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Analysis of Ca^{2+} fluxes and Ca^{2+} pools in pancreatic acini

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[Plate 1]

$^{45}Ca^{2+}$ movements have been analysed in dispersed acini prepared from rat pancreas in a quasi-steady state for $^{45}Ca^{2+}$. Carbamyl choline (carbachol; Cch) caused a quick $^{45}Ca^{2+}$ release that was followed by a slower $^{45}Ca^{2+}$ 'reuptake'. Subsequent addition of atropine resulted in a further transient increase in cellular $^{45}Ca^{2+}$. The data suggest the presence of a Cch-sensitive 'trigger' pool, which could be refilled by the antagonist, and one or more intracellular 'storage' pools. Intracellular Ca^{2+} sequestration was studied in isolated acini pretreated with saponin to disrupt their plasma membranes. In the presence of $^{45}Ca^{2+}$ (1 μM), addition of ATP at 5 mM caused a rapid increase in $^{45}Ca^{2+}$ uptake exceeding the control by fivefold. Maximal ATP-promoted Ca^{2+} uptake was obtained at 10 μM Ca^{2+} (half-maximal at 0.32 μM Ca^{2+}). In the presence of mitochondrial inhibitors it was 0.1 μM (half-maximal at 0.014 μM). $^{45}Ca^{2+}$ release could still be induced by Cch but the subsequent reuptake was missing. The latter was restored by ATP and atropine caused further $^{45}Ca^{2+}$ uptake. Electron microscopy showed electron-dense precipitates in the rough endoplasmic reticulum of saponin-treated cells in the presence of Ca^{2+}, oxalate and ATP which were absent in intact cells or cells pretreated with A23187. The data suggest the presence of a plasma membrane-bound Cch-sensitive 'trigger' Ca^{2+} pool and ATP-dependent Ca^{2+} storage systems in mitochondria and rough endoplasmic reticulum of pancreatic acini. It is assumed that Ca^{2+} is taken up into these pools after secretagogue-induced Ca^{2+} release.

Introduction

Calcium ions can be considered as intracellular trigger for secretagogue-induced enzyme secretion from the exocrine pancreas. Indirect evidence suggests that secretagogues increase cytoplasmic free Ca^{2+} concentration by releasing calcium from a cellular trigger store (Gardner *et al.* 1975; Renckens *et al.* 1978; Stolze & Schulz 1980) and by increasing the plasma membrane permeability to Ca^{2+} with a consequent increased calcium influx into the cell (Kondo & Schulz 1976*a*, *b*). The pool from which trigger Ca^{2+} is released and the pool into which the inflowing Ca^{2+} is stored have not yet been identified. However, present knowledge indicates that Ca^{2+} pools involved in Ca^{2+} redistribution are plasma membrane, endoplasmic reticulum and mitochondria (Clemente & Meldolesi 1975; Dormer & Williams 1981; Petersen & Iwatsuki 1978; Petersen & Ueda 1976; Ponnappa *et al.* 1981).

Previous studies suggested that the pool from which Ca^{2+} is released (trigger Ca^{2+} pool) is different from the one that it is taken up into again during stimulation (intracellular Ca^{2+} buffering pool) (Stolze & Schulz 1980). We now report that emptying and refilling of the Ca^{2+} trigger pool could also be observed in cells that had been treated with saponin. By this procedure pores of 8–10 nm in diameter are formed in plasma membranes that become permeable to ions and small molecules. The data indicate that the trigger Ca^{2+} pool is located in the plasma

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membrane. With the same preparation intracellular Ca^{2+} buffering was also studied. Ca^{2+} uptake could be induced in saponin-treated cells with ATP into rough endoplasmic reticulum and mitochondria as visualized by electron microscopy. Thus both organelles could be considered as intracellular Ca^{2+} buffering pools. Complementary studies were performed on subfractions of pancreatic tissue. ATP-promoted Ca^{2+} uptake could be shown into membrane vesicles enriched in the plasma membrane marker enzyme, Na^{+}–K^{+} ATPase, indicating an ATP-dependent Ca^{2+} extrusion mechanism in the same organelles. We were not able, however, to show an ATP-dependent Ca^{2+} uptake into isolated rough endoplasmic reticulum. Similarly, the trigger Ca^{2+} pool, from which Ca^{2+} is released by secretagogues, has not yet been localized in subfractions of pancreatic tissue.

Secretagogue-induced Ca^{2+} movements in 'intact' pancreatic acinar cells

Secretagogues of enzyme secretion such as cholecystokinin–pancreozymin (CCK–Pz), its C-terminal octapeptide (CCK-8) as well as the neurotransmitter acetylcholine and its analogue carbamyl choline (Cch) increase Ca^{2+} efflux from acinar cells during the intial few minutes of stimulation. This phase of Ca^{2+} release is followed by Ca^{2+} reuptake due to increased Ca^{2+} influx during sustained stimulation. These conclusions have been derived from studies on $^{45}Ca^{2+}$ fluxes in isolated acinar cells and acini at both 'steady-state' and 'Ca^{2+} net flux' conditions.

Ca^{2+} *tracer exchange*

When isolated acinar cells were preincubated in the presence of secretagogues and calcium for 1 h until a quasi-stationary state was reached, addition of trace amounts of $^{45}Ca^{2+}$ at time 0 caused increased $^{45}Ca^{2+}$ uptake in the presence of secretagogues (figure 1). Since the intial rate of $^{45}Ca^{2+}$ influx was increased, it was concluded that secretagogues increase unidirectional Ca^{2+} influx into the cells (Kondo & Schulz 1976*a*). Similar data were obtained for unidirectional $^{45}Ca^{2+}$ efflux (Kondo & Schulz 1976*b*). Assuming increased cytoplasmic free Ca^{2+} concentrations in the presence of secretagogues, the finding of increased Ca^{2+} exchange at steady state indicates that the Ca^{2+} permeability of the plasma membrane, the activity of plasma membrane-bound extrusion mechanisms, or both, are augmented. Since a primary increase in Ca^{2+} extrusion would lead to a decrease rather than to an increase in cytoplasmic Ca^{2+} concentration, it is more probable that secretagogues increase the Ca^{2+} permeability of the plasma membrane, which secondarily leads to increased Ca^{2+}-pump activity.

Ca^{2+} *net flux*

Measurements of calcium net fluxes are best performed by monitoring changes of total concentration of cellular calcium. However, since these changes are sometimes very small and hard to detect, ^{45}Ca has been used to monitor cellular calcium (Gardner *et al.* 1975; Stolze & Schulz 1980).

Figure 2 shows an experiment in which isolated acini had been preloaded with $^{45}Ca^{2+}$ for 45 min until a stationary state for the tracer was reached. Assuming that under these conditions the specific activity of ^{45}Ca is the same in all readily exchangeable cell compartments, the rate of ^{45}Ca flow can be taken to indicate calcium net flow. Addition of the secretagogue caused a quick $^{45}Ca^{2+}$ release followed by reuptake. As shown in curve *a*, prestimulated cells could

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not be restimulated to release $^{45}Ca^{2+}$ a second time (Gardner *et al.* 1975; Stolze & Schulz 1980). However, when the antagonist was added subsequently, $^{45}Ca^{2+}$ uptake rapidly increased above the control and a second release was then possible (curve *b*) (Stolze & Schulz 1980, Wakasugi *et al.* 1981*b*). These data were interpreted to mean that in the first phase of stimulation Ca^{2+} is released from a 'trigger pool' into the cytosol, increasing its free Ca^{2+} concentration, and subsequently extruded from the cell. In the second phase Ca^{2+} influx into the cell is augmented owing to the increased Ca^{2+} permeability of the plasma membrane, and calcium is taken up into an intracellular located 'storage pool'.

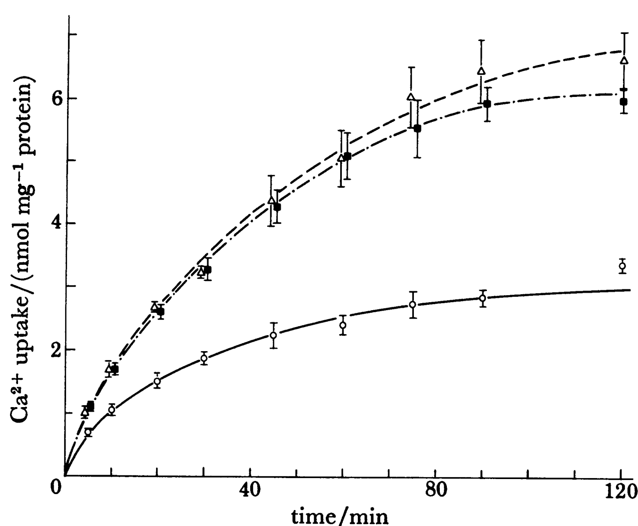

FIGURE 1. $^{45}Ca^{2+}$ uptake into isolated pancreas cells at steady-state and zero net flux conditions. Cells were preincubated for 60 min with Ca^{2+} (1.25 mM) in the presence or absence of secretagogues. At time 0, $^{45}Ca^{2+}$ in trace amounts was added. △, In the presence of CCK-Pz (86 nM); ■, in the presence of carbamyl choline (10 μM); ○, control. (From Kondo & Schulz (1976*a*).)

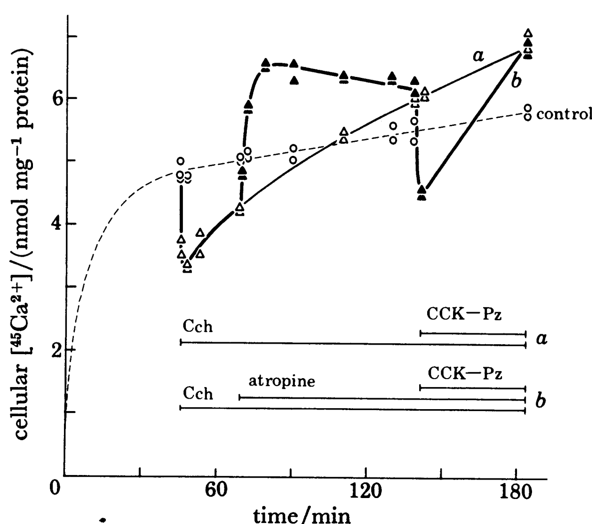

FIGURE 2. Effect of acute stimulation on cellular $^{45}Ca^{2+}$ content in Ca^{2+} net flux conditions. Cells were preloaded with $^{45}Ca^{2+}$ (1.25 mM) for 45 min until a steady state was reached. At indicated times, test substances were added. Curve *a*, successive additions of 0.1 mM carbamylcholine (Cch) and 0.1 μM cholecystokinin–pancreozymin (CCK–Pz). Curve *b*, successive addition of Cch and CCK–Pz with an interposed step of 10 μM atropine administration. ○, Control. (From Stolze & Schulz (1980).)

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ATP-PROMOTED $^{45}Ca^{2+}$ UPTAKE INTO 'LEAKY' ACINI

To investigate intracellular Ca^{2+} uptake and to localize Ca^{2+} sequestration, we treated isolated acini with saponin to disrupt their plasma membranes. As shown in other tissues this treatment makes the plasma membrane permeable to small ions and molecules by the interaction of saponin with cholesterol and formation of holes of 8–10 nm in a micellar-type arrangement (Bangham & Horne 1962; Blaustein *et al.* 1978*a*, *b*; Dourmashkin *et al.* 1962; Endo *et al.* 1977; Glauert *et al.* 1962). Cholesterol-poor membranes such as those of the endoplasmic reticulum and mitochondria (Korn 1966) should be less affected. The increase in plasma membrane permeability was estimated by measuring the uptake of trypan blue and the release of the cytosolic enzyme lactate dehydrogenase that were increased by 95–100% and 80%, respectively, after treatment with saponin for 10 min.

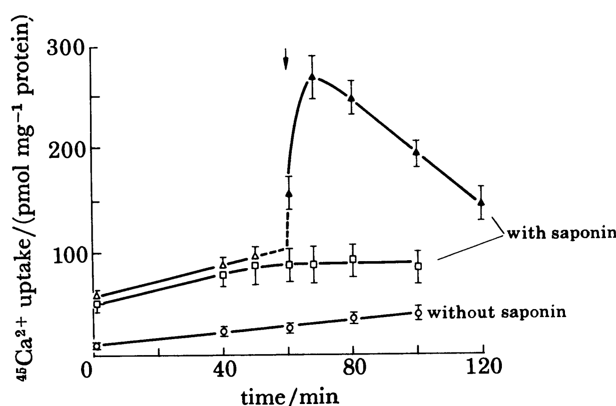

FIGURE 3. Effect of ATP on $^{45}Ca^{2+}$ uptake. Acini were preincubated for 10 min with (△, □) or without (○) saponin (45 µg/ml). At time 0, $^{45}Ca^{2+}$ (1 µM) was added; 60 min later 5 mM ATP (▲) was added (arrow) to saponin-treated acini. □, ○, No ATP added. Experiments show the means ± s.e. from 13 experiments (each point is taken in triplicate).

As shown in figure 3, the addition of ATP (5 mM) increased $^{45}Ca^{2+}$ uptake by four to fivefold over the control, whereas addition of ATP to 'intact' cells had only a very small effect (Wakasugi *et al.* 1981*a*). ADP also increased uptake by about 60% of the value obtained with ATP. Studies with the adenylate kinase inhibitor diadenosyl pentaphosphate suggest, however, that the effect of ADP might be due to the conversion of ADP into ATP. Other nucleotides such as ITP, UTP, CTP, GTP as well as the ATP analogues AMP-PNP and AMP-PCP (figure 4) (Wakasugi *et al.* 1981*a*) had no effect on $^{45}Ca^{2+}$ uptake. Also shown in figure 4 is the effect of the Ca^{2+} ionophore A23187. Since the effect of ATP was completely abolished in the presence of the ionophore it can be assumed that ATP-promoted uptake occurs into a vesicular space. Further support for an ATPase involved in ATP-promoted $^{45}Ca^{2+}$ uptake was obtained from the observation that uptake was abolished in the absence of Mg^{2+} (Wakasugi *et al.* 1981*a*).

Evidence for mitochondrial and non-mitochondrial Ca^{2+} *sequestration*

With increasing $^{45}Ca^{2+}$ concentrations of the incubation medium, free $[Ca^{2+}]$ being adjusted with EGTA, ATP-promoted Ca^{2+} uptake increased and reached a maximal value at 10 µM (figure 5). In the presence of the mitochondrial inhibitors oligomycin and antimycin A, $^{45}Ca^{2+}$

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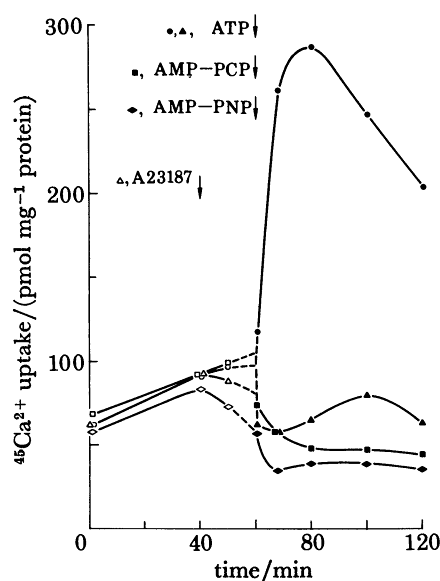

FIGURE 4. Effect of A23187 on ATP-promoted $^{45}Ca^{2+}$ uptake and of non-hydrolysable ATP analogues on $^{45}Ca^{2+}$ uptake. Cells were pretreated for 10 min with saponin (45 μg/ml) and at time 0, $^{45}Ca^{2+}$ (1 μM) was added. ●, Addition of Na_2ATP (5 mM) at 60 min; △, addition of A23187 (△, 1 μM) at 40 min and subsequent addition of Na_2ATP (▲, 5 mM) at 60 min; ◆, addition of adenylyl imidodiphosphate (tetralithium salt) (AMP–PNP); ■, of adenylyl (β, γ-methylene)-diphosphonate (tetralithium salt) (AMP–PCP) at 60 min. One experiment.

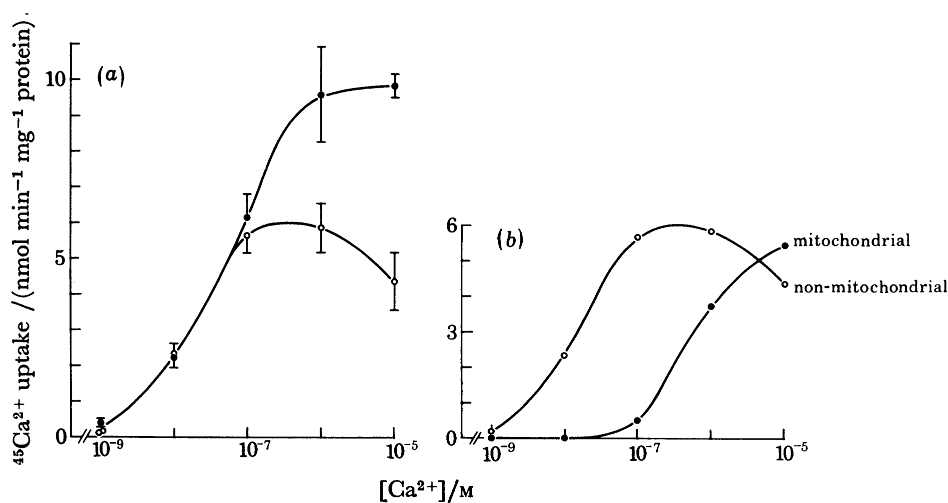

FIGURE 5. (*a*) Effect of different Ca^{2+} concentrations on ATP-promoted $^{45}Ca^{2+}$ uptake. Free Ca^{2+} concentration was adjusted with EGTA. ●, Without mitochondrial inhibitors; ○, in the presence of oligomycin and antimycin A (10 μM each). Values are the means ± s.e. of three to six experiments. (*b*) Replot of curves from (*a*). Curve for 'mitochondrial Ca^{2+} uptake' was obtained by subtracting the Ca^{2+} uptake in the presence of inhibitors (○ in (*a*)) from that in the absence of inhibitors (● in (*a*)).

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uptake was inhibited at higher Ca^{2+} concentrations, resulting in a concentration–response curve with maximal ATP-promoted $^{45}Ca^{2+}$ uptake at 100 nM and an apparent K_m value between 10 and 100 nM. These data suggest that at least two Ca^{2+}-sequestration sites are involved: mitochondria that take up Ca^{2+} if the free Ca^{2+} concentration is higher than 100 nM, and a non-mitochondrial one that regulates free Ca^{2+} concentration at lower levels, starting to take up between 1 and 10 nM and being saturated at 100 nM.

Localization of Ca^{2+} *uptake by electon microscopy*

To locate the site of Ca^{2+} uptake we have incubated saponin-treated acini in the presence of calcium, ATP and oxalate to obtain Ca oxalate precipitates at the site of ATP-dependent Ca^{2+} transport. As shown in figure 6, plate 1, electron-dense precipitates can be seen in the rough endoplasmic reticulum as well as in mitochondria. These precipitates have been confirmed by laser microprobe mass analysis to be areas of higher calcium content than in areas without precipitates (Wakasugi *et al.* 1981 *a*). It therefore appears from these experiments that the non-mitochondrial site of Ca^{2+} uptake is the rough endoplasmic reticulum.

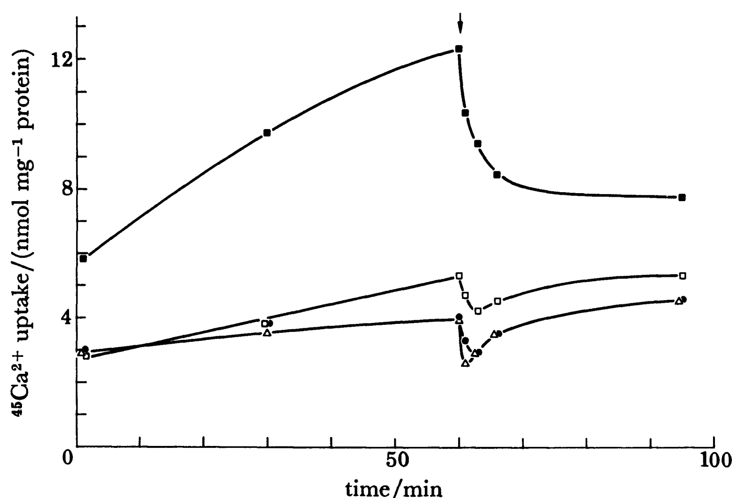

FIGURE 7. Effect of carbachol (Cch) on $^{45}Ca^{2+}$ uptake into 'intact' and saponin-treated acini. ■, Acini had been preincubated for 10 min with saponin (45 μg/ml). At time 0, $^{45}Ca^{2+}$ (1 μM) was added. ●, Saponin-treated acini that had been recentrifuged in the presence of incubation buffer to remove saponin. □, Saponin-untreated 'intact' acini. △, Saponin-untreated acini recentrifuged in incubation buffer. Arrow, addition of carbachol (10 μM).

Effect of carbachol

It is of interest whether these Ca^{2+}-uptake sites visualized in the presence of ATP are identical to those into which Ca^{2+} is taken up in the second phase of stimulation with secretagogues of enzyme secretion. We have therefore studied secretagogue-induced $^{45}Ca^{2+}$ movements in acini treated with saponin. Since these cells are open for substances of high molecular mass such as lactate dehydrogenase, it can be assumed that effects of secretagogues seen in these cells are restricted to the secretagogue–receptor interaction located on the plasma membrane and not to intracellular messengers.

Figure 7 shows carbachol-induced $^{45}Ca^{2+}$ movements in 'intact' acini and 'resealed' acini compared with saponin-treated acini. This experiment demonstrates that saponin-treated acini react like untreated acini when saponin has been washed away. It can therefore be assumed

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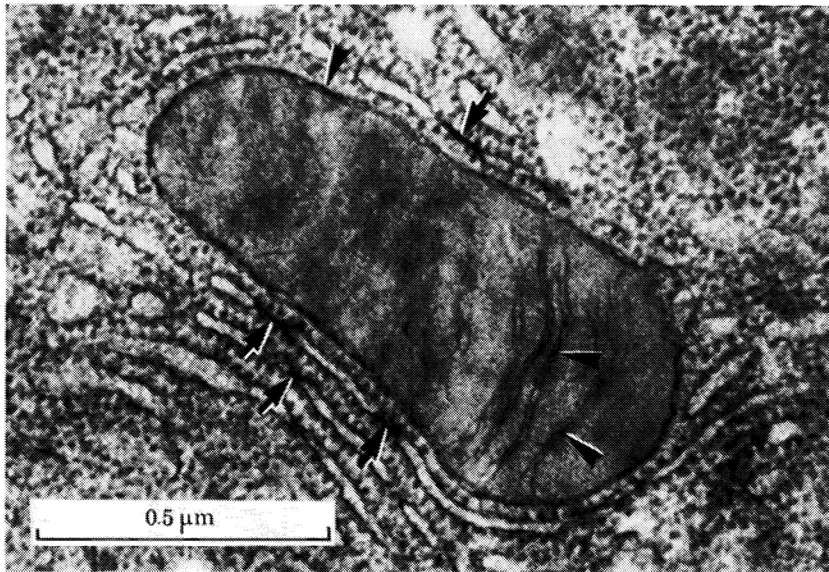

FIGURE 6. Section through a rat pancreatic acinar cell pretreated with saponin. The incubation medium contained potassium oxalate, ATP (5 mM), and calcium (0.1 mM). Electron-dense precipitates are formed in the cisternae of the rough endoplasmic reticulum (arrows) and in the envelope and cristae of mitochondria (arrowheads).

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that the formation of holes due to the binding of saponin to cholesterol of the plasma membrane is a reversible process. In the presence of 100 μM ^{45}Ca in the incubation medium (figure 7), $^{45}Ca^{2+}$ uptake is higher than at 1 μM (figure 1). This might be due to the inability of the cells to keep a low intracellular free Ca^{2+} concentration when the extrusion mechanism became ineffective. The addition of carbachol resulted in a quick $^{45}Ca^{2+}$ release that was not followed by $^{45}Ca^{2+}$ reuptake in saponin-treated acini. We interpret these data to mean that $^{45}Ca^{2+}$ release occurred from the plasma membrane as a consequence of direct secretagogue-receptor

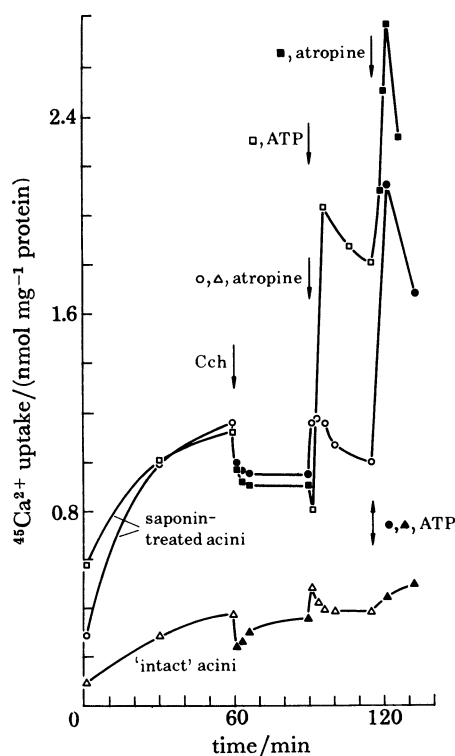

FIGURE 8. Effect of successive additions of carbachol (10 μM), atropine (10 μM; ○, △, ■) and ATP (5 mM; □, ●, ▲) on $^{45}Ca^{2+}$ uptake in saponin-treated acini (upper two curves) and untreated acini (lower curve). Acini had been preincubated with saponin (45 μg/ml) for 10 min. At time 0, $^{45}Ca^{2+}$ (100 μM) was added.

interaction and not of intracellular mediation of a second messenger. Missing $^{45}Ca^{2+}$ reuptake could be due to a lack of intracellular ATP in these open cells. The higher $^{45}Ca^{2+}$ release seen in saponin-treated acini than in 'intact' acini might be a consequence of missing $^{45}Ca^{2+}$ reuptake, since both $^{45}Ca^{2+}$ release and $^{45}Ca^{2+}$ reuptake at net flux conditions can be explained with two simultaneously occurring opposing $^{45}Ca^{2+}$ movements of the extent shown in figure 7 for 'release' and in figure 1 for 'uptake'. The overlap of both unidirectional Ca^{2+} efflux and influx curves should result in a net flow curve similar to that observed in intact acini.

When ATP was added to saponin-treated acini after the addition of carbachol, rapid $^{45}Ca^{2+}$ uptake could be observed. Subsequent addition of atropine, the antagonist of carbachol, resulted in further $^{45}Ca^{2+}$ uptake (figure 8). This experiment further supports our conclusion drawn from the experiment shown in figure 2 that a pool from which Ca^{2+} had been released by secretagogue ('trigger Ca^{2+} pool') could be refilled by action of the antagonist, whereas in the second phase of stimulation Ca^{2+} is taken up into an intracellular pool other than the

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'trigger pool'. This second pool appears to be ATP dependent and might be mitochondria and rough endoplasmic reticulum, as concluded from experiments with inhibitors and electron microscopy as described before.

$^{45}Ca^{2+}$ UPTAKE EXPERIMENTS ON CELLULAR SUBFRACTIONS

We have tried to confirm the sites of ATP-induced Ca^{2+} uptake as visualized in saponin-treated cells by studies on subfractions obtained by zonal centrifugation after homogenization of pancreatic tissue (Milutinović *et al.* 1977). As shown in figure 9, in the presence of mitochondrial inhibitors, ATP-induced $^{45}Ca^{2+}$ uptake was found only in a fraction enriched in plasma-membrane marker enzymes (F_I) and to a small extent in the rough endoplasmic reticulum (F_{II}). Correlation of Ca^{2+} uptake with marker enzymes of plasma membranes and endoplasmic reticulum made it likely that Ca^{2+} uptake occurred into plasma membrane vesicles (F_I) and that Ca^{2+} uptake into F_{II} was due to contamination of this fraction with plasma membranes (data not shown).

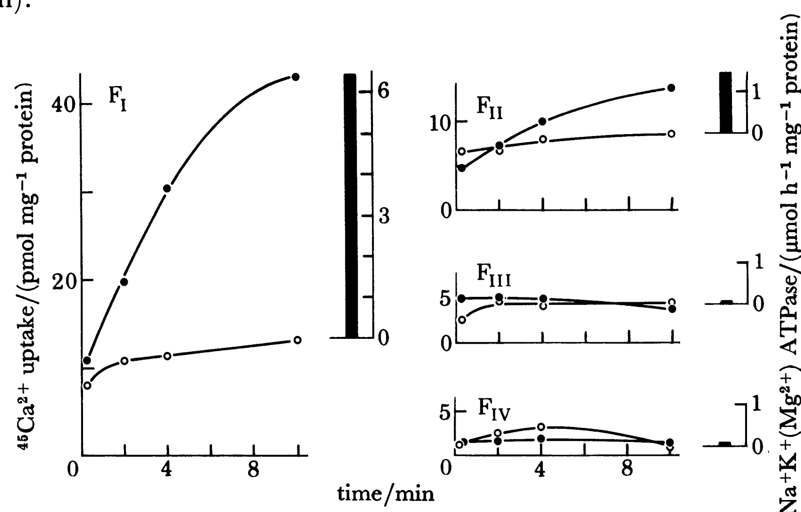

FIGURE 9. $^{45}Ca^{2+}$ uptake into isolated subfractions of pancreatic tissue (for method see Milutinović *et al.* (1977)) in the presence (●) or absence (○) of ATP (5 mM). F_I, F_{II}, F_{III}, F_{IV} refer to 'plasma membranes', 'rough endoplasmic reticulum', 'mitochondria', and 'zymogen granules', respectively.

These data are in contrast to those obtained from experiments in saponin-treated cells that showed Ca oxalate precipitates in the rough endoplasmic reticulum in the presence of ATP. We do not yet know why we were not able to demonstrate ATP-promoted Ca^{2+} uptake into isolated rough endoplasmic reticulum. The reason could be a lack of closed vesicles after preparation. However, a Mg^{2+}-dependent Ca^{2+} ATPase has also not been found in the rough endoplasmic reticulum, whereas it was present in 'plasma membranes' (data not shown). We therefore assume that a cofactor, necessary for ATP-dependent Ca^{2+} transport was lost during preparation. This, however, is not likely to be calmodulin, since this did not induce Ca^{2+} uptake in the rough endoplasmic reticulum fraction in the presence or absence of ATP (data not shown). On the other hand, we have found ATP-promoted Ca^{2+} uptake located in plasma membranes. This observation could mean that in addition to the 'trigger Ca^{2+} pool' from which Ca^{2+} is released our ATP-dependent Ca^{2+} pool is also located in plasma membranes. It seems

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to be more likely, however, that we have demonstrated the Ca^{2+}–Mg^{2+} ATPase responsible for ATP-dependent Ca^{2+} extrusion located in inside-out vesicles from the plasma membrane.

Although we have not yet been able to localize either the trigger Ca^{2+} pool or the Ca^{2+} storage pool in pancreatic subfractions, experiments on saponin-treated cells indicate that the former is the plasma membrane, whereas the latter are rough endoplasmic reticulum and mitochondria. More refined techniques than those applied by us should probably be able to decide their exact location.

References (Schulz *et al.*)

Bangham, A. D. & Horne, R. W. 1962 Action of saponin on biological cell membranes. *Nature, Lond.* **196**, 952–953.

Blaustein, M. P., Ratzlaff, R. W., Kendrick, N. C. & Schweitzer, E. S. 1978*a* Calcium buffering in presynaptic nerve terminals. I. Evidence for involvement of a nonmitochondrial ATP-dependent sequestration mechanism. *J. gen. Physiol.* **72**, 15–41.

Blaustein, M. P., Ratzlaff, R. W. & Schweitzer, E. S. 1978*b* Calcium buffering in presynaptic nerve terminals. II. Kinetic properties of the nonmitochondrial Ca sequestration mechanism. *J. gen. Physiol.* **72**, 43–66.

Clemente, F. & Meldolesi, J. 1975 Calcium and pancreatic secretion-dynamics of subcellular calcium pools in resting and stimulated acinar cells. *Br. J. Pharmac.* **55**, 369–379.

Dormer, R. L. & Williams, J. A. 1981 Secretagogue-induced changes in subcellular Ca^{2+} distribution in isolated pancreatic acini. *Am. J. Physiol.* **240**, G130–140.

Dourmashkin, R. R., Dougherty, R. M. & Harris, R. J. C. 1962 Electron microscopic observations on Rous sarcoma virus and cell membranes. *Nature, Lond.* **194**, 1116–1119.

Endo, M., Kitazawa, T., Yagi, S., Iino, M. & Katuka, Y. 1977 Some properties of chemically skinned smooth muscle fibers. In *Excitation–contraction coupling in smooth muscle* (ed. R. Casteels, T. Godfraind & J. C. Ruegg), pp. 199–209. Amsterdam: Elsevier/North-Holland Biomedical Press.

Gardner, J. D., Conlon, T. P., Klaeveman, H. L., Adams, T. D. & Ondetti, M. A. 1975 Action of cholecystokinin and cholinergic agents on calcium transport in isolated pancreatic acinar cells. *J. clin. Invest.* **56**, 366–375.

Glauert, A. M., Dingle, J. T. & Lucy, J. A. 1962 Action of saponin on biological cell membranes. *Nature, Lond.* **196**, 953–955.

Kondo, S. & Schulz, I. 1976*a* Calcium ion uptake in isolated pancreas cells induced by secretagogues. *Biochim. biophys. Acta* **419**, 76–92.

Kondo, S. & Schulz, I. 1976*b* Ca^{2+} fluxes in isolated cells of rat pancreas. Effect of secretagogues and different Ca^{2+} concentration. *J. Membrane Biol.* **29**, 185–203.

Korn, E. D. 1966 Structure of biological membrane. *Science, N.Y.* **153**, 1491–1498.

Milutinović, S., Sachs, G., Haase, W. & Schulz, I. 1977 Studies on isolated subcellular components of cat pancreas. I. Isolation and enzymatic characterization. *J. Membrane Biol.* **36**, 253–279.

Petersen, O. H. & Iwatsuki, N. 1978 The role of calcium in pancreatic acinar cell stimulus-secretion coupling: an electrophysiological approach. *Ann. N.Y. Acad. Sci.* **307**, 599–617.

Petersen, O. H. & Ueda, N. 1976 Pancreatic acinar cells: the role of calcium in stimulus–secretion coupling. *J. Physiol., Lond.* **254**, 583–606.

Ponnappa, B. C., Dormer, R. L. & Williams, J. A. 1981 Characterization of an ATP-dependent Ca^{2+} uptake system in mouse pancreatic microsomes. *Am. J. Physiol.* **240**, G122–129.

Renckens, B. A. M., Schrijen, J. J., Swarts, H. G. P., De Pont, J. J. H. H. M. & Bonting, S. L. 1978 Role of calcium in exocrine pancreatic secretion. IV. Calcium movements in isolated acinar cells of rabbit pancreas. *Biochim. biophys. Acta* **544**, 338–350.

Stolze, H. & Schulz, I. 1980 Effect of atropine, ouabain, antimycin A, and A23187 on 'trigger Ca^{2+} pool' in exocrine pancreas. *Am. J. Physiol.* **238**, G338–348.

Wakasugi, H., Kimura, T., Haase, W., Kribben, A., Kaufmann, R. & Schulz, I. 1981*a* Calcium uptake into acini from rat pancreas: evidence for intracellular ATP-dependent calcium sequestration. *J. Membrane Biol.* (Submitted.)

Wakasugi, H., Stolze, H., Haase, W. & Schulz, I. 1981*b* Effect of La^{3+} on secretagogue-induced Ca^{2+} fluxes in rat isolated pancreatic acinar cells. *Am. J. Physiol.* **240**, G281–G289.

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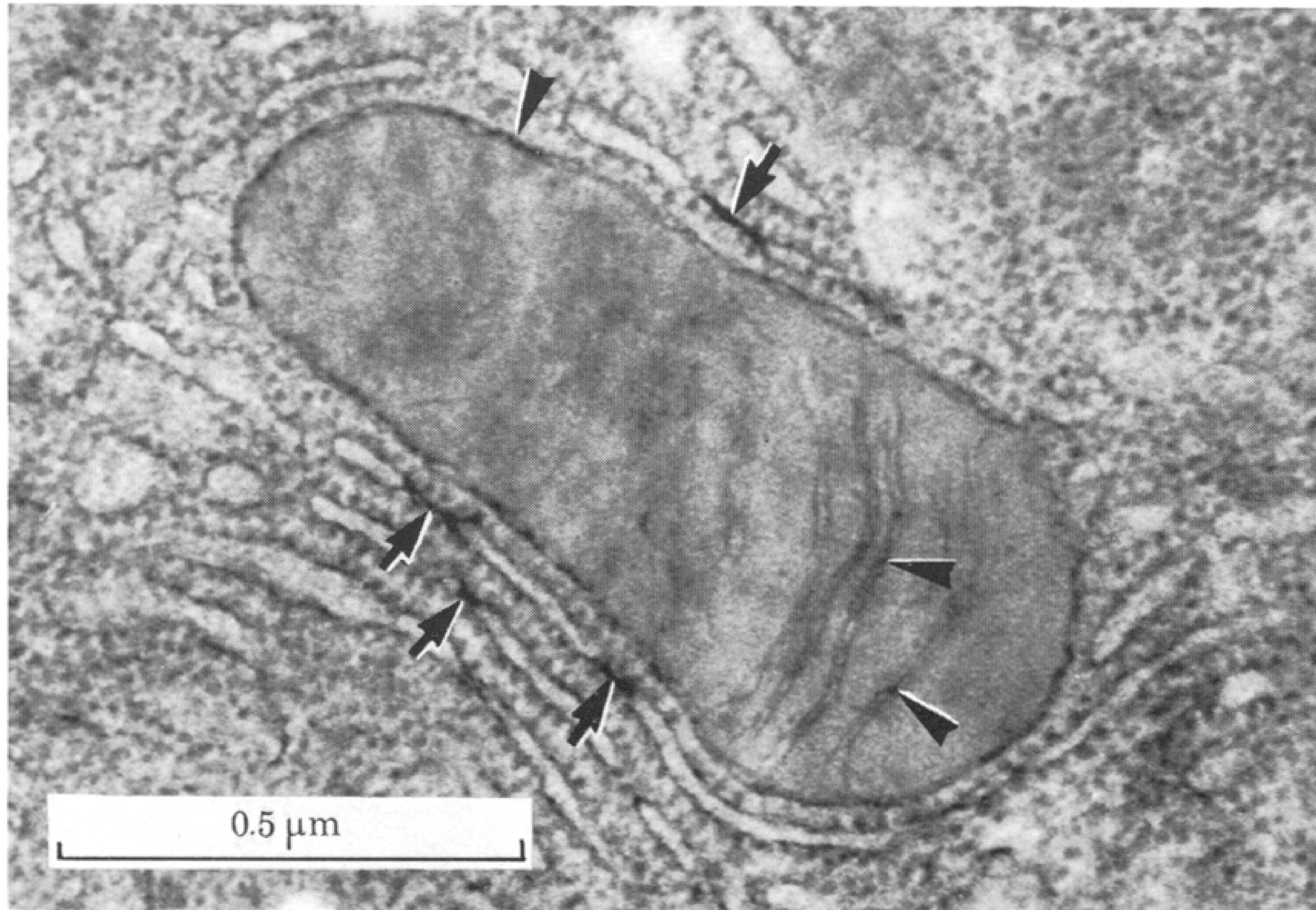

FIGURE 6. Section through a rat pancreatic acinar cell pretreated with saponin. The incubation medium contained potassium oxalate, ATP (5 mM), and calcium (0.1 mM). Electron-dense precipitates are formed in the cisternae of the rough endoplasmic reticulum (arrows) and in the envelope and cristae of mitochondria (arrowheads).