Calcium dependence of calcium extrusion and calcium uptake in mouse pancreatic acinar cells

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1. The droplet technique was used to investigate the calcium dependence of calcium extrusion from pancreatic acinar cells with preserved intracellular environments. The calcium dependence of calcium extrusion indicated a strong co-operativity (Hill coefficient, 3). The half-maximal rate of calcium extrusion occurred at an intracellular free calcium concentration ($[Ca^{2+}]_i$) of approximately 200 nM. At $[Ca^{2+}]_i$ levels higher than 400 nM the calcium extrusion mechanism was almost completely saturated.

2. The rate of $[Ca^{2+}]_i$ recovery was measured with the same cells under conditions where both calcium extrusion and calcium reuptake occurred simultaneously and under conditions when calcium reuptake was prevented and recovery depended entirely upon calcium extrusion. The rate of $[Ca^{2+}]_i$ recovery due to calcium reuptake displayed a very sharp dependence on $[Ca^{2+}]_i$. The rate of $[Ca^{2+}]_i$ recovery due to reuptake increased approximately 10 times (from 4·3 to 44·1 nM s^{-1}) for an increase of $[Ca^{2+}]_i$ of only 100 nM (from 120 to 220 nM).

3. With a decrease of $[Ca^{2+}]_i$ the ratio of rate of calcium extrusion to rate of calcium uptake into internal stores increased, indicating that extrusion plays a more important role at low $[Ca^{2+}]_i$ levels. Data for $[Ca^{2+}]_i$ recovery rates due to extrusion and due to reuptake allowed us to evaluate the absolute rate of calcium translocation into the internal stores during the recovery process. When $[Ca^{2+}]_i = 350$ nM the total (i.e. bound and free) calcium concentration in the cytosol decreased by approximately 100 μM s^{-1} due to calcium uptake into internal stores. The rate of uptake was approximately 20 times slower when $[Ca^{2+}]_i = 120$ nM.

Cytosolic Ca^{2+} signals evoked by hormones or neurotransmitters are initiated by the opening of Ca^{2+} channels in the membranes of intracellular store and/or in the plasma membrane. Since prolonged cytosolic Ca^{2+} elevations are generally undesirable (Nicotera, Bellomo & Orrenius, 1992) and repetitive signals are needed (Petersen, Petersen & Kasai, 1994) rapid recovery of the normal cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) following the rise occurs. The most important transport events removing Ca^{2+} from the cytosol are mediated by Ca^{2+} ATPases. This paper reports the results of an investigation into the properties of Ca^{2+} ATPases in internal stores and those in the plasma membrane, and attempts to assess the relative contribution of each of these mechanisms in calcium recovery following stimulation with agonists.

We have previously reported direct measurements of Ca^{2+} extrusion made simultaneously with measurements of $[Ca^{2+}]_i$ in single pancreatic acinar cells using the droplet technique (Tepikin, Voronina, Gallacher & Petersen, 1992*a*, *b*; Tepikin, Llopis, Snitsarev, Gallacher & Petersen, 1994).

In this study we used the droplet technique to characterize the dependence of calcium extrusion on the intracellular Ca^{2+} concentration. The synchronous measurements of the intracellular calcium concentration and Ca^{2+} extrusion in agonist-stimulated pancreatic acinar cells allowed us to investigate the calcium dependence of calcium extrusion in cells with preserved intracellular environments. We now describe experiments in which we have assessed $[Ca^{2+}]_i$ recovery and Ca^{2+} extrusion under conditions where both Ca^{2+} extrusion and reuptake into internal stores occurred as well as in situations where $[Ca^{2+}]_i$ recovery was solely dependent on extrusion. In this way we have been able to quantify the Ca^{2+} dependence of the extrusion mechanism

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and assess Ca^{2+} reuptake into stores and extrusion at *different levels of* $[Ca^{2+}]_i$.

METHODS

Preparation of fura-2-loaded pancreatic acinar cells

Pancreata were obtained from adult male mice killed by cervical dislocation. Isolated single acinar cells and small clusters of acinar cells were prepared using collagenase digestion of the gland. The cells were loaded with fura-2 by incubation with fura-2 AM (Molecular Probes). Detailed procedures of isolation and loading have been described previously (Toescu, Lawrie, Petersen & Gallacher, 1992). Following loading, cells were washed by centrifugation and maintained in the Ca^{2+}-containing solution (1 mM $CaCl_2$) prior to the start of experiments. All experiments were performed at a temperature of 25 °C.

Measurement of $[Ca^{2+}]_i$ and the extracellular Ca^{2+} concentration ($[Ca^{2+}]_o$) in cell suspensions

Fura-2-loaded cells were suspended in a nominally Ca^{2+}-free solution containing 0·1 μM of the Ca^{2+} indicator Calcium Green-5N (Molecular Probes) in a quartz fluorimetric cuvette. The volume of the cuvette was 1·7 ml and the cell density was approximately 2 million cells ml^{-1}. The suspension was stirred constantly. The substantial difference between the excitation spectra of fura-2 and Calcium Green-5N allowed intracellular and extracellular calcium levels to be monitored simultaneously. The fluorescence of the cell suspension was recorded with a spectrofluorimeter (Perkin Elmer, model LS50B; Beaconsfield, Bucks, UK) using the excitation wavelengths 340, 380 and 490 nm (Fast Filer Accessory for Perkin Elmer spectrofluorimeter) and the emission wavelength 540 nm. One of the advantages of using calcium green 5N as the extracellular indicator is that it has a relatively low affinity for calcium. Its K_d value of 12 μM is higher than that of fura-2 and fluo-3. Calcium concentrations in cuvette experiments were substantially smaller (10–40 μM) than the level of saturation of Calcium Green-5N.

Measurements of $[Ca^{2+}]_i$ and $[Ca]_o$ in droplet experiments

A cluster of six to twelve acinar cells loaded with fura-2 was maintained in a small droplet containing a nominally Ca^{2+}-free solution and 100 μM of the Ca^{2+} indicator fluo-3. In order to decrease the ratio of the volume of the droplet to the volume of the cells and to improve the resolution of the records of calcium extrusion, clusters of acinar cells were used in this study as opposed to single cells which were employed in the experiments described in previous papers (Tepikin *et al.* 1992*a*, *b*). Ratios of volumes (volume of droplet divided by volume of cells) in this study varied in different experiments from 17 to 49. The fluorescence of the droplet system was recorded with a microspectrofluorimeter (SPEX, Glen Spectra, Stanmore, Middlesex, UK) using excitation wavelengths of 380 and 490 nm and the emission wavelength 530 nm. The volume of cells and the volume of droplet were measured in each experiment. These data allow us to calculate the changes of total (i.e. both bound and free) calcium in the cells that occur due to extrusion. Details of droplet formation, estimation of volumes of the cells and the volume of the droplet, delivery of agonists and calculation of intracellular and extracellular calcium concentrations have been described previously (Tepikin *et al.* 1994).

Measurements of $[Ca^{2+}]_i$ in cells placed in a superfusion chamber were performed by the usual ratiometric technique (Grynkiewicz, Poenie & Tsien, 1985). A system of taps allowed changes of extracellular solution within 3 s. The data are presented in the paper as means ± standard error of the mean (S.E.M.).

RESULTS

Ca^{2+} dependence of calcium extrusion

Experiments were performed on small clusters of pancreatic acinar cells using the droplet technique. Stimulation with supramaximal doses of cholecystokinin (CCK; approximately 1 nM) resulted in a fast elevation of $[Ca^{2+}]_i$ followed by recovery to prestimulation levels. The increase in $[Ca^{2+}]_i$ was accompanied by Ca^{2+} extrusion (Fig. 1*A*). In Fig. 1*B* the after-spike part of the $[Ca^{2+}]_i$ curve (lower trace) is plotted together with the rate of calcium extrusion (upper trace). The rate of calcium extrusion was calculated as the derivative of the *extracellular* calcium concentration multiplied by the ratio of the volume of the droplet to the volume of the cells and expressed as a loss of intracellular calcium in micromoles per litre per minute.

The plot of the rate of calcium extrusion against the corresponding $[Ca^{2+}]_i$ provides information on the Ca^{2+} dependence of calcium extrusion (Fig. 1*C*) into nominally Ca^{2+}-free solution. In Fig. 2 the rate of calcium extrusion is expressed as a fraction of the maximal rate (at $[Ca^{2+}]_i = 450$ nM – a point which is close to saturation, which was reached in all experiments). This data presentation was used in order to decrease the scattering of experimental points by making the results independent of the imprecision of measurements of cell and droplet volumes. The rate of calcium extrusion at $[Ca^{2+}]_i = 450$ nM was 380 ± 80 μM min^{-1}. The rates of calcium extrusion at rest (before stimulation) were analysed separately. The resting $[Ca^{2+}]_i$ in eight experiments (measured in the same clusters of cells placed in droplets prior to the application of CCK) was 70 ± 9 nM, and the calcium extrusion rate at rest was 2·6 ± 0·6% of the rate of calcium extrusion at $[Ca^{2+}]_i = 450$ nM.

The Ca^{2+} dependence of calcium extrusion (Fig. 2) indicates a low rate of calcium extrusion across the plasma membrane at low (close to resting) levels of $[Ca^{2+}]_i$ with a substantial increase in the intensity of extrusion for small increases in $[Ca^{2+}]_i$ and saturation of calcium extrusion at $[Ca^{2+}]_i$ higher than 450 nM. Analysis of the kinetics of this process for data presented in Fig. 2 demonstrated strong co-operativity with a Hill coefficient of 3·0 ± 0·3.

Role of calcium extrusion in the recovery phase of $[Ca^{2+}]_i$ elevated by supramaximal doses of CCK

During supramaximal stimulation by CCK or acetylcholine (ACh) a substantial amount of cellular calcium (more than 0·5 mM) is extruded (Tepikin *et al.* 1992*a*). It would therefore appear that calcium extrusion plays a central role in establishing the shape of calcium recovery after

supramaximal agonist stimulation in pancreatic acinar cells. To check this, we performed experiments aimed at assessing the content of the internal Ca^{2+} stores after supramaximal stimulation by CCK, since another contributor to the recovery process could be Ca^{2+} uptake into internal stores. These experiments (data not shown) were performed in the cuvette of a spectrofluorimeter on suspensions of cells. Experiments performed in this way have the advantage of allowing the introduction of lipophilic reagents (e.g. thapsigargin, antimycin A, carbonylcyanide *p*-(trifluoromethoxy)phenylhydrazone (FCCP)) to the cells without the possibility of loss of reagent by absorption into the mineral oil that surrounds a microdroplet. The application of CCK induced a substantial transient elevation of $[Ca^{2+}]_i$ accompanied by a marked calcium extrusion. The subsequent addition of a high concentration of thapsigargin induced only a small ($12{\cdot}1 \pm 1{\cdot}2\%$ of CCK-induced $[Ca^{2+}]_i$ rise, $n = 12$) elevation of $[Ca^{2+}]_i$.

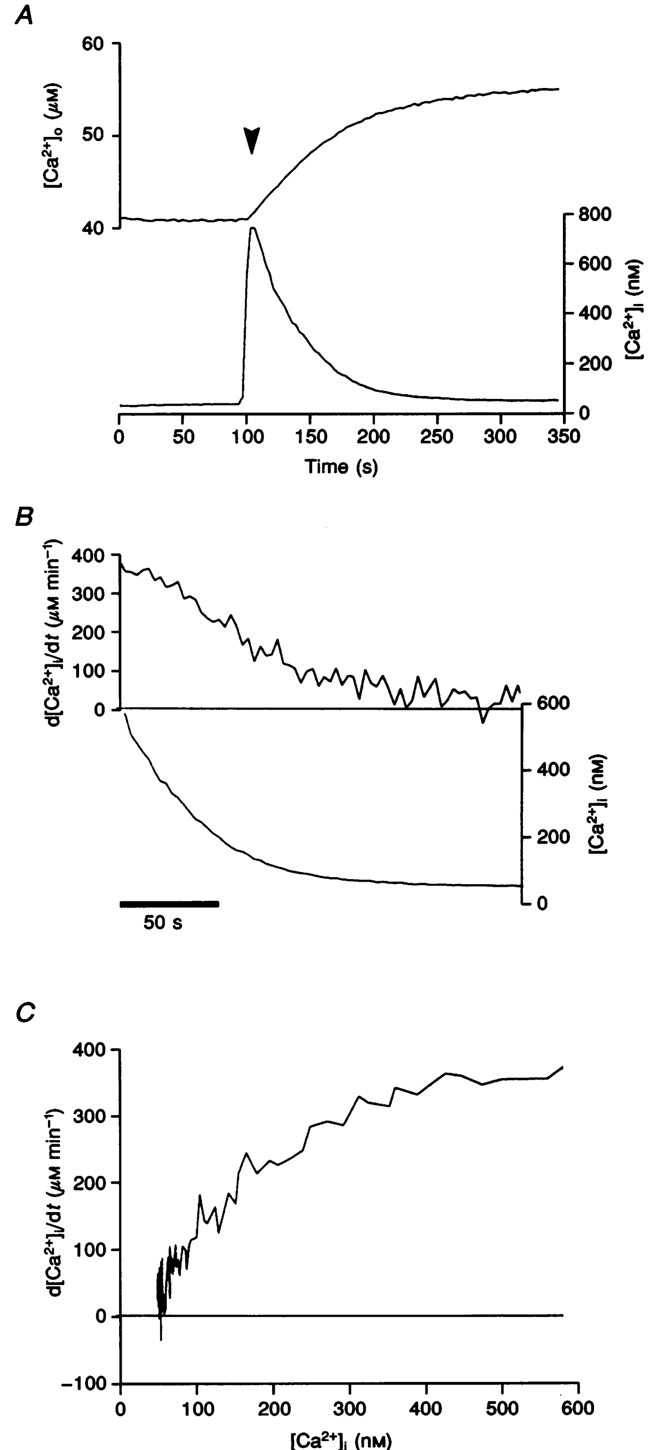

Figure 1. The Ca^{2+} dependence of Ca^{2+} extrusion from a cluster of pancreatic cells

A, changes of $[Ca^{2+}]_i$ (lower trace, right axis) and $[Ca]_o$ in droplet solution (upper trace, left axis) as a result of stimulation by a high dose of CCK (the time of CCK application is shown by the arrowhead). *B*, the rate of Ca^{2+} extrusion (upper curve, left axis) and recovery of $[Ca^{2+}]_i$ (lower curve, right axis). *C*, curve illustrating Ca^{2+} dependence of Ca^{2+} extrusion rate.

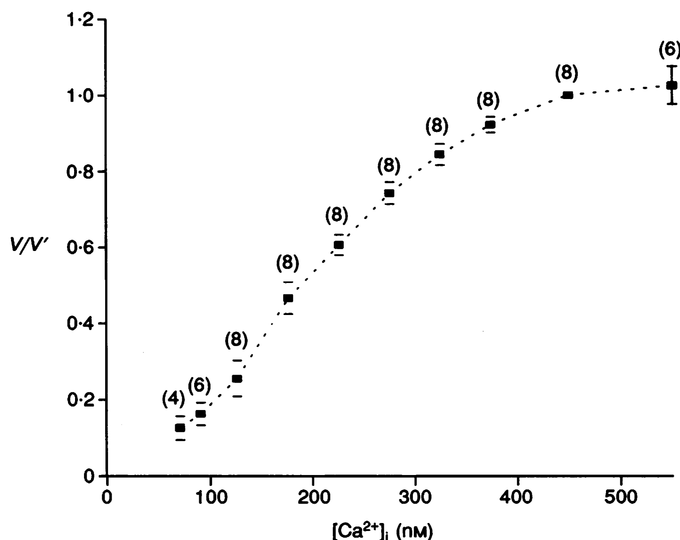

Figure 2. $[Ca^{2+}]_i$ dependence of Ca^{2+} extrusion as a function of $[Ca^{2+}]_i$

The relative rate of Ca^{2+} extrusion (V/V' where V' is the extrusion rate at $[Ca^{2+}]_i = 450$ nM) plotted as a function of $[Ca^{2+}]_i$. Values in the graph represent means ± S.E.M.

It was important also to check whether the thapsigargin-induced elevation of intracellular calcium was accompanied by a substantial calcium extrusion. The resolution of cuvette measurements was lower than that of the droplet technique; however, whilst it was possible to detect a substantial elevation in the extracellular calcium concentration due to the application of CCK, any calcium extrusion during a subsequent addition of thapsigargin was small and usually below the limit of resolution.

The addition of the mitochondrial inhibitors antimycin A (an antibiotic that inhibits electron flow between cytochromes b and c_1; $n = 6$) or FCCP (a protonophore; $n = 3$) after thapsigargin also produced only a small and slowly developing elevation of $[Ca^{2+}]_i$ with no detectable extrusion. The very small $[Ca^{2+}]_i$ responses evoked by high concentrations of thapsigargin and the mitochondrial inhibitors, when applied after a supramaximal dose of CCK, indicate that under these conditions the major mechanism responsible for the recovery of $[Ca^{2+}]_i$ to basal levels is calcium extrusion because the endoplasmic reticulum (ER) is leaky and $[Ca^{2+}]_o$ is low.

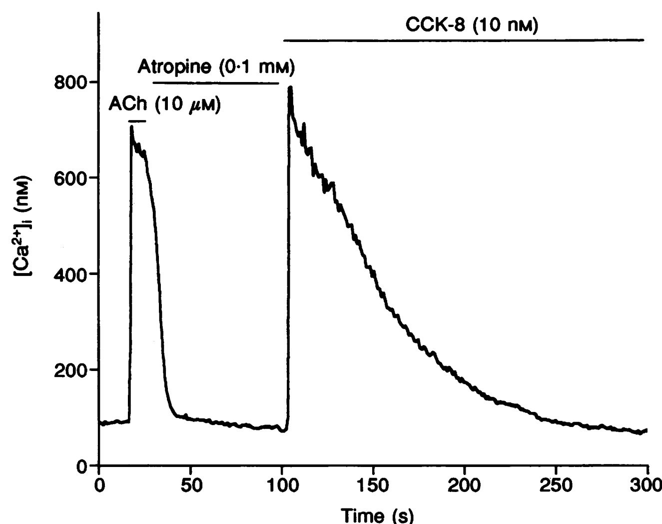

Figure 3. $[Ca^{2+}]_i$ recovery following cessation of muscarinic receptor activation and during continuing CCK stimulation

Changes of $[Ca^{2+}]_i$ induced by subsequent applications of ACh, atropine and CCK.

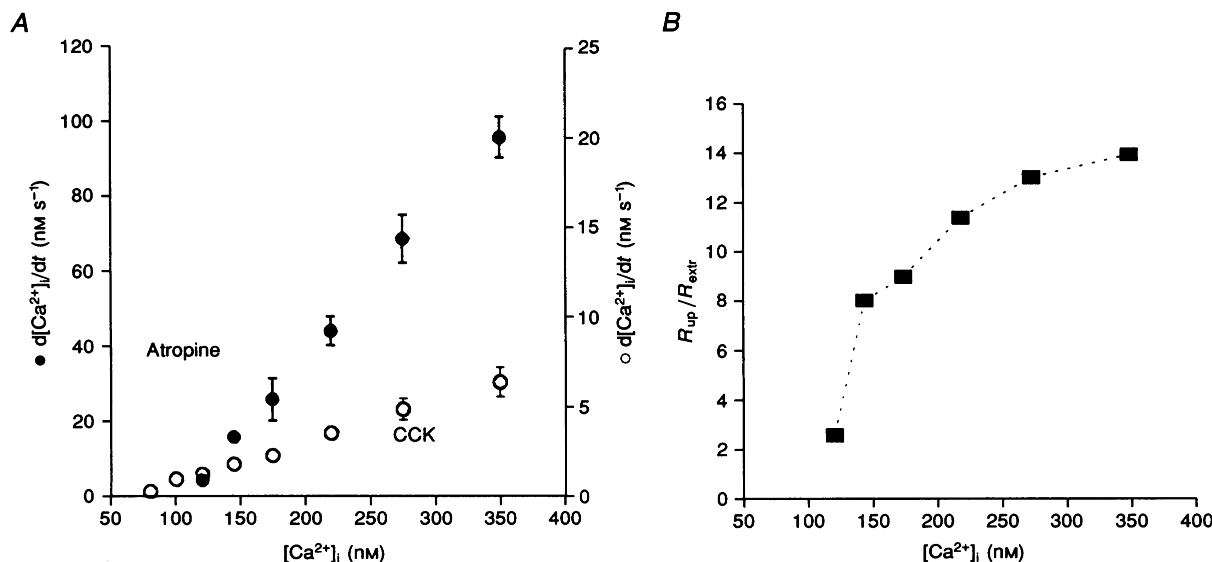

Figure 4. Ca^{2+} dependence of $[Ca^{2+}]_i$ recovery following abolition of ACh stimulation (by atropine) and during continuing CCK stimulation

A, Ca^{2+} dependence of $[Ca^{2+}]_i$ recovery following abolition of ACh stimulation by atropine (●) and during continuing CCK stimulation (○). Means ± S.E.M. from 6 experiments. *B*, ratio of recovery rates R_{up}/R_{extr}, where R_{up} is the recovery rate due to Ca^{2+} uptake into internal stores:

$$R_{up} = d[Ca^{2+}]_i/dt_{Atr} - d[Ca^{2+}]_i/dt_{CCK},$$

and R_{extr} is the recovery rate due to Ca^{2+} extrusion:

$$R_{extr} = d[Ca^{2+}]_i/dt_{CCK}.$$

Atr is recovery after atropine addition in continuous presence of ACh, CCK is recovery in continuous presence of the supramaximal CCK concentration. Values of derivatives in this formula were taken at the same calcium concentration.

Ca^{2+} dependence of Ca^{2+} reuptake from the cytoplasm

The application of a supramaximal concentration of ACh results in an elevation of $[Ca^{2+}]_i$ that slowly (after 200–300 s) recovers to the basal level (Tepikin *et al.* 1992*a*). The application of atropine during ACh stimulation (Fig. 3) resulted in rapid recovery of $[Ca^{2+}]_i$ to the prestimulation level. The rate of atropine-induced recovery was much faster than the rate of recovery during the subsequent sustained stimulation with a supramaximal dose of CCK (Figs 3 and 4*A*). The rate of recovery is represented by the derivative of $[Ca^{2+}]_i$ (i.e. $d[Ca^{2+}]_i/dt$) plotted against the corresponding value of $[Ca^{2+}]_i$ in Fig. 4*A*. The atropine-induced recovery presumably occurs in conditions where the calcium release channels in the internal stores are closed and the internal calcium store is therefore able to transport calcium from the cytoplasm. Recovery in the continuous presence of CCK occurs when the internal store is leaky due to sustained opening of Ca^{2+} release channels and recovery depends mainly on extrusion. The much higher rate of atropine-induced recovery, compared with the recovery during continuing CCK stimulation, implies that the main mechanism responsible for this process is Ca^{2+} uptake into internal stores. The plot of the Ca^{2+} dependence of Ca^{2+} uptake into internal stores (filled circles in Fig. 4*A*) indicates a very sharp rise in the rate of uptake when $[Ca^{2+}]_i$ is elevated above the resting level. The rate of Ca^{2+} uptake increased approximately 10 times (from 4·3 to 44·1 nM s^{-1} for an increase in $[Ca^{2+}]_i$ of only 100 nM (from 120 to 220 nM).

DISCUSSION

The droplet technique, which was employed for measurements of calcium extrusion, allowed us for the first time to measure the Ca^{2+} dependence of calcium extrusion in mammalian cells with a normal (preserved) intracellular environment (Figs 1 and 2). Analysis of the Ca^{2+} dependence of calcium extrusion from pancreatic acinar cells indicates that this process does not follow classical Michaelis–Menten kinetics. The evaluation of the kinetics for calcium extrusion for the data presented in Fig. 2 demonstrated a strong degree of co-operativity (Hill coefficient, 3·0 ± 0·3). Sigmoidal shapes of curves describing the calcium dependence of Ca^{2+}-ATPase activity (Hill coefficient, > 1) have been reported for other cell types

(Carafoli, 1991). One possible explanation for such a shape could be the activation of ATPases by calmodulin. Indeed dramatic changes in the activity of Ca^{2+}-ATPases due to calmodulin stimulation have been reported in work performed on plasma membrane vesicles obtained from pancreatic acinar cells (Ansah, Molla & Katz, 1984). Half-maximal activation of Ca^{2+} extrusion occurs at a $[Ca^{2+}]_i$ of approximately 200 nM. Calcium-ATPases of the plasma membrane usually have a high affinity for calcium ($K_m < 0.5\ \mu M$) in the optimally activated state (Carafoli, 1992).

It has been proposed that the calcium extrusion rate may be independent of $[Ca^{2+}]_i$ (Zhang, Zhao, Loessberg & Muallem, 1992). Our results (Fig. 2) show that the calcium extrusion rate is indeed independent of $[Ca^{2+}]_i$ in part of the physiological range (> 450 nm).

In the droplet technique extrusion is measured into an extracellular solution which has a low calcium concentration i.e. a few micromolar extracellular free calcium as opposed to the physiological concentration of 1 mM. This raises the question as to whether the extrusion rate is altered by the presence or absence of extracellular calcium. The results of studies with radioactive calcium ($^{45}Ca^{2+}$) monitoring calcium extrusion from pancreatic acinar cells indicate that calcium extrusion does not change significantly with changes of extracellular calcium concentration from physiological concentration to nominally calcium-free solution (Kondo & Schulz, 1976). A similar finding was described by Muallem, Beeker & Pandol (1988). The same studies also addressed the question of calcium influx into pancreatic acinar cells. In particular it was shown that a decrease of extracellular free calcium concentration from 2 mM to 50 μM (a value which is still larger than in the extracellular droplet solution) results in elimination of calcium influx (Muallem *et al.* 1988). These findings, along with the fact that the extracellular Ca^{2+} concentration in the droplet is not only low but also buffered by 100 μM fluo-3, indicate that the droplet technique does allow selective measurements of calcium extrusion and that the rate of calcium extrusion measured using the droplet technique should not be substantially different from the rate of extrusion at physiological extracellular Ca^{2+} concentrations.

The hormone- or neurotransmitter-evoked cytosolic Ca^{2+} signal in pancreatic acinar cells is primarily due to the messenger-mediated opening of Ca^{2+} release channels in internal stores (Petersen *et al.* 1994). When the agonist activation is interrupted by the application of an

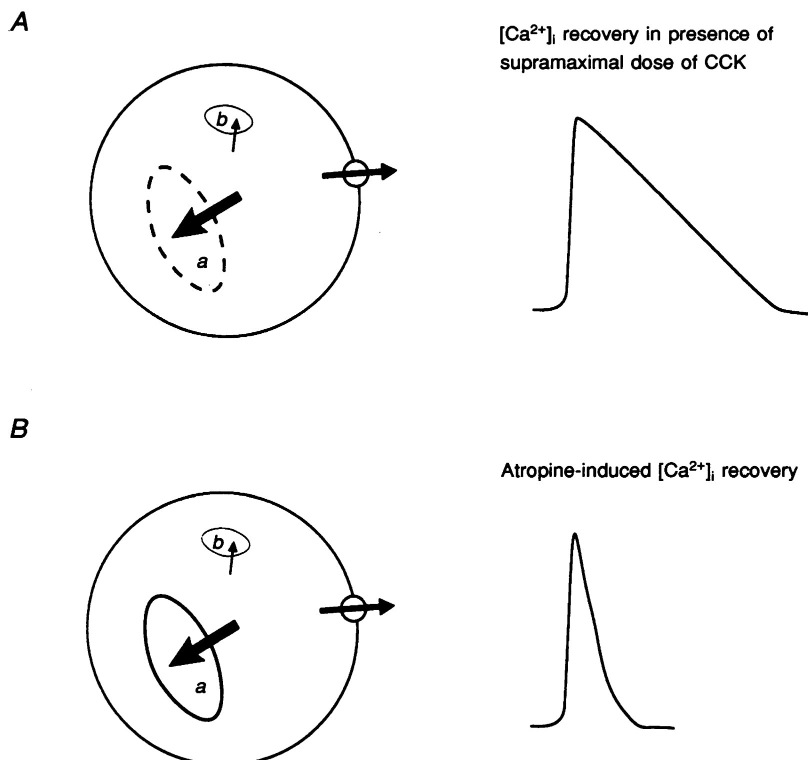

Figure 5. Model describing components of $[Ca^{2+}]_i$ recovery

a, CCK releasable calcium store. *b*, small calcium store non-releasable by CCK but releasable by thapsigargin. *A*, schematic drawing of the status of internal stores and the $[Ca^{2+}]_i$ recovery process in the presence of a supramaximal concentration of CCK. *B*, schematic drawing of the status of internal stores and the $[Ca^{2+}]_i$ recovery process induced by application of atropine in the presence of ACh.

appropriate antagonist, the enhanced messenger level would be expected to decrease rapidly to prestimulus levels (the rate of IP_3 breakdown is fast – its degradation time is in the region of 1 s; Allbritton, Meyer & Stryer, 1992) and the Ca^{2+} release channels should therefore close. This would enable Ca^{2+} pumps to effectively transport Ca^{2+} back into the stores, but Ca^{2+} extrusion to the external solution would also occur (Figs 3 and 5*B*). This is probably the simplest explanation for the atropine-induced recovery. If, on the other hand, agonist stimulation is maintained, there is still recovery to the resting $[Ca^{2+}]_i$, but it occurs much more slowly (Figs 3 and 5*A*). In this case, the Ca^{2+} release channels should remain open due to maintained, elevated messenger levels, and therefore the Ca^{2+} pump-mediated reuptake into the stores is counteracted by rapid loss. The reuptake mechanism is therefore in this case ineffective and Ca^{2+} removal from the cytosol will almost exclusively occur by extrusion. The experiment in which a high dose of thapsigargin was applied following supramaximal agonist stimulation indicates that there is in such a case very little releasable calcium left in the stores after recovery.

In the case of calcium reuptake, formal calculations of affinity, co-operativity and maximal rate were not possible since the cytosolic Ca^{2+} binding capacity is unknown, but the shape of the curve illustrating the Ca^{2+} dependence of reuptake (Figs 3 and 4*A*) indicates a very steep Ca^{2+} dependence (the rate of reuptake increases more than 10 times for a less than 2-fold increase in $[Ca^{2+}]_i$ from 120 to 220 nM).

In our experiments the application of thapsigargin after a CCK-induced calcium response (in the continuous presence of a supramaximal dose of CCK) has shown that very little calcium is left in the internal stores after CCK-induced calcium release and recovery. In previous studies we have shown that the amount of calcium lost from the cells into the extracellular medium of the droplet due to supramaximal hormone stimulation corresponds to the loss of most of the mobilizable cellular calcium (Tepikin *et al.* 1992*a*, *b*). Together these data indicate that the calcium recovery during stimulation by a supramaximal dose of CCK occurs mainly by calcium extrusion (Fig. 5*A*). In other words the rate of recovery in the presence of a supramaximal dose of CCK ($d[Ca^{2+}]_i/dt_{CCK}$) is the recovery rate determined by extrusion (R_{extr}), i.e.

$$R_{extr} = d[Ca^{2+}]_i/dt_{CCK}.$$

Since atropine-induced recovery is much faster than recovery in the presence of a supramaximal dose of CCK and application of a supramaximal dose of CCK following atropine-induced recovery elicits a second Ca^{2+} response of the same order of magnitude as that evoked initially by ACh (Fig. 3), it seems likely that the atropine-induced recovery is due mainly to Ca^{2+} reuptake into internal stores (Fig. 5*B*). However, this calcium recovery will contain a component that is due to extrusion (Fig. 5*B*); this component can be subtracted, i.e. the rate of calcium recovery mediated by uptake into internal stores (R_{up}) can be calculated as:

$$R_{up} = d[Ca^{2+}]_i/dt_{Atr} - d[Ca^{2+}]_i/dt_{CCK}.$$

(Values of derivatives in this formula are calculated for the same $[Ca^{2+}]_i$.) The ratio of recovery rates is the rate of recovery due to calcium divided by the recovery rate due to calcium extrusion:

$$R_{up}/R_{extr} = (d[Ca^{2+}]_i/dt_{Atr} - d[Ca^{2+}]_i/dt_{CCK})/d[Ca^{2+}]_i/dt_{CCK}.$$

Figure 4*B* shows that with a decrease of $[Ca^{2+}]_i$, the ratio of recovery rates increases. At $[Ca^{2+}]_i = 350$ nM the ratio of recovery rates was 14; with a decrease of intracellular calcium to 120 nM the ratio of recovery rates decreased to 2·5. This indicates a relatively more important role for calcium extrusion mechanisms in regulating $[Ca^{2+}]_i$ at close to resting $[Ca^{2+}]_i$ levels.

At high values of $[Ca^{2+}]_i$, calcium uptake into stores can be much faster than calcium extrusion, but in this condition the calcium uptake mechanism is partially shunted by calcium release occurring from the same store. The slow CCK-induced calcium transients (Tepikin *et al.* 1992*b*; Petersen *et al.* 1994) are examples of such an effect. The recovery after a spike (especially the recovery after the first spike) is usually substantially slower (some times more than 5 times slower) than recovery after the application of atropine in the presence of ACh (Yule, Lawrie & Gallacher, 1991; Tepikin *et al.* 1992*b*). This indicates that even at high $[Ca^{2+}]_i$ values, the role of the plasma membrane Ca^{2+}-ATPases can be very important in determining the amplitude of $[Ca^{2+}]_i$ spikes and the shape of the calcium recovery.

The available data allow us to estimate an important parameter – the rate of calcium uptake into internal stores. The droplet technique allows us to calculate the rate of calcium extrusion; for instance when $[Ca^{2+}]_i = 350$ nM, the extrusion rate was approximately 340 μM min^{-1} (this means that the total – i.e. both bound and free – calcium concentration in the cell decreases by 340 μM each minute due to extrusion). From the atropine data we also know that the recovery due to calcium uptake at this $[Ca^{2+}]_i$ is 14 times faster than the recovery due to calcium extrusion (see above and Fig. 4*B*). In other words we know the extrusion rate and we know that uptake into internal stores is 14 times more powerful. To find the rate of calcium uptake into internal stores it is possible simply to multiply the rate of calcium extrusion by the ratio of the recovery rates: 340 μM min^{-1} × 14 = 5 mM min^{-1} or 80 μM s^{-1} (this means that the total – i.e. both bound and free – calcium concentration in the cytosol decreases 80 μM s^{-1} due to calcium uptake into internal stores). This calculation is

based on the assumption that the volume of the cytosol is close to the total volume of the cell and that the high affinity calcium buffer is located mainly in the cytosol of the cell. This estimation can be refined further by using the data published by Bolender (1974) which describe the volumes of the different cellular compartments in pancreatic acinar cells. The volumes of cytosol and nucleoplasm together constitute 62% of the volume of the cell, whilst the volume of the rough endoplasmic reticulum (RER; probably the main calcium store) constitutes 22%. This means that the volume from which calcium uptake occurs during the atropine-induced recovery constitutes 62% of the cellular volume, and it means that the changes in total cytosolic calcium concentration due to calcium uptake should be somewhat larger than estimated above. At $[Ca^{2+}]_i = 350$ nM correcting the data for the volume of cytosol, the uptake rate can be estimated to be 130 μM s^{-1}; $[Ca^{2+}]_i = 120$ nM gives approximately a 20 times slower rate of uptake of 7 μM s^{-1}. The volume of the reticulum is approximately one-third that of the cytosol, so assuming that calcium uptake occurs into the reticulum, the rate of change of the total calcium concentration in the endoplasmic reticulum should be 3 times faster (in absolute value) than in the cytosol. Therefore at $[Ca^{2+}]_i = 350$ nM the calcium concentration in the reticulum should increase at a rate of 130 μM s^{-1} × 3 = 390 μM s^{-1} due to uptake from the cytosol.

The reliability of these estimations depends on how well the data on intracellular volumes obtained for the guinea-pig pancreas (Bolender, 1974) can be extrapolated to the mouse pancreas, on whether the nucleoplasm is calcium permeable or not, and on whether the RER is the only store involved in calcium uptake.

We have assumed that the high affinity calcium buffer is localized mainly in the cytoplasm of the cell; this assumption seems reasonable since the free calcium concentration in the cytoplasm is usually much lower than in the calcium stores. However, data regarding the exact distribution of this high affinity calcium buffer in pancreatic acinar cells is not available. We consider that this estimation is useful since it shows how substantial the calcium fluxes are, which can be produced by mechanisms of calcium uptake into internal stores (approximately 100 μM total cytosolic calcium per second).

The very high rate of calcium uptake into the internal stores is probably important for organizing localized Ca^{2+} signalling events. The short-lasting calcium transients found, in this cell type, to be localized at the secretory pole (Petersen *et al.* 1994; Thorn, Gerasimenko & Petersen, 1994) presumably require localized calcium release and a very intense reuptake mechanism in order to prevent $[Ca^{2+}]_i$ build-up in this region to levels sufficient to initiate a calcium wave by calcium-induced calcium release.

The main mobilizable intracellular Ca^{2+} store in the pancreatic acinar cell is generally regarded as being the rough endoplasmic reticulum, although there is now evidence for localization of Ca^{2+} release channels in the inner nuclear membrane of liver cells (Gerasimenko, Gerasimenko, Tepikin & Petersen, 1995) and in the secretory granules of insulin-secreting cells (Blondel *et al.* 1994). In guinea-pig pancreatic acinar cells, RER accounts for 22% of the cellular volume and the surface area of RER was found to be 12·5 times higher than the surface area of the plasma membrane (Bolender, 1974). This means that although the stores can transport Ca^{2+} faster than the plasma membrane, the rate of transport per unit surface area even at moderate levels of $[Ca^{2+}]_i$ is approximately equal for the plasma membrane and the endoplasmic reticulum. It is possible that due to the limited rate of calcium diffusion there are localized regions with higher or lower than the cellular average rates of uptake and extrusion measured in this study.

The secretory pole of the pancreatic acinar cells contains a much lower density of endoplasmic reticulum than the basolateral part. This may indicate that the role of the plasma membrane ATPases in regulation of calcium in the secretory pole is of particular importance.

We found that the calcium transporting systems of both the plasma membrane and the internal stores are almost silent at low (close to the resting) calcium levels and undergo a very sharp activation with a relatively small rise in $[Ca^{2+}]_i$ above the resting level.

The difference in $[Ca^{2+}]_i$ between a resting and a stimulated pancreatic acinar cell is between 100 and 800 nM (usually there is no more than a 10-fold elevation in $[Ca^{2+}]_i$; Williams & Yule, 1993). This is a much smaller difference than is the case for muscle cells (Konishi & Baylor, 1991), chromaffin cells (Augustine & Neher, 1992) and many other excitable cell types. This may indicate that in pancreatic acinar cells a particularly efficient mechanism for maintaining $[Ca^{2+}]_i$ is necessary in order to prevent undesirable activation of Ca^{2+}-dependent processes. The high co-operativity of Ca^{2+} activation of calcium reuptake and calcium extrusion is undoubtedly very important for the effective control of the resting $[Ca^{2+}]_i$ in this cell type. The steep $[Ca^{2+}]_i$ dependence of calcium pumping can explain the relative insensitivity of the resting $[Ca^{2+}]_i$ in this cell type to changes in the extracellular calcium concentration (Muallem, Schoeffield, Fimmel & Pandol, 1988), equality of $[Ca^{2+}]_i$ before and after stimulation by $[Ca^{2+}]_i$ releasing agonists (Yule *et al.* 1991) and the stability of calcium recovery levels between spikes of transient calcium oscillations (Yule *et al.* 1991).

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