield free Ca^{2+} concentrations ranging from 0.003 to 4.36 μM. Free calcium concentrations were determined as described earlier (Helman et al., 1986).

Initial rates were calculated from linear regression of the values obtained for 0, 10 and 20 sec of assay. Each time point was assayed in duplicate or triplicate. Where indicated, SEM or SD shows the experimental variations obtained between different membrane preparations. Only averages (from triplicate samples) are shown for representative (of 3 to 6 individual) experiments. Correlation coefficients between 0.98 and 0.99 were routinely obtained for the transport data. Specific conditions for each experiment have been indicated in the Figure legends. Statistical analyses were performed using the Student's *t*-test.

Fluorescence Measurement

Fluorescence was measured using an SLM 8000 fluorimeter (microprocessor controlled). The cuvette temperature was maintained at 37°C and the sample was stirred continuously. Membrane potential (interior negative) was monitored as the quenching of the fluorescence of the dye, di-S-C2-(5), with excitation at 590 nm and emission at 670 nM. A 4-nm bandwidth was used in all the experiments. Specific details of the experiments are given in the Figure legend.

Other Methods

Protein was measured using the Bio-Rad protein assay kit with bovine serum albumin as the standard.

Table 1. Effect of oxalate and phosphate on ATP-dependent calcium transport in basolateral membrane vesicles[a]

Additions	% Calcium accumulation
None	100
5 mM oxalate	98
5 mM phosphate	150

[a] Experimental conditions were similar to that described for Fig. 1. The calcium accumulated at 5 min (steady state) without any additions was considered as 100%. Control membranes accumulated 21 ± 2.5 nmol Ca^{2+}/mg protein (n = 4). 5 mM K-oxalate or 5 mM K-phosphate were included in the assay medium as indicated. Ca^{2+} accumulation in the absence of ATP was not changed with these inclusions (*data not shown*).

Results

ATP-Dependent $^{45}Ca^{2+}$ Transport in Basolateral Membrane Vesicles

Inverted BLMV isolated from rat parotid gland exhibit Ca^{2+} transport activity, which has an obligatory requirement on Mg^{2+} and ATP (Fig. 1). When ATP and Mg^{2+} are present in the assay medium, the $^{45}Ca^{2+}$ accumulated by BLMV is increased 8- to 10-fold as compared to that in the absence of ATP. Typically, maximal activation is achieved around 0.5 mM ATP, while half-maximal activation is seen between 0.1 and 0.2 mM ATP (*data not shown*). All experiments described herein were performed in the presence of 1 mm ATP. When Mg^{2+} is excluded from the assay medium the $^{45}Ca^{2+}$ uptake is similar to that obtained in the absence of ATP. This background level of $^{45}Ca^{2+}$ incorporation is similar to that obtained either when A23187(5 μM) is added to vesicles loaded with Ca^{2+} (as shown in Fig. 1) or when it is present from the beginning of the assay (*not shown*). When A23187 is added to the vesicles in the absence of ATP, there is a slight increase in $^{45}Ca^{2+}$ incorporation, as reported earlier for these vesicles (Takuma et al., 1985).

It had been shown earlier, that the $^{45}Ca^{2+}$ loaded into BLMV could be released by including >30 mM Na^+ in the extravesicular medium (Takuma et al., 1985), indicating the presence of a Na^+/Ca^{2+} exchange system in the same membrane vesicles. This phenomenon is a characteristic of plasma membrane vesicles (Rega, 1986). The data shown in Table 1 provide further evidence that the Ca^{2+} transport activity described above is located in a plasma membrane system. We have examined the ability of oxalate and phosphate to stimulate Ca^{2+} transport. Neither the initial rate of $^{45}Ca^{2+}$ uptake

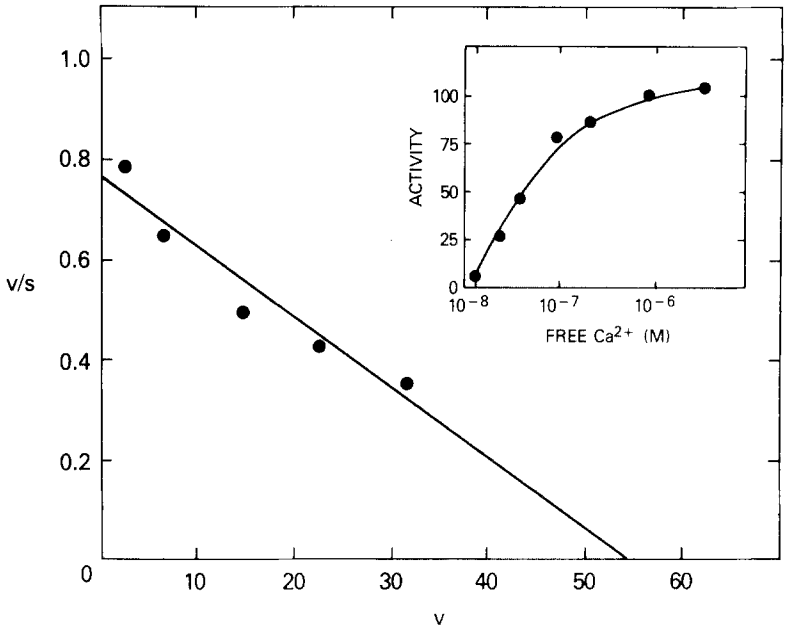

Fig. 2. Kinetic parameters of ATP-dependent Ca^{2+} uptake. General assay conditions were as described for Fig. 1. Protein concentration was 50 μg/ml. Ca^{2+}-EGTA buffers were used to obtain the desired free Ca^{2+} concentration (3 nM to 4.36 μM). Initial rates obtained at the various free $[Ca^{2+}]$ have been plotted according to the Eadie-Hoffstee method. The data represent results from two experiments performed with different membrane preparations, each point being assayed in triplicate. Units for x and y axis values given in the Figure are: v, nmol Ca^{2+} per mg protein per min; s, free Ca^{2+} (nM) in the assay medium. Inset figure shows the initial rate of Ca^{2+} transport as a function of the free $[Ca^{2+}]$ in the assay medium. The data have been normalized to the rate of Ca^{2+} transport at V_{max} (100% = ~54 nmol/mg protein/min)

nor the amount accumulated after 5 min is altered when 5 mM oxalate is included in the assay medium. Inclusion of 5 mM phosphate in the assay medium does not alter the initial rate of Ca^{2+} transport but does increase the steady-state level by about 50%. A microsomal membrane preparation obtained from the same tissue shows five- to eightfold stimulation of $^{45}Ca^{2+}$ accumulation in the presence of 5 mM oxalate under similar conditions (*data not shown*). In addition 50 to 60% of the initial rate of ATP-dependent Ca^{2+} transport in BLMV could be inhibited by ~50 μM orthovanadate, after a 15-min preincubation (*data not shown*).

Kinetics of Basolateral Membrane Vesicle Ca^{2+} Transport

The Ca^{2+} transport activity in BLMV was measured at various Ca^{2+} concentrations, buffered by EGTA, in the assay medium. These data are shown in Fig. 2. The parotid BLMV ATP-dependent Ca^{2+} transport mechanism exhibits a very high affinity for Ca^{2+}, (K_m ~ 72 nM) and a high Ca^{2+} transport capacity (V_{max} ~ 54 nmol per mg protein per min) achieved at >1 μM Ca^{2+} (inset Fig. 2). Studies with intact parotid cells indicate that resting free $[Ca^{2+}]_i$ levels are ~140 to 180 nM (by quin 2 fluorescence) and reach maximal levels of ~700 nM Ca^{2+} after stimulation (Ambudkar et al., 1988). All further experiments for this study were conducted at a relatively high concentration of total Ca^{2+} (10 μM, calculated free Ca^{2+} = 4.76 μM) without EGTA buffering.

Effect of K^+-Diffusion Potentials on the ATP-Dependent Ca^{2+} Transport

The experiments described above (Figs. 1 and 2) were carried out under KCl gradient conditions, i.e., extravesicular medium contained 150 mM KCl, 1 mM Mg-gluconate, 10 mM Tris-HEPES, pH 7.5, while the intravesicular medium contained 300 mM mannitol, 10 mM Tris-HEPES, pH 7.5. When the vesicles are equilibrated in the 150-mM KCl medium by incubation at 25 to 28°C for 1 hr, there is an increase, 119% ± 7 (mean ± SD, $P < 0.005$, $n = 5$) in the initial rate of Ca^{2+} transport (*data not shown*). The maximum amount of Ca^{2+} loaded (i.e. at steady state) is the same in both KCl equilibrium and gradient conditions. This increased initial rate of Ca^{2+} transport is not due to the presence of intravesicular Mg^{2+} since similar equilibration in the mannitol medium (plus Mg^{2+}) does not stimulate calcium transport (*data not shown*). Also, equilibration with KCl medium (minus $MgCl_2$) does not alter the stimulation of Ca^{2+} transport activity as long as Mg^{2+} is present in the assay medium (*data not shown*).

When valinomycin (5 μM) is added to the vesicles in the presence of an inwardly directed KCl gradient (Fig. 3*A*), an inhibition of the Ca^{2+} transport activity is observed. Valinomycin allows an electrogenic influx of K^+ into the vesicles, resulting in a positive (inside) membrane potential. The inhibition of Ca^{2+} uptake observed in the presence of valinomycin, therefore, suggests that the Ca^{2+} flux in the presence of ATP results in a transfer of posi-

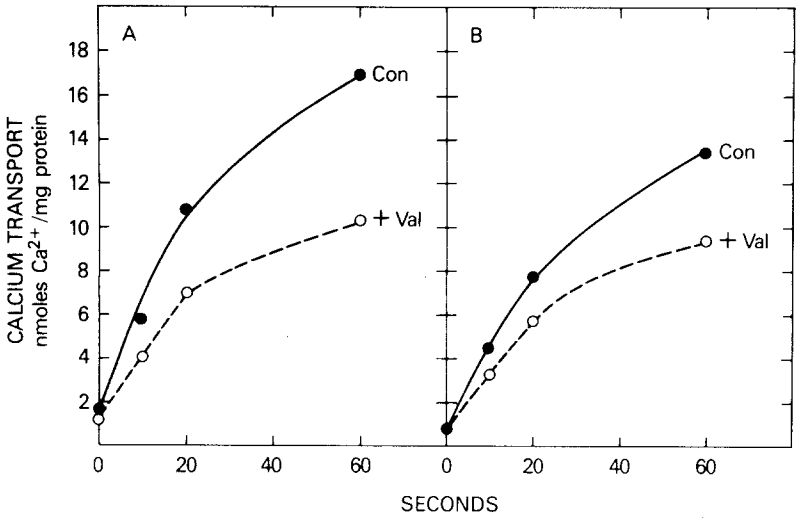

Fig. 3. Effect of valinomycin on the ATP-dependent Ca^{2+} transport in K^+-containing media. Parotid BLMV, containing 300 mM mannitol, 10 mM Tris-HEPES, pH 7.5, were assayed in media containing either 150 mM KCl (*A*) or 150 mM K-gluconate (*B*), and 1 mM Tris-ATP, 1 mM Mg-gluconate, 10 mM Tris-HEPES, pH 7.5. 5 μM ethanolic solution of valinomycin (dotted lines) or an equivalent volume of ethanol (solid lines) were added to membrane vesicles in the reaction media and vortexed. The reaction was initiated 5 sec later by the addition of ATP and Ca^{2+}. Other assay conditions were as described in Materials and Methods. Data are the mean values of triplicate determinations in a representative experiment of five performed with different membrane preparations

tive charge to the interior of the vesicle. This would be inhibited by a preimposed (i.e. by valinomycin) positive charge inside the vesicle. Valinomycin had no effect on $^{45}Ca^{2+}$ flux in the absence of ATP (*not shown*). Moreover, when $^{45}Ca^{2+}$ uptake was measured after equilibration in KCl medium or in the absence of KCl (i.e. in 300 mM mannitol or 300 mM sucrose medium), valinomycin had no effect on either the initial rates or steady-state levels of Ca^{2+} transport (*data not shown*). These results suggest that valinomycin (5 μM) does not directly inhibit the Ca^{2+} transporter. When Cl^- was replaced with an impermeant anion, gluconate, valinomycin induced a similar inhibition of transport (shown in Fig. 3*B*). This suggests that the permeability of these membranes to Cl^- is relatively low. However, the initial rate of the ATP-dependent Ca^{2+} transport measured in the K-gluconate medium is ~35% lower than that in a KCl medium suggesting a Cl^- requirement for maximal Ca^{2+} transport activity.

The results presented above indicate that the ATP-dependent Ca^{2+} transport system could be electrogenic. In order to directly examine the effect of membrane potential on calcium transport, K diffusion potentials were created in the presence of K^+ gradients and valinomycin. Since the presence or absence of Cl^- did not change the valinomycin effect (Fig. 3, *A* and *B*), 150 mM Cl^- was present in all media used for examining membrane-potential effects. Vesicles were equilibrated with 150 mM KCl medium, by incubation at 25 to 28°C for 1 hr, and then diluted into 150 mM KCl medium or 150 mM TMA-Cl medium. 5 μM valinomyin was then added which induced zero or negative (interior) membrane potential, respectively, in the two dilution media. In order to obtain positive (interior) membrane potential, vesicles were equilibrated with 150 mM TMA-Cl and valinomycin and then diluted into medium containing 150 mM KCl. Typically, 5 μl of vesicles were diluted into 200 μl of medium, creating a 40-fold concentration gradient. The ability to generate membrane potential (negative inside) was checked by measuring the fluorescence of the dye di-S-C2-(5) under the conditions described above. However, positive (interior) membrane potential cannot be detected by di-S-C2-(5) and our attempts to use oxonol dyes were unsuccessful. As seen in Fig. 4(*A*), when KCl-loaded vesicles were diluted into TMA-Cl medium an immediate, sharp quenching of the fluorescence was seen only when valinomycin was added indicating the formation of negative (interior) membrane potential. When the dilution medium was 150 mM KCl, no change in fluorescence was seen. The quenched level of fluorescence was steady for a fairly long time (~5 min) and could be reversed by increasing the $[K^+]$ in the extravesicular medium. FCCP did not reverse either the di-S-C2-(5) quenching when added after valinomycin or the valinomycin-induced decrease in $^{45}Ca^{2+}$ uptake (Table 2). In the fluorescence assay system described above, significant dissipation of the preimposed membrane potential could not be observed by initiating calcium transport, unless fairly high concentrations of Ca^{2+} were used (>100 μM total Ca^{2+}). We therefore measured $^{45}Ca^{2+}$ uptake using the conditions to generate membrane potentials described above.

The effects of negative and positive (interior) membrane potentials on ATP-dependent $^{45}Ca^{2+}$ uptake into the parotid BLMV can be seen in Fig. 4(*B*). Compared to Ca^{2+} transport in KCl-equilibrated vesicles (KCl_o/KCl_i), those with a negative (interior) membrane potential ($KCl_i/TMA\text{-}Cl_o$) show higher initial rates of $^{45}Ca^{2+}$ uptake 154% ± 21

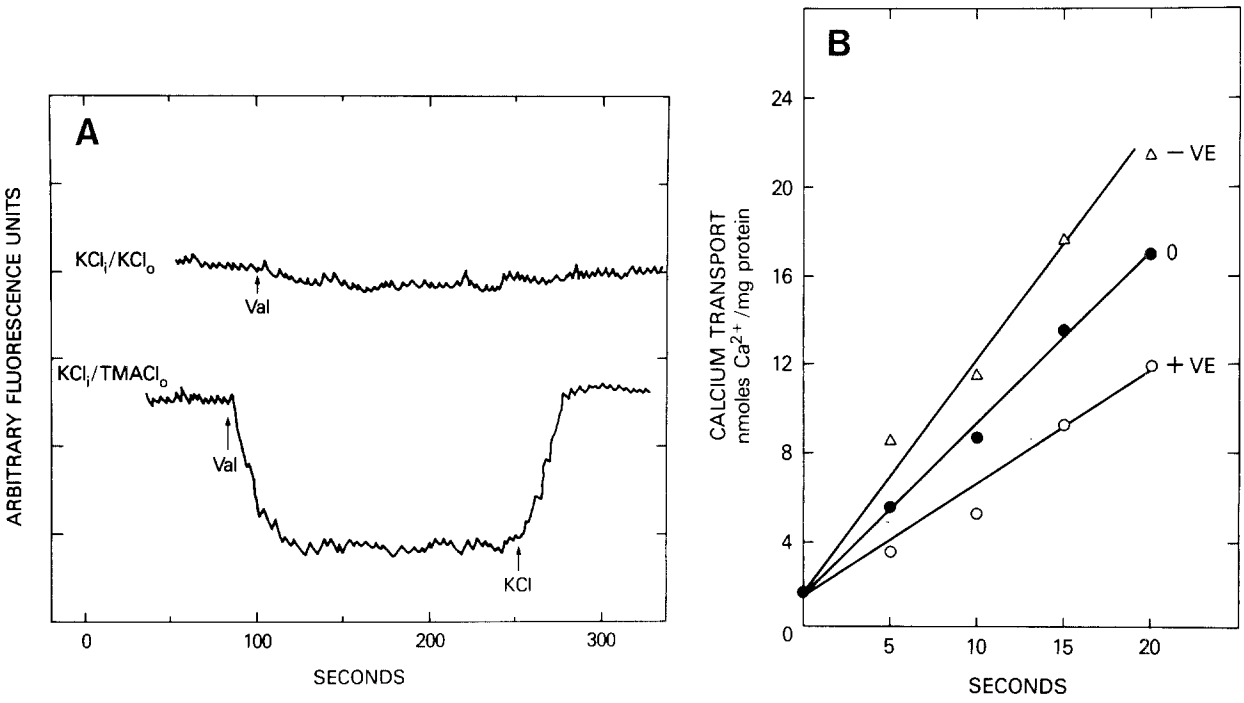

Fig. 4. (*A*) Valinomycin-induced generation of a negative (interior) membrane potential. Negative (interior) membrane potential was monitored as the quenching of the fluorescence of the dye di-S-C2-(5). In these experiments, membranes containing 300 mM mannitol, 10 mM Tris-HEPES, pH 7.5, were incubated in a medium containing 150 mM KCl, pH 7.5 for 1 hr at 25°C. 50 μl of the membrane (10 μg protein) was then added to a cuvette with 1.95 ml of media containing either 150 mM TMA-Cl, 10 mM Tris-HEPES, pH 7.5 (lower trace), or 150 mM KCl, 10 mM Tris-HEPES, pH 7.5 (upper trace) at 37°C and continuously stirred and 2 μM di-S-C2-(5) was added. 5 μM valinomycin was added as an ethanolic solution where indicated (4 μl ethanol). Fluorescence measurements were made as described in Materials and Methods with excitation and emission wavelengths of 590 and 670 nM. The "0" time fluorescence level was comparable in assays where the dye was added before or after the membranes. In control experiments, additions of 4 μl ethanol to the assay did not alter the fluorescence. KCl was added to the extravesicular medium to dissipate the membrane potential (lower trace). (*B*) Effects of K^+-diffusion potentials on ATP-dependent Ca^{2+} transport. Membrane vesicles, containing 300 mM mannitol and 10 mM Tris-HEPES, pH 7.5, were incubated in medium containing either 150 mM KCl, 10 mM Tris-HEPES, pH 7.5, or 150 mM TMA-Cl, 10 mM TRIS-HEPES, pH 7.5, for 1 hr, at 25°C. Membranes (5 μl, containing 10 μg protein) were then diluted 40 times into assay medium containing 10 μM Ca^{2+} (Total), 1 mM Tris-ATP, 1 mM Mg-gluconate, 10 mM Tris-HEPES, pH 7.5, and either 150 mM KCl or 150 mM TMA-Cl. The membrane potentials imposed by these manipulations are: KCl-loaded/TMA-Cl (-ve, negative interior); TMA-Cl-loaded/KCl (+ve, positive interior); KCl-loaded/KCl (no membrane potential). Details of Ca^{2+} uptake assay and initial rate determinations were similar to that described in Materials and Methods. Data are the mean values of triplicate determinations in one representative experiment of three to four performed with different membrane preparations

(mean ± SD); while those with a positive (interior) potential (TMA-Cl_i/KCl_o) show lower uptake rate 76.7% ± 4 (mean ± SD). These differences are statistically significant ($P < 0.01$, $n = 4$ and 3, respectively). Therefore, the total change in Ca^{2+} transport rate that is induced by changing the membrane potential from relatively positive to relatively negative is about ~86% ($P < 0.01$). The $^{45}Ca^{2+}$ transport measured in the absence of ATP is not affected under these conditions (*data not given*). Moreover, the data in Fig. 4(*A*) show that during the time required to measure initial $^{45}Ca^{2+}$ transport rate (~1 min), the established membrane potential is maintained.

Effect of a pH Gradient on ATP-Dependent Ca^{2+} Transport

ATP-dependent Ca^{2+} transport activity can be observed in the absence of KCl, i.e. in a mannitol medium. However, in mannitol medium, the initial rate of Ca^{2+} transport is ~60% less than that seen in the KCl-containing medium. A similar decrease is also observed in the steady-state level of uptake. Figure 5 compares the Ca^{2+}-transporting activity obtained in the presence of either 150 mM KCl or 300 mM mannitol at pH 7.5. This observation is similar to that reported for ATP-dependent Ca^{2+} transport in other plasma membranes (Penniston, 1983)

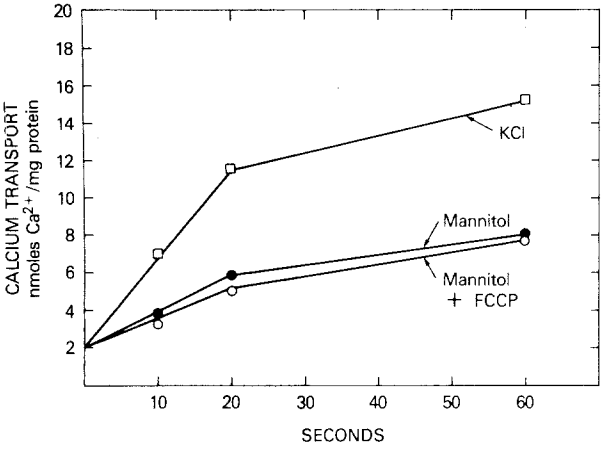

Fig. 5. ATP-dependent Ca^{2+} uptake in a KCl-free medium. Membrane vesicles were added to assay media containing 12.5 μM Ca (total), 1 mM Mg-gluconate, 1 mM Tris-ATP, 10 mM Tris-HEPES, pH 7.5, and either 150 mM KCl or 300 mM mannitol. Other assay conditions were similar to that described for Fig. 1. 5 μM FCCP (ethanolic solution, 5 μl ethanol) was added to the assay indicated (○). 5 μl ethanol was added in control experiments with KCl (□) and mannitol (●). Transport was initiated by addition of ATP and Ca^{2+} 5 sec after the addition of ethanol or FCCP

Table 2. Effect of valinomycin and FCCP on the initial rates of ATP-dependent Ca^{2+} transport in BLMV[a]

	% Ca^{2+} transport
Control	100
+Valinomycin	76 ± 8 (n = 5)
+FCCP	102 ± 3 (n = 5)
+Valinomycin +FCCP	74 ± 6 (n = 4)

[a] Experimental conditions were similar to those described for Fig. 3. 5 μM valinomycin and 5 μM FCCP were used as ethanolic solutions where indicated. Control experiments contained an equal volume of ethanol (ethanol volume was <1% of the total volume). Data are expressed as the initial rate of Ca^{2+} transport in control membranes which was ~24 ± 6 nmol Ca^{2+}/mg protein/min (n = 4).

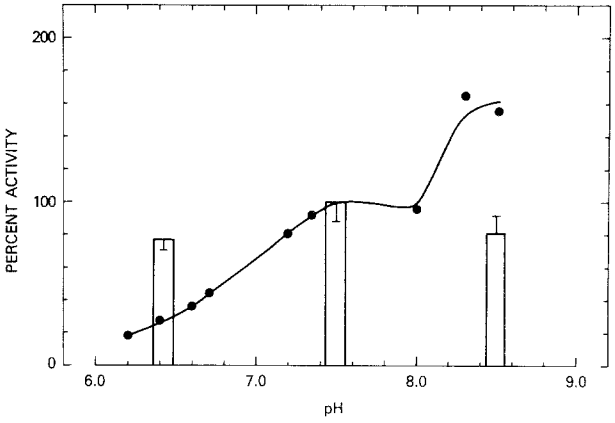

Fig. 6. Effect of pH on ATP-dependent Ca^{2+} transport in a KCl-free medium. Membrane vesicles containing 300 mM mannitol, 10 mM Tris-HEPES, pH 7.5, were incubated in a medium containing 300 mM mannitol, 50 mM Tris-MES, pH 7.5 for 1 hr at 25°C, and then added to assay media containing 12.5 μM Ca^{2+} (total), 1 mM Mg-gluconate, 1 mM Tris-ATP, 300 mM mannitol, 50 mM Tris-MES adjusted to various pH from 6.2 to 8.5. Initial rate of Ca^{2+} transport at each pH was determined as described in Materials and Methods and has been shown in the Figure (●—●). The bars show the initial rates of Ca^{2+} uptake when the intravesicular pH was equal to the extravesicular pH. $6.4_o/6.4_i$, $7.5_o/7.5_i$, $8.5_o/8.5_i$ relative to the rate at pH 7.5. These data are presented as the mean ± SEM of three experiments

and suggests that KCl stimulates a basal level of calcium transport. The activity in the mannitol medium, therefore, reflects the level of Ca^{2+} transport achieved in the absence of any effects due to K^+ or Cl^-. Addition of 5 μM FCCP during Ca^{2+} transport in mannitol medium has no effect on both the level of Ca^{2+} accumulation and the initial transport rates (Fig. 5). Similar experiments carried out using an impermeant ionic medium (NMDG-gluconate) instead of mannitol, gave comparable results (*data not shown*).

In order to examine the effects of pH on Ca^{2+} transport, independent of secondary ion movements, all the experiments described below were performed in mannitol media in the absence of any K^+ and Cl^-. In the data shown in Fig. 6, we have examined the effect of pH and pH gradients on this basal level of Ca^{2+} transport. When equilibrated in mannitol media, at pH 6.4, 7.4 and 8.5, by incubation for 1 hr at 25°C, maximal Ca^{2+} transport activity was seen at pH 7.4, with slight (~20%) decreases in the initial rates of Ca^{2+} transport at pH 6.4 and 8.5. However, when the intravesicular pH was kept at pH 7.4 and the pH of the extravesicular medium was adjusted between 6.2 to 8.5, marked effects were observed on the initial rates of calcium transport (Fig. 6). A steady level of transport rate was seen between pH 7.4 to 8.0 while at lower extravesicular pH (6.5 to 6.2), there is a 60 to 80% reduction in calcium transport and at higher extravesicular pH (8.3 to 8.5) there is a ~60% stimulation of the Ca^{2+} transport activity. Addition of 5 μM FCCP does not alter the effects of the pH gradients on initial Ca^{2+} transport activity (*data not shown*). Figures 7(*A*) and 7(*B*) show the calcium transport activity obtained at pH equilibrium (8.5_i to 8.5_o and 6.4_i to 6.4_o). When oppositely directed pH gradients are imposed, there is an inverse effect on the cal-

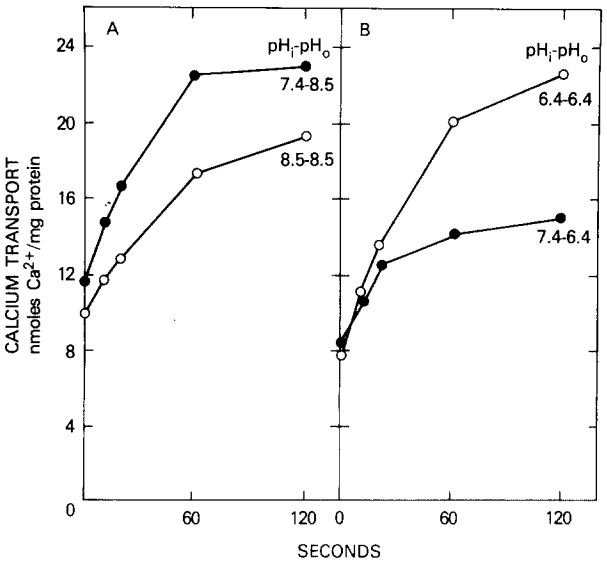

Fig. 7. Effect of pH gradient on ATP-dependent Ca^{2+} transport. All the conditions were as described for Fig. 6. Membranes were equilibrated in media containing 300 mM mannitol, 50 mM Tris-MES adjusted to pH 6.4, 7.5, and 8.5 (pH_i) and assayed in media containing 1 mM Mg-gluconate, 1 mM Tris-ATP, 25 μM $^{45}Ca^{2+}$ (total), 300 mM mannitol, 50 mM Tris-MES at pH 8.5 (*7A*) or 6.4 (*7B*) as indicated in the figure (pH_o). Other details of the assay were as described in Materials and Methods

cium transport activity. An inwardly directed pH gradient (6.4_o to 7.4_i) induces an inhibition of calcium transport (*7B*) while an outwardly directed gradient (8.5_o to 7.4_i) induces a stimulation (*7A*). When calcium transport was measured under exactly the same conditions, in the absence of ATP (*data not shown*), there was no effect of an inwardly directed (6.4_o to 7.4_i) pH gradient on the level of $^{45}Ca^{2+}$ in the vesicles. When the extravesicular medium is more alkaline (8.5_o to 7.4_i) there is a slight increase (<10%) in the background level of Ca^{2+} bound ($^{45}Ca^{2+}$ uptake at 0 min), that is seen both in the absence (*not shown*) and presence of ATP (as seen in Fig. 7*A*). In the absence of ATP, when the level of $^{45}Ca^{2+}$ retained by the vesicles is corrected for this "extra" Ca^{2+} binding, there is no change between the $^{45}Ca^{2+}$ incorporation under the conditions 8.5_o to 7.4_i and 8.5_o to 8.5_i. Also, this increase in the background $^{45}Ca^{2+}$ level is related to the $[Ca^{2+}]$ in the assay medium (*data not shown*). This suggests that there is no ATP-independent Ca^{2+}/H^+ countertransport activity under the conditions of our assay.

In order to look at the effects of an outwardly directed H^+ gradient on ATP-dependent Ca^{2+} transport in a KCl medium, KCl-loaded vesicles were diluted into TMA-Cl or KCl media in the presence of nigericin. In the presence of a K^+ gradient, nigericin induces an electroneutral K^+/H^+ exchange, making the intravesicular medium acidic (outwardly directed H^+ gradient), without changing membrane potential. Under these conditions, we observed a ~30% decrease in the steady-state level of Ca^{2+} accumulation (*see* Table 3) without any effect on the initial rates of transport. The $^{45}Ca^{2+}$ incorporation in the absence of ATP was similar in the two media (*data not shown*). If the BLMV contained a Ca^{2+}/H^+ exchange system in addition to the ATP-dependent $^{45}Ca^{2+}$ transporter we would expect to observe an additional accumulation of ^{45}Ca under these conditions. However, apparently H^+ cannot successfully substitute for intravesicular K^+ in achieving optimal ATP-dependent Ca^{2+} accumulation.

Table 3. Effect of intravesicular H^+ on ATP-dependent calcium transport in BLMV[a]

Media		% Ca Transport	
Incubation	Assay	Initial rate	Accumulation at 2 min
KCl	KCl	100	100
KCl	TMA-Cl	105 ± 6	75 ± 3

[a] Membranes were incubated with KCl in the presence of nigericin (0.2 μM) for 1 hr at 25°C. Aliquots were then diluted either into assay media containing 150 mM KCl or 150 mM TMA-Cl, 1 mM Tris-ATP, 1 mM Mg-gluconate, 10 μM $^{45}Ca^{2+}$ (total). Other details of assays were as described for Fig. 1. Values are the mean ± SEM of two experiments with different membrane preparations; each time point was assayed in triplicate. The mean value for initial rate in the KCl medium was 24 nmol/mg/min, and the Ca^{2+} accumulation was 21 nmol/mg protein.

Discussion

The data presented in this paper provide a detailed characterization of the ATP-dependent Ca^{2+} transporter found in the basolateral plasma membrane of the rat parotid gland. Calcium transport in parotid BLMV exhibits an obligatory requirement for ATP and Mg^{2+}, shows vanadate sensitivity, has a high affinity for Ca^{2+} and fairly large capacity for Ca^{2+} transport under V_{max} conditions. Oxalate, an anion which stimulates ATP-dependent Ca^{2+} flux in the endoplasmic reticulum, has no effect on Ca^{2+} flux into parotid BLMV. The K_m (70 nM) and V_{max} (54 nmol/mg protein/min) values obtained for the Ca^{2+} transport activity, are very similar to those reported for a membrane preparation from rat pancreas, another exocrine gland (Rega, 1986). It has been previously reported by our laboratory that the ATP-

dependent Ca^{2+} transport in parotid BLM vesicles was optimally active in a K^+ medium as compared to a Na^+ medium, and that Na^+ could discharge the accumulated Ca^{2+} in the vesicles. Similar observations have been made for ATP-dependent Ca^{2+} transporters in plasma membranes from other sources (Penniston, 1983; Rega, 1986).

A majority of the reports on ATP-dependent Ca^{2+} transport in plasma membranes, have addressed regulation of the kinetic properties of the Ca^{2+} transporter by calmodulin. Relatively few studies have been directed towards characterization of the electrical properties of the Ca^{2+} flux and several conflicting mechanisms have been proposed. It appears that the inherent conductive pathways for anions and cations in various membranes could contribute significantly towards the expression of an electrogenic Ca^{2+} flux and may therefore explain some observed functional discrepancies. Bayerdorffer et al. (1985) have reported that the ATP-dependent Ca^{2+} transporter in membrane vesicles isolated from rat pancreas mediates an electrogenic flux of Ca^{2+}. Detailed studies regarding the nature of the ATP-dependent Ca^{2+} flux have been done with the erythrocyte plasma membrane Ca^{2+} transport system, where intact cells, resealed ghosts, inside-out membrane vesicles, as well as reconstituted systems have been used. Nevertheless, reports regarding whether the ATP-dependent Ca^{2+} transporter in the erythrocyte plasma membrane mediates an electrogenic flux of Ca^{2+} are still conflicting. For example, Waisman et al. (1981) and Rossi and Schatzmann (1982) earlier suggested that the Ca^{2+} flux was electrogenic, while, Niggli, Sigel and Carafoli (1982) using the reconstituted Ca^{2+} transporter, and Smallwood et al. (1983) using membrane vesicles, have provided evidence for an electroneutral $Ca^{2+}/2H^+$ exchange mechanism during ATP-dependent Ca^{2+} transport. There is only one report demonstrating an electrogenic Ca^{2+} flux in the endoplasmic reticulum of a "nonexcitable" tissue, rat liver (Muallem et al., 1985). Similar controversies still exist regarding the ATP-dependent Ca^{2+} transporter in sarcoplasmic reticulum from skeletal muscle, one of the best characterized ATP-dependent Ca^{2+} transport systems. Thus, some studies have suggested that this flux of Ca^{2+} is electrogenic (Zimniak & Racker, 1978; Beeler, 1980) though in situ, it may appear electroneutral due to secondary ion fluxes which provide charge compensation (Meissner, 1981). Other studies have suggested that the ATPase could itself behave as a Ca^{2+}/H^+ exchanger (Chiesi & Inesi, 1980; Ueno & Sekine, 1981) or a Ca^{2+}/K^+ exchanger (Haynes, 1982) and thus mediate an electroneutral Ca^{2+} flux.

Based on the results presented herein we propose that during ATP-dependent Ca^{2+} uptake in rat parotid basolateral membrane vesicles there is a transfer of electrical charge. The strongest evidence for this is our observation that the initial rate of Ca^{2+} transport can be significantly altered by preimposed membrane potentials. A negative (inside) K^+ diffusion potential stimulates the transport rate while positive (inside) K^+ diffusion potential inhibits Ca^{2+} transport. This suggests that the Ca^{2+} flux results in accumulation of positive charges in the vesicle lumen. Since this effect is not observed in the presence of an outwardly directed K^+ gradient, in the absence of valinomycin, it is not an effect of the K^+ gradient in itself, but rather due to the clamping of membrane potential by valinomycin, a situation which was confirmed using the fluorescent dye di-S-C2-(5). Bayerdörffer et al. (1985) had reported that in rat pancreatic basolateral membrane vesicles, valinomycin was not necessary to clamp the membrane potential, suggesting the rat pancreas to possess an inherently K^+ permeant membrane. The rat pancreatic acinar cell membranes possess a nonselective cation conductance, which is Ca^{2+} activated but not voltage dependent. Rat parotid plasma membranes possess a K^+-selective conductive pathway which is gated by both Ca^{2+} and voltage (positive inside) (Petersen & Maruyama, 1984).

Proton movement has also been associated with ATP-dependent Ca^{2+} transport in several systems. In erythrocyte plasma membranes, Niggli et al. (1982) have suggested that the Ca^{2+}-ATPase carries out an electroneutral ATP-dependent Ca^{2+}/H^+ exchange reaction, while Villalobo and Roufogalis (1986) have suggested this to be an electrogenic ATP-dependent, countertransport of Ca^{2+} and H^+. In addition a H^+ effect on the hepatic plasma membrane Ca^{2+} transport has been shown (Kraus-Friedmann et al., 1982). Meissner (1981) had reported earlier that the ATP-dependent Ca^{2+} transport in skeletal muscle sarcoplasmic reticulum was affected by pH gradients and that proton movements accompanied Ca^{2+} transport. The results described in this paper show that the initial rate of Ca^{2+} transport can be altered by imposing a pH gradient across the membrane. The effect was independent of direct effects on the transport due to the pH. The initial rate of Ca^{2+} transport was higher when both the intravesicular and extravesicular pH = 6.4 than when the extravesicular pH = 6.4 and intravesicular pH = 7.4. The reverse situation was true at pH 8.5 (*see* Figs. 6 and 7). These effects could also be explained by a Ca^{2+}/H^+ exchange mediated by the ATP-dependent Ca^{2+} transporter itself or by a Ca^{2+}/nH^+ exchanger in addition to the ATP-dependent transporter in the same membrane vesicle. Two points argue against the latter interpretation of our

data: (1) no increase was seen in Ca^{2+} accumulation in the absence of ATP, when intravesicular H^+ was increased by diluting K^+-loaded vesicles into K^+-free medium in the presence of nigericin, and (2) no stimulation of ATP-dependent Ca^{2+} flux was seen under the same conditions. Moreover, since the experiments involving pH gradients were performed in the absence of K^+ and Cl^-, in a mannitol-containing assay medium, we can eliminate direct pH effects on either K^+ or Cl^- flux. The lack of an effect of FCCP on ATP-dependent Ca^{2+} transport (as shown in Table 2) also would argue against the involvement of a H^+ flux during the ATP-dependent Ca^{2+} transport, though not conclusively establish the lack of a Ca^{2+}/H^+ exchange activity. Niggli et al. (1982) when using inverted membrane vesicles from erythrocytes had observed that FCCP did not alter Ca^{2+} flux. However, when using a reconstituted system from the same membrane, they detected a definite FCCP effect on the ATP-dependent Ca^{2+} transport, providing evidence for a Ca^{2+}/H^+ exchange mechanism.

Given the present data, we cannot conclusively differentiate effects due to a H^+ gradient-induced membrane potential from those due to ΔpH. However, we have observed a small change in membrane potential (quenching of di-S-C2-(5)) when an outwardly directed pH gradient, 7.4_i to 8.5_o, was established (*data not shown*). At present we are unable to measure a Ca^{2+}-dependent ATP hydrolysis coupled to Ca^{2+} transport in these membranes, which further complicates this issue. This problem is mainly a result of high background levels of K^+-dependent phosphatase in these membranes, which has an extremely low sensitivity to ouabain as well as digoxin, or digitoxigenin (Ambudkar, *unpublished data*). Therefore, though our data show that ATP-dependent Ca^{2+} transport in rat parotid BLMV can be modulated by the membrane potential, indicating a charge transfer, we presently cannot unequivocally establish whether the Ca^{2+} uptake, per se is electrogenic or is coupled electroneutrally to the electrogenic transport of another ion. Further studies, aimed towards reconstituting the Ca^{2+} transport activity in a lipid vesicle system and establishing the associated ATP hydrolytic activity, will provide a more complete understanding of this transport system, especially the effects due to electrically coupled ion fluxes.

Even though a direct role for the ATP-dependent Ca^{2+} transport in the Ca^{2+} mobilizing events following receptor activation of the parotid cell has not yet been established, several considerations suggest that the activity represents a physiologically relevent system for Ca^{2+} efflux from the cell. Measurements of $[Ca^{2+}]_i$ in parotid cells using Quin 2 (Ambudkar et al., 1988) have shown that the resting $[Ca^{2+}]_i$ is about 150 nm, which is close to the K_m of the transporter. After neurotransmitter (epinephrine, carbamylcholine) stimulation, the $[Ca^{2+}]_i$, though four- to fivefold higher, is still below the $[Ca^{2+}]$ required for V_{max} (achieved at $[Ca^{2+}] > 1$ μM). Based on the data presented here we propose that if the membrane potential or pH gradient were altered at the basolateral plasma membrane, the Ca^{2+} transport activity could potentially be altered. Depending on the direction of the shift in activity, neurotransmitter-simulated $[Ca^{2+}]_i$ elevation would either be sustained longer or dissipated faster, thus conceivably modulating the secretory events. A possible direct regulation of the ATP-dependent Ca^{2+} transporter (increase in K_m and V_{max}) by agents which elevate cyclic AMP was suggested in an earlier report by Helman et al. (1986). Together, these observations suggest a broad regulatory range for the ATP-dependent Ca^{2+}-transporting system in rat parotid basolateral membrane.

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References

Ambudkar, I.S., Kuyatt, B.L., Roth, G.S., Baum, B.J. 1988. Modification of ATP-dependent Ca^{2+} transport in rat parotid basolateral membranes during aging. *Mech. Ageing Dev. (in press)*

Bayerdörffer, E., Eckhardt, L., Haase, W., Schulz, I. 1985. Electrogenic calcium transport in plasma membrane of rat pancreatic acinar cells. *J. Membrane Biol.* **84:**45–60

Beeler, T.J. 1980. Ca^{2+} uptake and membrane potential in sarcoplasmic reticulum vesicles. *J. Biol. Chem.* **255:**9156–9161

Carafoli, E., Zurini, M. 1982. The Ca^{2+} pumping ATPases of plasma membranes. Purification, reconstitution and properties. *Biochim. Biophys. Acta* **683:**288–301

Chiesi, M., Inesi, G. 1980. Adenosine triphosphate-dependent fluxes of manganese and hydrogen ions in sarcoplasmic reticulum vesicles. *Biochemistry* **19:**2912–2918

Feinstein, M., Schramm, M. 1970. Energy production in rat parotid gland. Relation to enzyme secretion and effects of calcium. *Eur. J. Biochem.* **13:**158–163

Haynes, D.H. 1982. Relationship between H^+, anion, and monovalent cation movements and Ca^{2+} transport in sarcoplasmic reticulum: Further proof of a cation exchange mechanism for the Ca^{2+}–Mg^{2+}-ATPase pump. *Arch. Biochem. Biophys.* **215:**444–461

Helman, J., Kuyatt, B.L., Takuma, T., Seligmann, B., Baum, B.J. 1986. ATP dependent calcium transport in rat parotid basolateral membrane vesicles. Modulation by agents which elevate cylic-AMP. *J. Biol. Chem.* **261:**8919–8923

Immelmann, A., Soling, H.D. 1983. ATP-dependent calcium sequestration and calcium/ATP stoichiometry in isolated microsomes from guinea pig parotid glands. *FEBS Lett.* **162:**406–410

Kanagasuntheram, P., Teo, T.S. 1982. Parotid microsomal calcium transport. *Biochem. J.* **208:**789–794

Kraus-Friedmann, N., Biber, J., Murer, H., Carafoli, E. 1982. Calcium uptake in isolated hepatic plasma-membrane vesicles. *Eur. J. Biochem.* **129:**7–12

Meissner, G. 1981. Calcium transport and monovalent cation and proton fluxes in sarcoplasmic reticulum vesicles. *J. Biol. Chem.* **256:**636–643

Muallem, S., Schoeffield, M., Pandol, S., Sachs, G. 1985. Inositol triphosphate modification of ion transport in rough endoplasmic reticulum. *Proc. Natl. Acad. Sci. USA* **82:**4433–4437

Niggli, V., Siegel, E., Carafoli, E. 1982. The purified Ca^{2+} pump of human erythrocyte membranes catalyze an electroneutral Ca^{2+}–H^+ exchange in reconstituted liposomal systems. *J. Biol. Chem.* **257:**2350–2356

Penniston, J. T. 1983. Plasma membrane Ca^{2+} ATPases as active Ca^{2+} pumps. *In:* Calcium and Cell Function. W.Y. Cheung, editor. Vol. IV, pp. 99–149. Academic, New York

Petersen, O.H., Maruyama, Y. 1984. Ca^{2+}-activated potassium channels and their role in secretion. *Nature (London)* **307:**693–696

Putney, J.W., Jr. 1986. Identification of cellular activation mechanisms associated with salivary secretion. *Annu. Rev. Physiol.* **48:**75–88

Rega, A.F. 1986. From the discovery of the Ca^{2+} pump in plasma membranes to the demonstration of its ubiquitousness *In:* Ca^{2+} Pump of Plasma Membranes, A.F. Rega and P.J. Garrahan, editors. pp. 45–58. CRC Press, Florida

Rossi, J.P., Schatzmann, F.C. 1982. Is the red cell calcium pump electrogenic? *J. Physiol. (London)* **327:**1–15

Schulz, I. 1980. Messenger role of calcium in the function of pancreatic acinar cells. *Am. J. Physiol.* **239:**G335–G347

Smallwood, J.L., Waisman, D.M., Lafreniere, D., Rasmussen, H. 1983. Evidence that the erythrocyte calcium pump catalyzes a Ca^{2+} : nH^+ exchange. *J. Biol. Chem.* **258:**11095–11097

Takuma, T., Kuyatt, B.L., Baum, B.J. 1985. Ca^{2+} transport in basolateral plasma membrane enriched vesicles from rat parotid glands. *Biochem. J.* **227:**239–245

Ueno, T., Sekine, T. 1981. Role of H^+ flux in active Ca^{2+} transport into sarcoplasmic reticulum vesicles. II. H^+ ejection during Ca^{2+} uptake. *J. Biochem.* **89:**1247–1252

Villalobo, A., Roufogalis, B.D. 1986. Proton countertransport by the reconstituted erythrocyte Ca^{2+}-translocating ATPase: Evidence using ionophoretic compounds. *J. Membrane Biol.* **93:**249–258

Waisman, D.M., Gimble, J.M., Goodman, D.B.P., Rasmussen, H. 1981. Studies of the Ca^{2+} transport mechanism of human erythrocyte inside out plasma membrane vesicles. III. Stimulation of the Ca^{2+} pump by anions. *J. Biol. Chem.* **256:**420–424

Zimniak, P., Racker, E. 1978. Electrogenicity of Ca^{2+} transport catalyzed by the Ca^{2+} ATPase from sarcoplasmic reticulum. *J. Biol. Chem.* **253:**4631–4637

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