THE ACTIVATION OF SEA URCHIN EGGS BY THE DIVALENT IONOPHORES A23187 AND X-537A

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SUMMARY: The divalent ionophores A23187 and X-537A induce parthenogenesis in sea urchin eggs. This results from their ability to mobilize intracellular Ca^{2+}, which is implicated in both artificial parthenogenesis as well as the natural fertilization process. A23187 causes expulsion of cortical granules and elevation of the fertilization membrane within 0.5-9 min followed by an initiation of cell cleavage. The broader spectrum ionophore X-537A is less potent, but the production of cytoplasmic aberrations are more apparent. In contrast to the sperm-activated egg, the initial phase of ionophore induced activation is accompanied either by relatively insignificant changes in membrane resistance, or an increase.

The ability of certain ionophores to transport divalent cations across membranes has recently been described (1-3). Ca^{2+} is a key biological control agent; accordingly these ionophores can perturb a wide variety of biological processes such as the release of Ca^{2+} from sarcoplasmic reticulum (4-6) and the release of histamine from secretory granules of mast cells (7,8) or acetylcholine at the neuromuscular junction (9) via Ca^{2+} mediated exocytosis. Since fertilization of sea urchin eggs has been associated with an increase in both the influx and net efflux of Ca^{2+} (10-12) and an intracellular release of this ion (13,14), we undertook to examine the effects of the divalent ionophores A23187 (1,2) and X-537A (2,3) on unfertilized sea urchin eggs.

MATERIALS AND METHODS

Gametes of the sea urchin Lytechinus variegatus were used within 6 hr of harvesting, and maintained at 23-24°C. The ionophores X-537A, nigericin, A23187, dianemycin, monensin and valinomycin were dissolved in a mixture of equal parts of dimethyl sulfoxide and EtOH, and 0.4 to 4 µl/per ml of sea water added immediately before use. Suitable controls established that the solvent alone had no electrophysiological or developmental effects.

Abbreviations: FM, fertilization membrane; E_m, membrane potential; R_m, specific membrane resistance in Ω cm^2; SW, sea water

For electrophysiological measurements, jelly-free eggs which attached tc the bottom of a plastic Petri dish were impaled using glass electrodes filled with 3 M KCl with tip resistances of 20 to 60 MΩ. Membrane potentials were recorded on a Texas Instruments Servo/Riter coupled to a WP instruments Model M4A electrometer. The same electrode served to pass hyperpolarizing current pulses of 2-10 X 10^{-10}A, 250 msec in duration, at a frequency of 1 to 5 per 10 sec through the egg membrane to the grounded medium.

Artificial sea water and other salt solutions (Table 1), buffered with 2.3 mM HCO^-_3 and 0.4 mM H_3BO_3, were adjusted to pH 8.4. Traces of free divalent cations in the K-Na medium were removed by adding 0.5 mM EGTA.

Table 1. Composition of sea water and other salt solutions, mmoles/litre

Medium	Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-	So_4^{2-}
SW	488	10	10	52	560	30
150 mM KSW	348	150	10	52	560	30
300 mM KSW	198	300	10	52	560	30
K-Na	164	450	0	0	552	30

RESULTS

Effects of A23187. A23187 at 0.5-50 µM activated virtually all unfertilized eggs suspended in sea water. At 5-10 µM the cortical granules were expelled with elevation of the fertilization membrane (FM) in 30-45 sec (Fig. 1), a response virtually indistinguishable from that induced by sperm.

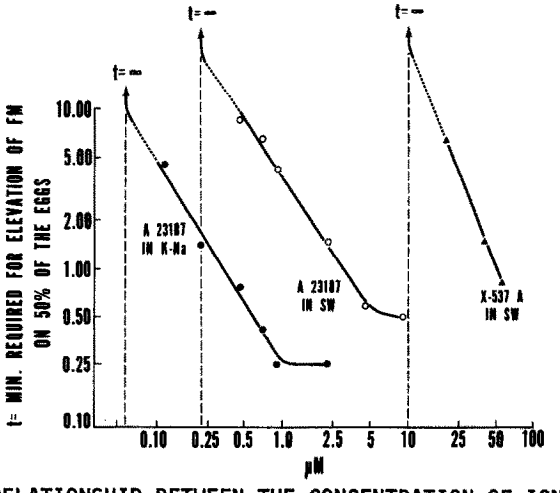

FIG. 1. RELATIONSHIP BETWEEN THE CONCENTRATION OF IONOPHORE AND THE TIME (t) REQUIRED FOR ELEVATION OF THE FERTILIZATION MEMBRANE, LOG-LOG PLOT. ▲—▲ X-537A in sea water. o—o A23187 in sea water. ●—● A23187 in K-Na medium.

Below 5 μM A23187, the elevation of the FM is retarded progressively, reaching 9 min at 0.5 μM. Within 10 to 15 min after FM elevation the hyaline layer is secreted, ultimately reaching a thickness of 1-2 μ. The egg nucleus gradually enlarges to approximately twice its initial diameter as it moves toward the egg center. Dissolution of the swollen egg nuclei occurs with remarkable synchrony in all eggs approximately 60 min after FM elevation. An aster appears at the site where the egg nucleus disappears, grows to fill the entire egg, and becomes exceptionally prominent. Approximately 2 hr after FM elevation, the majority of the eggs undergo cleavage-like figures. Few of the eggs fail to show cleavage forms; in these cases multiple nuclei can be seen in the cytoplasm, and several cycles of reformation and dissolution of the nuclei may ensue.

Effects of X-537A. X-537A in sea water also induces the cortical reaction, but the minimum effective concentration (20 μM) is 40 times that of A23187 (Fig. 1). At concentrations of X-537A which cause elevation of the FM, hyaline layer formation takes place, but within 30 min the eggs become progressively more granular and opaque, with cytolysis ensuing in 2-3 hr. However, if the eggs exposed to X-537A are washed in sea water within 5 min after elevation of the FM, the coagulative changes are minimized. The egg nuclei can then be observed to enlarge and move to the egg center, with dissolution of the egg nuclear membrane at about 60 min. No asters could be seen, possibly due to the opacity of the cytoplasm. In 2 to 3 hr cleavage furrows developed in 25-45% of the eggs.

Response of the E_m and R_m. Fig. 2, lower panel, Expt A depicts the response in E_m and R_m to sperm activation. The first response phase is depolarization associated with transient polarity reversal and a marked fall in R_m. The peak of potential reversal (+17 to +21 mV) is consistent, regardless of the variable initial E_m (15). The second phase is a repolarization to -74 to -76 mV associated with an initial rise, then fall in R_m. The alteration of E_m by the addition of 150 and 300 mM KSW (Fig. 2, lower

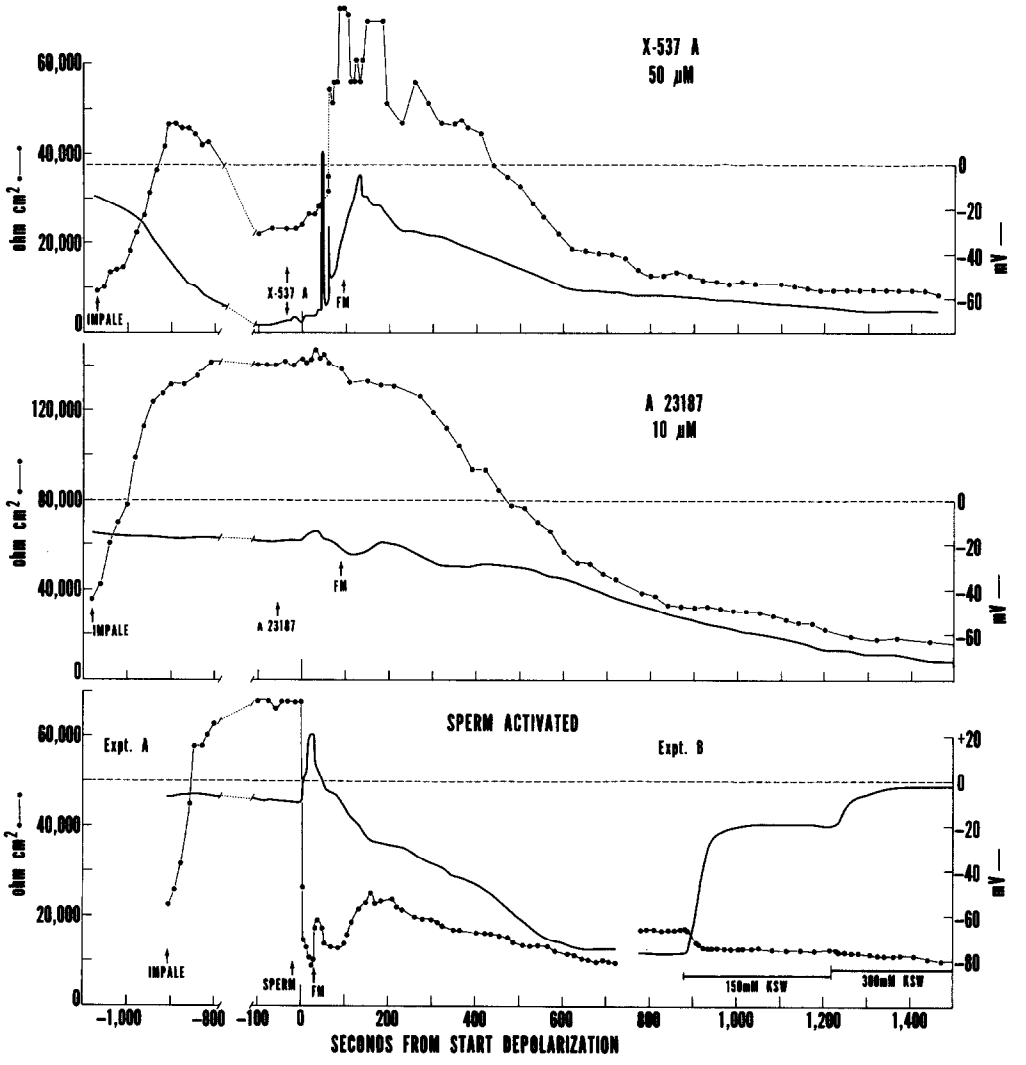

FIG. 2. CHANGES IN THE E_m AND R_m OF EGGS FOLLOWING ACTIVATION BY SPERM, A23187 AND X-537A. Time measured from the start of depolarization (0 time). ●——● R_m in Ω cm2, ordinates on left, ——— E_m in mV, ordinates on right. In each Expt the unfertilized egg is impaled (↑), the E_m and R_m recorded until stabilized, then sperm, or ionophore added (↑), subsequent time of elevation of FM (↑) also shown.

Lower panel: Sperm activated egg. Expt A depicts the pattern up to 12 min after insemination when polarization is essentially complete, thereafter no significant changes occur through 25 min. Curves for Expt B start after the egg has fully polarized, to demonstrate the depolarizing effect of adding KSW (the earlier part of the record not shown was the same as Expt A).

Middle panel: Egg activated by 10 µM A23187.

Upper panel: Egg activated by 50 µM X-537A. During the spike-like depolarizations following addition of the ionophore, R_m measurements could not be made (indicated by the dotted line).

panel, Expt B) follows the expected values for a K^+ dependent potential consistent with a marked increase in K^+ permeability upon fertilization (16-18).

Fig. 2, middle panel, is representative of activation by 10 μM A23187. Although an initial depolarization phase occurs, the changes in E_m and R_m are small, in contrast to sperm activation. In 9 experiments the average value of the E_m at the peak of depolarization was -10 mV and the maximum ΔR_m was $\pm$ 20%. The subsequent polarization phase developed essentially as with sperm activation. The fully polarized egg responded to KSW exactly as expected for high K^+ permeability.

For eggs activated with 50 μM X-537A the changes observed during the depolarization phase were of two types. Activation often proceeded as with the lower doses of A23187. Equally prevalent was the response pattern illustrated in the upper panel of Fig. 2. Superimposed on the slower depolarization phase were 2-3 sudden depolarization spikes lasting 2-3 sec, with an occasional reversal of potential attaining +2 to +7 mV; R_m increased three-fold during this interval. The extent of the slow depolarization was large, presumably a consequence of the exceptionally high initial E_m in these cases. However, the average E_m attained at the height of depolarization was essentially the same as for A23187 activated eggs; neither ionophore caused a potential reversal of the magnitude achieved with sperm activation.

X-537A shows a stronger tendency than most ionophores to crystallize from dilute aqueous solutions. It is possible that when X-537A produced effects resembling those of A23187, its effective concentration fell during the time required to change the fluid around the impaled egg; the response pattern seen in Fig. 2, upper panel, may reflect the effects of higher effective doses of X-537A due to metastable supersaturation.

Other ionophores. To ascertain the dependency of ionophore activation on divalent cation selectivity, the effects of dianemycin, monensin, nigericin and valinomycin, which are highly selective for monovalent cations (19), were also examined. In the concentration ranges 5-140 μM these ionophores failed to activate eggs; however, except for valinomycin, they did cause an

opacity and coagulated appearance within 2-3 hr reminiscent of that obtained with X-537A.

Effects of ionophores in the absence of Ca^{2+} and Mg^{2+}. In the Ca^{2+} and Mg^{++} -free K^{+} -Na^{+} medium A23187 was even more potent in eliciting the cortical reaction than in sea water (c.f. Fig. 1). Moreover, the eggs undergo full activation including aster formation. Pure 600 mM K^{+} or Na^{+} also support the cortical response, however, only in the latter case does activation proceed to the dissolution of the egg nucleus. X-537A is also effective in the K-Na medium, however, there is no augmentation of its potency as in the case of A23187. This could be accounted for by the divalent ions in the sea water complexing the A23187 in the aqueous phase thereby limiting its effective availability for entering the eggs.

DISCUSSION

The divalent ionophores have proved valuable tools for studying the role of Ca^{2+} in fertilization. They are relatively inert chemically save for their remarkable ability to translocate cations across membranes (19). Although divalent ionophores do function with Mg^{2+} (1-3,6), there is little precedent for this ion playing a prominent cellular control function. The inability of the other ionophores tested, which are highly selective for monovalent ions (19), to activate eggs also supports the involvement of Ca^{2+}. In addition, the greater potency of A23187 over X-537A parallels the relative ability of these ionophores to release Ca^{2+} from sarcoplasmic reticulum (6).

The expulsion of cortical granules by divalent ionophores is analogous to other exocytotic processes triggered by these agents (7-9). With eggs the Ca^{2+} must arise from an intracellular pool since activation by ionophores can take place in the absence of external divalent cations. A23187 is known to initiate a Ca^{2+} mediated response in frog eggs in a Ca^{2+} free medium (20). Moreover, we have shown that the initial phases of ionophore-induced activation are not accompanied by any marked increase in membrane permeability.

We therefore conclude that divalent ionophores mediate the release of intracellular Ca^{2+} stores which initiates: (1) expulsion of cortical granules,

and (2) activation of cytoplasmic and nuclear components. However, during the initial phase of sperm activation an analogous release of intracellular Ca^{2+} could be triggered by an inward surge of Na^{+} resulting from the marked increase in Na^{+} permeability (16,21).

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REFERENCES

1. Reed, P.W. and Lardy, H.A. (1972) J. Biol. Chem. 248, 2670-6977.
2. Pressman, B.C. (1973) Federation Proc. 32:1698-1703.
3. Pressman, B.C. and N.T. deGuzman (1974) Annals N.Y. Acad. Sci. 227, 308-391.
4. Scarpa, A., Baldassare, J. and Inesi, G. (1972) J. Gen. Physiol. 60, 735-749.
5. Entman, M.L., Gillette, P.C., Wallick, E.T., Pressman, B.C., and Schwartz, A. (1972) Biochem. Biophys. Res. Commun. 48, 847-852.
6. Caswell, A.H., and Pressman, B.C. (1972) Biochem. Biophys. Res. Commun. 49, 292-298.
7. Foreman, J.C., Mongar, J.L., and Gomperts, B.D. (1973) Nature 245, 249-251.
8. Cochrane, D.E., and Douglas, W.W. Proc. Nat. Acad. Sci. 71, 408-412.
9. Kita, H. and Van der Kloot, W.G. (1973) The Physiologist 16, 365.
10. Azarnia, R. and Chambers, E.L. (1969) Biol. Bull. 137, 391-392.
11. Azarnia, R. and Chambers, E.L. (1970) Biol. Bull. 139, 413-414.
12. Chambers, E.L., Azarnia, R. and McGowan, W.E. (1970) Biol. Bull. 139, 417-418.
13. Mazia, D. (1937) J. Comp. Physiol. 10, 291-304.
14. Nakamura, M. and Yasumasu, I. (1974) J. Gen. Physiol. 63, 374-388.
15. Ito, S. and Yoshioka, K. (1972) Exptl. Cell Res. 72, 547-551.
16. Steinhardt, R.A., Lundin, J. and Mazia, D. (1971) Proc. Nat. Acad. Sci., 68, 2426-2430.
17. Chambers, E.L. and Chambers, R. (1949) Am. Naturalist 83, 269-284.
18. Chambers, E.L. (1949) Biol. Bull. 97, 251-252.
19. Pressman, B.C. (1973) in Inorganic Review Biochemistry (Eichorn, G.L., ed.) pp. 203-226, Elsevier, Amsterdam, New York.
20. Schroeder, T.E. and Strickland, D.L. (1974) Exptl. Cell Res. 83, 139-142.
21. Chambers, E.L. (1972) The Physiologist 15, 103.