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CHARACTERIZATION OF THE SUBMANDIBULAR GLAND MICROSOMAL CALCIUM TRANSPORT SYSTEM

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Calcium accumulation by submandibular gland microsomes (first described by Selinger and Naim, ((1970) Biochim. Biophys. Acta 323, 337–341) has been further characterized. Accumulation was concentration dependent, had a K_m of 25 μM added calcium and a V_{max} of 12 nM calcium/mg protein per min. No accumulation was observed in the presence of either the calcium ionophore A23187, or the detergent Triton X-100 (0.05%). The divalent cations Sr^{2+} and Mn^{2+} inhibited accumulation competitively with K_i values of 67 μM and 200 μM, respectively. The effect of various enzyme inhibitors were tested on the microsomal calcium transport system and it was found that chlorpromazine, trifluoperazine, and DIDS all inhibited. The mitochondrial transport inhibitors ruthenium red and CCCP had no effect on transport. Experiments directed at clarifying the cellular location of the system are described. It was found that the membrane vesicles responsible for transport show different purification properties than the membrane vesicles which contain the standard enzyme markers for total and rough endoplasmic reticulum, Golgi apparatus, plasma membrane, and lysosomes. These conclusions are based upon experiments using three properties for membrane purification, density, size, and electrophoretic mobility. Three possible explanations of the results are given and it is suggested that the calcium transporting membrane represents a specialized region of one of the cellular organelles. The significance of the results in: (1) understanding the biochemical properties of the submandibular gland microsomal calcium transport system, (2) clarifying the cellular location of the system, and (3) clarifying the function of the system in salivary secretion are discussed.

Introduction

It has become clear in recent years that calcium ions have a critical role in cellular metabolism [1,2]. This is especially true in salivary glands, where changes in the level of the cytosolic calcium concentration can lead to several separate effects. These effects include: (1) Calcium influx in response to hormone stimulation at the α-adrenergic and cholinergic receptors lead to ion and water movement into the lumen of the tissue [3]. (2) Hormonal stimulation at the β-adrenergic receptors of the acinar cells leads to protein release by the cells [4], and there is evidence that calcium release from intracellular stores plays a role in this process [5]. (3) Calcium ions are packaged into the zymogen granules of the acinar cells for release

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Abbreviations: CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; DIDS, 4,4′-diisothiocyanostilbene-2,2′-disulfonate; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

into the lumen of the gland during exocytosis [6].

Batzri et al. [4] have shown that single salivary gland acinar cells contain cholinergic, α-adrenergic, and β-adrenergic receptors. It is unclear, then, how a single cellular process (changes in the level of cytosolic calcium) can mediate more than one cellular response. In other words, how can it be that, on the one hand, changes in cytosolic calcium lead to protein release and no ion movements (in response to β-adrenergic activation), while, on the other hand, changes in cytosolic calcium lead to ion movements yet only a small amount of protein release (α-adrenergic and cholinergic activation). The solution of this apparent paradox will require a clearer understanding of which of the cellular membranes regulates the movement of calcium into and out of the cytoplasm.

Recently, it has been observed that the microsomal fraction of rat salivary glands contains an ATP-dependent calcium transport system [7]. The microsomal fraction is an operational term designating the most slowly sedimenting membranes from a tissue homogenate, and while most of the membrane found in this fraction is derived from the endoplasmic reticulum, a significant fraction of both the plasma membrane and Golgi membrane sediment with the microsomes [8]. The microsomal fraction also contains membrane vesicles derived from secretory granules, mitochondria [8], lysosomes [8], and coated vesicles [9].

In this report, the system is characterized with an eye toward those properties expected of a membrane system involved in cytosolic calcium regulation. In addition, we describe experiments directed at clarifying the cellular location of the rat submandibular gland microsomal calcium transport system. The results should help to clarify the function of the microsomal calcium transport system in salivary secretion.

Materials and Methods

A. Isolation of the microsomal pellet

The microsomal pellet was isolated in the following manner. Glands were collected and placed into fractionation media 5 ml/g containing 0.5 M sucrose, 1 mM EDTA, 8 mM Hepes (pH 7.2). All operations were carried out at 4°C. The tissue was minced, homogenized with 20 strokes of a motor driven teflon pestle, and centrifuged in a Beckman JA-20 rotor at 2000 rpm for 10 min. The pellet was resuspended in fractionation medium (2.5 ml/g tissue) by use of two strokes of the homogenizing pestle, and recentrifuged at 2000 rpm for 10 min. The resulting pellets were discarded. The two supernatants were combined and centrifuged at 12000 rpm for 10 min to obtain the mitochondrial pellet. The supernatant was centrifuged at 31000 rpm for 45 min in a Sorvall A841 rotor to obtain the microsomal pellet.

B. Measurement of calcium uptake.

Calcium uptake media consisted of 100 mM KCl, 5 mM $MgCl_2$, 5 mM sodium oxalate, 1 μM CCCP, 50 μm $CaCl_2$ (0.2 μCi ^{45}Ca/ml), and 8 mM Hepes (pH 7.0). Uptake studies were initiated by the addition of 50 μg of membrane protein in 0.1 ml of fractionation medium − EDTA to 1.9 ml of uptake media. At the appropriate time points thereafter, the reaction was terminated by filtration through HA millipore filters (0.45 μ). The filters were washed with 10 ml fractionation media − EDTA, dried overnight, placed in 10 ml of scintillation fluid (15.16 g omnifluor/8 pints toluene) and counted in a Beckman LS-235 scintillation counter.

C. Sucrose gradient centrifugation

Purification of the organelle membrane vesicles contained in the microsomal fraction by sucrose gradient centrifugation was carried out using a Beckman SW50.1 rotor. The microsomal fraction was resuspended at 5–10 mg/ml in fractionation media and 2 ml of the microsomal suspension was layered over 4 ml of a preformed discontinuous sucrose gradient. Details of the composition of the gradients as well as length of time and speed of centrifugation are given in the figure captions. Unless otherwise stated, all gradient fractions contained 1 mM EDTA and 8 mM Hepes (pH 7.0). All centrifugation (at 4°C) was carried out with minimal rotor acceleration and with the brake set to the 'off' position. At the end of the centrifugation, the membranes were removed with the use of a J-shaped pipette and each fraction was diluted 10-fold with fractionation media. The fractions were then pelleted by centrifugation at 40000 rpm

for 1 h in a Sorvall A841 rotor and resuspended in 0.5 ml fractionation media – EDTA.

The distribution of the membrane enzyme markers between the fractions obtained was plotted as histograms with the heights of the histograms determined às:

$$\text{Height} = 100 \times \frac{\text{total activity of enzyme in fraction}}{\text{total activity of enzyme in microsomes}} (\%).$$

D. Curtain flow electrophoresis

Purification of the organelle fractions contained in the microsomal fraction by curtain flow electrophoresis was carried out on a DESAGA 48 free-flow electrophoresis machine. The flow buffer for the experiment consisted of 0.45 M sucrose, 1 mM Hepes (pH 7.0 adjusted with Tris-HCl), and 0.25 mM Na_2EDTA, and the electrode buffer consisted of 12 mM NaCl. The high voltage was set at 1.2 kV which corresponded to a current of 50 mA. The dose pump was set at 8 and discharge pump set at 30. Microsomes were suspended at 10 mg/ml of fractionation media and 2 ml was used for each batch. The fractions collected from each batch were pooled as described in the figure captions, pelleted, and resuspended in 0.5 ml fractionation medium – EDTA.

The distribution of the membrane enzyme markers between the fractions obtained was plotted as histograms with the heights of the histograms determined as:

$$\text{Height} = 100 \times \frac{\text{total activity of enzyme in fraction}}{\text{total activity of enzyme in microsomes}} (\%).$$

E. Enzymatic assays

Protein concentration was measured by the method of Lowry et al. [10]. RNA was measured by the method of Schneider [11].

5'-Nucleotidase activity was measured by following the release of phosphate from AMP as described [12].

NADH-cytochrome *c* reductase, NADPH-cytochrome *c* reductase, and succinate dehydrogenase were measured by following the reduction of cytochrome *c* in the presence of substrate at 550 nM [13–15].

Galactosyltransferase activity was assayed by measuring the transfer of [^{14}C]-galactose from UDPgalactose to *N*-acetylglucosamine [16].

Amylase activity was measured by the method of Bernfield [17].

Acid phosphastase activity was measured as described by De Duve and coworkers [18,19].

Results

I. Characterization of calcium transport

Michaelis-Menten analysis of calcium transport into the microsomal fraction was carried out and the results are shown in Fig. 1. It can be seen that the concentration of medium calcium which gives one-half maximal rates of accumulation is 25 μM. It would be expected that this value overestimates the concentration of ionized calcium which gives

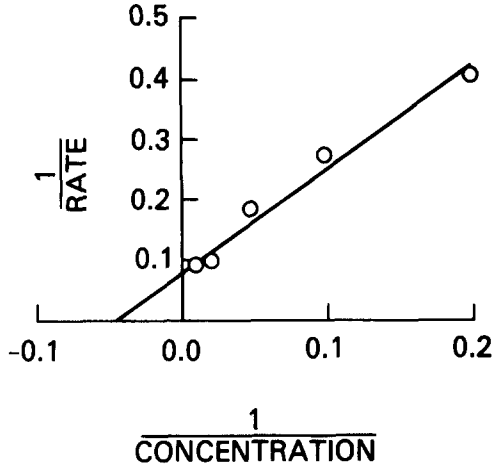

Fig. 1. Michaelis-Menten analysis of transport. Calcium accumulation was measured in samples containing 5, 10, 20, 50 and 100 μM $CaCl_2$ and ten samples were incubated for each calcium concentration. Membrane protein concentration was 50 μg/ml. At 1, 2, 5, 20 and 60 min after the initiation of transport, duplicate samples were filtered for each concentration. From the amount of radioactivity bound to the filters at each time point, it was possible to extrapolate the initial rate of calcium transport for each concentration of calcium. The reciprocal of the rates of transport was plotted vs. the reciprocal of calcium concentration to yield the Lineweaver-Burk plot. The rate of calcium transport is expressed as nmol Ca^{2+}·(mg protein)$^{-1}$·min^{-1} and concentration as μM.

one-half maximal rates of transport because of calcium binding to ATP, oxalate, and membranes in the uptake medium. The work of Hasselbach and Makinose [20], who found that the apparent solubility product of the total concentration of calcium and oxalate in solutions containing 5 mM magnesium and ATP is 100-times higher than the solubility product of calcium oxalate in water, leads to the conclusion that no precipitation should occur between calcium and oxalate in the medium. The K_m value is in the range one expects for the concentration of free calcium in the cell cytoplasm [21,22].

It is of interest to know whether calcium accumulation by microsomes represents binding of calcium to the microsomal membrane or transport of cation to the interior of the vesicles. Both the detergent Triton X-100 (0.05%) and the calcium-proton antiport A23187 (1.0 μg/ml) in the presence of the protonophore CCCP (1 μM) inhibit sequestration some 95% (data not shown). The concentration of detergent is high enough to make the microsomal membrane leaky to calcium but at the same time is low enough to leave the membrane vesicles intact [23]. This data suggests that calcium sequestration by microsomes represent transport of cation to the vesicle interior.

Fig. 2 shows that both Sr^{2+} and Mn^{2+} inhibit calcium transport by microsomes with Sr^{2+} being the more effective of the two. Michaelis-Menten analysis of calcium transport in the presence of the other divalent cations shows that Mn^{2+} and Sr^{2+} inhibit transport competitively with K_i values of 67 μM for Sr^{2+} and 220 μM for Mn^{2+} (data not shown). Theses results, together with the results demonstrating a low K_m of the system for calcium, suggest that the microsomal fraction contains specific membrane binding sites for calcium as opposed to ATP-dependent calcium binding to the exterior of the microsomal membrane.

Various inhibitors of other ion transport systems were tested for their effects on calcium transport into the microsomal fraction of rat submandibular glands.

Diisothiocyanodisulfonic stilbene (DIDS) is known to interact with clusters of positive charges in membranes [24]. It is a well known inhibitor of anion transport in red blood cells [24]. Fig. 3 shows the effect of various concentrations of DIDS

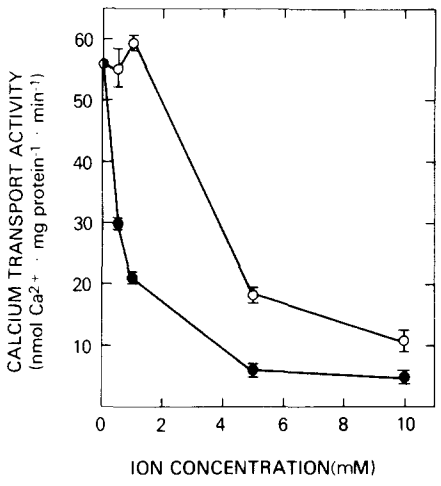

Fig. 2. Inhibition of calcium accumulation by Sr^{2+} and Mn^{2+}. Calcium accumulation was measured under standard assay conditions for 30 min in media containing various concentrations of Sr^{2+} and Mn^{2+}. The amount of membrane protein added to each sample was 25 mg/ml. The amount of calcium accumulated is plotted vs. the amount of Sr^{2+} or Mn^{2+} added. ●——●, Sr^{2+}; ○——○, Mn^{2+}.

upon calcium transport into the microsomal fraction of rat submandibular glands. It can be seen that DIDS has a strong inhibitory effect and maxi-

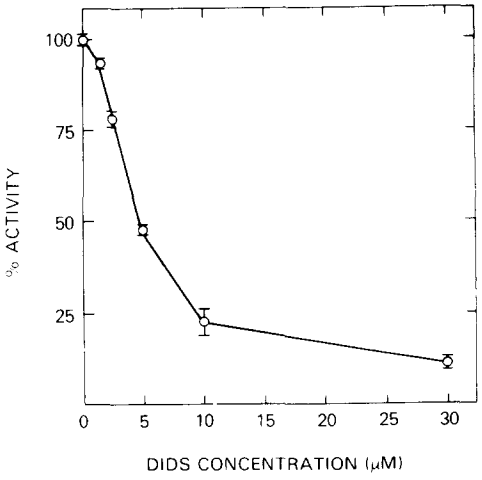

Fig. 3. The effect of DIDS on calcium uptake. Calcium accumulation was allowed to proceed under standard assay conditions for 20 min in uptake media containing various amounts of DIDS. The amount of membrane protein added to each sample was 50 μg/ml. The percent of calcium transported relative to the amount transported in media containing to DIDS is plotted at each DIDS concentration.

mally inhibits transport at 30 μM.

Neither Ruthenium red (1 μM) nor CCCP (1 μM) inhibit calcium accumulation in the microsomal fraction (data not shown). The agents are potent inhibitors of mitochondrial calcium transport [25,26] and these results demonstrate that mitochondrial contamination in the microsomal fraction is not responsible for calcium transport.

Fig. 4 shows that the antipsychotic drugs chlorpromazine and trifluoperazine inhibit calcium transport in the microsomal system. Similar results have been found previously with a number of calcium dependent enzymes including the calcium transport system derived from sarcoplasmic reticulum [27]. It has been suggested that the drugs act via inhibiting the calcium regulatory protein calmodulin [28]. If the microsomal fraction contains endogenous calmodulin, and if this protein effects calcium transport, then it is possible that the antipsychotics are acting on the microsomal membrane via the calcium regulatory protein.

II. Attempts at localizing the calcium-transport system

The experiments described below were designed to identify the organelle membrane responsible for calcium transport. The approach taken was to follow both the membrane responsible for calcium transport and various organelle membrane enzyme markers through different purification procedures. If it was found that the membrane responsible for calcium transport copurifies with one of the membrane enzyme markers, then it could be concluded that both the transport system and the enzyme marker lie in the same membrane and the organelle responsible for transport could be identified. Experiments utilizing purification based upon membrane vesicle density, size, and electrophoretic mobility are described.

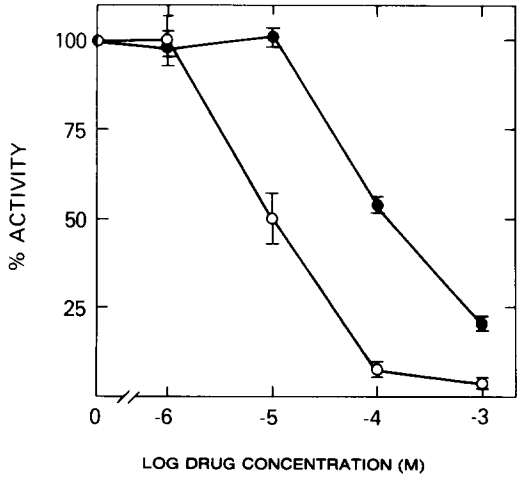

Fig. 4. The effects of trifluoperazine and chlorpromazine on calcium transport. Accumulation of calcium was measured under standard conditions for 20 min in media containing various concentrations of trifluoperazine or chlorpromazine. The percentage of calcium accumulated relative to the amount accumulated in media containing no drug is plotted at each trifluoperazine or chlorpromazine concentration, (○——○), Trifluoperazine; (●——●), chlorpromazine. The amount of membrane protein added to each sample was 50 μg.

A. Purification based upon vesicle density. Fig. 5 (Expt. 1) summarizes the results of isopycnic sedimentation of the microsomal fraction on a discontinuous sucrose gradient. Details of the experimen-

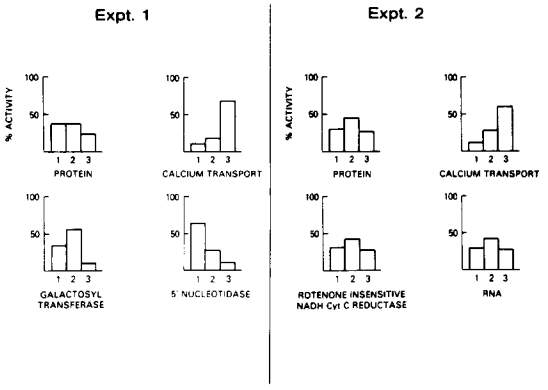

Fig. 5. Separation of the microsomal vesicles by isopycnic sedimentation. The microsomal fraction (10 mg protein in 2.0 ml) was layered over a discontinuous sucrose gradient consisting of 2.0 ml of 1.1 M sucrose layered over 2.0 ml of 1.25 M sucrose. All fractions contained 8 mM Hepes (pH 7.0) and 1 mM EDTA. The gradient was centrifuged in a Beckman SW 50.1 rotor at 31000 rpm for 12 h. Each of the interfaces between the sucrose layers were removed, diluted with 20 ml fractionation medium − EDTA, and pelleted by centrifugation at 40000 rpm for 1.0. h. Each pellet was then resuspended in 0.5 ml fractionation medium − EDTA. The membrane which sedimented to but did not penetrate the 1.1 M sucrose layer of the gradient was fraction 1; fraction 2 contained the membrane which sedimented to the 1.1–1.25 M sucrose interface, and fraction 3 contained the material, which pelleted during centrifugation. For each membrane enzyme marker assay, samples of constant volume were assayed from each fraction. The histograms indicate the percent of activity of the enzyme marker found in a particular fraction relative to the total amount layered on the sucrose gradient.

tal procedure are described in Materials and Methods. It can be seen that the bulk of the membranes, containing 5′-nucleotidase (membrane enzyme marker for plasma membrane), are less dense than 1.1 M sucrose, and the bulk of the membranes, containing galactosyltransferase activity (Golgi apparatus), are less dense than 1.25 M sucrose. On the other hand, 60% of the calcium transporting membrane sediments to the 1.25–1.40 M sucrose interface. Thus, based upon density, the membrane vesicles responsible for calcium transport are distinct from the membranes containing the traditional enzyme markers for plasma membrane, and Golgi apparatus.

Fig. 5 (Expt. 2) shows that similar results are obtained when the distribution of the calcium transporting membrane is compared to the distribution of enzyme markers for rough and total endoplasmic reticulum after isopycnic centrifugation. While there appears to be little purification of both RNA (rough endoplasmic reticulum) and rotenone insensitive NADH-cytochrome *c* reductase (total endoplasmic reticulum) compared to the distribution of bulk protein, the bulk of membrane which transports calcium sediments through 1.25 M sucrose.

B. Purification based upon vesicle size. The organelle membrane vesicles contained in the microsomal fraction can be separated by differential centrifugation of the postmitochondrial supernatant. This technique takes advantage of variations in vesicle size and the results of such an experiment are given in Table I. While 49% of the membrane, contained in the post-mitochondrial supernatant, which is responsible for transport, sediments at 20000 rpm × 15 min, only 33% of the rotenone insensitive NADH-cytochrome *c* reductase activity does so. Thus, under the homogenization and fractionation conditions used, the membrane vesicles, which contain the calcium transport system, are on the average larger than the vesicles, which contain rotenone insensitive NADH-cytochrome *c* reductase activity.

This distinction between the calcium transporting fraction and the endoplasmic reticulum fraction with regard to size and density is shown more clearly in Fig. 6. The microsomal fraction was sedimented on a discontinuous sucrose gradient for a period of time less than that required to reach equilibrium. While the bulk of the calcium-transporting membrane was found in fraction 3, 50% of the rotenone-insensitive cytochrome *c* reductase activity was found in fraction 1. Fig. 6 also shows that the calcium-transporting membrane has different purification properties than lysosomes (acid phosphatase).

TABLE I

SEPARATION OF THE MICROSOMAL MEMBRANE VESICLES BY DIFFERENTIAL CENTRIFUGATION

Three rats were used for this experiment. The post-mitochondrial supernatant was sedimented at 20000 rpm × 15 min in a Sorvall A-841 rotor. The pellet was suspended in 1.0 ml fractionation medium – EDTA and the supernatant was sedimented at 31000 rpm × 30 min. The pellet was resuspended in 1.0 ml fractionation medium – EDTA and the supernatant was sedimented at 40000 rpm for 1 h. The resulting pellet was resuspended in fractionation medium – EDTA. The % activity of a particular enzyme in a fraction represents:

$$100 \times \frac{\text{total activity of enzyme in fraction}}{\text{total activity of enzyme in microsomes}} (\%).$$

Fraction	% Activity			
	Transport	Protein	Rotenone-sensitive NADH-cytochrome *c* reductase	Succinate dehydrogenase
20000 × 15 min	49.1	30.3	33.7	62.2
31000 × 30 min	32.7	37.6	37.7	26.9
40000 × 1 h	18.2	32.1	28.6	10.9

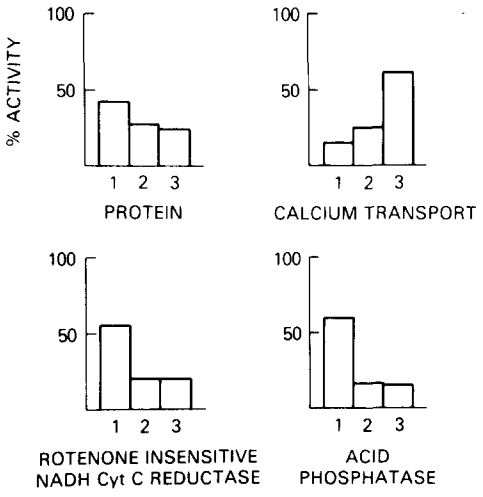

Fig. 6. Separation of the microsomal fraction on a discontinuous sucrose gradient. The protocol for this experiment was identical to that described in Fig. 5 except that the gradient was centrifuged for 4 h.

C. Separation based upon electrophoretic mobility. Heidrich et al. [29] have described how curtain flow electrophoresis can be used to separate

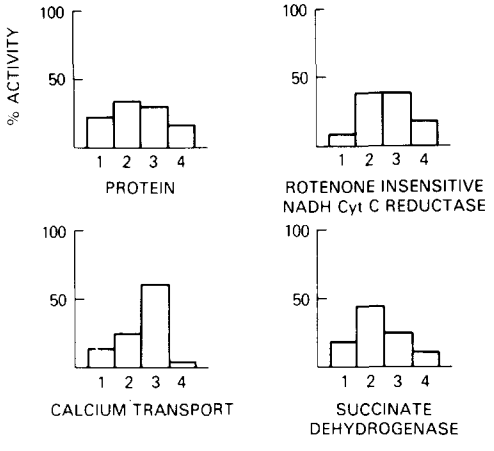

Fig. 7. Separation of the microsomal vesicles by curtain flow electrophoresis. Microsomal membranes (20 mg in 2.0 ml) were separated by curtain flow electrophoresis as described in Materials and Methods. Fraction 1 contained the membranes in collecting tubes 47–56 (collecting tube 1 being at the cathode); fraction 2: collecting tubes 57–61; fraction 3: collecting tubes 62–66; fraction 4: collecting tubes 67–77. The fractions collected were pelleted by centrifugation at 31000 rpm for 1.0 h, and resuspended in 0.5 ml fractionation medium – EDTA. The histograms in the figure plot the percent of activity of the enzyme marker found in a particular fraction relative to the total amount used in the experiment.

organelle membranes contained in the microsomal fraction of rat liver homogenates. Fig. 7 shows the results of separation of microsomal membrane vesicles derived from rat submandibular gland preparations by this technique. The results are consistant with the results described above. The membrane, responsible for calcium transport, can be purified at the expense of membrane vesicles, which contain standard organelle membrane markers for endoplasmic reticulum.

Discussion

Data has been presented which suggest that the submandibular gland microsomal calcium transport system shows the following properties: (1) the system has a K_m for calcium which is similar to the concentration of calcium in the cell cytoplasm, (2) the system transports calcium across the membrane of the microsomes to the interior of the vesicles, (3) the microsomal membranes contain specific binding sites for calcium, and (4) the system is inhibited by substances known to inhibit other ion transport systems. These properties are to be expected of a system which plays a role in vivo in helping to regulate the level of cytosolic calcium ions. It must be kept in mind that other cellular organelles, including mitochondria, which contain both influx [30] and efflux [31,32] systems, and the cellular membrane [33] contain calcium transport systems. The relationship between the microsomal system and the other calcium transport system remain unclear.

The results of three approaches to organelle purification (density, size, and electrophoretic mobility) demonstrated that the membrane responsible for transport is distinct from the membrane which contain standard markers for endoplasmic reticulum, plasma membrane, Golgi apparatus, and lysosomes.

There are several possible explanations for the results. Perhaps the most obvious is that the organelle responsible for calcium tansport is different from the better characterized organelles mentioned above. Several new organelles have recently been discovered populating most cells including the acinar cells of salivary glands. These include coated vesicles [34], transitional elements between the endoplasmic reticulum and Golgi

aparatus [35], and immature zymogen granules [36]. It would be expected that under the homogenization scheme used, each of these membranes would sediment in the microsomal fraction, and the possibility exists that one of these organelles contains the calcium transport system.

A second possible explanation for the results discussed above is that while the calcium transporting system is located within the membrane of one of the more thoroughly characterized organelles, only one of the many types of cells within the rat submandibular gland contains the system. If the purification properties of the organelles from this particular cell differ from those of the other cell types, then results similar to those obtained would occur. The rat submandibular gland contains acinar, intercalated duct, striated duct, and myoepithelial cells [6].

A third possible explanation for the results obtained is that one of the membrane enzyme markers does not accurately represent the organelle for which it was used because the enzyme is not uniformly distributed throughout the organelle.

Suppose, for example, that the calcium transporting system is part of the Golgi apparatus and that galactosyltransferase is not uniformly distributed throughout this organelle. Upon homogenization, two populations of Golgi vesicles will be formed: one containing both the calcium transporting system and galactosyltransferase and a population, which just contains the transport system. If the properties of these two membrane vesicles are different, then galactosyltransferase will not accurately represent the Golgi apparatus and results similar to those obtained and described above could be observed. A similar argument, to that given for galactosyltransferase, will show that if the calcium transporting system is not uniformally distributed throughout a particular organelle, similar results will be observed.

There is evidence from a number of tissues that specialized regions of organelle membranes do exist, and this is especially true for exocrine cells. Hand and Oliver [36] have characterized a region of the parotid gland endoplasmic reticulum named GERL which may be involved in transporting newly synthesized proteins into lysosomes. Hand [37] has reported that the parotid gland contains subsurface cisterns of the endoplasmic reticulum which are in apposition to the surface membrane just below or very near synapses that release secretogogues. Cytochemical evidence exists for progressive elaboration of cell coat material across the stacked cisterns of the Golgi apparatus [38]. The cell coat material represents carbohydrate composition and the results suggest a stepwise assembly of coat material within the Golgi.

One must conclude also that the cell membrane of the acinar cells within this tissue contains specialized regions since there is both a serosal side (which contains the receptors for neurotransmitters [37] and a luminal side which contains the proteins mediating exocytosis and ion release from the cells [35].

The results presented above are not sufficiently by themselves to determine which of these explanations is the correct one. It is thus not currently possible to determine, from the data, the localization of the calcium transporting membrane within the cells of the submandibular gland. The answer to this question will have to wait for (1) more sophisticated techniques of membrane separation and (2) a better understanding of the structure, composition, and function of the various organelle membranes contained within the cell.

The possibility that the microsomal calcium transport system is contained in a specialized region of one of the cellular organelles is particularly interesting when one considers the role played by calcium in the hormonally stimulated secretion of saliva. As discussed in the Introduction, the response of acinar cells to α-adrenergic and cholinergic activation is different from the response of the cells to β-adrenergic activation. Yet changes in the level of cytosolic calcium seem to mediate both responses. Putney et al. [39] have explained this apparent paradox (that one cellular event can mediate two separate hormone responses) by proposing that there exists in the acinar cell cytoplasm two separate regions, and that the level of calcium in these regions vary differently in response to hormone activation. Thus, α-adrenergic and cholinergic activations, which lead to an increased calcium influx from the blood, would lead to an increased cytosolic calcium concentration near the serosal plasma membranes of the cells, but the calcium concentration near the lumen of the cell would remain the same. α-adren-

ergic activation leads to calcium release from intracellular stores, and Putney et al. [39] propose that this would increase the concentration of calcium near the lumen of the cell and not the serosal region. Putney et al. [39] add that an increased level of calcium near the serosal side of acinar cells leads to K^+ efflux by the cells, and that an increased level of calcium at the lumen side leads to exocytosis. In order that this model be correct, it need no be assumed that the two regions of the cytosol are completely isolated from one another by membranes. It has been shown that large transient variations in Ca^{2+} concentration are possible across the cytosol of a cell containing mitochondria [40]. Such effects would be enhanced however if the cytosolic regions in question were at least partially isolated from other parts of the cytosol by the membrane of a cellular organelle such as endoplasmic reticulum, for example, to prevent rapid Ca^{2+} diffusion into the bulk of the cytosol. If the membrane of the cellular organelle, responsible for partially isolating one region of the cytosol from the rest, contained a calcium sequestering system, such as the ones studied here, the effects could be even more precisely controlled and there is some cytochemical evidence supporting such a model. Hand [37] has observed in the acinar cells of rat parotid glands subsurface cisterns of endoplasmic reticulum which are in apposition to the surface membrane and just beneath or very near synapses which release secretagogues. Similar observations have been made in muscle [41] and nerve cells [42], and in both these systems it has been suggested that the subsurface cisterns of membranes function in calcium storage and released. It would be intriguing if the regions of endoplasmic reticulum observed by Hand [37] function in an identical manner in salivary glands.

Acknowledgements

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