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Calcium transport mechanisms in basolateral plasma membrane-enriched vesicles from rat parotid gland

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Ca^{2+} transport was studied by using basolateral plasma membrane vesicles from rat parotid gland prepared by a Percoll gradient centrifugation method. In these membrane vesicles, there were two Ca^{2+} transport systems; Na^+/Ca^{2+} exchange and ATP-dependent Ca^{2+} transport. An outwardly directed Na^+ gradient increased Ca^{2+} uptake into vesicles, while an inwardly directed Na^+ gradient inhibited Ca^{2+} uptake. Ca^{2+} efflux from Ca^{2+}-preloaded vesicles was stimulated by an inwardly directed Na^+ gradient. However, Na^+/Ca^{2+} exchange did not show any 'uphill' transport of Ca^{2+} against its own gradient. ATP-dependent Ca^{2+} transport exhibited 'uphill' transport. An inwardly directed Na^+ gradient also decreased Ca^{2+} accumulation by ATP-dependent Ca^{2+} uptake. The inhibition of Ca^{2+} accumulation was proportional to the external Na^+ level. Na^+/Ca^{2+} exchange was inhibited by monensin, tetracaine and chlorpromazine, whereas ATP-dependent Ca^{2+} transport was inhibited by orthovanadate, tetracaine and chlorpromazine. Oligomycin had no effect on either system. These results suggest that in the parotid gland cellular free Ca^{2+} is extruded mainly by an ATP-dependent Ca^{2+} transport system, and Na^+/Ca^{2+} exchange may modify the efficacy of that system.

It is widely recognized that the intracellular free Ca^{2+} level plays a crucial role in various cellular functions. In mammalian cells the cytosolic free Ca^{2+} level is regulated by several mechanisms including active Ca^{2+} transporting systems such as the ATP-dependent Ca^{2+} pump and Na^+/Ca^{2+} exchange (Shamoo & Ambudkar, 1984). ATP-dependent Ca^{2+} transport has been demonstrated in various cell types in both plasma membranes and the endoplasmic reticulum. On the other hand, an active 'uphill' transport of Ca^{2+} by Na^+/Ca^{2+} exchange has been detected only in plasma membranes of nerve cells (Mullins, 1979; Gill *et al.*, 1981) and heart cells (Reeves & Sutko, 1979). The physiological significance of Na^+/Ca^{2+} exchange as found in other cells is still unknown.

In salivary glands various secretagogues, including cholinergic, adrenergic and peptidergic agonists, stimulate Ca^{2+} fluxes across plasma membranes during salivary secretion (Butcher & Putney, 1980). The subcellular distribution of Ca^{2+} transport systems has been studied in rat submandibular and parotid glands and these studies showed that the microsomal fraction of each gland has an ATP-dependent Ca^{2+} transport system (Selinger *et al.*, 1970; Bonis & Rossignol, 1982; Kanagasuntheram & Teo, 1982; Terman & Gunter, 1983; Hayakawa *et al.*, 1983). Thus far, however, Na^+/Ca^{2+} exchange in a plasma membrane fraction has not been studied.

Recently we prepared basolateral membrane vesicles with an active amino acid transport system from rat parotid gland by a Percoll gradient centrifugation method (Takuma & Baum, 1985). In the present study we have evaluated both the ATP-dependent Ca^{2+} transport and Na^+/Ca^{2+} exchange systems in this preparation. Our studies suggest that the Na^+/Ca^{2+} exchange system may be involved in the regulation of cellular Ca^{2+} level by modifying the efficacy of ATP-dependent Ca^{2+} transport.

Materials and methods

Materials

Animals used in these experiments were male, 350g Wistar strain rats purchased from Harlan/

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Sprague–Dawley. Rats were fed *ad libitum* and killed between 09:30 and 10:30h. [^{45}Ca]Cl_2 was obtained from Amersham International. ATP (dibarium salt), oligomycin, ouabain, orthovanadate, tetracaine hydrochloride and chlorpromazine were from Sigma. A23187 and monensin were from Calbiochem. Percoll was from Pharmacia. All other reagents utilized were the highest grade commercially available.

Preparation of basolateral membrane vesicles

The basolateral plasma membrane vesicles were prepared as described previously (Takuma & Baum, 1985). Briefly, parotid glands were homogenized with 0.25M-sucrose containing 0.1mM-phenylmethanesulphonyl fluoride and 10mM-Tris/HCl, pH7.6, in a Polytron homogenizer. The homogenate (approx. 10%, w/v) was centrifuged at 2600*g* for 15min and the resulting supernatant was centrifuged at 23500*g* for 20min. This supernatant was discarded and the pellet was homogenized with the same sucrose buffer in a Teflon/glass homogenizer. The homogenate was mixed with Percoll (final concentration 12%, v/v), and centrifuged at 41700*g* for 30min. The distribution of cellular membranes in the Percoll gradient was followed by assaying various marker enzymes. For the present report only the distribution of K^+-dependent phosphatase and NADPH:cytochrome *c* reductase are given. Previously we have published a more detailed marker enzyme profile (Takuma & Baum, 1985). K^+-dependent phosphatase ($Na^+ + K^+$-ATPase) was determined as described by Arvan & Castle (1982) and NADPH:cytochrome *c* reductase was measured as described by Sottocasa *et al.* (1967). A basolateral plasma membrane enriched fraction (upper half except top clear fraction) was collected and washed three times with loading buffer (150mM-NaCl or KCl, 10mM-Hepes/Tris, pH7.5) by centrifuging at 41700*g* for 15min. The membrane vesicles were finally suspended in a small volume of loading buffer, and preincubated at 37°C for 10min prior to transport experiments.

Ca^{2+} transport

The transport of Ca^{2+} was measured by rapid filtration through a Millipore filter (HA, 0.45μm). For Na^+/Ca^{2+} exchange, membrane vesicles (10μl, 25–40μg of protein) were incubated with 90μl of reaction mixture containing 10μM-$^{45}CaCl_2$, 150mM-NaCl or KCl and 10mM-Hepes/Tris, pH7.5, at 37°C for the indicated period. For ATP-dependent Ca^{2+} transport, 1mM-ATP and 1mM-$MgCl_2$ were added to the above mixture. The incubation was terminated by adding 2ml of ice-cold buffer containing 150mM-KCl and 5mM-Hepes/Tris, pH7.5, passed over a Millipore filter, and washed three times with 2ml of the same buffer. The filters were placed in vials containing 10ml of Beckman Ready-Solv and radioactivity was determined by liquid-scintillation spectrometry. All determinations were carried out in triplicate in at least two separate experiments and average values were always within ±5%. Protein was determined by the method of Lowry *et al.* (1951) with bovine serum albumin as a standard.

Results

Characterization of vesicles

We have previously reported (Takuma & Baum, 1985) the distribution of marker enzymes in parotid membrane fractions after Percoll gradient centrifugation. For the present study we deemed it especially important to assess the level of membranes derived from the endoplasmic reticulum contaminating our basolateral membrane preparation. This was not done earlier, but it is relevant since previous studies on parotid Ca^{2+} transport mechanisms primarily employed microsomal membrane preparations enriched in endoplasmic reticulum (e.g. Selinger *et al.*, 1970; Kanagasuntheram & Teo, 1982; Bonis & Rossignol, 1982). The enrichment factors and percentage recoveries of the marker enzymes, for basolateral membrane and endoplasmic reticulum in our preparation, are shown in Table 1. It is quite clear that the Percoll-gradient prepared parotid membranes used here for Ca^{2+} transport studies are enriched with respect to K^+-dependent phosphatase (basolateral membrane) but not NADPH:cytochrome *c* reductase (endoplasmic reticulum).

Na^+/Ca^{2+} exchange

For detection of Na^+/Ca^{2+} exchange, basolateral plasma membrane vesicles were preloaded with 150mM of NaCl or KCl, and incubated with 10μM-$^{45}CaCl_2$ in the presence or absence of Na^+ gradient between the intra- and extra-vesicular fluid. As shown in Fig. 1(*a*), an outwardly directed Na^+ gradient (Na^+_i/K^+_o) stimulated Ca^{2+} uptake by about 25% above control levels (Na^+_i/Na^+_o). Conversely, an inwardly directly Na^+ gradient (K^+_i/Na^+_o) decreased Ca^{2+} uptake by 20% compared with the control (K^+_i/K^+_o) (Fig. 1*b*).

We next examined whether Na^+/Ca^{2+} exchange can stimulate the efflux of Ca^{2+} from vesicles. Vesicles were passively loaded with $^{45}Ca^{2+}$ by incubating with 100μM-$^{45}CaCl_2$ and 150mM-KCl for 30min at 37°C, and aliquots (10μl) were incubated with 90μl of 150mM-KCl or NaCl. As shown in Fig. 2, an inwardly directed Na^+ gradient stimulated Ca^{2+} efflux by 30% over controls.

Although the Na^+ gradient enhanced Ca^{2+} fluxes across membrane vesicles in these experi-

Table 1. *Distribution of protein and enzyme activities*

Each fraction was prepared as described in the Materials and methods section. Enzyme data indicate the relative specific activities (enrichment factors) compared with the homogenate fraction and are the average of two separate experiments. The percentage recovery of enzymes, or protein, in each fraction, is given by the number in parentheses. Effective recovery refers to total enzyme activity recovered from the Percoll gradient compared with the amount of activity placed on the gradient, as described by Scalera *et al.* (1980). A detailed distribution of marker enzymes in these fractions was previously reported by us (Takuma & Baum, 1985).

Fraction	Protein (mg)	Relative specific activity	
		K^+-dependent phosphatase	NADPH:cytochrome *c* reductase
Homogenate	119	1	1
	(100)	(100)	(100)
Pellet (2600*g*)	38	1.48	0.36
	(32.0)	(47.7)	(11.5)
Supernatant (23500*g*)	66.2	0.56	1.32
	(55.7)	(31.0)	(73.6)
Crude membrane	10.3	2.62	1.42
	(8.7)	(22.9)	(12.2)
Basolateral membrane-enriched	1.2	9.55	0.71
	(1.0)	(9.6)	(0.7)
Luminal membrane-enriched	2.86	1.76	1.62
	(2.4)	(1.8)	(3.8)
Remaining fractions	1.28	1.29	1.16
	(1.1)	(1.4)	(1.3)
Effective recovery (%)	51.8	56.7	47.7

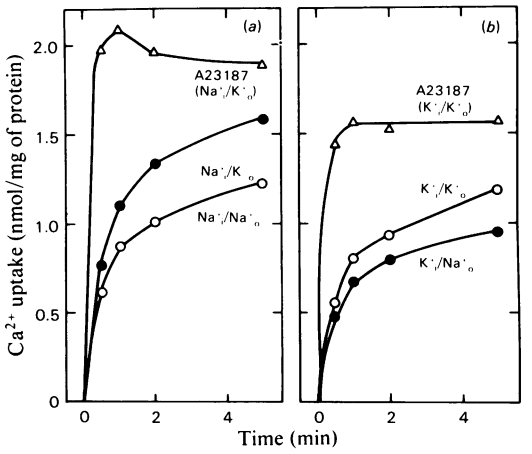

Fig. 1. *Ca^{2+} uptake by basolateral plasma membrane vesicles from rat parotid gland*

Vesicles were preloaded with 10mM-Hepes/Tris buffer, pH 7.5, and 150mM-NaCl (*a*) or KCl (*b*). Aliquots of vesicles (10μl) were incubated with 90μl of incubation mixture containing 10μM-$^{45}CaCl_2$, 10mM-Hepes/Tris, pH 7.5, 150mM-KCl or NaCl, with or without 5μM-A23187 at 37°C for the indicated period. The radioactive Ca^{2+} accumulated in vesicles was measured by a rapid filtration method.

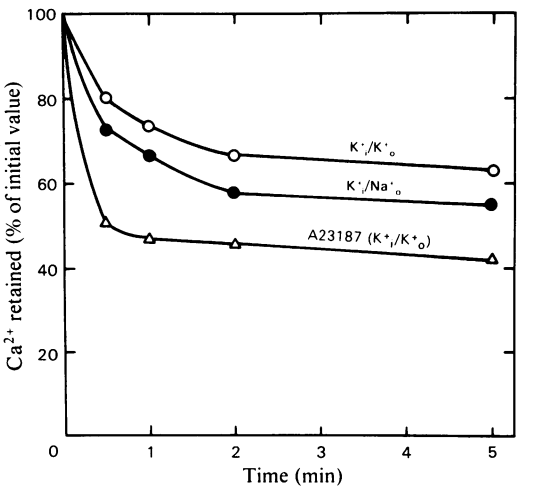

Fig. 2. *Ca^{2+} efflux from parotid basolateral plasma membrane vesicles*

Vesicles were preloaded with 100μM-$^{45}CaCl_2$, 150mM-KCl and 10mM-Hepes/Tris, pH 7.5, at 37°C for 30min. Aliquots (10μl) were incubated with 90μl of 150mM-KCl or NaCl, 10mM-Hepes/Tris, pH 7.5, in the presence or absence of 5μM-A23187 at 37°C for the indicated period.

ments, the Ca^{2+} level stimulated by a Na^+ gradient did not exceed the equilibrium level observed when membranes were incubated in the presence of the divalent cation ionophore, A23187 (Figs. 1 and 2). To examine whether Na^+/Ca^{2+} exchange has the ability to transport Ca^{2+} against its own gradient, vesicles were preincubated with 30μM-

$^{45}CaCl_2$ and 150mM-NaCl for 30min at 37°C, and aliquots (10μl) were incubated with 90μl of 10μM-$^{45}CaCl_2$ in 150mM-NaCl or KCl. As shown in Fig. 3, an outwardly directed Na^+ gradient maintained the intravesicular Ca^{2+} level for at least 6min, but could not exceed this initial Ca^{2+}-level. Without the presence of a Na^+ gradient, $^{45}Ca^{2+}$ was gradually lost from vesicles (Na^+_i/Na^+_o, Fig. 3). If in addition there was no $^{45}Ca^{2+}$ in the incubation buffer, loss of $^{45}Ca^{2+}$ from vesicles was accelerated considerably.

ATP-dependent Ca^{2+} transport

ATP-dependent Ca^{2+} transport was measured by the same method as Na^+/Ca^{2+} exchange except for the addition of 1mM-$MgCl_2$ and 1mM-ATP to the incubation mixture. As shown in Fig. 4, the membrane vesicles accumulated Ca^{2+} rapidly over the Ca^{2+} level obtained in the presence of A23187 (i.e. equilibrium level). Addition of A23187 to vesicles that had taken up $^{45}Ca^{2+}$ for 1 min resulted in the rapid loss of accumulated $^{45}Ca^{2+}$ and the attainment of equilibrium uptake levels.

ATP-dependent Ca^{2+} uptake was saturable. The K_m value for added Ca^{2+} was 33μM and the $V_{max.}$ was 17nmol/min per mg of protein (Fig. 5). These are very close to values reported previously for ATP-dependent Ca^{2+} uptake in microsomal fractions from rat parotid (15.4μM and 21.5nmol/min per mg of protein) (Kanagasuntheram & Teo, 1982) and submandibular (25μM and 12nmol/min per mg of protein) (Terman & Gunter, 1983) glands. The present work, to compare with these earlier studies, did not examine uptake with respect to free Ca^{2+} levels. Based on reports evaluating ATP-dependent Ca^{2+} uptake in plasma membranes from other tissues (e.g. Gmaj *et al.*, 1979; Van Heeswijk *et al.*, 1984), it is likely that K_m values with respect to free Ca^{2+} would be significantly lower than concentrations for added Ca^{2+} given here.

As seen in Fig. 4, an inwardly directed Na^+ gradient also inhibited (by about 30%) the accumulation of Ca^{2+} by the ATP-dependent Ca^{2+} uptake system. Since ouabain had no effect on this process (results not shown), the inhibition was not due to the consumption of ATP by the $Na^+ + K^+$-ATPase. Also, we did not detect any additive effect of Na^+/Ca^{2+} exchange and ATP-dependent Ca^{2+} transport, i.e. no further Ca^{2+} uptake occurred in the presence of outwardly directed Na^+ gradient and ATP (results not shown).

The Na^+-induced inhibition of ATP-dependent Ca^{2+} uptake was Na^+-concentration-dependent

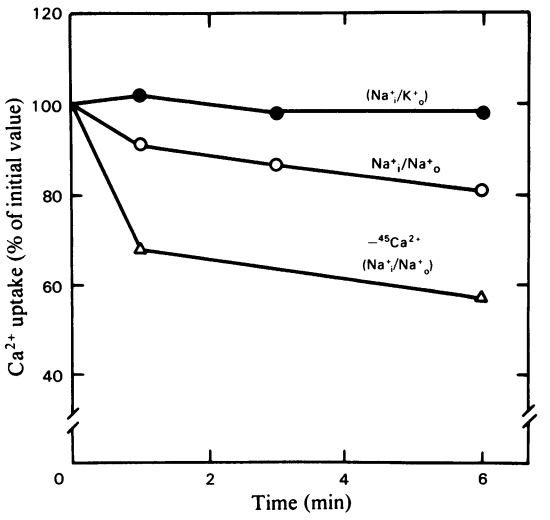

Fig. 3. *Ca^{2+} fluxes in parotid basolateral plasma membrane vesicles*
Vesicles were preloaded with 30μM-$^{45}CaCl_2$, 150mM-NaCl and 10mM-Hepes/Tris, pH7.5, at 37°C for 30min. Aliquots (10μl) were incubated with 90μl of 150mM-KCl or NaCl, 10mM-Hepes/Tris, pH7.5, with or without 10μM-$^{45}CaCl_2$ at 37°C for the indicated period.

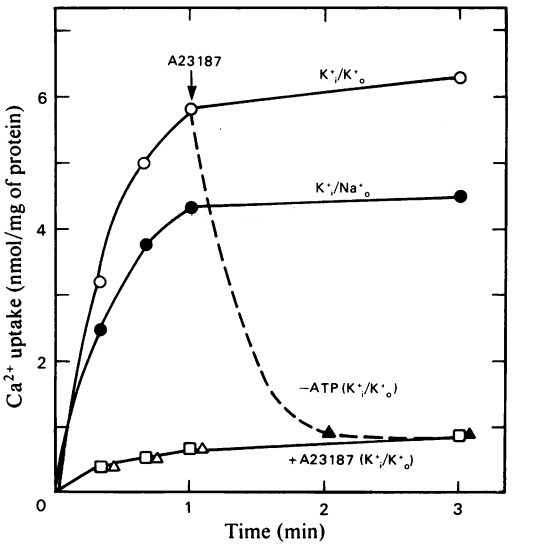

Fig. 4. *ATP-dependent Ca^{2+} uptake in parotid basolateral membrane vesicles*
Vesicles were preloaded with 150mM-KCl and 10mM-Hepes/Tris, pH7.5. Aliquots (10μl) were incubated with 90μl of incubation mixture containing 10μM-$^{45}CaCl_2$, 150mM-KCl or NaCl, 1mM-ATP, 1mM-$MgCl_2$, 10mM-Hepes/Tris, pH7.5, at 37°C for the indicated period. Notation used: −ATP, ATP was deleted from the above mixture; +A23187, 5μM-A23187 was added to the above complete mixture.

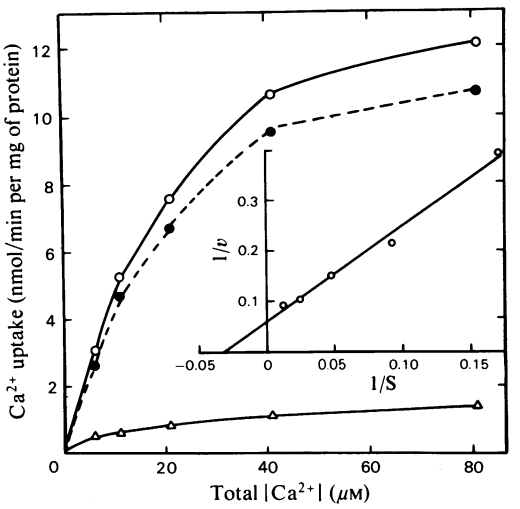

Fig. 5. *Effect of Ca^{2+} concentration on ATP-dependent Ca^{2+} uptake by parotid basolateral plasma membrane vesicles*

Vesicles were preloaded with 150mM-KCl and 10mM-Hepes/Tris, pH7.5. Aliquots (10μl) were incubated with 90μl of 5μM-$^{45}CaCl_2$, 150mM-KCl, 1mM-ATP, 1mM-$MgCl_2$, 10mM-Hepes/Tris, pH7.5, and increasing concentrations of non-radioactive $CaCl_2$ at 37°C for 30s. ○, total uptake; ●, ATP-dependent uptake; △, ATP-independent uptake.

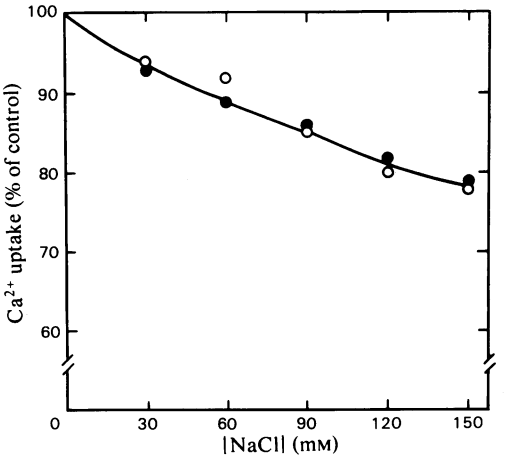

Fig. 6 *Effect of extravesicular Na^+ concentration on ATP-dependent Ca^{2+} uptake and Na^+/Ca^{2+} exchange*

Vesicles were preloaded with 150mM-NaCl and 10mM-Hepes/Tris, pH7.5, for Na^+/Ca^{2+} exchange, and with 150mM-KCl and 10mM-Hepes/Tris, pH7.5, for ATP-dependent Ca^{2+} uptake. Vesicles (10μl) were incubated with 90μl of incubation mixture containing 10μM-$^{45}CaCl_2$, 10mM-Hepes/Tris, pH7.5, 150–0mM-KCl and 0–150mM-NaCl, at 37°C for 1min for Na^+/Ca^{2+} exchange. For ATP-dependent Ca^{2+} uptake, 1mM-ATP and 1mM-$MgCl_2$ and 1mM-ouabain were added to the above incubation mixture. ○, ATP-dependent Ca^{2+} uptake; ●, Na^+/Ca^{2+} exchange. Data are expressed as a percentage of control values (that in the presence of 150mM-KCl and 0mM-NaCl) in the incubation mixture.

(Fig. 6). This was examined by an increasing inwardly directed Na^+ gradient. Simultaneously the effect of decreasing the outwardly directed Na^+ gradient on Ca^{2+} uptake mediated by Na^+/Ca^{2+} exchange was also examined. The inhibition of Na^+/Ca^{2+} exchange was also proportional to the concentration of Na^+ in the extravesicular solution and $^{45}Ca^{2+}$ uptake was decreased in a similar fashion to the ATP-dependent uptake. (Fig. 6).

Effects of various agents on Na^+/Ca^{2+} exchange and ATP-dependent Ca^{2+} transport

To characterize further Na^+/Ca^{2+} exchange and ATP-dependent Ca^{2+} transport in this basolateral plasma membrane fraction, effects of various agents on the two processes were examined (Table 2). For the Na^+/Ca^{2+} exchange process, maximum inhibition was achieved by 150mM-Na^+ (i.e. no Na^+ gradient) and by 3mM-tetracaine. Oligomycin, an inhibitor of ATP-dependent Ca^{2+} uptake in mitochondria, had no effect on Ca^{2+} uptake by either process in basolateral membrane vesicles. Ouabain also was without effect, suggesting that 1mM-ATP was adequate to offset any ATP utilization by $Na^+ + K^+$-ATPase. In the absence of Mg^{2+}, A23187 and orthovanadate stimulated Ca^{2+} uptake by Na^+/Ca^{2+} exchange. However, in the presence of 1mM-Mg^{2+}, the effect of A23187 on this system was greatly diminished and that of orthovanadate completely disappeared (results not shown). A23187 markedly reduced ATP-dependent Ca^{2+} uptake (i.e. in the presence of 1mM-Mg^{2+} plus 1mM-ATP) and similarly orthovanadate substantially inhibited this Ca^{2+} transport system. Chlorpromazine (10μM) had no effect on either process, but showed an inhibitory effect on both systems at 100μM. Tetracaine also had a strong inhibitory effect on the ATP-dependent Ca^{2+} transport mechanism. Monensin inhibited only Na^+/Ca^{2+} exchange.

Discussion

Previous studies of Ca^{2+} transport in parotid membrane vesicles have employed microsomal preparations (e.g. Selinger *et al.*, 1970; Bonis & Rossignol, 1982; Kanagasuntheram & Teo, 1982)

Table 2. *Effects of various agents on Na^+/Ca^{2+} exchange and ATP-dependent Ca^{2+} transport*
Basolateral plasma membrane vesicles were preloaded with 150mM-NaCl and 10mM-Hepes/Tris, pH 7.5, for Na^+/Ca^{2+} exchange and with 150mM-KCl and 10mM-Hepes/Tris, pH 7.5 for ATP-dependent Ca^{2+} uptake. Vesicles (10μl) were incubated with 90μl of 10μM-$^{45}CaCl_2$, 150mM-KCl, 10mM-Hepes/Tris, pH 7.5, and various agents at 37°C for 1 min for Na^+/Ca^{2+} exchange. In 'NaCl' KCl was replaced by 150mM-NaCl. For ATP-dependent Ca^{2+} uptake, 1mM-ATP and 1mM-$MgCl_2$ were added to the incubation mixture. Results are the mean value of two separate experiments performed in triplicate. Control values were 0.95nmol/min per mg of protein for Na^+/Ca^{2+} exchange and 6.85nmol/min per mg of protein for ATP-dependent Ca^{2+} uptake.

	Na^+/Ca^{2+} exchange (% of control)	ATP-dependent Ca^{2+} transport (% of control)
Control (150mM-KCl)	100	100
NaCl (150mM)	75	73
A23187 (5μM)	175	9
Oligomycin (10μg/ml)	96	93
Monensin (10μM)	88	96
Ouabain (1mM)	97	95
Orthovanadate (1mM)	139	42
Tetracaine (3mM)	72	26
Chlorpromazine (100μM)	86	52

which contained an ATP-dependent system. Such preparations may contain membranes from several sources, including plasma membrane and endoplasmic reticulum. It is likely however that these earlier studies examined an ATP-dependent Ca^{2+} transport system derived from endoplasmic reticulum. For example, Ca^{2+} transport activity paralleled the activity distribution of NADPH:cytochrome *c* reductase, a marker of endoplasmic reticulum, quite closely (Kanagasuntheram & Teo, 1982). Also inhibitors of mitochondrial transport, such as Ruthenium Red and oligomycin, were without effect on these systems. The characteristics of transport activity in all three studies were also similar and both Bonis & Rossignol (1982) and Kanagasuntheram & Teo (1982) conclude that the microsomal ATP-dependent Ca^{2+}-transport system was endoplasmic reticulum-associated.

The present study has employed different membrane preparation procedures to obtain a vesicle fraction enriched in basolateral plasma membranes. This preparation was about 10-fold enriched in K^+-dependent phosphatase, a convenient marker for plasma membrane $Na^+ + K^+$-ATPase in ouabain-resistant rat tissues (Arvan & Castle, 1982). Conversely, the preparation was relatively depleted with respect to the endoplasmic reticulum marker, NADPH:cytochrome *c* reductase. Although it is inappropriate to conclude that this preparation is free of endoplasmic reticulum-derived membranes, for several reasons it is clear that the ATP-dependent Ca^{2+} transport system which we have studied is quite different from that studied previously. First, Na^+/Ca^{2+} exchange has so far been ascribed only to plasma membranes and mitochondria. Our preparation is depleted with respect to cytochrome oxidase (Takuma & Baum, 1985) and Ca^{2+} transport is insensitive to oligomycin, thus indicating no mitochondrial involvement. Since, as shown in Fig. 4, an inwardly directed Na^+ gradient inhibited ATP-dependent Ca^{2+} accumulation, the Na^+/Ca^{2+} exchange and the ATP-dependent system were present in the same (at least partially) vesicles. Finally, the ATP-dependent Ca^{2+} transport systems studied by Bonis & Rossignol (1982) and Kanagasuntheram & Teo (1982) were both activated by Na^+, not inhibited by Na^+ as demonstrated herein. In aggregate, such reasoning leads to the conclusion that the present study has evaluated Ca^{2+} transport in a different cellular membrane system than earlier studies and that the present membrane vesicles were enriched in basolateral plasma membranes.

In the present study the capacity of Ca^{2+} transport by Na^+/Ca^{2+} exchange was too small to accumulate Ca^{2+} in the membrane vesicles above an equilbrium level. So it seems unlikely that Na^+/Ca^{2+} exchange plays an important role in extruding cellular free Ca^{2+} against its own steep gradient between the cytosol and the extracellular fluid. The 'uphill' transport of Ca^{2+} by Na^+/Ca^{2+} exchange has been so far reported only in plasma membranes of nerve cells and mammalian heart cells (Mullins, 1979; Gill *et al.*, 1981; Reeves & Sutko, 1979). In these cells Na^+/Ca^{2+} exchange has a capacity for Ca^{2+} transport comparable with that of the ATP-dependent system. Conversely, Na^+/Ca^{2+} exchange in plasma membranes of kidney (Gmaj *et al.*, 1979; Van Heeswijk *et al.*, 1984), small intestine (Hildmann *et al.*, 1982; Ghijsen *et al.*, 1983) and smooth muscle (Grover *et*

al., 1983; Morel & Godfraind, 1984) has only limited capacity compared with ATP-dependent transport and a physiological role for Na^+/Ca^{2+} exchange in these cells has not been proposed.

The effects of the various modifying agents tested here on Na^+/Ca^{2+} exchange and ATP-dependent Ca^{2+} transport were essentially the same as observed in other cells (Gill *et al.*, 1981; Ueda, 1983). Interestingly, chlorpromazine at 100 μM inhibited both Na^+/Ca^{2+} exchange and ATP-dependent Ca^{2+} uptake. The latter effect is consistent with the purported regulation of ATP-dependent Ca^{2+} transport by calmodulin in various cell types (Moore & Kraus-Friedman, 1983; Famulski & Carafoli, 1984). However, it is unknown whether the inhibition of Na^+/Ca^{2+} exchange by chlorpromazine in parotid basolateral membranes also involves a calmodulin-dependent process, as has been reported for cardiac sarcolemma (Caroni & Carafoli, 1983).

The ATP-dependent Ca^{2+} transport system ($Ca^{2+}+Mg^{2+}$-ATPase) has a specific orientation on each membrane. In the plasma membrane, Ca^{2+} is transported unidirectionally from the inside to the outside of cells. On the contrary, the Na^+/Ca^{2+} exchanger has no intrinsic direction of Ca^{2+} transport (Gill *et al.*, 1981). Therefore, if the concentration of Na^+ increases in the cytosol, Na^+/Ca^{2+} exchange would decrease Ca^{2+} efflux from cells. As shown in Fig. 6, Na^+/Ca^{2+} exchange and ATP-dependent Ca^{2+} transport were proportionally decreased with the increase in Na^+ concentration in the extravesicular solution. In this case a decrease in the latter is more important than that in the former, since 20% of ATP-dependent Ca^{2+} transport is, in absolute terms, a much larger value than 20% of Na^+/Ca^{2+} exchange.

Many hormones and neurotransmitters modulate the permeability of plasma membranes to Na^+, and increase cytosolic Na^+ concentration in various cell types, including salivary glands (Putney, 1979). In these cells, a Na^+/Ca^{2+} exchange process may reduce the efficacy of Ca^{2+} extrusion by the ATP-dependent system, and thus prolong the duration of a high free Ca^{2+} level in the cytosol. Indeed the importance of Na^+ for hormone action has been reported in various cell types (Williams, 1975; Watson *et al.*, 1981; Connolly *et al.*, 1984). Additional study is needed to clarify the role of these transport systems in secretory responses.

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