Calcium-ion-transporting activity in two microsomal subfractions from rat pancreatic acini

Modulation by carbamylcholine

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1. Two microsomal subfractions from isolated rat pancreatic acini were produced by centrifugation through a discontinuous sucrose density gradient and characterized by biochemical markers. The denser fraction (SF2) was a highly purified preparation of rough endoplasmic reticulum; the less-dense fraction (SF1) was heterogeneous and contained Golgi, endoplasmic reticulum and plasma membranes. 2. $^{45}\text{Ca}^{2+}$ accumulation in the presence of ATP and its rapid release after treatment with the bivalent-cation ionophore A23187 were demonstrated in both fractions. The pH optimum for active $^{45}\text{Ca}^{2+}$ uptake was approx. 6.8 for the rough endoplasmic reticulum (SF2) and approx. 7.5 for SF1. 3. Initial rate measurements were used to determine the affinity of the rough-endoplasmic-reticulum uptake system for free Ca^{2+} . An apparent K_m of $0.16 \pm 0.06 \mu\text{M}$ and V_{max} of $21.5 \pm 5.6 \text{ nmol of Ca}^{2+}/\text{min per mg of protein}$ were obtained. $^{45}\text{Ca}^{2+}$ uptake by SF1 was less sensitive to Ca^{2+} , half-maximal uptake occurring at $1\text{--}2 \mu\text{M}$ -free Ca^{2+} . 4. When fractions were prepared from isolated acini stimulated with $3 \mu\text{M}$ -carbamylcholine, $^{45}\text{Ca}^{2+}$ uptake was increased in the rough endoplasmic reticulum. The increased uptake was due to a higher V_{max} with no significant change in K_m . No effect was observed on $^{45}\text{Ca}^{2+}$ uptake by SF1. 5. In conclusion, two distinct non-mitochondrial, ATP-dependent calcium-uptake systems have been demonstrated in rat pancreatic acini. One of these is located in the rough endoplasmic reticulum, but the precise location of the other has not been determined. We have shown that the Ca^{2+} -transporting activity in the rough endoplasmic reticulum may have an important role in maintaining the cytosolic free Ca^{2+} concentration in resting acinar cells and is involved in Ca^{2+} movements which occur during stimulation of enzyme secretion.

Stimulation of enzyme release from both rat and mouse pancreatic acinar cells by cholinergic agents has been shown to be accompanied by a rise in the cytosolic free Ca^{2+} concentration (O'Doherty & Stark, 1982; Dormer, 1983). Membrane Ca^{2+} pumps in plasma membrane, endoplasmic reticulum, Golgi fractions and mitochondria have all been suggested as regulators of the cytosolic free Ca^{2+} concentration. In the erythrocyte, the plasma-membrane Ca^{2+} pump is of major importance in this function (Schatzmann, 1982), and high-affinity Ca^{2+} -transporting ATPases have been

described in the plasma membrane of various cell types (Penniston, 1982). However, in cells such as the pancreatic acinar cell, the contribution of active Ca^{2+} pumps in intracellular membranes must be considered. In addition, although it is widely accepted that intracellular Ca^{2+} stores are involved in release and re-uptake of Ca^{2+} during stimulation of enzyme secretion, the nature of this store(s) is still unclear (Case, 1978; Williams, 1980; Petersen, 1982).

A study using the fluorescent probe chlorotetracycline (Chandler & Williams, 1978*a,b*) suggested that an intracellular site, requiring metabolic energy for sequestering Ca^{2+} , released membrane-bound Ca^{2+} in response to cholinergic stimulation. It has been shown that pancreatic

Abbreviations used: KHB, Krebs–Henseleit (1932) bicarbonate buffer; Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid.

acinar cells possess at least two distinct ATP-dependent Ca^{2+} -sequestering systems, one of which is mitochondrial (Argent *et al.*, 1975; Lucas *et al.*, 1978; Ponnappa *et al.*, 1981) and the other non-mitochondrial (Lucas *et al.*, 1978; Ponnappa *et al.*, 1981; Dormer & Richardson, 1982). We also showed that microsomal membranes lost Ca^{2+} during stimulation of enzyme release from mouse pancreatic acini (Dormer & Williams, 1981) and suggested that they represented the site of intracellular Ca^{2+} release.

In addition, their Ca^{2+} -transporting activity was increased when isolated from stimulated acini rather than from controls (Ponnappa & Williams, 1981; Ponnappa *et al.*, 1981). Since the microsomal fraction is a mixture of membranes from the endoplasmic reticulum, Golgi apparatus and plasma membrane (Dormer & Williams, 1981; Richardson & Dormer, 1983), it is essential to define which of these is involved in the Ca^{2+} movements occurring during stimulation of secretion.

In the present study we have isolated two microsomal subfractions having distinct Ca^{2+} -sequestering properties. We have shown that one of these uptake activities is located in the rough endoplasmic reticulum and is modulated during cholinergic stimulation of secretion from pancreatic acini.

Experimental

Materials

$^{45}\text{CaCl}_2$ (10–40 mCi/mg of Ca) was obtained from The Radiochemical Centre, Amersham, Bucks., U.K. The following were purchased from Sigma: soya-bean trypsin inhibitor (type 1-S), collagenase (type III, fraction A), hyaluronidase (type I), ATP (disodium salt from equine muscle; vanadium-free), cytochrome *c* (type III), NADPH (type X), imidazole (grade 1), benzamidine, carbamylcholine chloride, DL-dithiothreitol, EGTA, L-glutamine, ouabain octahydrate and thiamin pyrophosphate (cocarboxylase). Bovine serum albumin (fraction V) was from Miles Laboratories, Eagle's minimal medium amino acid supplement, minus glutamine, from Gibco Bio-Cult, Paisley, Renfrewshire, Scotland, U.K., ionophore A23187 from Boehringer, and *p*-methylaminophenol sulphate from Kodak Limited, Kirkby, Liverpool, U.K. All other reagents were AnalR grade from BDH.

Preparation of pancreatic acini

Pancreatic acini were prepared by the procedure of Williams *et al.* (1978), with some modifications (Dormer, 1983). Briefly, pancreas from overnight-starved male Wistar rats were digested in KHB containing soya-bean trypsin inhibitor

(0.1 mg/ml), collagenase (75 units/ml) and hyaluronidase (520–550 units/ml).

The tissue was dissociated by passage through polypropylene pipettes and the acini purified by centrifugation at 50g for 4 min through KHB containing 4% (w/v) bovine serum albumin. Acini were suspended in KHB containing 1% (w/v) bovine serum albumin and preincubated for 30 min at 37°C. The suspension was then centrifuged and the acini were homogenized as outlined below. In studies where acini were exposed to secretagogues, they were stimulated with a concentration of carbamylcholine (3 μM) which produced maximal release of amylase (results not shown).

Preparation of microsomal subfractions

Isolated pancreatic acini (approx. 10^6 acini/ml) were homogenized in 0.30M-sucrose buffered with 10mM-Hepes, pH 7.4, containing 1mM-benzamidine, 2mM- NaN_3 and 5mM-dithiothreitol. Homogenization was carried out by eight strokes (no twisting) of an all-glass Potter-Elvehjem homogenizer. The homogenate was then centrifuged at 1000g for 10 min and the resulting supernatant for 10 min at 12000g. A 1 ml portion of this post-mitochondrial supernatant was underlayered with 3 ml of buffer (100mM-KCl/5mM- MgCl_2 /2mM- NaN_3 /100mM-imidazole, pH 6.8 at 4°C) containing 8.5% (w/w) sucrose. This was underlayered with 40% (w/w) sucrose and then by 60% (w/w) sucrose in buffer. The gradient was then centrifuged for 2 h at 100000g using a 3 × 25 ml swing-out rotor in an MSE Superspeed 50 centrifuge. The fractions at the 8.5%/40% (SF1) and 40%/60% (SF2) sucrose interfaces were removed and diluted to 1.0 or 1.5 ml with buffer. All the above operations were carried out at 4°C.

Measurement of $^{45}\text{Ca}^{2+}$ -uptake activity

This was done by a modification of the method of Ponnappa *et al.* (1981). The incubation mixture consisted of 100mM-imidazole (adjusted to pH 6.8 or 7.5, at 37°C), 5mM- MgCl_2 , 2mM- NaN_3 , 100mM-KCl, 0.25M-sucrose and the required concentration of CaCl_2 or Ca^{2+} /EGTA buffer (10mM-EGTA plus various amounts of added $^{45}\text{CaCl}_2$ of specific radioactivity 3.1 mCi/mmol). Membranes (30–50 μg of protein for SF1 or 50–75 μg of protein for SF2, in a final volume of 2 ml) were added to this mixture, and passive uptake measured by filtration of a 1 ml portion. ATP (5mM) was added to the remaining 1 ml for subsequent measurement of active uptake. Samples were filtered through 0.45 μm -pore-size Millipore filters presoaked with 100mM-imidazole (pH 7.0 at 4°C)/5mM- MgCl_2 /2mM- NaN_3 /100mM-KCl/0.25M-sucrose and washed within 30 s with 5 ml of this buffer. The filters were dissolved in 1 ml of 2-methoxyethanol

and 9 ml of scintillant [2 vol. of toluene: 1 vol. of Triton X-100 containing 2.67 g of 2,5-diphenyl-oxazole/litre and 66.7 mg of 1,4-bis-(5-phenyl-oxazol-2-yl)benzene/litre] was added. The radioactivity was measured on the ¹⁴C channel of a Packard Tri-Carb liquid-scintillation counter with an efficiency of approx. 80%.

Calculation of free Ca²⁺ concentrations

Free Ca²⁺ concentrations in media containing several interacting ligands (EGTA, ATP, NaN₃, and imidazole) and ions (Ca²⁺, Mg²⁺, Na⁺, K⁺) were calculated by using an iterative program run on the Welsh National School of Medicine computer. Association constants were taken from Sillen & Martell (1964).

Enzyme and other assays

(Na⁺ + K⁺)-activated ATPase was measured by the method of Wizemann *et al.* (1974) with some modifications: samples were pretreated for 30 min at room temperature in 200 mM-Tris/HCl, pH 7.0, containing 0.4 mg of sodium deoxycholate/ml and 2 mM-EDTA. Portions were then transferred to tubes containing 200 mM-Tris/HCl (pH 7.0)/3 mM-MgATP/100 mM-NaCl/5 mM-KCl, with and without 2 mM-ouabain at 37°C. After 60 min the reaction was stopped with 0.2 M-HClO₄ and, after 10 min at 4°C, centrifuged (2 min in an Eppendorf microcentrifuge), and the supernatant assayed for P_i by the method of LeBel *et al.* (1978). Thiamin pyrophosphatase was measured by the method of Meldolesi *et al.* (1971), except that the reaction was stopped with 0.2 M-HClO₄ acid, and the P_i produced assayed by the method of LeBel *et al.* (1978). Cyanide-insensitive NADPH-dependent cytochrome *c* reductase was assayed as described by Sottocasa *et al.* (1967). RNA was extracted from the fractions as described by Munro & Fleck (1966) and determined by its A₂₆₀. Protein was determined by the method of Lowry *et al.* (1951), with sample preparation by the method of Bensadoun & Weinstein (1976) to remove sucrose and dithiothreitol. Bovine serum albumin was used as a standard.

Results

Stability of ⁴⁵Ca²⁺ uptake by microsomal membranes

Microsomal membranes were isolated from homogenates prepared in the presence or absence of 5 mM-dithiothreitol, by centrifugation of the postmitochondrial supernatant for 1 h at 100 000 *g*. ⁴⁵Ca²⁺ uptake was assayed immediately and after storage for 18 h at 4°C. Membranes prepared in the presence of dithiothreitol retained approx. 40% of the initial activity after 18 h, whereas, in the

absence of dithiothreitol, only 2% of the activity remained. Membranes stored for 5 h at 4°C in the presence of dithiothreitol retained more than 90% of the original activity. Owing to this relative instability of ⁴⁵Ca²⁺ uptake, we previously attempted to rapidly subfractionate microsomal membranes using Percoll density gradients (Richardson & Dormer, 1983). This approach was not pursued, since Percoll inhibited ⁴⁵Ca²⁺ uptake, but, on the basis of the Percoll densities at which ⁴⁵Ca²⁺ uptake was detected, we were able to devise a discontinuous sucrose density gradient which achieved maximum separation of subcellular components as rapidly as possible. The total time taken to isolate the two subfractions described below, was 3–3.5 h and thus rapid enough for them to retain full activity.

Distribution of biochemical markers

The postmitochondrial supernatant contained plasma membrane, Golgi apparatus and endoplasmic reticulum as assessed by the activities of marker enzymes: (Na⁺ + K⁺)-activated ATPase, thiamin pyrophosphatase and NADPH-cytochrome *c* reductase respectively (Meldolesi *et al.*, 1971). The percentages of the total activity of these enzymes in the homogenate recovered in the postmitochondrial supernatant is shown in Table 1, line 1. The recovery of markers from the postmitochondrial supernatant (Table 1, line 2) reflects only material collected from the two interfaces, no attempt being made to fractionate the gradient quantitatively.

Subfraction 2 (SF2) was enriched in markers for the rough endoplasmic reticulum (NADPH-cytochrome *c* reductase and RNA), but contained very low amounts of the plasma-membrane and Golgi markers, which, in some experiments, were undetectable. By contrast, subfraction 1 (SF1) contained more than 90% of the recovered (Na⁺ + K⁺)-activated ATPase and thiamin pyrophosphatase, together with some rough-endoplasmic-reticulum markers. Thus SF2 can be considered to be a highly purified preparation of rough endoplasmic reticulum, whereas SF1 was a heterogeneous fraction.

Characterization of ⁴⁵Ca²⁺ uptake by microsomal subfractions

ATP-dependent ⁴⁵Ca²⁺ uptake in both SF1 and SF2 was characterized by rapid uptake within the first minute (Fig. 4) followed by a plateau phase reached between 5 and 10 min after ATP addition (Fig. 1). Fig. 1 also shows that all of the ⁴⁵Ca²⁺ taken up by both fractions in the presence of ATP could be released by addition of the bivalent-cation ionophore A23187. The ⁴⁵Ca²⁺ remaining after 2 min was equivalent to the passive uptake.

Table 1. Distribution of marker-enzyme activities, RNA and protein between subcellular fractions isolated from rat pancreatic acini

Isolated acini were homogenized and fractionated as described in the Experimental section. Results are means $\pm$ S.E.M. for the number of observations denoted in parentheses. Enrichment is defined as specific activity (units/mg of protein) in the fraction divided by specific activity of the homogenate.

	(Na ⁺ + K ⁺)- activated ATPase	Thiamin pyro- phosphatase	NADPH- cytochrome c reductase	RNA	Protein
Recovery in the post-mitochondrial supernatant (% of total in the homogenate)	7.4 $\pm$ 0.7 (4)	30.0 $\pm$ 6.2 (4)	18.1 $\pm$ 3.1 (4)	29.3 $\pm$ 2.5 (4)	22.0 $\pm$ 2.7 (4)
Recovery in the two subfractions (% of total in the postmitochondrial supernatant)	94.7 $\pm$ 32.5 (5)	7.7 $\pm$ 0.8 (5)	27.2 $\pm$ 6.2 (5)	47.8 $\pm$ 6.9 (5)	19.9 $\pm$ 2.0 (5)
Percentage distribution of recovered activity in:					
SF1	97.5 $\pm$ 1.3 (7)	91.6 $\pm$ 3.7 (7)	20.7 $\pm$ 4.7 (7)	54.6 $\pm$ 1.7 (6)	58.8 $\pm$ 4.9 (7)
SF2	2.5 $\pm$ 1.3 (7)	8.4 $\pm$ 3.7 (7)	78.9 $\pm$ 4.5 (7)	45.4 $\pm$ 1.7 (6)	41.2 $\pm$ 4.9 (7)
Enrichment:					
SF1	3.57 $\pm$ 1.44 (4)	1.19 $\pm$ 0.20 (4)	0.46 $\pm$ 0.12 (4)	2.60 $\pm$ 0.42 (4)	—
SF2	0.11 $\pm$ 0.07 (4)	0.19 $\pm$ 0.08 (4)	1.53 $\pm$ 0.18 (4)	2.80 $\pm$ 0.23 (4)	—

In the present study we have characterized the microsomal subfractions by measuring initial rates of $^{45}\text{Ca}^{2+}$ uptake (1 min after ATP addition). This obviated the use of a precipitating anion such as oxalate, since, in pancreatic microsomal vesicles, we have found that oxalate caused a large increase in the passive uptake of $^{45}\text{Ca}^{2+}$ (results not shown).

The pH-dependence of $^{45}\text{Ca}^{2+}$ uptake by SF2 is shown in Fig. 2. Maximum uptake occurred at pH 6.8. $^{45}\text{Ca}^{2+}$ uptake by SF1 was less dependent on pH and was optimal at approx. pH 7.5 (results not shown).

The Ca^{2+} -sensitivity of each uptake system was investigated (Fig. 3). In both cases uptake reached a maximum rate, but was inhibited at higher Ca^{2+} concentrations. The values for uptake by SF2, up to the maximum, fitted a straight-line double-reciprocal plot (Fig. 3, inset). The mean apparent K_m from four separate determinations was $0.16 \pm 0.06 \mu\text{M}$, with a maximum velocity of $21.5 \pm 5.6 \text{ nmol of } \text{Ca}^{2+} / \text{min per mg of protein}$. The results demonstrate that SF1 was less sensitive to Ca^{2+} than was SF2, half-maximal uptake occurring between 1 and $2 \mu\text{M}$.

Effect of cholinergic stimulation of pancreatic acini

We have previously shown (Ponnappa *et al.*, 1981) that the microsomal fraction isolated from mouse pancreatic acini stimulated by physiological secretagogues had higher $^{45}\text{Ca}^{2+}$ -uptake activity than that isolated from control acini. We have investigated whether this also occurs in rat pancreatic acini and which of the two subfractions

Fig. 1. $^{45}\text{Ca}^{2+}$ uptake by SF1 and SF2: effect of ionophore A23187 on accumulated $^{45}\text{Ca}^{2+}$

$^{45}\text{Ca}^{2+}$ uptake was measured as described in the Experimental section using a Ca^{2+} /EGTA buffer giving $0.7 \mu\text{M}$ -free Ca^{2+} at pH 6.8. Passive uptake was measured and ATP added at 5 min. (●, ○, SF1; ▲, △, SF2). At the point marked by the vertical arrows either $5 \mu\text{M}$ -A23187 in dimethyl sulphoxide (○, △) or the equivalent volume (1% v/v) of dimethyl sulphoxide (●, ▲) were added. Points are means $\pm$ S.E.M. for four observations.

shows this effect. As shown in Fig. 4, $^{45}\text{Ca}^{2+}$ uptake by SF2 was increased by prior exposure of intact acini to $3 \mu\text{M}$ -carbamylcholine for 20 min,

but uptake by SF1 was unaffected. In four experiments, the maximum velocity of uptake in SF2 was increased from 24.56 ± 1.60 to 34.56 ± 5.60 nmol of Ca²⁺/min per mg of protein ($P < 0.05$ for differ-

Fig. 2. pH-dependence of initial velocity of ⁴⁵Ca²⁺ uptake by SF2

⁴⁵Ca²⁺ uptake was measured as described in the Experimental section. Buffers of different pH were made up by the addition of various amounts of HCl at 37°C. Total Ca²⁺ was 20 μM (no EGTA), giving a final free Ca²⁺ concentration of approx. 2 μM. Points are means ± S.E.M. for four observations of uptake measured 1 min after ATP addition and corrected for passive uptake.

ence from control). Uptake of ⁴⁵Ca²⁺ by unfractionated postmitochondrial supernatant was increased by a similar magnitude, from 2.77 ± 0.66 to 4.51 ± 0.53 nmol/min per mg of protein ($n = 4$; $P < 0.05$ for difference from control). We could detect no significant change in the affinity for Ca²⁺ of uptake by SF2 from control or stimulated acini: in four experiments, half-maximal uptake occurred at 0.40 ± 0.08 μM- and 0.44 ± 0.08 μM-free Ca²⁺ in membranes from control and stimulated acini respectively.

Discussion

Earlier studies of Ca²⁺ transport by non-mitochondrial membranes, isolated from mouse or guinea-pig pancreas, used microsomal fractions derived from the postmitochondrial supernatant by differential centrifugation (Lucas *et al.*, 1978; Ponnappa *et al.*, 1981). This did not separate the different membrane types sufficiently to distinguish which possessed Ca²⁺-transport activity. In the rat and cat pancreas, previous attempts to measure Ca²⁺ uptake in either microsomal or rough-endoplasmic-reticulum fractions were unsuccessful (Argent *et al.*, 1975; Kribben *et al.*, 1983). However, it has been shown that, in saponin-permeabilized rat pancreatic acinar cells, calcium oxalate precipitated in the rough endoplasmic reticulum in the presence of ATP (Waka-

Fig. 3. Dependence on free Ca²⁺ concentration of initial velocity of ⁴⁵Ca²⁺ uptake by SF1 and SF2

⁴⁵Ca²⁺ uptake was measured as described in the Experimental section by using a series of Ca²⁺/EGTA buffers at pH 7.5 for SF1 (●) and pH 6.8 for SF2 (▲). Points are means ± S.E.M. for four observations of uptake measured 1 min after ATP addition and corrected for passive uptake. The inset shows a double-reciprocal plot of ⁴⁵Ca²⁺ uptake by SF2 utilizing values up to the maximum. The line was fitted by linear-regression analysis ($r = 0.99$, $K_m = 0.21$ μM, $V_{max} = 22.73$ nmol/min per mg).

Fig. 4. Time course of $^{45}\text{Ca}^{2+}$ uptake by SF1 and SF2 isolated from control or carbamylcholine-treated acini. Isolated acini were preincubated for 30 min at 37°C , centrifuged and resuspended in $\text{KHB} \pm 3 \mu\text{M}$ -carbamylcholine. After 20 min at 37°C , the acini were centrifuged, homogenized and fractionated as described in the Experimental section. $^{45}\text{Ca}^{2+}$ uptake was measured as described in the Experimental section by using a Ca^{2+} /EGTA buffer, giving $0.7 \mu\text{M}$ -free Ca^{2+} at pH 6.8 (●, ○, SF1; ▲, △, SF2). ○, △ denote uptake by SF1 and SF2 from stimulated acini and ●, ▲ from control acini. Points are means $\pm$ S.E.M. for four observations.

sugi *et al.*, 1982). The two subfractions we have isolated showed distinct Ca^{2+} -uptake activities; SF2 contained virtually no marker-enzyme activities for plasma membrane and Golgi apparatus and was enriched in both NADPH-cytochrome *c* reductase activity and RNA. Thus our evidence is strongly in favour of a major Ca^{2+} -uptake activity being located in the rough endoplasmic reticulum. It has recently been shown that mouse pancreatic acini also possess Ca^{2+} -uptake activity in the rough endoplasmic reticulum, on the basis of

deposition of calcium oxalate precipitates inside morphologically defined rough-endoplasmic-reticulum vesicles (Preissler & Williams, 1983).

The properties of $^{45}\text{Ca}^{2+}$ uptake by SF1, which contained Golgi, endoplasmic reticulum and plasma membrane, distinguished it from that of the rough endoplasmic reticulum (SF2). This activity was not modulated by treatment of acini with carbamylcholine. Further studies are required to define precisely the location and function of this Ca^{2+} -transporting activity.

SF2, the rough-endoplasmic-reticulum fraction, has an apparent K_m for free Ca^{2+} of $0.16 \mu\text{M}$; thus, at $0.4 \mu\text{M}$, the resting free Ca^{2+} concentration of the acinar cell cytosol (O'Doherty & Stark, 1982), the pump would be working at approx. 75% of its maximum capacity. This is consistent with the rough endoplasmic reticulum being able to sequester Ca^{2+} actively in the resting cell, where it would function either as a Ca^{2+} store or in the packaging of secretory proteins.

The increased rate of Ca^{2+} uptake by SF2, isolated from carbamylcholine-treated acini demonstrates its importance in the Ca^{2+} movements which occur during stimulation of enzyme secretion. The intracellular site of Ca^{2+} action in stimulating secretion is not known, but presumably this involves its binding to specific Ca^{2+} -binding proteins. Calmodulin has been shown to be present in pancreatic acinar cells, by its activation both of phosphodiesterase (Vandermeers *et al.*, 1977) and of erythrocyte ($\text{Ca}^{2+} + \text{Mg}^{2+}$)-activated ATPase (Dormer & Richardson, 1982). At an estimated concentration of $5 \mu\text{M}$ it may account for a large proportion of the cytosolic Ca^{2+} -binding activity (Vandermeers *et al.*, 1977). In the stimulated acinar cell the cytosolic Ca^{2+} concentration is of the order of $2\text{--}4 \mu\text{M}$ (O'Doherty & Stark, 1982; Dormer, 1983), at which level calmodulin would be expected to be at least 90% saturated with Ca^{2+} (Scharff, 1981). A decrease in the cytosolic Ca^{2+} concentration to $0.4 \mu\text{M}$ would result in the removal of 60–70% of the bound Ca^{2+} or 2–3 mol/mol of calmodulin. Thus, assuming a cytosolic volume of approx. 1 pl, the rough-endoplasmic-reticulum pump would have to remove at least 15 amol of Ca^{2+} /cell to revert to the resting state. From the recoveries of NADPH-cytochrome *c* reductase, RNA and protein in SF2, it can be estimated that the rough endoplasmic reticulum contains at least 25% of the total cellular protein. At the rate of Ca^{2+} uptake of 20 nmol/min per mg of protein the pump is capable of removing 5–8 fmol of Ca^{2+} from the cytosol in 1 min, and could, therefore, be expected to fulfil such a function.

In summary, we have demonstrated two distinct Ca^{2+} -transporting activities in membranes of the

rat pancreatic acinar cell. One of these, the rough-endoplasmic-reticulum pump, may have an important role in maintaining the cytosolic free Ca²⁺ at the resting level and could function as a Ca²⁺ store. In addition, it is clearly implicated in the Ca²⁺ movements that occur during stimulation of enzyme secretion and has the capacity to restore the cytosolic free Ca²⁺ from the stimulated to the resting level.

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