

Regulation of Cortical Vesicle Exocytosis in Sea Urchin Eggs by Inositol 1,4,5-Trisphosphate and GTP-binding Protein

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Abstract. To investigate the roles of inositol 1,4,5-trisphosphate (InsP₃) and guanyl nucleotide binding proteins (G-proteins) in the transduction mechanism coupling fertilization and exocytosis of cortical vesicles in sea urchin eggs, we microinjected InsP₃ and guanyl nucleotide analogs into eggs of *Lytechinus variegatus*. Injection of 28 nM InsP₃ caused exocytosis. However, if the egg was first injected with EGTA ([Ca_i] ≤ 0.1 μM; EGTA = 1.6 mM), InsP₃ injection did not cause exocytosis, supporting the hypothesis that InsP₃ acts by causing a rise in intracellular free calcium. Injection of 28 μM guanosine-5'-0-(3-thiotri-

phosphate) (GTP-γ-S), a hydrolysis-resistant analog of GTP, caused exocytosis, but exocytosis did not occur if the egg was pre-injected with EGTA. Injection of 3 mM guanosine-5'-0-(2-thiodiphosphate) (GDP-β-S), a metabolically stable analog of GDP, prevented sperm from stimulating exocytosis. However, injection of GDP-β-S did not prevent the stimulation of exocytosis by InsP₃. These results suggested the following sequence of events. The sperm activates a G-protein, which stimulates production of InsP₃. InsP₃ elevates intracellular free calcium, which causes exocytosis.

FERTILIZATION of the sea urchin egg causes a transient increase in intracellular free calcium [Ca_i]¹ due to calcium release from intracellular stores (11, 37), probably from the endoplasmic reticulum (12). The rise in calcium is first detectable in the sea urchin *Arbacia punctulata* about 23 s after fertilization and propagates around the egg as a wave (11). At the same time, cortical vesicles begin to fuse with the plasma membrane, and exocytosis proceeds as a wave around the egg from the site of sperm fusion (5, 22, 27, 32). The exocytosis results in the elevation of the fertilization envelope, which prevents the entry of additional sperm (34). The regulation of this exocytosis is not fully understood, but the rise in calcium is required since injection of EGTA prevents sperm from stimulating exocytosis (45), whereas buffering [Ca_i] above ~0.3 μM causes exocytosis (18).

Before the onset of exocytosis there is an increase in the amounts of polyphosphoinositide lipids present in sea urchin eggs (40; see also 23). This is of particular interest because inositol 1,4,5-trisphosphate (InsP₃) is produced by hydrolysis of phosphatidylinositol 4,5-bisphosphate (TPI) (20), and InsP₃ has been shown to release calcium from intracellular stores of

many cells, including pancreatic acinar cells (38), *Limulus* photoreceptors (3, 9), frog eggs (4), and sea urchin eggs (6). Radiolabeling with [³H]inositol has shown an increase in the production of InsP₃ in sea urchin eggs by 1-min postfertilization (23). When injected into eggs of the sea urchin *Lytechinus pictus*, InsP₃ causes exocytosis (43); similar results have been obtained with starfish and frog eggs (4, 30). In this study, we tested the hypothesis that InsP₃ acts by stimulating the release of calcium from within the egg, by injecting InsP₃ into sea urchin eggs in which [Ca_i] was buffered at a low level: no exocytosis would be expected in these eggs if InsP₃ acts by stimulating release of calcium.

How might the fertilizing sperm regulate the production of InsP₃? Studies of stimulus-response coupling in other cells (e.g., *Limulus* photoreceptor, blowfly salivary gland, human neutrophil, rat hepatocyte) have indicated that a guanyl nucleotide binding protein (G-protein) may regulate the production of InsP₃ (2, 7, 8, 13, 14, 25, 41). Further evidence that G-proteins are involved in pathways leading to exocytosis comes from investigations of mast cells (15, 16). In this study, we tested the possibility that a G-protein might be an intermediate in the pathway leading to exocytosis in sea urchin eggs by microinjecting eggs with analogs of GTP and GDP. Guanosine-5'-0-(3-thiotriphosphate) (GTP-γ-S) is a hydrolysis-resistant analog of GTP that binds with high affinity to the regulatory site of G-proteins, thereby stabilizing the G-protein in its active form (28). Exocytosis after microinjection of

¹ Abbreviations used in this paper: BAPTA, 1,2-bis (0-aminophenoxy)ethane-*N,N,N',N'*-tetraacetic acid; [Ca_i], intracellular free calcium; GDP-β-S, guanosine-5'-0-(2-thiodiphosphate); GTP-γ-S, guanosine-5'-0-(3-thiotriphosphate); G-protein, guanyl nucleotide binding protein; KAsp, potassium aspartate; InsP₃, inositol-1,4,5-trisphosphate; pl, picoliter; TPI, phosphatidylinositol 4,5-bisphosphate.

GTP- γ -S into sea urchin eggs would suggest the involvement of a G-protein in the regulation of exocytosis. Guanosine-5'-0-(2-thiodiphosphate) (GDP- β -S) is a metabolically stable analog of GDP which acts as a competitive inhibitor of GTP at the regulatory site of G-proteins (10). Inhibition of sperm-stimulated exocytosis by injection of GDP- β -S would likewise suggest the involvement of a G-protein in the regulatory pathway. We also combined injections of these guanyl nucleotides with injections of EGTA and InsP₃, in order to investigate the sequence of events leading to exocytosis.

Materials and Methods

Gametes

Sea urchins (*Lytechinus variegatus*) were obtained from S. J. Decker (Hollywood, FL). Eggs and sperm were collected using electrical stimulation. For insemination, 100 μ l of a 1:100 dilution of dry sperm in natural sea water was added to the injection chamber, resulting in a final dilution of sperm of ~1:1,000. All experiments were conducted at 20–22°C.

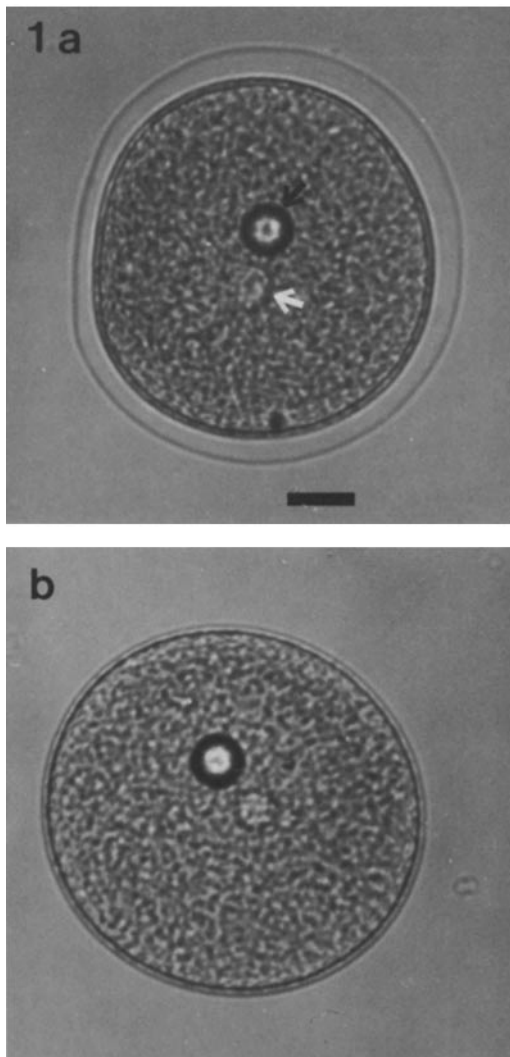


Figure 1. Fertilization envelope elevation caused by injection of InsP₃. (a) An egg which was injected with 7 pl of 2 μ M InsP₃, in 100 mM KAsp/10 mM Hepes buffer (pH 7.0), resulting in a final concentration of InsP₃ of 28 nM. The injected oil droplet (black arrow) and female pronucleus (white arrow) are visible. (b) A control egg which was injected with 7 pl of 100 mM KAsp/10 mM Hepes buffer (pH 7.0). Bar, 25 μ m.

Microinjection

The microinjection method was as described by Hiramoto (19) and Kiehart (24). The injection chamber was made by attaching a small rectangle of coverslip glass to another coverslip with a spacer of double-stick tape, leaving a gap in which the eggs were held. This assembly was attached, rectangle side down, to the upper surface of a plastic support slide. Another coverslip was attached to the bottom of the support slide, and the completed chamber was filled with natural sea water. The chamber was placed on the stage of an upright Zeiss microscope, in such a way that a micropipette could be brought in from one side. Cells were viewed using a 10 \times objective and were photographed on Kodak 35-mm Technical Pan film (TP 2415) (Eastman Kodak Co., Rochester, NY). A glass micropipette, drawn on a pipette puller from a Pyrex glass capillary of 1.0 mm O.D. (World Precision Instruments, Inc., New Haven, CT), was backfilled with a small drop of mercury. The micropipette was fitted into a microinstrument holder (E. Leitz, Inc., Rockleigh, NJ) attached to a micromanipulator. A micrometer syringe (Gilmont Instruments, Inc., Great Neck, NY) containing mineral oil was connected to the pipette holder by way of Teflon tubing.

A glass capillary was filled with the fluid to be injected, capped with vegetable oil and attached to the support slide. The tip of the micropipette was broken back on the edge of the capillary to a diameter of \sim 1 μ m. Pressure was applied to drive the mercury to the tip, and the tip was moved into the oil in the capillary. A volume of oil equal to the volume of fluid to be injected was taken into the pipette. The pipette was moved into the injection fluid, loaded with the desired volume, and then capped with oil. The fully loaded pipette was pushed into an egg and the cap of oil and aqueous solution were injected. The pipette was rapidly pulled out, and the remaining volume of oil was expelled into the seawater. The radius of this oil drop was measured to determine the volume injected. In practice, each pipette was precalibrated with oil, allowing predetermined volumes to be loaded into the pipettes. Volumes injected were 3–14 picoliters (pl), which represents 0.6–2.8% of the total egg volume of \sim 500 pl (egg diameter is \sim 100 μ m).

Solutions

InsP₃ was generously provided to us by Dr. R. F. Irvine (Department of Biochemistry, AFRC Institute of Animal Physiology, Babraham, Cambridge CB2 4AT, England). InsP₃, GTP- γ -S (Boehringer Mannheim Biochemicals, Indianapolis, IN), and GDP- β -S (Boehringer Mannheim Biochemicals) were dissolved in a buffer of 100 mM potassium aspartate (KAsp) and 10 mM Hepes (pH 7.0). CaEGTA/EGTA solutions were prepared by mixing stocks of CaEGTA (0.2 M) and EGTA (0.2 M) in the ratios given in the tables and figure legends. CaEGTA stock was made by mixing equal volumes of EGTA and calcium chloride. All CaEGTA and EGTA solutions also contained 0.2 M Pipes, and were adjusted to pH 7.0 with KOH. For some experiments we used a derivative of EGTA, 1,2-bis(0-aminophenoxy)ethane-*N,N,N',N'*-tetraacetic acid (BAPTA). CaBAPTA/BAPTA solutions (Gallard-Schlesinger Chemical Mfg Corp., Carle Place, NY) were prepared by mixing stocks of CaBAPTA (0.2 M) and BAPTA (0.2 M) in a ratio of 1:3 and did not contain Pipes; pH was adjusted to 7.0 with KOH.

Results

Injection of InsP₃ Caused Exocytosis

Injection of eggs of the sea urchin *Lytechinus variegatus* with InsP₃ at a final concentration of \sim 28 nM, caused complete exocytosis of cortical vesicles, indicated by full elevation of the fertilization envelope in 40/40 trials (Fig. 1a; Table I). Elevation began a few seconds after the injection, often proceeding from several points on the egg surface. If the injection site was in the cortex of the egg, rather than in the center, elevation was often first observed near the injection site.

For the interpretation of subsequent experiments, it was important to determine the concentration dependence of the InsP₃ effect (Fig. 2). A final concentration of InsP₃ of 28 nM resulted in exocytosis in 100% of cases. With final concentrations of InsP₃ below 28 nM, the fraction of eggs with fully elevated envelopes decreased. Some eggs exhibited partial elevation, that is elevation around only a portion of the egg surface. 50% of the eggs showed full elevation with a final InsP₃ concentration of 5–10 nM. At a final concentration of 0.2 nM, no elevation was seen. These results are similar to

Table I. Effects of Calcium Buffers on *InsP₃* Stimulation of Exocytosis

Solution*	Volume	Stock [‡]	Final [§]	Fraction of envelopes elevated
1 <i>InsP₃</i>	7 pl	2 μ M	28 nM	40/40
2a KAsp/Hepes	7 pl	100 mM/10 mM	1.4 mM/0.14 mM	0/10
2b <i>InsP₃</i>	7 pl	2 μ M	28 nM	10/10
3a Pipes	4 pl	0.2 M	1.6 mM	0/10
3b <i>InsP₃</i>	7 pl	2 μ M	28 nM	10/10
4a EGTA	4 pl	0.2 M	Ca ²⁺ <0.1 μ M EGTA = 1.6 mM	0/10
4b <i>InsP₃</i>	7 pl	2 μ M	28 nM	0/10
5a CaEGTA/EGTA	4 pl	0.2 M	Ca ²⁺ = 0.1 μ M EGTA = 1.6 mM	0/10
5b <i>InsP₃</i>	7 pl	2 μ M	28 nM	0/10
6a CaEGTA/EGTA	4 pl	0.2 M	Ca ²⁺ = 0.1 μ M EGTA = 1.6 mM	0/10
6b CaEGTA	4 pl	0.2 M	Ca ²⁺ = 0.5 μ M EGTA = 3.2 mM	10/10
7a CaBAPTA/BAPTA	4 pl	0.2 M	Ca ²⁺ = 0.25 μ M BAPTA = 1.6 mM	0/5
7b <i>InsP₃</i>	7 pl	2 μ M	28 nM	0/5

* For double injections, *a* indicates the first injection, and *b* the second. Solutions of *InsP₃* also contained 100 mM KAsp and 10 mM Hepes, pH 7.0. Solutions of EGTA and CaEGTA also contained 0.2 M Pipes, pH 7.0. Solutions of KAsp/Hepes and Pipes were adjusted to pH 7.0.

[‡] Stocks of CaEGTA/EGTA and CaBAPTA/BAPTA contained a 1:3 mixture of the two components, each at 0.2 M.

[§] Final concentrations of Ca²⁺ in EGTA solutions were calculated as described by Hamaguchi and Hiramoto (18). Final concentrations of Ca²⁺ in BAPTA solutions were calculated using an equilibrium constant of 0.76 μ M, as determined for the closely related compound Fura-2, at an ionic strength of 250 mM (reference 17).

^{||} Envelopes scored as elevated were fully elevated; no partial elevations were observed.

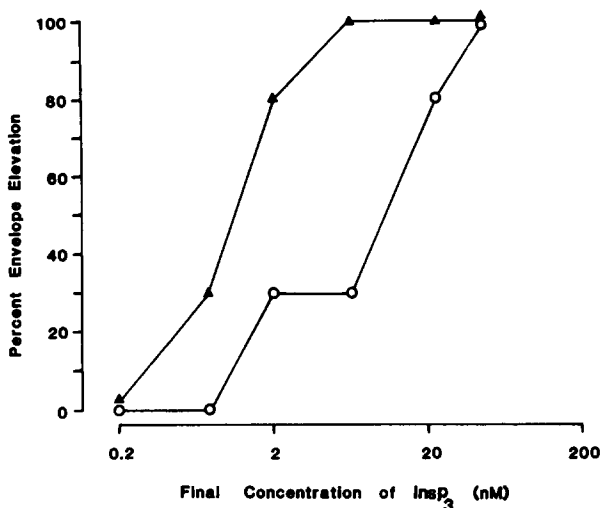


Figure 2. Concentration dependence of *InsP₃*-induced envelope elevation. The open circles represent the percentage of eggs whose envelopes elevated around the entire egg, as in Fig. 1a, and the triangles represent the percentage of eggs showing either partial or complete envelope elevation. Percentages at each concentration represent the results from 10 injected eggs, except for those at 0.2 nM *InsP₃*, which are for six eggs, and for those at 28 nM *InsP₃*, which are for 40 eggs.

those of Whitaker and Irvine (43), who found 50% of the eggs of *L. pictus* elevated envelopes with a final *InsP₃* concentration of ~4 nM.

Injection of a comparable amount of buffer only did not result in exocytosis (Fig. 1b; Table I). When injection of buffer was followed by injection of *InsP₃* in buffer, the fraction of eggs elevating envelopes was 100% (Table I). These observations indicate that envelope elevation results from injection of *InsP₃*, and not buffer, and that 100% elevation can be achieved using a final *InsP₃* concentration of 28 nM.

Intracellular Calcium Buffers Inhibited the Stimulation of Exocytosis by *InsP₃*

If the free calcium level of a sea urchin egg is buffered below 0.1 μ M, sperm can no longer stimulate exocytosis (18, 45). To test the hypothesis that *InsP₃* causes exocytosis by raising the $[Ca_i]$, we pre-injected eggs with buffer to maintain $[Ca_i]$ below 0.1 μ M, and then injected *InsP₃*. Injection of 4 pl of 0.2 M EGTA (final EGTA = 1.6 mM), followed by injection of 7 pl of 2 μ M *InsP₃* (final *InsP₃* = 28 nM), resulted in no envelope elevation (Table I). Since injection of EGTA might lower $[Ca_i]$ to an unphysiological and possibly damaging level, we injected eggs with a mixture of CaEGTA and EGTA calculated to maintain $[Ca_i]$ at 0.1 μ M (assuming no effect of the cell's inherent calcium buffering capacity). When such eggs were subsequently injected with *InsP₃*, fertilization envelope elevation did not occur (Fig. 3a; Table I). However, if the EGTA-injected egg was given a second injection of CaEGTA calculated to bring $[Ca_i]$ to 0.5 μ M, envelope elevation occurred (Fig. 3b, Table I). These results indicated that buffering $[Ca_i]$ at 0.1 μ M prevents the stimulation of exocytosis by *InsP₃*. The effect cannot be explained by a deleterious effect of the EGTA or double-injection procedure.

To minimize effects of the release of protons when EGTA binds Ca²⁺, we included Pipes buffer in our EGTA solutions (pH 7.0). Control injections of the Pipes buffer alone did not prevent *InsP₃* from causing envelope elevation (Table I). We also injected eggs with BAPTA, a derivative of EGTA which does not release protons upon chelating calcium (39). After injection of a mixture of CaBAPTA and BAPTA which buffered $[Ca_i]$ at ~0.25 μ M, *InsP₃* injection did not stimulate exocytosis (Table I).

Injection of GTP- γ -S Caused Exocytosis

To investigate the possibility that a G-binding protein might be a component of the stimulatory pathway leading to exo-

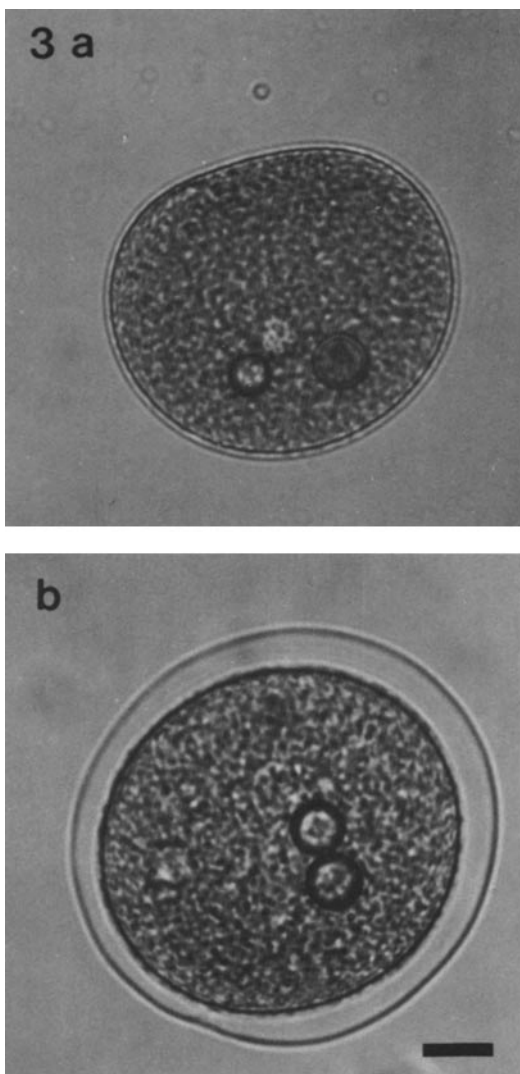


Figure 3. Inhibition of InsP_3 -induced exocytosis by pre-injection with CaEGTA buffer. (a) This egg was pre-injected with 4 pl of 0.2 M CaEGTA/0.2 M EGTA, mixed in the ratio of 1:3, to buffer free calcium at $0.1 \mu\text{M}$ (1.6 mM EGTA). It was then injected with 7 pl of $2 \mu\text{M}$ InsP_3 , in 100 mM KAsp/10 mM HEPES, resulting in a final InsP_3 concentration of 28 nM. The two oil droplets indicate a double injection. (b) This egg was pre-injected with 4 pl of 0.2 M CaEGTA/0.2 M EGTA, mixed in the ratio of 1:3, to buffer free calcium at $0.1 \mu\text{M}$ (1.6 mM EGTA). It was then injected with 4 pl of 0.2 M CaEGTA, to raise the free calcium to $0.5 \mu\text{M}$. After the initial injection, no envelope elevation was seen; after the second injection however, the envelope elevated fully. Bar, 25 μm .

cytosis after fertilization, we microinjected eggs with GTP- γ -S, a hydrolysis-resistant analog of GTP. Injection of GTP- γ -S at a final concentration of $28 \mu\text{M}$ caused complete elevation of the fertilization envelope in 10/10 trials (Fig. 4a; Table II). Elevation sometimes began within a few seconds after injection, but in other trials it began after a delay of up to 1–2 min. The concentration dependence of the GTP- γ -S stimulation of exocytosis is shown in Fig. 5. With a final concentration of GTP- γ -S of $28 \mu\text{M}$, envelopes elevated fully on 100% of the eggs. With a final concentration of GTP- γ -S of $3 \mu\text{M}$, envelopes elevated fully on 50% of the eggs. At final concentrations of below $0.4 \mu\text{M}$, no elevation, partial or complete, was seen. These results indicate that a G-binding

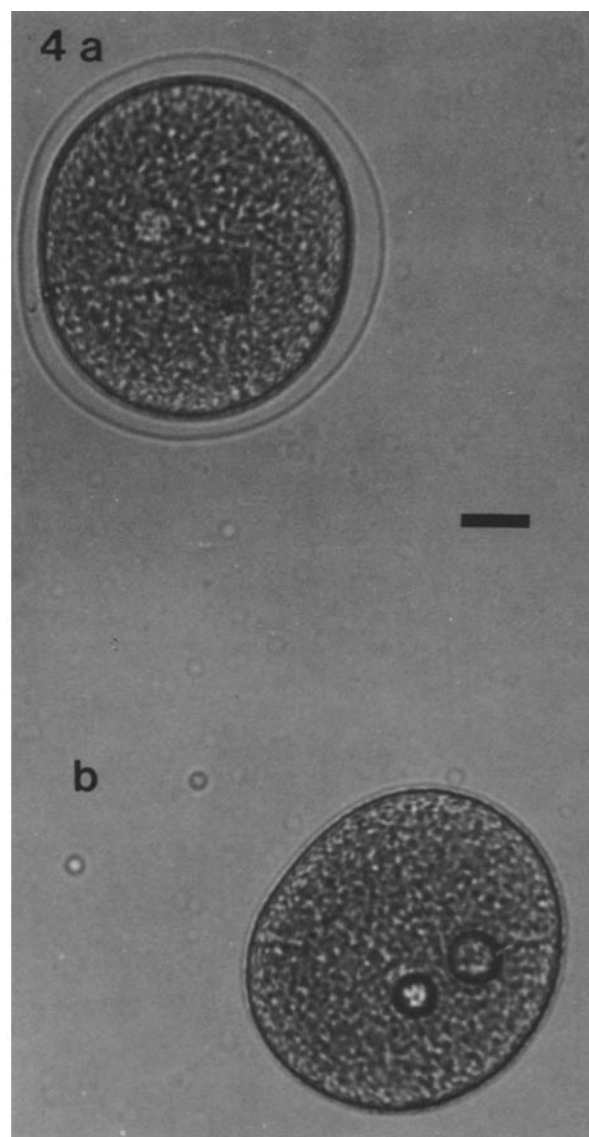


Figure 4. Calcium-dependent exocytosis stimulated by GTP- γ -S. (a) An egg which was injected with 7 pl of 2 mM GTP- γ -S (GTP- γ -S = $28 \mu\text{M}$). GTP- γ -S stock also contained 100 mM KAsp and 10 mM HEPES, pH 7.0. (b) An egg which was first injected with 4 pl of a 1:3 mixture of 0.2 M CaEGTA and 0.2 M EGTA ($\text{Ca}^{2+} = 0.1 \mu\text{M}$; EGTA = 1.6 mM), and then injected with 7 pl of 2 mM GTP- γ -S (GTP- γ -S = $28 \mu\text{M}$). Bar, 25 μm .

Table II. Effects of GTP- γ -S and GDP- β -S on Exocytosis

Solution*	Volume	Stock	Final	Fraction of envelopes elevated†
1 GTP- γ -S	7 pl	2 mM	$28 \mu\text{M}$	10/10
2a CaEGTA/EGTA	4 pl	0.2 M	$\text{Ca}^{2+} = 0.1 \mu\text{M}$ EGTA = 1.6 mM	0/10
2b GTP- γ -S	7 pl	2 mM	$28 \mu\text{M}$	0/10
3a GDP- β -S	3 pl	0.5 M	3 mM	0/20
3b Sperm	—	—	—	0/20
4a GDP- β -S	3 pl	0.5 M	3 mM	0/12
4b InsP_3	7 pl	$2 \mu\text{M}$	28 nM	11/12

* For double injections, a indicates the first injection, and b the second. GTP- γ -S and GDP- β -S stock solutions were made in 100 mM KAsp and 10 mM HEPES, pH 7.0. Other solutions as in Table I.

† Envelopes scored as elevated were fully elevated; one case of partial elevation was seen (4b). 11/12 elevated fully, 1 elevated partially.

protein may be involved in the pathway leading to exocytosis at fertilization; injection of GTP- γ -S presumably results in activation of this protein.

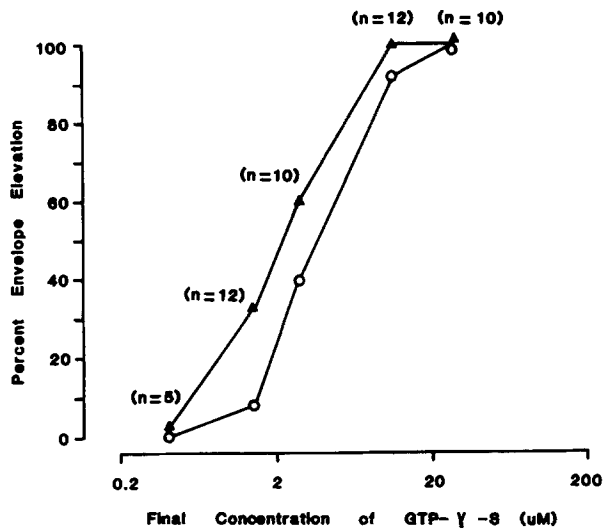


Figure 5. Concentration dependence of GTP- γ -S-induced exocytosis. Open circles represent the percentage of eggs showing completely elevated envelopes; triangles represent the percentage showing any elevation. The number of trials at each concentration is indicated in parentheses.

Intracellular Calcium Buffers Inhibited the Stimulation of Exocytosis by GTP- γ -S

If GTP- γ -S stimulates exocytosis by the same pathway as InsP₃, pre-injection of eggs with calcium buffers should prevent GTP- γ -S from stimulating exocytosis. To test this hypothesis, eggs were injected with CaEGTA/EGTA buffer, resulting in a [Ca_i] of 0.1 μ M (Table II). Upon subsequent injection of GTP- γ -S, no envelope elevation was seen (Fig. 4b; Table II). These results suggest that both InsP₃ and GTP- γ -S increase the level of intracellular free calcium, which thereby leads to exocytosis.

Injection of GDP- β -S Inhibited the Stimulation of Exocytosis by Fertilization

If a G-binding protein is involved in the transduction mechanism leading to exocytosis, then injection of GDP- β -S, a metabolically stable analog of GDP and a competitive inhibitor of GTP at the regulatory site of the G-binding protein, might block exocytosis. To test this hypothesis, eggs were injected with GDP- β -S and subsequently inseminated. Injection of GDP- β -S at a final concentration of 3 mM caused no envelope elevation (Table II), and no change in egg morphology. When sperm were added in sufficient concentration to cause envelope elevation in all neighboring eggs, 0/20 eggs injected with GDP- β -S elevated envelopes (Fig. 6a, Table II). At concentrations below 3 mM, GDP- β -S did not prevent

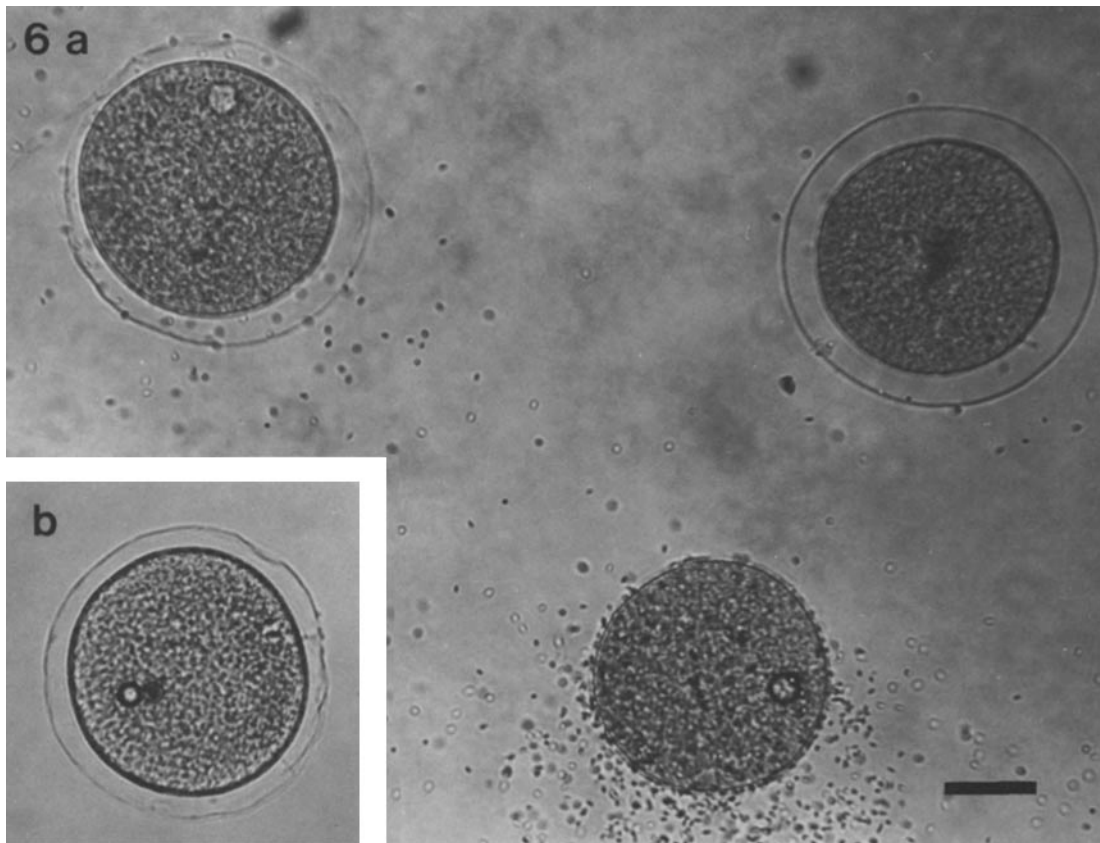


Figure 6. GDP- β -S effects on exocytosis stimulated by sperm or InsP₃. (a) The lower egg was injected with 3 μ l of 0.5 M GDP- β -S (GDP- β -S = 3 mM); the two upper eggs are uninjected controls. After insemination, both control eggs elevated fertilization envelopes, but the injected egg did not. The specks around the eggs are sperm heads. The GDP- β -S stock also contained 100 mM KAsp and 10 mM HEPES. (b) An egg which was injected with 3 μ l of 0.5 M GDP- β -S (GDP- β -S = 3 mM), and which was then injected with 7 μ l of 2 μ M InsP₃ (InsP₃ = 28 nM). The fertilization envelope elevated after the injection of InsP₃. Bar, 50 μ m.

sperm from stimulating exocytosis, whereas at a final concentration of 3 mM, GDP- β -S completely prevented sperm from stimulating exocytosis (Fig. 7).

GDP- β -S Did Not Inhibit the Stimulation of Exocytosis by InsP₃

If the step in the transduction pathway involving the G-binding protein precedes the InsP₃-dependent step, then injection of InsP₃ into eggs previously injected with GDP- β -S should result in exocytosis. When such eggs were injected with InsP₃, envelopes elevated in 12/12 trials (Fig. 6*b*; Table II). The envelopes elevated fully in 11/12 trials and partially in the other. This result indicates that although GDP- β -S blocks sperm from stimulating exocytosis, it does not block the stimulation by InsP₃.

When eggs were injected with GDP- β -S, then inseminated, and then injected with InsP₃, only 1 out of 7 eggs elevated an envelope. This somewhat surprising result indicates that although fertilization does not induce exocytosis in a GDP- β -S-injected egg, it alters the egg's ability to respond to InsP₃. In summary, our injection results suggest that a G-binding protein participates in the transduction pathway between fertilization and exocytosis, and does so before the InsP₃-dependent step.

Discussion

Injection of InsP₃ into unfertilized sea urchin eggs at a final concentration of 28 nM caused exocytosis of cortical vesicles. This effect of InsP₃ was blocked by buffering intracellular free calcium at or below 0.1 μ M, suggesting that InsP₃ is acting by releasing calcium. A similar result has been reported for starfish eggs (30). Injection of 28 μ M GTP- γ -S stimulated exocytosis, and this effect was also blocked by buffering [Ca_i] at 0.1 μ M. Thus both agents appear to cause exocytosis by releasing calcium. GDP- β -S (3 mM) prevented sperm but not InsP₃ from stimulating exocytosis, suggesting that the step involving the G-binding protein occurs before the InsP₃-dependent step.

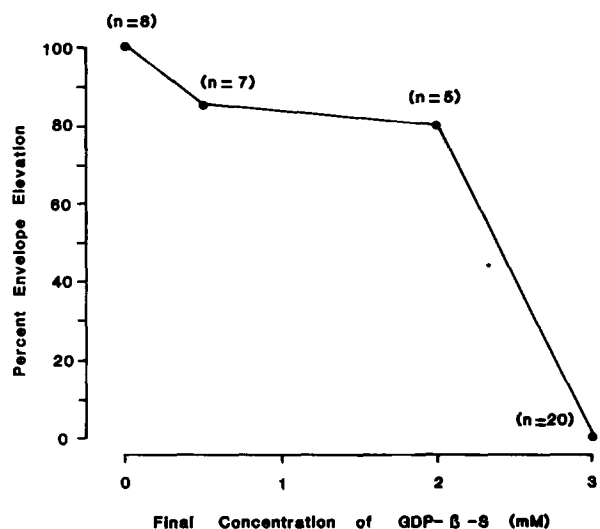


Figure 7. Concentration dependence of the inhibition of exocytosis by GDP- β -S. Eggs were injected with GDP- β -S at various concentrations, and then inseminated. The GDP- β -S stock also contained 100 mM KAsp and 10 mM Hepes, pH 7.0. The number of trials at each concentration is indicated in parentheses.

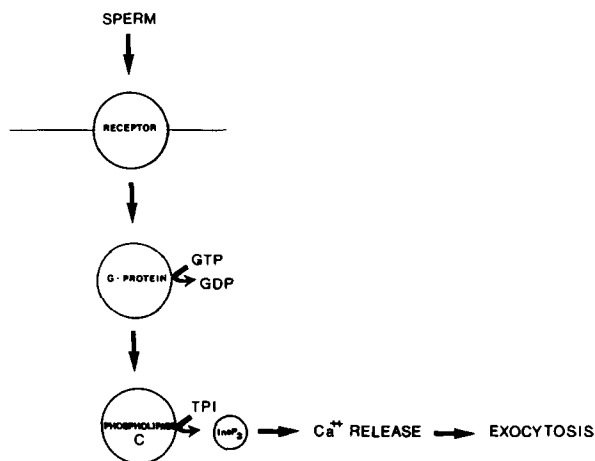


Figure 8. Pathway by which sperm might cause exocytosis of cortical vesicles in the sea urchin egg.

These results led us to propose the model in Fig. 8. The sperm, acting at the plasma membrane, stimulates a G-protein, perhaps by way of a receptor in the plasma membrane. The active form of the protein (with GTP bound) stimulates the production of InsP₃. InsP₃ then causes the release of calcium from intracellular stores, and the elevated calcium causes fusion of vesicles with the plasma membrane as well as initiating other aspects of further embryonic development.

Exocytosis proceeds from the site of sperm egg fusion around the surface of the egg (5, 32). How could the model explain propagation? If the initial stimulation of InsP₃ production was close to the site of sperm fusion, levels of InsP₃ would elevate initially in that region and lead to a local release of calcium. As proposed by Whitaker and Irvine (43), the increased level of calcium might then promote the production of InsP₃ in adjacent regions of the egg, possibly through stimulation of phospholipase C (20); this could lead to further release of calcium. Alternatively, propagation of the exocytotic wave might be a consequence of calcium-stimulated calcium release, as suggested by Ridgway et al. (31) and Busa et al. (4).

Does the egg contain enough TPI to generate levels of InsP₃ sufficient to cause exocytosis? An unfertilized sea urchin egg contains about 6 μ M TPI (40). This seems sufficient, since microinjection of ~5–10 nM InsP₃ causes exocytosis. Increased production of cytosolic InsP₃ prior to or concomitant with exocytosis remains to be demonstrated, although a rise in InsP₃ production at 1 min postfertilization has been observed (23).

How might production of InsP₃ be increased after fertilization? The fact that TPI of the unfertilized egg can be labeled with ³²P indicates turnover of this lipid (40). Hydrolysis of TPI is therefore occurring at some basal rate, presumably by way of the phosphodiesterase, phospholipase C. Thus one possible mechanism which could lead to increased production of InsP₃ would be to increase the amount of substrate for this enzyme. Consistent with this hypothesis, there is an increase in the amount of TPI present in the sea urchin egg before exocytosis (40). The G-protein could regulate the production of TPI by interaction with the appropriate kinases. Alternatively the activity of phospholipase C could be increased. Phospholipase C activity could be regulated by the G-protein, a proposal consistent with the model presented by Berridge and Irvine (2).

How InsP_3 might release calcium from stores within the egg, or how calcium release is linked to exocytosis is not known. Although calcium is required for exocytosis in the sea urchin egg, it is not by itself sufficient. ATP is also required (1), calmodulin may (26, 35, 42), or may not (29) be involved. Other evidence suggests the importance of proteins (21, 33) and vesicle swelling (44) in the pathway between the rise in calcium and exocytosis.

The sequence of events leading to calcium release in the sea urchin egg at fertilization is important not only for exocytosis but also for further embryonic development (36). The results presented in this paper provide evidence for the involvement of a G-protein and InsP_3 in this sequence.

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