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A Cytosolic Sperm Protein Factor Mobilizes Ca^{2+} from Intracellular Stores by Activating Multiple Ca^{2+} Release Mechanisms Independently of Low Molecular Weight Messengers*

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Ca^{2+} oscillations can be induced in mammalian eggs and somatic cells by microinjection of a cytosolic sperm protein factor. The nature of the sperm factor-induced Ca^{2+} signaling was investigated by adding sperm protein extracts to homogenates of sea urchin eggs, which contain multiple classes of Ca^{2+} release mechanisms. We show that the sperm factor mobilizes Ca^{2+} from non-mitochondrial Ca^{2+} stores in egg homogenates after a distinct latency. This latency is abolished by preincubation of sperm extracts with egg cytosol. The preincubation step is highly temperature-dependent and generates a high molecular weight, protein-based Ca^{2+}-releasing agent that can also mobilize Ca^{2+} from purified egg microsomes. This Ca^{2+} release appears to be mediated via both inositol 1,4,5-trisphosphate and ryanodine receptors, since homologous desensitization of these two release mechanisms by their respective agonists inhibits further release by the sperm factor. However, sperm factor-induced Ca^{2+} release by these channels is independent of inositol 1,4,5-trisphosphate or cADPR since antagonists of either of these two messengers did not block the Ca^{2+} release effected by the sperm factor. The sperm protein factor may cause Ca^{2+} release via an enzymatic step that generates a protein-based Ca^{2+}-releasing agent.

At fertilization in mammals and some marine invertebrates, the sperm activates the egg by inducing a series of Ca^{2+} oscillations involving release from intracellular stores (1, 2). In eggs, the propagation of Ca^{2+} waves is thought to be mediated by Ca^{2+}-induced Ca^{2+} release operating via inositol 1,4,5-trisphosphate ($InsP_3$)[1]-sensitive Ca^{2+} channels (1, 3, 4), possibly in concert with ryanodine receptors (RyRs) (3–5). The sequence of events leading to the initiation of Ca^{2+} oscillations is unclear (6). One hypothesis for signaling at fertilization proposes that sperm-egg fusion allows the diffusion of a soluble cytosolic sperm factor into the egg cytoplasm which leads to the activation of Ca^{2+} release (7, 8). This is consistent with the finding that sperm-egg membrane fusion occurs before Ca^{2+} release in the eggs of sea urchins and mice (9, 10). Furthermore, injecting cytosolic sperm extracts into mouse, hamster, bovine, human, and nemertean worm eggs cause Ca^{2+} oscillations similar to those seen at fertilization in each species (7, 11–15). Injecting intact sperm also leads to Ca^{2+} oscillations in human and nemertean worm eggs (16, 17). The factor that causes these Ca^{2+} oscillations is only found in sperm, is only effective when injected into the egg, and has been shown to be a high molecular weight protein (7, 12–15). Ca^{2+} oscillation-inducing activity has been correlated with a soluble protein made up from 33-kDa subunits, which is concentrated inside the sperm head around the site where it fuses with the egg (18). However, it is unclear how this protein might mediate Ca^{2+} changes in eggs. In mouse eggs, the sperm factor-induced Ca^{2+} oscillations are maintained for short periods in Ca^{2+}-free media, suggesting that the sperm factor initiates release of Ca^{2+} from intracellular stores (11). However, there has been no direct demonstration that the sperm factor can trigger Ca^{2+} release from intracellular stores. Nor is it known if the sperm factor could activate both the $InsP_3$- and ryanodine-sensitive mechanisms that are implicated in some eggs at fertilization (3–5).

In this study, we have tested the hypothesis that sperm factor-induced Ca^{2+} signaling is based on its ability to mobilize Ca^{2+} from internal stores by directly adding boar sperm extracts to sea urchin egg homogenates, which can accumulate Ca^{2+} in the presence of an ATP-regenerating system and release Ca^{2+} via multiple Ca^{2+} release channels.

MATERIALS AND METHODS

Sperm extracts were prepared from the cytosol of boar sperm as described previously (7). This involved washing boar sperm into a KCl-based buffer (120 mM KCl, 20 mM HEPES, 1 mM EDTA, pH 7.5), lysing sperm by two cycles of freeze-thawing and then spinning lysates at 100,000 × *g* for 1 h at 4 °C. The supernatant from the high speed spin was then concentrated on Centricon C-30 membranes (Amicon Ltd, Stonehouse, Gloucestershire, United Kingdom (UK)). In some experiments, the supernatant was rediluted and concentrated several times to isolate the fraction of boar sperm cytosol that contained proteins greater than 30 kDa. The protein concentration of these extracts was 10–35 mg/ml, depending upon the batch of preparation. They were frozen and kept at −70 °C until the day of use. Each particular set of experiments (*e.g.* dose-response relationships, desensitization experiments) was carried out with the same batch of extract. Homogenates (2.5%) of unfertilized *Lytechinus pictus* eggs (Marinus, Inc., Long Beach, CA) were prepared as described previously (19), and Ca^{2+} loading was achieved by incubation at 17 °C for 3 h in an intracellular medium consisting of 250 mM potassium gluconate, 250 mM *N*-methylglucamine, 20 mM HEPES, pH 7.2, 1 mM $MgCl_2$, 1 mM ATP, 10 mM phosphocreatine, 10 unit/ml creatine phosphokinase, 1 μg/ml oligomycin, 1 μg/ml antimycin, 1 mM sodium azide, 3 μM fluo-3. Free Ca^{2+} concentration was measured by monitoring fluorescence intensity at excitation and emission wavelengths of 490 and 535 nm, respectively. Fluorimetry was performed at 17 °C using 500 μl of homogenate in a

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[1] The abbreviations used are: $InsP_3$, inositol 1,4,5-trisphosphate; RyR, ryanodine receptor; cADPR, cyclic adenosine diphosphate ribose.

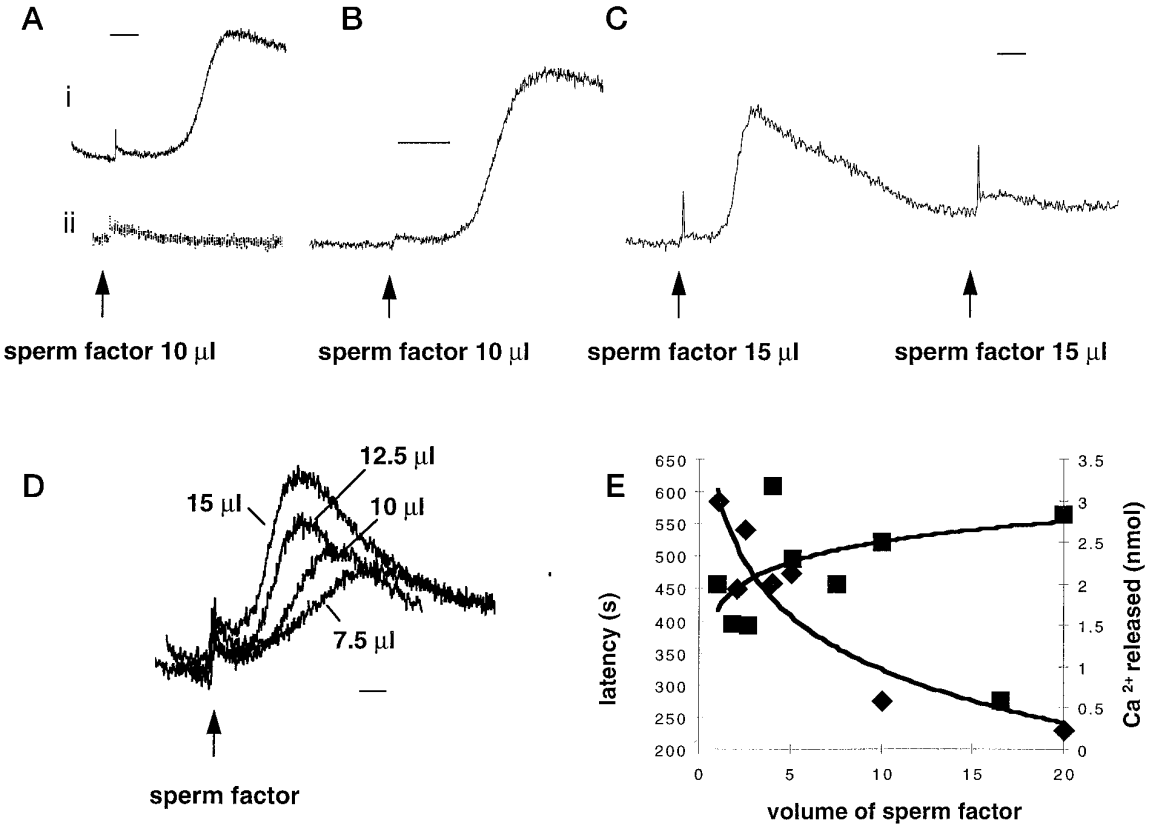

FIG. 1. **Sperm factor mobilizes Ca^{2+} from sea urchin and *Xenopus* egg homogenates.** *A*, *i*, the effect of the sperm factor (10 μl) on Ca^{2+} release from sea urchin homogenates (2.5%) at 17 °C measured by changes in fluo-3 fluorescence. Sperm factor solution was added at the time indicated by the *arrow*. *A*, *ii*, the effect of trypsin-pretreated sperm factor on Ca^{2+} release under the same conditions as in *i*. *B*, sperm factor mobilizes Ca^{2+} from *Xenopus* egg homogenates. The effect of sperm factor (10 μl) on Ca^{2+} release from *Xenopus* egg homogenates (10%) at 22 °C measured by changes in fluo-3 fluorescence. Sperm factor solution was added at the time indicated by the *arrow*. The *horizontal bars* indicate 5 min. *C*, effect of two subsequent additions of sperm factor on Ca^{2+} release from 2.5% sea urchin egg homogenates. After the first challenge with sperm factor (15 μl) followed by resequestration of Ca^{2+} a second addition of sperm factor (15 μl) failed to induce a second Ca^{2+} release. *D*, typical responses illustrating the dose-response relationships between varying the volume of sperm factor added to four separate cuvettes on fluo-3 fluorescence traces. Increasing volumes of sperm factor resulted in shortened latencies to the initiation of Ca^{2+} release and also to a higher maximal Ca^{2+} release. *E*, graphical summary of the effect of varying the volumes of sperm factor additions on the latencies (*diamonds*) and magnitude of Ca^{2+} release (*squares*). The amount of Ca^{2+} released was calibrated from fluorescent traces by adding known amounts of Ca^{2+} to a new cuvette containing 2.5% sea urchin egg homogenate from the same batch of extract. The *horizontal bars* indicate 5 min.

Perkin-Elmer LS-50B fluorimeter. Additions were made in 5-μl volumes, except where stated otherwise, and all chemicals were added in intracellular medium containing 10 μM EGTA and continuously stirred. Basal concentrations of Ca^{2+} were typically between 100 and 150 nM. Sequestered Ca^{2+} was determined by monitoring decrease in fluo-3 fluorescence during microsomal loading and by measuring Ca^{2+} release in response to ionomycin (5 μM) and was constant between experiments. For each experiment, standard Ca^{2+} calibration was performed by adding known amounts of Ca^{2+} (range of 0–8 nmol) to homogenates and monitoring fluorescence changes. *Xenopus* egg homogenates were prepared and used by a modified protocol of the above as described previously (4). Microsomes were purified from 50% egg homogenates by the Percoll density centrifugation method described previously (20). Briefly, a fractionating buffer was prepared by diluting Percoll stock to 25% in a modified intracellular medium (333 mM *N*-methylglucamine, 333 mM potassium acetate, 27 mM HEPES, 1.3 mM $MgCl_2$, pH titrated to 7.2 with acetic acid). This was then supplemented with 0.5 mM ATP, 2 units/ml creatine phosphokinase, 4 mM phosphocreatine, 10 μM EGTA. 1 ml of 50% egg homogenate was layered on 9 ml of this solution and centrifuged at 27,000 × *g* for 30 min at 15 °C. The top fraction (1 ml) was collected and represented the supernatant fraction. The Ca^{2+}-storing, $InsP_3$, and cADPR-sensitive microsomes formed a distinct tight band halfway down the tube. 1 ml of this was collected using a disposable syringe to puncture the vessel wall. The fractions were aliquoted and stored at −70 °C until use.

Sperm factor extracts were incubated with sea urchin egg cytosol supernatant or microsomal fractions prepared on Percoll gradients as described above, in a 1:2 ratio, respectively, at 24 °C, except where stated otherwise. Aliquots of the resulting mixtures were added by pipette to sea urchin egg homogenates or microsomal fractions to examine their Ca^{2+} releasing activities. In some cases, the resulting mixtures were further fractionated using a series of 3-, 10-, and 100-kDa cut-off filters (Amicon), in which the mixture was placed on top of the filtration columns and microcentrifuged for 10–30 min at 4 °C. Both resulting filtrate and retentate fractions from each of these columns were tested for their Ca^{2+} releasing activities. Sperm factor extracts or fractions derived from incubation and filtration protocols were treated with by dilution into buffer containing trypsin (100 μg/ml) for 30 min at room temperature, before reconcentration back to the original volume (7). Aliquots of the resulting mixture were then tested for activity in the egg homogenates and microsomal fractions. 8-NH_2-cADPR and fluo-3 were from Molecular Probes; fluo-3 was from Calbiochem (Molecular Probes); all other chemicals were from Sigma. Chemicon cut-off filters were obtained from Amicon, Beverly, MA.

RESULTS AND DISCUSSION

Diluted sea urchin egg homogenates (2.5% v/v) containing the fluorescent Ca^{2+} indicator, fluo-3, and mitochondrial inhibitors were allowed to sequester Ca^{2+} in the presence of an ATP-regenerating system over 3 h at 17 °C. After a stable base line of fluorescence had been attained, aliquots of soluble cytoplasmic (>30 kDa) extracts from boar sperm were added to the cuvette. Fig. 1*A* (*i*) shows that a delayed and transient increase in the fluorescence signal occurred after sperm extract addition, indicating that the sperm extracts contained a Ca^{2+} mobilizing activity. The latencies of Ca^{2+} release were at least 100 s, and after this release the Ca^{2+} was resequestered (Fig. 1*A*, *i*). A protein component rather than a small molecular

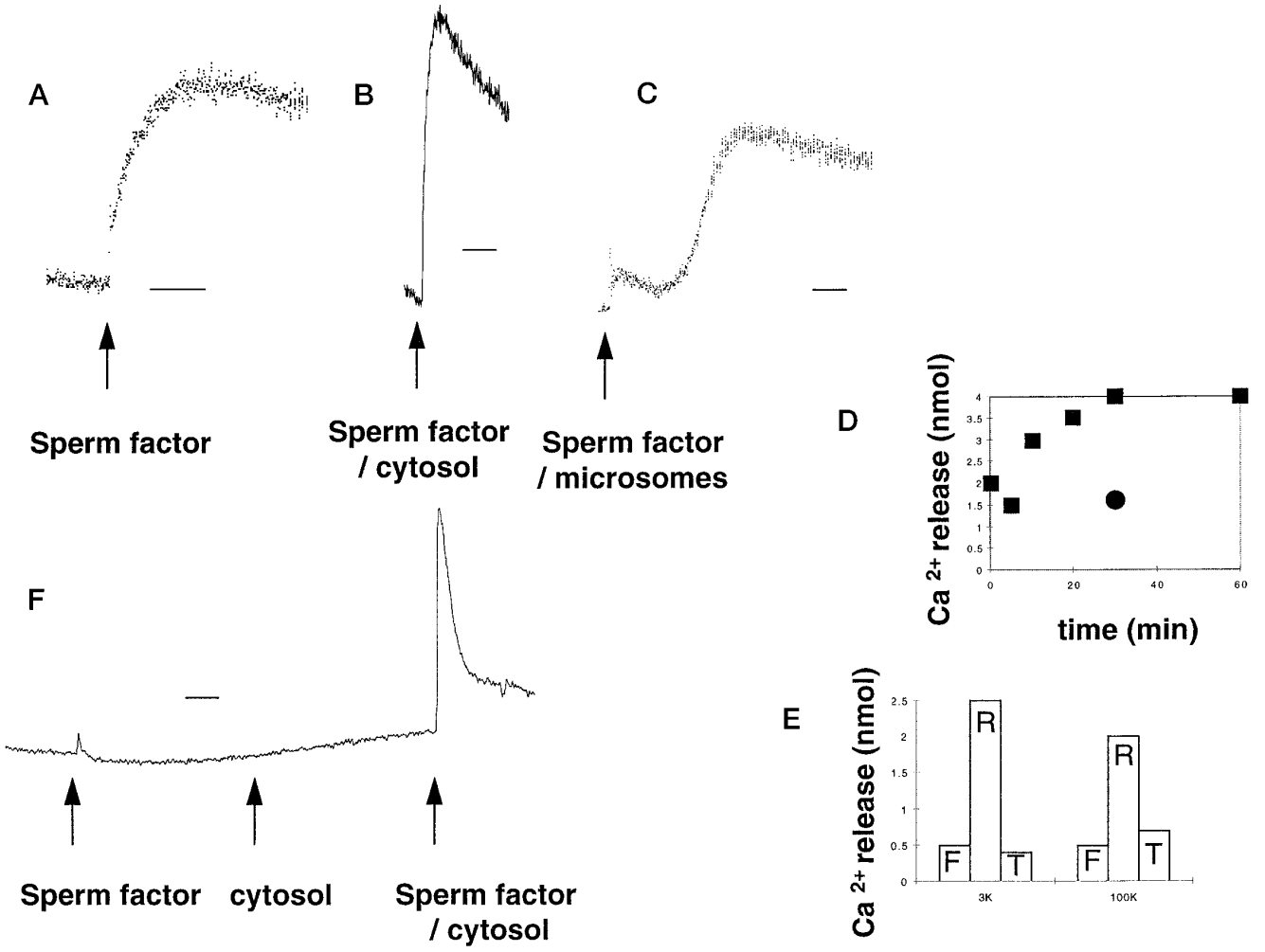

FIG. 2. **Requirement for cytosolic factors for sperm factor-induced Ca^{2+} release.** *A*, sperm factor-induced Ca^{2+} release from a concentrated (50%) sea urchin egg homogenate. Sperm factor (10 μl) caused an immediate rise in fluorescence with no discernible latency. *B*, preincubation of the sperm factor with supernatant from Percoll-fractionated sea urchin egg homogenate abolishes the latency and increases Ca^{2+} release. 10 μl of sperm factor was incubated for 30 min with 20 μl of egg supernatant, and then the mixture was added to 2.5% egg homogenates. Up to 50 μl of supernatant alone kept at room temperature for 30 min had no discernible effect on Ca^{2+} release (data not shown). *C*, 10 μl of sperm factor was incubated for 30 min with 20 μl of egg microsomes and then the total mixture was added to 2.5% egg homogenates. A prominent latency to Ca^{2+} release was observed similar to Fig. 1*A* without any preincubation or additions of egg fractions. *D*, time dependence of sperm factor incubation on the amount of Ca^{2+} released (*squares*) by sperm factor:supernatant mixtures in a 1:2 ratio, respectively, for a total addition of 30 μl. Incubations were performed at room temperature. The effect of lowering the temperature to 4 °C is shown for a single time point (*circle*) at 20 min. *E*, size fractionation of sperm factor/supernantant incubates on their Ca^{2+} releasing properties. Sperm factor (60 μl) was incubated with supernatant (120 μl) as in *C* and then separated using 3-kDa or 100-kDa cut-off filters. 30 μl of either filtrate (*F*) or retentate (*R*) were then tested for Ca^{2+} release activities. The most active fractions were the 3-kDa and 100-kDa retentates. However, when aliquots of both these fractions were incubated with trypsin (*T*), their Ca^{2+} mobilizing activities were lost. *F*, the effect of sperm factor on Ca^{2+} release from Percoll-purified microsomes. Sperm factor alone was unable to release Ca^{2+} from 5% microsomal suspensions. However, sperm factor preincubated with supernatant as in *B* elicited a large release. Supernatant cytosolic fractions (20 μl) had no effect on Ca^{2+} release *per se*. The *horizontal bars* indicate 5 min.

weight component was likely to be responsible for this effect since sperm extracts preincubated with trypsin at room temperature abolished the sperm extract-induced Ca^{2+} release (Fig. 1*A*, *ii*), but the same homogenate still responded with a characteristically rapid Ca^{2+} release to $InsP_3$ or cADPR (data not shown), known low molecular weight Ca^{2+} mobilizing messengers active in this system (19, 21). Fig. 1*B* shows that sperm extract also induced Ca^{2+} release from *Xenopus* egg homogenates (4) after a latency similar to that seen in sea urchin egg homogenates, indicating that the effects of the sperm factor are not specific to the sea urchin egg system. The release mechanism activated by the sperm factor underwent a desensitization process since subsequent application of sperm factor following Ca^{2+} resequestration failed to induce a significant second Ca^{2+} increase (Fig. 1*C*). Sperm extract-induced Ca^{2+} release was dependent on the amount of sperm factor added, with the magnitude of Ca^{2+} release increasing and latency decreasing at higher concentrations (Fig. 1, *D* and *E*).

The latent period suggested that sperm factor-induced Ca^{2+} release is indirect and that a biochemical reaction is initiated by the sperm factor perhaps requiring cofactors in the homogenate either as substrates or components of the signaling pathway. We examined the effect of sperm extract on Ca^{2+} release from different dilutions of egg homogenates since these would contain varying concentrations of cofactors that might be necessary for sperm extract-induced Ca^{2+} release. At higher homogenate concentrations (*e.g.* 50% v/v), the latency for sperm extract-induced Ca^{2+} release for the same volume of sperm extract addition was reduced (Fig. 2*A*), when compared with that in the standard 2.5% (v/v) assay system in Fig. 1. These data suggest that egg homogenate components play a facilitatory role in the sperm factor-sensitive Ca^{2+} release mechanism.

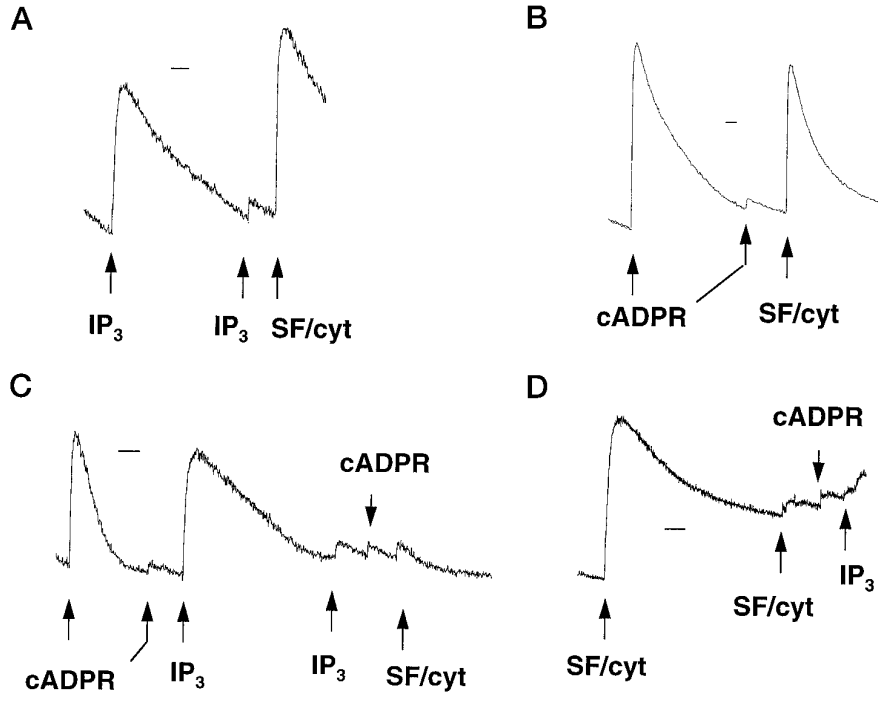

FIG. 3. **The interactions between $InsP_3$ and cADPR-induced Ca^{2+} release and Ca^{2+} release by preincubated sperm factor with supernatant.** *A*, prior release and desensitization of egg homogenates to $InsP_3$ (1 μM) still allowed Ca^{2+} release by the sperm factor/supernatant (30 μl, total addition) mixtures preincubated for 30 min in a 1:2 ratio at room temperature. Similar results were also seen for homogenates previously desensitized to cADPR by two sequential additions of cADPR (500 nM) to release by sperm factor/supernatant (30 μl) (*B*). The *horizontal bars* indicate 5 min. *C*, the effect of desensitizing homogenates to both $InsP_3$ and cADPR by two sequential additions of $InsP_3$ (1 μM) and cADPR (500 nM) rendered the homogenates unable to respond to supernatant-preactivated sperm factor (30 μl) prepared as described above. *D*, egg homogenates desensitized to Ca^{2+} release by supernatant-preactivated sperm factor rendered them insensitive to subsequent addition of either cADPR (500 nM) or $InsP_3$ (1 μM).

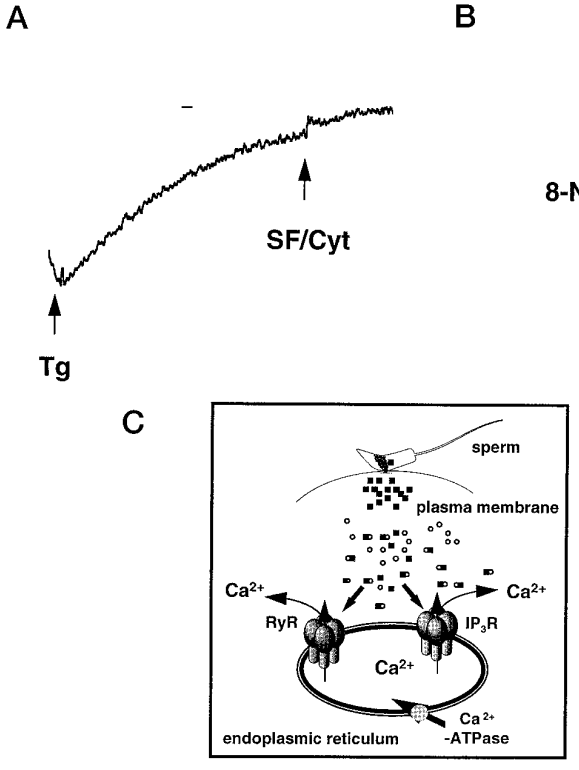

FIG. 4. **The effect of inhibitors of microsomal Ca^{2+} storage and release on Ca^{2+} release by sperm factor.** *A*, pretreatment of sea urchin egg homogenates with the microsomal Ca^{2+}ATPase inhibitor, thapsigargin (10 μM) depleted Ca^{2+} stores and rendered them insensitive to sperm factor (10 μl). *B*, incubation of egg homogenates with antagonists of $InsP_3$Rs, heparin (500 μg/ml) or cADPR-induced Ca^{2+} release, 8-NH_2-cADPR (10 μM), which block release by $InsP_3$ (1 μM) and cADPR (500 nM), respectively (data not shown; Ref. 3), did not affect subsequent Ca^{2+} release by supernatant-preactivated sperm factor (30 μl). The *horizontal bars* indicate 5 min. *C*, scheme for sperm factor-induced Ca^{2+} release from intracellular stores at fertilization. The sperm protein (possibly oscillin) is represented by *filled squares* and is introduced into the egg on sperm-egg fusion. It then diffuses throughout the cytoplasm, where it interacts enzymatically with cytoplasmic protein(s) (*unfilled circles*). The complex then activates both $InsP_3$Rs and RyRs by a mechanism independent of $InsP_3$ or cADPR, resulting in Ca^{2+} mobilization from intracellular stores.

Preincubation of sperm extract with egg cytosol (homogenate supernatant) before addition to 2.5% homogenates substantially reduced the latency and greatly increased the magnitude of response to sperm extract (Fig. 2*B*). In contrast, preincubation with purified particulate/microsomal fractions did not have any effect, suggesting that the egg components responsible for the facilitatory role are likely to be soluble (Fig. 2*C*). The enhancement of the sperm factors' Ca^{2+} mobilizing actions was time-dependent and significantly reduced by incubations of sperm extracts and egg cytosol at 4 °C (Fig. 2*D*). The Q_{10} of the reaction was investigated by incubating 10 μl of sperm factor with 20 μl of egg supernatant for 15 min at 17 °C or at 7 °C. The mixture was then added to sea urchin egg homogenates (2.5% v/v) and Ca^{2+} release monitored at 17 °C. The latency to release was 319 ± 28 s (n = 3; S.E.) and 690 ± 49 s (n = 3; S.E.) at 17 °C and 7 °C, respectively. These data suggest a Q_{10} of ~2.2 for the latency and imply that an enzymatic reaction is rate-limiting between the sperm factor and soluble egg components (22). The Q_{10} is similar to that of 2.3 for the latent period at fertilization in sea urchin eggs (23, 24).

Addition of boar sperm extract did not cause Ca^{2+} release from purified microsomal preparations (Fig. 2*F*), despite the

fact that these preparations responded to $InsP_3$ and cADPR (data not shown). However, when boar sperm extract was preincubated with egg cytosolic fractions for 30 min (as above) and then added to microsomes immediate Ca^{2+} release occurred, while egg cytosolic fractions were not able themselves to cause Ca^{2+} release (Fig. 2*F*). These data suggested that the preincubation of boar sperm extract with an egg cytosolic protein leads to the formation of an active principle that can directly trigger Ca^{2+} release via channels in the endoplasmic reticulum or microsomal fractions. The active principle in egg cytosol was in excess of 100 kDa since rapid Ca^{2+} releasing activity generated by incubation of sperm extract with supernatant fractions was apparent only with retentate from 100 kDa cut-off filters rather than the filtrate (Fig. 2*E*). This argued against the idea that the sperm factor exerts its effects by generating small molecular intracellular messengers such as $InsP_3$ or cADPR. Furthermore, it is unlikely that sperm extract interacts with large molecules to produce these low molecular weight intermediates which could trigger Ca^{2+} release since size filtration (100-kDa cut-off filter) of the sperm extract preincubated with egg cytosol for up to 60 min resulted in the active fraction being retained by the filter (>100 kDa) and did not appear in the filtrate (<100 kDa) (Fig. 2*E*). That an additional protein is required for sperm extract action is supported by the finding that transient treatment of egg cytosol with agarose-trypsin beads abolished the enhancing effect of egg cytosol on sperm factor-induced Ca^{2+} release (Fig. 2*E*). A role for protein kinases in the the preincubation reaction did not seem likely, because the preincubation reaction still gave rise to a rapid Ca^{2+}-releasing agent when it was carried out in the presence of a 10 μM concentration of the potent protein kinase inhibitor staurosporine, or the absence of any added ATP (data not shown).

We examined which Ca^{2+} release channels mediated sperm extract-induced Ca^{2+} release. Ca^{2+} release in sea urchin egg homogenates by $InsP_3$ and cADPR can be fully inactivated by prior stimulation with a maximal concentration of each respective agonist (25). Fig. 3*A* shows the effect of prior desensitization to $InsP_3$ on the cytosol-preactivated, sperm factor-induced Ca^{2+} release, and Fig. 3*B* shows the same for cADPR-inactivated homogenates. Sperm extract-induced Ca^{2+} release still occurred in homogenates refractory to either of these agents. However, if both $InsP_3$ and cADPR mechanisms are inactivated in the same homogenate, the sperm factor-induced Ca^{2+} release was abolished (Fig. 3*C*). In addition, prior exposure of the homogenates to cytosol-preactivated sperm factor desensitized the microsomes to both $InsP_3$ and cADPR (Fig. 3*D*). Previous studies indicate that $InsP_3$ and cADPR are located on substantially overlapping Ca^{2+} pools and that lack of response to one agent probably reflects a desensitization of the Ca^{2+} release mechanism rather than pool depletion (19, 26). Additionally, a distinct Ca^{2+} pool that is sensitive to nicotinic acid adenine dinucleotide phosphate does not increase in size after mobilization of Ca^{2+} by the other two agents (25, 26). It is thus thought that after desensitization of cADPR- and $InsP_3$-sensitive Ca^{2+} release mechanisms, the pool expressing these channels is still able to sequester Ca^{2+}. These data therefore suggest that sperm extract can trigger Ca^{2+} release by activating both $InsP_3$Rs and RyRs. The effects of sperm factor resemble those of the sulfhydryl reagent thimerosal, which can stimulate both $InsP_3$Rs and RyRs (27, 28) and shares the ability to mimic fertilization and trigger sustained Ca^{2+} oscillations in a variety of mammalian eggs (8).

To confirm that the sperm factor induces Ca^{2+} release by activating multiple Ca^{2+} release mechanisms present on internal stores, we tested the effects of the sperm factor on homogenates pretreated with thapsigargin (10 μM). Thapsigargin is a potent microsomal Ca^{2+}-ATPase inhibitor and functionally removes Ca^{2+} stores and renders sea urchin egg microsomes insensitive to $InsP_3$ and cADPR (25, 26). Fig. 4*A* shows that the sperm factor-induced Ca^{2+} release in egg homogenates was abolished by pretreatment with thapsigargin. However, a combination of heparin plus 8-NH_2-cADPR, which competitively blocks both $InsP_3$Rs and RyRs to activation by $InsP_3$ and cADPR, respectively (3), did not affect egg cytosol-preincubated, sperm factor-induced Ca^{2+} release (Fig. 4*B*).

Our data support the view that the sperm extract modulates Ca^{2+} release via $InsP_3$Rs and RyRs by a novel mechanism that is independent of low molecular weight messengers such as $InsP_3$ or cADPR. These features of the sperm factor do not correlate with the 23-kDa tr-kit sperm protein, which has been suggested to generate $InsP_3$-induced Ca^{2+} release in mouse eggs (29). The active component causing Ca^{2+} oscillations in the sperm extract fraction used is correlated with a high molecular mass complex composed of 33-kDa subunits which has been named oscillin and has 53% amino acid sequence homology with an *Escherichia coli* enzyme glucosamine-6-phosphate isomerase (18). It is not clear how this class of enzyme might be linked to Ca^{2+} release channel activation. However, the features of the sperm factor-induced Ca^{2+} mobilization in egg homogenates suggest a novel signaling mechanism and further studies should reveal any possible connection with this class of enzymes. In view of the ability of the sperm factor to trigger distinct Ca^{2+} oscillations in somatic cells (30, 31), it is possible that it may also reveal a novel component of cellular Ca^{2+} oscillators.

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REFERENCES

1. Miyazaki, S., Shirakawa, H., Nakada, K., and Honda, Y. (1993) *Dev. Biol.* **158,** 62–78
2. Swann, K., and Ozil, J. P. (1994) *Int. Rev. Cytol.* **152,** 183–222
3. Lee, H. C., Aarhus, R., and Walseth, T. J. (1993) *Science* **261,** 352–355
4. Galione, A., McDougall, A., Busa, W. B., Willmott, N., Gillot, I., and Whitaker, M. (1993) *Science* **261,** 348–352
5. Jones, K. T., Carroll, J., and Whittingham, D. G. (1995) *J. Biol. Chem.* **270,** 6671–6677
6. Whitaker, M., and Swann, K. (1993) *Development* **117,** 1–12
7. Swann, K. (1990) *Development* **110,** 1295–1302
8. Swann, K., and Lai, F. A. (1997) *Bioessays* **19,** 371–378
9. McCulloh, D. H., and Chambers, E. L. (1992) *J. Gen. Physiol.* **99,** 137–175
10. Lawrence, Y., Whitaker, M., and Swann, K. (1997) *Development* **124,** 233–241
11. Swann, K. (1994) *Cell Calcium* **15,** 331–339
12. Homa, S. T., and Swann, K. (1994) *Human Reprod.* **9,** 2356–2361
13. Wu, H., He, C. L., and Fissore, R. A. (1996) *Mol. Reprod. Dev.* **46,** 176–189
14. Palermo, G. D., Averch, O. M., Colombero, L. T., Wu, H., Wolny, Y. M. Fissore, R. A., and Rosenwaks, Z. (1997) *Mol. Human Reprod.* **3,** 367–374
15. Stricker, S. A. (1997) *Dev. Biol.* **186,** 185–201
16. Tesarik, J., Sousa, M., and Testart, J. (1994) *Human Reprod.* **9,** 511–518
17. Stricker, S. A. (1996) *Dev. Biol.* **176,** 243–263
18. Parrington, J., Swann, K., Shevchenko, V. I., Sesay, A. K., and Lai, F. A. (1996) *Nature* **379,** 364–368
19. Dargie, P. J., Agre, M. C., and Lee, H. C. (1990) *Cell Regul.* **1,** 279–290
20. Clapper, D. L., Walseth, T. F., Dargie, P. J., and Lee, H. C. (1987) *J. Biol. Chem.* **262,** 9561–9568
21. Galione, A., Lee, H. C., and Busa, W. B. (1991) *Science* **253,** 1143–1146
22. Gutfreund, H. (1996) *Kinetics for the Life Sciences*, Cambridge University Press, Cambridge, UK
23. Allen, R. D., and Griffen, J. L. (1958) *Exp. Cell Res.* **15,** 163–173
24. Dale, B. D., deFelice, L. J., and Taglietti, V. (1978) *Nature* **275,** 217–219
25. Genazzani, A. A., and Galione, A. (1997) *Trends Pharmacol. Sci.* **18,** 108–110
26. Genazzani, A. A., and Galione, A. (1996) *Biochem. J.* **315,** 721–725
27. Kaplin, A. I., Ferris, C. D., Volgmaier, S. M., and Snyder, S. H. (1994) *J. Biol. Chem.* **269,** 28972–28978
28. Abramson, J. J., Zable, A. C., Favero, T. C., and Salama, G. (1995) *J. Biol. Chem.* **270,** 29644–29647
29. Sette, C., Bevilacqua, A., Mangia, F., Geremia, R., and Rossi, P. (1997) *Development* **124,** 2267–2274
30. Currie, K. P. M., Swann, K., Galione, A., and Scott, R. H. (1992) *Mol. Biol. Cell* **3,** 1415–1425
31. Berrie, C. P., Cuthbertson, K. R. S., Parrington, J., Lai, F. A., and Swann, K. (1996) *Biochem. J.* **311,** 369–372