Calcium Uptake and Release by Isolated Cortices and Microsomes from the Unfertilized Egg of the Sea Urchin *Strongylocentrotus droebachiensis*

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Abstract. Isolated cortices from unfertilized sea urchin eggs sequester calcium in an ATP-dependent manner when incubated in a medium containing free calcium levels characteristic of the resting cell (~0.1 μM). This ATP-dependent calcium uptake activity was measured in the presence of 5 mM Na azide to prevent mitochondrial accumulation, was increased by oxalate, and was blocked by 150 μM quercetin and 50 μM vanadate (known inhibitors of calcium uptake into the sarcoplasmic reticulum). Cortical regions preloaded with ^{45}Ca in the presence of ATP were shown to dramatically increase their rate of calcium efflux upon the addition of (*a*) the calcium ionophore A23187 (10 μM), (*b*) trifluoperazine (200 μM), (*c*) concentrations of free calcium that activated cortical granule exocytosis, and (*d*) the calcium mobilizing agent inositol trisphosphate.

This pool of calcium is most likely sequestered in the portion of the egg's endoplasmic reticulum that remains associated with the cortical region during its isolation. We have developed a method for obtaining a high yield of purified microsomal vesicles from whole eggs. This preparation also demonstrates ATP-dependent calcium sequestering activity which increases in the presence of oxalate and has similar sensitivities to calcium transport inhibitors; however, the isolated microsomal vesicles did not show any detectable release of calcium when exposed to inositol trisphosphate.

ARLY observations by Mazia (20) on frozen-thawed preparations of *Arbacia* eggs suggested a release of bound calcium on fertilization. The existence of intracellular calcium stores responsible for calcium release in sea urchin eggs has been supported by studies showing parthenogenic activation by a calcium ionophore (5, 38) and normal fertilization (4, 27) in calcium-free seawater. The fact that this release of calcium from internal stores leads to the elevation of cytosolic free calcium was demonstrated using the calcium-sensitive photoprotein aequorin (8, 12, 29), and more recently the fluorescent calcium probe Fura-2 (23). This increase in free calcium appears to be the primary event triggering, directly or indirectly, the various processes associated with activation of the egg, e.g., cortical granule exocytosis, the respiratory burst, the increase in intracellular pH, and increases in DNA and protein synthesis (10).

In this study, calcium transport is described in two preparations from unfertilized eggs: isolated cortical regions and purified microsomal vesicles. Evidence will be presented in support of the endoplasmic reticulum (ER)[1] being the calcium sequestering system in both preparations as is the case in a number of other cell systems (2).

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1. *Abbreviations used in this paper:* ER, endoplasmic reticulum; IP3, inositol trisphosphate.

Materials and Methods

Preparation of Eggs

Strongylocentrotus droebachiensis (from the coast of Maine, Ocean Resources, Peaks Island, ME) were induced to shed mature eggs by intracoelomic injection of 0.5 M KCl. The suspension of eggs in artificial seawater (465 mM NaCl, 55 mM MgCl$_2$, 11 mM CaCl$_2$, 10 mM KCl, and 2.5 mM NaHCO$_3$, pH 7.8 [1]) was titrated with 0.1 M HCl to pH 5 for 2 min to remove the jelly coat and returned to pH 7.8 with 0.1 M Tris. The eggs were then washed several times and resuspended at a 1/5 (vol/vol) ratio of eggs to artificial seawater.

Preparation of Cortices

Isolated, immobilized cortices were prepared at 4°C following the method of Vacquier (31) in the buffer described by Baker and Whitaker (1). To enable quantitative measurements of calcium uptake and release, cortical lawns of equal sizes were prepared in 24-well Costar tissue culture plates (16.4-mm well diam). 200 μl of a solution of polylysine (200,000 mol wt) in distilled water (0.1 mg/ml) was pipetted into each well and allowed to stand for 1 h, after which the wells were rinsed free of the solution with distilled water and allowed to dry. 0.5-ml samples of the dejellied egg suspension were then layered into each polylysine-treated compartment. After allowing 5 min for attachment of the eggs, the seawater was flushed away with 10 ml of 10 mM EGTA in buffer A (500 mM glycine, 220 mM K glutamate, 10 mM NaCl, 10 mM MgCl$_2$, 5 mM Na azide [pH 6.7], adapted from [1]). The bulk of the eggs were then sheared from their attached cortical regions by gently spraying the surface of

the plate with the overlying solution. Immediately after each shearing procedure, the cell debris was flushed away with 20 ml of the same buffer. Under the light microscope the cortical lawns were devoid of any attached intact eggs.

Calcium Uptake into Cortical Lawns

Experiments were done at 20°C. All assays were done in buffer A, which contained 5 mM Na azide to block mitochondrial calcium uptake. To limit total calcium, and hence maximize specific radioactivities for ^{45}Ca, a ratio of 0.05 mM Ca (5 μCi $^{45}Ca/ml$) to 0.5 mM EGTA in buffer A was used unless otherwise indicated. This Ca/EGTA ratio corresponded to a calculated free calcium concentration of ~0.1 μM (11). Incubation media containing 10 mM ATP also included an additional 10 mM MgCl$_2$ (1). Details of other conditions are given in the Results section. After incubations in which the cortices were loaded with ^{45}Ca, the preparations were flushed three times with 20 ml of 1 mM EGTA in buffer A to remove loosely bound calcium. After removing the last wash, 0.2 ml of 1 M NaOH was applied to the preparation to liberate all bound calcium, and subsequently transferred to a vial for scintillation counting. The NaOH extraction removed 95% of the bound calcium. Duplicate determinations varied no more than 5%.

We ensured in earlier (unpublished) experiments on eggs of the sea urchin *Arbacia punctulata* that calcium was being sequestered into components of the cortex in these preparations and not into a sealed compartment which might be formed between the cortex and the plastic base. Cortical lawns from *Arbacia* eggs were prepared on permeable bases (1-cm diam nitrocellulose filters, 0.45-μm pore size) or plastic coverslips cut to an equivalent dimension. Their ATP-dependent calcium uptake activities were compared under otherwise identical conditions. No significant difference in the specific activity of the uptake was found. Since *A. punctulata* and *S. droebachiensis* cortices respond similarly in their calcium uptake characteristics, these experiments indicate that no calcium is sequestered into a sealed compartment between the cortex and the base in these preparations.

Calcium Release Studies

Since measured calcium accumulation is the result of its uptake and release, it is important to also establish the rate of calcium release under the various experimental conditions. Calcium release was measured in cortical lawns by first loading the lawns with ^{45}Ca according to the standard conditions described above. After washing the preparations free of loosely bound calcium with buffer A containing 1 mM EGTA, samples of the overlying medium were periodically removed and their ^{45}Ca content measured to determine the rate of calcium release. At the end of the time course the remaining bound radioactivity was removed by adding 200 μl of 1 N NaOH to the lawns.

Isolation of Endoplasmic Reticulum

All steps were done at 4°C. Dejellied eggs were resuspended three times in calcium-free artificial seawater, and finally, resuspended in buffer B (0.5 M KCl, 10 mM MgCl$_2$, 10 mM 2-[N-morpholino]propane sulfonic acid, 10 mM EGTA, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, and 5 mM benzamidine [pH 7.0]) as a 1/10 (vol/vol) suspension of eggs in buffer. The eggs were then homogenized manually with 10 strokes of a 40 ml Potter-Elvehjem type teflon homogenizer. Sequential fractions were separated by differential centrifugation using a Sorvall RC2-B centrifuge with an SS-34 rotor. The pellets from each spin consisted primarily of the indicated organelles: 200 g for 3 min (cortices), 3,000 g for 20 min (yolk), and 15,000 g for 20 min (mitochondria). The 15,000 g supernatant was spun at 100,000 g for 30 min in a Beckman L3-50 ultracentrifuge (Beckman Instruments, Inc., Palo Alto, CA) with a 50.2 Ti fixed angle rotor to pellet the microsomes. The microsomal fraction packed loosely over a denser, more aggregated pellet. The upper portion was carefully separated from the lower pellet, layered on a 0.3 M sucrose, 0.7 M glycine solution at pH 7, and centrifuged at 100,000 g for 30 min. The recovered pellets were stored at −80°C (modified from reference 15).

Electron Microscopy of Microsomal Vesicles

The loose microsomal pellet from the first 100,000 g spin was resuspended in 0.5 M KCl, 0.1 M Na cacodylate, and 3% glutaraldehyde (pH 7.4) at a concentration of 0.5 mg protein in 10 ml. After fixing for 30 min the microsomes were centrifuged at 10,000 g for 1 h (3), and the resulting pellet was treated with osmium, and dehydrated and embedded in araldyte for thin sectioning. 800-Å sections were then stained in uranyl acetate and lead citrate (13) and the electron micrographs taken with an accelerating voltage of 80 kV in a Philips EM 300 electron microscope.

Enzyme Activities of Microsomal Fraction

Calcium-dependent ATPase activity was measured by adding 20-μl aliquots of the microsomal pellet to 0.98-ml aliquots of buffer A + 10 mM K oxalate, 10 mM MgATP, and Ca/EGTA buffers (1 mM EGTA) to give various free calcium concentrations (calculated according to Fabiato and Fabiato [11]). Duplicate 100-μl aliquots were removed at 10, 20, and 30 min and assayed for free phosphate according to the method of Marsh (19).

Glucose-6-phosphatase activity was measured by adding 0.1 ml of microsomes to 0.9 ml of buffer B, 1% Triton X-100, and 20 mM glucose-6-phosphate. After allowing 30 min for the microsomal vesicles to become fully permeable, 100-μl samples were removed at 10-min intervals to determine free phosphate (19).

Cytochrome *c* oxidase (32) and NADPH cytochrome *c* reductase (17) activities were also determined.

Calcium Uptake and Release by the Microsomal Fraction

Microsomal vesicles (15 μg protein/ml) were incubated in buffer A containing 0.5 mM EGTA and 0.05 mM ^{45}Ca (free calcium ~0.1 μM) in the presence or absence of ATP (see Results). At the times indicated 200-μl samples were transferred onto nitrocellulose filters (0.45-μm pore size) under suction and washed with 5 ml of buffer A + 1 mM EGTA. The ^{45}Ca retained was determined by scintillation counting of the filters.

Calcium release experiments were done by preloading the microsomal vesicles for 1 h under the conditions described above. Duplicate 200-μl samples were taken (as above) immediately before the addition of calcium transport inhibitors to the experimental samples. Duplicate samples of control and experimental solutions were taken at 5 and 10 min thereafter.

Protein Determinations

Representative samples of cortical preparations were solubilized by gently agitating with 200 μl of 1% SDS for 10 min. The protein contents of solubilized cortical preparations or untreated microsomal vesicles were determined by the method of Lowry et al. (18).

Results

Calcium Uptake into Isolated Cortical Lawns and Microsomal Preparations

When isolated cortical lawns were incubated in the presence and absence of ATP at ~0.1 μM free calcium (50 μM $^{45}Ca/$ 500 μM EGTA), the ^{45}Ca was found to accumulate in an energy-dependent manner (Fig. 1 *a*). In the absence of oxalate, calcium uptake by the lawns began to saturate after 40 min, whereas in the presence of oxalate calcium uptake was linear for up to 2 h. The isolated microsomal preparation showed very similar kinetics (Fig. 1 *b*), except for the expected increase in specific activity (note difference in Y axis scale). Enhanced uptake of calcium in the presence of oxalate is a property commonly observed in preparations containing calcium sequestering ER but is seldom observed with calcium uptake by plasma membrane vesicles (36).

Calcium Uptake and Release in the Presence of Calcium Transport Inhibitors

Active calcium uptake was determined at 0.1 μM free calcium, as described above, in the presence of a number of substances known to influence calcium transport. Quercetin and sodium orthovanadate are two compounds shown to inhibit calcium uptake in the sarcoplasmic reticulum by inhibiting translocation of the calcium pump (28) and by stabilizing the translocated form (21), respectively. As shown in Table I, these compounds markedly reduced calcium transport by both the
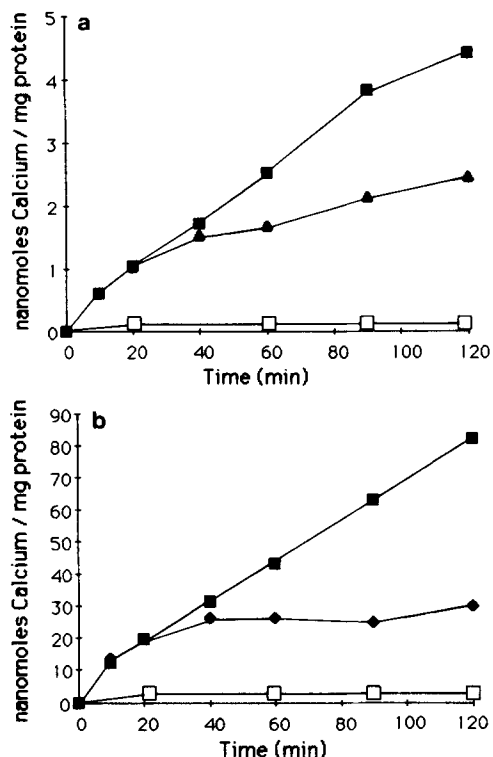
Figure 1. (a) Calcium uptake into cortical lawns (at ~0.1 μM free calcium) in the presence (▲) and absence (□) of 10 mM MgATP, and the presence of 10 mM MgATP and 10 mM K oxalate (■). (b) Calcium uptake into isolated microsomes (15 μg protein/ml) under conditions identical to those described for the uptake of calcium in cortical lawns (Fig. 1a). Presence (◆) and absence (□) of 10 mM MgATP and the presence of 10 mM MgATP and 10 mM K oxalate (■).

Table I. Inhibitors of Calcium Uptake in Cortical Lawns and Microsomes

	Calcium uptake (% of control)	
Inhibitor (concentration)	Cortical lawns	Microsomes
Control	100	100
Control − ATP	7.9	10.1
Vanadate (50 μM)	11.6	15.9
Quercetin (150 μM)	10.1	17.0
Trifluoperazine (10 μM)	82.6	48.8
Trifluoperazine (200 μM)	21.3	13.7
A23187 (10 μM)	27.7	18.2

Calcium uptake into cortical lawns or isolated microsomes in the presence of 10 mM ATP (Control) and in its absence (Control − ATP), and in the presence of 10 mM ATP plus various calcium transport inhibitors. Incubations were done for 60 min in buffer A with free calcium ~0.1 μM. Values are expressed as a percent of control ATP-dependent calcium uptake.

cortical lawns and the microsomal vesicles, but had no effect on the rate of calcium release (data not shown).

Trifluoperazine (10 μM) had a slight inhibitory effect on the cortical lawns and a larger effect on the microsomes (a discrepancy that will be referred to in the Discussion). On the other hand, a high concentration of trifluoperazine (200 μM) reduced the apparent calcium uptake in both preparations to a level comparable to that found in the presence of calcium ionophore A23187 (10 μM).

The latter reductions in net uptake were due to a marked

increase in the release of ^{45}Ca from the lawn preparations. Trifluoperazine had a considerable effect on the permeability of the cortical lawns to calcium, with 200 μM trifluoperazine producing a rate of calcium release equivalent to that of the ionophore A23187 (10 μM). Similar effects have been associated with ultrastructural changes in vesicle membranes at high trifluoperazine concentrations (14). Trifluoperazine and A23187 also reduce the apparent calcium uptake in the microsomes (Table I) by increasing their rate of calcium release (~90% of the initial calcium load released within 5 min).

## Comparison of Calcium Transport in Cortical Lawns before and after Cortical Granule Fusion

If cortical lawns are exposed to high levels of free calcium, the cortical granules will fuse to the plasma membrane in a manner similar to cortical granule exocytosis during fertilization (34, 37). Active calcium uptake by cortical lawns briefly exposed to 18 μM free calcium to fuse their cortical granules was compared with that of lawns with intact granules. The lawns with fused cortical granules sequestered far less calcium than did those with intact cortical granules (Fig. 2).

Calcium-induced Calcium Release in Cortical Lawns

Cortical lawns with intact cortical granules were loaded with ^{45}Ca (0.1 μM free concentration) for 30 min, washed free of unbound calcium, and calcium efflux was measured after exposure to various free concentrations of calcium buffered with 10 mM EGTA (11). As shown in Fig. 3, the cortical lawns exposed to lower free calcium concentrations, which did not undergo noticeable cortical granule exocytosis, showed a slow rate of calcium release. Those lawns exposed to 10 and 18 μM free calcium were observed to rapidly undergo cortical granule fusion, and showed an accelerated rate of ^{45}Ca release.
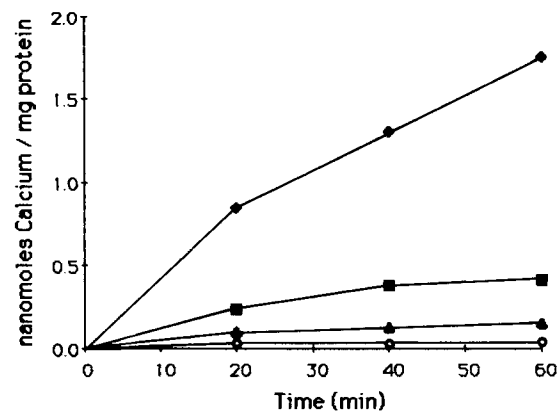
Figure 2. Calcium uptake into intact cortical lawns (unfused) and cortical lawns whose cortical granules have been fused by prior exposure to 18 μM free calcium (fused). Unfused −ATP (○); unfused +ATP (◆); fused −ATP (▲); fused +ATP (■). Cortical lawns were exposed for 1 min to buffer A containing either EGTA or 18 μM free calcium. Lawns exposed to the higher calcium concentration underwent rapid cortical granule fusion. The overlying solutions were then suctioned off and replaced first by a wash of buffer A containing 0.05 mM ^{40}Ca/0.5 mM EGTA (~0.1 μM free calcium) with no ATP, and finally buffer A containing 0.05 mM ^{45}Ca/0.5 mM EGTA with or without 10 mM MgATP, as indicated, for the uptake measurements.
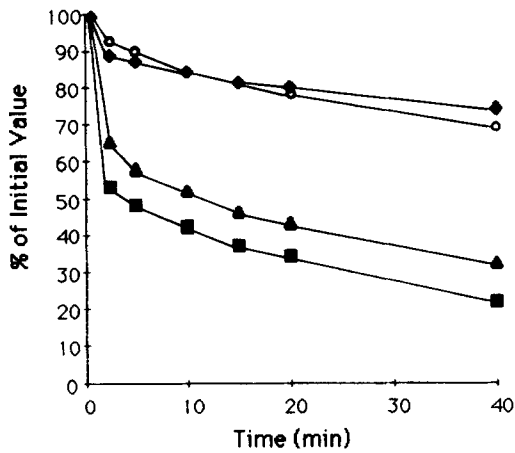
Figure 3. Calcium release by cortical lawns (preloaded with ^{45}Ca [~0.1 μM] in buffer A and 10 mM ATP for 30 min) exposed to 0.1 μM (O), 4.0 μM (◆), 10 μM (▲), and 18 μM (■) free calcium. At 10 μM and 18 μM free calcium the rate of calcium release increased and cortical granule fusion proceeded to completion.
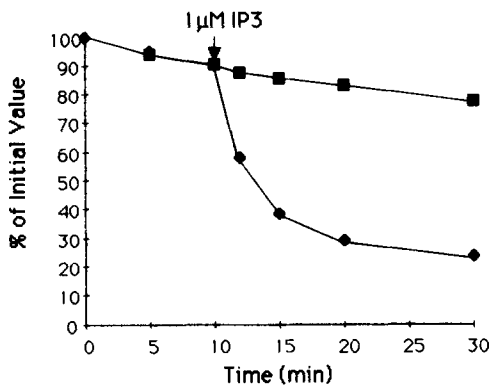

Figure 4. The effect of IP3 (1 μM) on the total ionophore releasable pool of ^{45}Ca. The differences between the 30-min points and the baseline represent the percent of ^{45}Ca released by 10 μM A23187 at that time. All determinations are standardized to the initial values for exchangeable calcium: control (■); addition of 1 μM IP3 at 10 min (◆).

IP3-dependent Release of Calcium from Cortical Lawns

IP3 is produced in vivo by the breakdown of polyphosphoinositides from the plasma membrane by phospholipases, certain of which can be activated by micromolar levels of free calcium (33). IP3 has in turn been shown to regulate the release of calcium from the ER of many cell types (2) and also to initiate the activation of sea urchin eggs (30, 35). In our preliminary studies IP3-dependent calcium release from cortical lawns was observed to occur under the same conditions as used in the preceding experiments. However, we modified this procedure to obtain an enhanced effect.

Cortical lawn preparations were isolated in buffer A containing 10 mM EGTA and 10 mM neomycin. They were then washed extensively with the same buffer minus the EGTA, after which 10 mM MgATP and ^{45}Ca (5 μCi/ml) were added for a 15-min incubation at room temperature. These modifications allowed us to load the cortical lawns with ^{45}Ca for a short period of time (15 min), at a relatively high free calcium concentration (~7 μM), since neomycin inhibits both
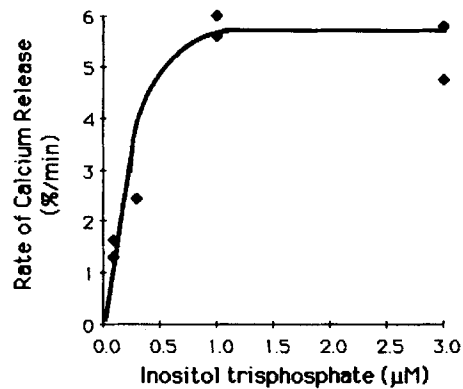
Figure 5. Release experiments were performed as in Fig. 4 with the addition of various concentrations of IP3. The rate of calcium release was extrapolated from the first 5 min of (linear) calcium efflux after the addition of IP3.
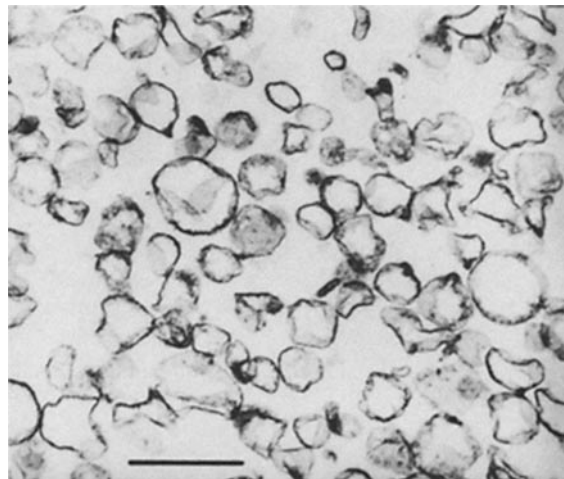
Figure 6. Transmission electron micrographs of isolated microsomal vesicles. Bar, 0.5 μm.

the cortical granule fusion and hydrolysis of polyphosphoinositides that ordinarily occur at this calcium level (33). After the incubation the preparation was flushed free of loosely bound ^{45}Ca with buffer A containing 1 mM EGTA and 0.1 mM ATP (no neomycin) at 4°C and maintained at this temperature. Aliquots (200 μl) of the overlying buffer were removed at intervals over a 30-min period. At the end of the experiment, 10 μM A23187 was added to release the remaining exchangeable calcium.

Fig. 4 shows release of calcium induced by 1 μM IP3. Since the overlying medium contained 1 mM EGTA, this release of calcium was not accompanied by cortical granule exocytosis. Increasing the IP3 concentration up to 10 μM did not increase the rate of calcium release. A dose response curve showed a half maximal response at ~400 nM IP3 with maximal calcium release occurring at 1 μM IP3 (Fig. 5).

Isolation and Characterization of Microsomal Vesicles from Sea Urchin Eggs

As seen by transmission electron microscopy (Fig. 6) the microsomal fraction was apparently free of mitochondria and consisted mainly of vesicles. The preparation also had negligible mitochondrial enzyme activity (<0.3% of the specific

activity of cytochrome c oxidase associated with the mitochondrial pellet) but demonstrated two ER marker activities (NADPH cytochrome c reductase = 33 nmol cytochrome c reduced/min per mg protein and glucose-6-phosphatase = 3.54 nmol phosphate released/min per mg protein), which could not be detected in other fractions. Microsomal ATPase activity was increased up to 10-fold by the presence of calcium. An Eadie-Hofstee plot of the dependence of ATPase activity on calcium showed a K_m of 0.85 μM free calcium and a V_{max} of 8.67 nmol PO_4 released/min per mg protein.

ATP-dependent calcium uptake into the vesicles was increased in the presence of 10 mM K oxalate as shown in Fig. 1 b. This ATP-dependent calcium transport activity was not affected by the inclusion of 5 mM Na azide (data not shown), confirming the lack of mitochondrial contamination. Under these conditions the rate of calcium uptake increased with increasing free calcium concentration until saturation was reached at ~6 μM. The saturated rate of uptake showed no decline at higher free calcium concentrations up to 18 μM, suggesting no calcium-induced calcium release. The addition of 10 μM IP3 to preloaded microsomes in the presence of 10 mM ATP did not produce any detectable increase in the rate of release of sequestered calcium.

Discussion

The structures for the sequestration and intracellular release of calcium in the egg remain ill-defined. The transient release of calcium on egg activation could be from an ER store analogous to the sarcoplasmic reticulum of muscle (9, 12). Ultrastructural studies of sea urchin eggs indicate an association of calcium with a tubular membranous system and large acidic vesicles in the cortical region (22). Schatten and Hemmer (26), however, have suggested that the cortical granules are the source of releasable calcium. This latter claim appears to gain support from our findings on a reduced calcium uptake by cortical lawns that have undergone cortical granule fusion compared to those with unfused granules (Fig. 2) and an increased release of calcium during cortical granule fusion (Fig. 3). These results, however, do not necessarily identify the cortical granules themselves as a primary calcium store since other structures in the cortical lawn preparation, such as ER (6, 24), also undergo considerable distortion during fusion (24). This would agree with the fact that in cortical lawns where nearly all of the cortical granules have undergone fusion, calcium accumulating activity is still present (Fig. 2). Changes in calcium transport by cortical components could also be caused by the degradative effects of proteases or phospholipases (33) released or activated during cortical granule fusion.

The sensitivity of the calcium pool to A23187 (Table I) indicates that this pool is in fact a vesicular store. In addition to A23187, high concentrations of trifluoperazine greatly increased the rate of calcium release from the cortical lawns. The greater inhibiting effect of low concentrations of trifluoperazine (10 μM) on microsomes compared to cortical lawns (Table I) may be due to the partitioning of this amphiphile into the membrane, making the effects of the drug partially dependent upon the differing amounts of membranes available in the two preparations.

Although some mitochondrial contamination is present in

isolated cortical lawns (24), we blocked calcium uptake into this organelle by including sodium azide in all incubation media. A number of our findings favor the hypothesis that most of the calcium sequestered by isolated cortices is in the ER. First, calcium uptake into cortical lawns is stimulated by oxalate (Fig. 1 a), a property commonly associated with sarcoplasmic and endoplasmic reticulum calcium uptake activity (36). Second, quercetin and vanadate, known inhibitors of calcium uptake by the sarcoplasmic reticulum of muscle (21, 28), inhibited calcium uptake in the cortices and isolated microsomes (Table I). Third, the calcium uptake properties of the cortices (Fig. 1 a) were similar to those of the isolated vesicles (Fig. 1 b) which, from the enzymatic studies, are likely to be derived from the ER. Finally, the IP3-induced release of calcium by the cortices (Figs. 4 and 5) suggests an effect on the ER, since this "messenger" has been shown to release calcium from a sea urchin egg ER fraction (7) as well as the ER of other tissues (2).

Sardet (24) and Chandler (6) have shown that the cortical region has an associated system of ER with specialized membrane varicosities. These membranes of the cortical region would be readily available to interact with IP3, hydrolyzed from polyphosphoinositides of the egg's plasma membrane during fertilization (16). The release of sequestered calcium from cortical lawns exposed to free calcium concentrations (10 and 18 μM) capable of initiating cortical granule exocytosis (Fig. 3), could also be an effect of IP3-mediated calcium channels, since these same levels of free calcium have been shown to activate hydrolysis of polyphosphoinositides associated with the plasma membrane of cortical lawns (33).

Recently Clapper and Lee (7) have shown an IP3-induced release of calcium in a microsomal fraction from eggs of the sea urchin *Lytechinus pictus*. The amount of IP3 required for half maximal release from their microsomal preparation (50–60 nM) is lower than that observed for the cortical preparation in this study (400 mM). The reason for this difference is not clear; however, the amount required for maximal release in both was ~1 μM.

In our microsomal preparation from *Strongylocentrotus droebachiensis* eggs, we could not detect any IP3-dependent calcium release. This difference in the response of the microsomal fraction to that of the cortical preparation or that of *L. pictus* microsomes may result from differences in the isolation procedures and/or in the measurement of calcium release. Our vesicle preparation, based on the method of Inoue and Yoshioka (15), involves the use of high ionic strength media, whereas the cortices were isolated at low ionic strength, as were the microsomes of Clapper and Lee (7). The high ionic strength medium was chosen because of higher yields of microsomes obtained, however, as noted by Sasaki and Epel (25), high ionic strength washes of cortices can remove protein constituents. One such component, removed during preparation of the microsomes, could be necessary for IP3-dependent release. Another possible reason for the apparent discrepancy between the effect of IP3 on microsomes in the present study compared with those of Clapper and Lee (7) is that the rate of calcium release in the present study was determined by monitoring the amount of ^{45}Ca remaining in the vesicles over a period of time, whereas Clapper and Lee (7) measured the free calcium of the extravesicular fluid as determined by calcium probes (a method potentially more

sensitive in preparations where many of the calcium sequestering vesicles present may be lacking calcium channels [7]).

Our present findings on two isolated preparations, cortical lawns and microsomes, are suggestive of a role for ER as the storage and release site of calcium in the sea urchin egg.

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