ADENYL CYCLASE IN PLASMA MEMBRANE PREPARATIONS OF SEA URCHIN EGGS AND ITS INCREASE IN ACTIVITY AFTER FERTILIZATION*

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It has been shown (cf. Robison et al., 1968) that adenyl cyclase is present in preparations of plasma membranes from cells of many kinds of animal tissues and that the specific hormones cause, in vivo or in vitro, increased production of cyclic AMP, which is often attributable to increased enzyme activity. The system also has been implicated in chemotactic phenomena (Konijn et al., 1968).

The process of fertilization is known to involve the interaction of specific receptors (fertilizin) on the plasma membrane of the egg with complementary receptors (antifertilizin) on that of the sperm (Tyler, 1965; Tyler and Tyler, 1966). It is of interest, then, to know if adenyl cyclase is present in membrane preparations from sea urchin eggs and if it increases in activity after fertilization. The present results answer both of these questions affirmatively.

Materials and Methods

Gametes of the sea urchin Lytechinus pictus were handled as described elsewhere (Tyler and Tyler, 1966), mechanical demembranation, rather than urea, being used to avoid possible denaturation effects. Incubations were

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terminated by rapid cooling of the eggs to 0-4°, and two washings with "cation-free" (to avoid enzyme activation (Williams et al., 1968)) cold homogenization buffer (0.05 M tris-HCl, pH 7.6; 0.25 M sucrose; 0.02 M theophylline and 0.005 M β-mercaptoethanol) followed immediately by homogenization with two volumes of the same buffer. "Membrane fractions" were obtained by pelleting twice at 2200 x g for 10 min, rinsing and resuspending. "Supernatant" represents the upper half of the fluid after a second centrifugation of the first supernatant.

Determination of cyclic AMP was done (cf. Streeto and Reddy, 1967) by paper (Whatman 3MM) chromatography. ^{3}H-cyclic AMP (0.1 μc, 80 μg) was used as carrier (recovery about 90%). Double isotope scintillation counting (efficiencies of 80% for ^{14}C and 6% for ^{3}H) was done directly on the paper sections. Protein was determined according to Lowry et al. (1951).

Results

Figure 1 shows the results of tests of adenyl cyclase activity of a

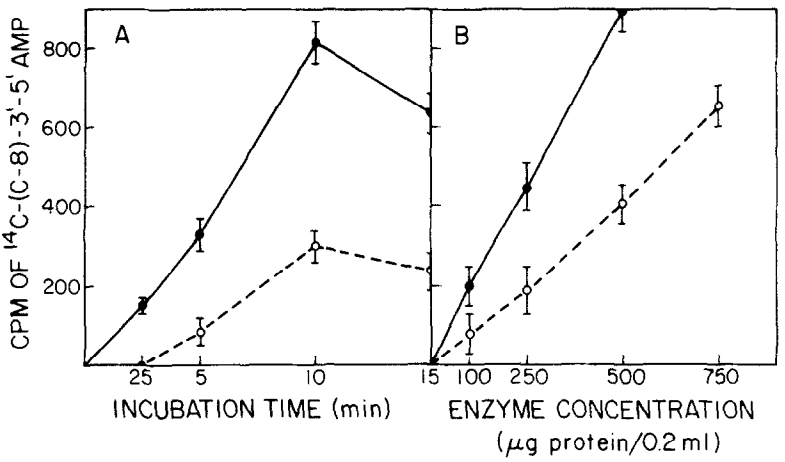

Fig. 1. Adenyl cyclase activity of membrane preparations (●—●) and supernatants (0---0) of fertilized (30 min) eggs of L. pictus. A, time course production (corrected for recovery) of cyclic AMP at 30°C in a system containing as μmoles/0.2 ml: 6, tris (pH 7.6); 1, $MgSO_4$;3, theophylline; 1, β-mercaptoethanol; 0.2, ATP (1 μc ^{14}C at C-8); 2, phosphoenol pyruvate; and 5 μg PEP-kinase, and 350 μg of enzyme preparations as protein. B, same system as A, for 10 min incubation with different amounts of enzyme preparation. Error bars represent duplicate tubes.

membrane preparation from fertilized eggs. The supernatant also shows activity, but much lower (1/4 to 1/3). Since it is not demonstrably free of membranes, this material, as in other work (e.g. Rosen and Rosen, 1968) may account for the activity. Optimum incubation time (Fig. 1A) is 10 min, which agrees with other preparations (Weiss and Costa, 1967). The subsequent decrease is attributable to hydrolysis of the cyclic AMP even in the presence of theophylline which does not completely inhibit cyclic phosphodiesterase (cf. Streeto and Reddy, 1967; Honda and Imamura, 1968). The 10 min values show linear increase with enzyme concentration (Fig. 1B). The optimum Mg^{++} concentration was found to be 5 mM.

Preparations from unfertilized eggs show very low activity. There is a gradual increase after fertilization (Fig. 2A), with a plateau after 30 minutes. Again, the supernatant shows much lower activity than the pellet. NaF, known to activate adenyl cyclase maximally, causes a large increase in the activity of the sea urchin preparations (Fig. 2B), those from unfertilized eggs reaching levels some 10 times the plateau level for the fertilized eggs. In the NaF-treated preparations the activity drops abruptly after fertilization to less than half the unfertilized egg value. Pellets from unfertilized eggs are yellow, while those from fertilized eggs are white and

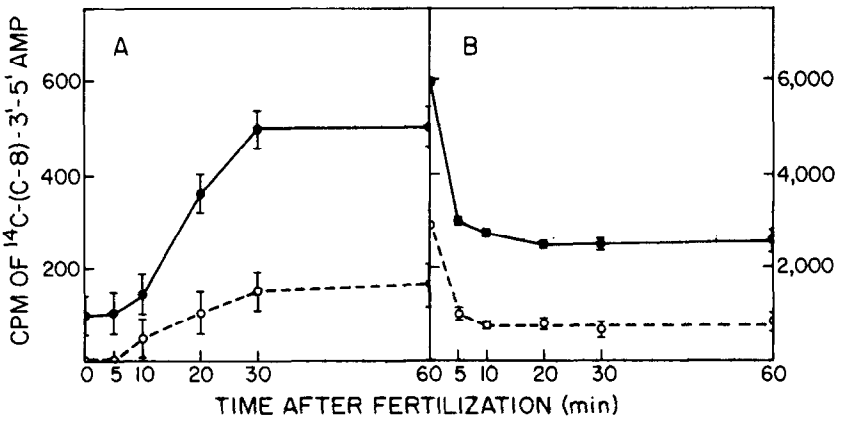

Fig. 2. Adenyl cyclase activity before and after fertilization without (Fig. 2A) and with (Fig. 2B) NaF at 10 mM. System, preparations (300 µg protein) and symbols as in Fig. 1.

much more densely packed. These and other known changes are indicative of redistribution of materials. However, the drop in adenyl cyclase activity is approximately the same in the supernatant as in the pellet.

Discussion

The finding of adenyl cyclase in membrane preparations of sea urchin eggs and its increase in activity after fertilization adds another to the many systems in which production of cyclic AMP seems to be involved in the response of target cells to their specific hormonal or other agent. In many systems, as noted above, cyclic AMP itself can evoke the response. In preliminary tests (mainly by Dr. G. S. Hand) with sea urchin eggs no activation has occurred, nor has the dibutyryl analog proven effective. The ineffectiveness may simply reflect impermeability of the unfertilized egg to such compounds.

Increase in enzyme activity following fertilization is gradual, according to the present data. This may imply that cyclic AMP production is not part of the triggering mechanism but contributes to later events. However, the possibility cannot be excluded that an initial small change in enzyme activity may suffice to initiate egg-activation events. Also, activities *in vivo* may be very different. Further, the extent to which phosphodiesterase may contaminate the various preparations is at present not known. In fact, as in experiments of others reporting increases in adenyl cyclase activity, this is one reservation attached to the conclusion that it is this enzyme itself that increases in activity after fertilization. From the shape of the rate curves it appears unlikely that variation in amount of associated phosphodiesterase can account for the differences at different stages. Also, the fact that with the NaF-activated samples the changes in activity after fertilization are in the opposite direction argues against that possibility.

The drop in activity of the NaF preparations after fertilization implies an actual loss of enzyme or, less likely, a decreased responsiveness to NaF.

A loss could be related to the breakdown of the cortical vesicles and subsequent decrease in total amount of membrane following fertilization. Membrane loss is inferred from the approximate constancy of surface area although cortical vesicle membranes are added to the plasma membrane. The behavior of the cortical vesicles upon fertilization (cf. Tyler and Tyler, 1966; Metz, 1967) has its counterpart in such apparently diverse phenomena as synaptic transmission and the release of insulin, in which membrane-bound vesicles fuse with the plasma membrane and discharge their contents externally. Interesting mechanisms have been proposed (Rasmussen and Tenenhouse, 1968) involving the action of cyclic AMP in these systems. For egg activation similar schemes might be based on an increase in adenyl cyclase activity upon interaction of the receptors of sperm and egg, or the action of parthenogenetic agents. Because of its role in parthenogenesis (Tyler, 1941), involvement of calcium release by decreased chelation by cyclic AMP is intriguing. The fertilizing sperm itself is known to respond to interaction with egg surface (fertilizin) by a rupture of its acrosomal vesicle. Unusually large amounts of cyclic AMP have been obtained (Butcher et al., 1965) from human sperm, the handling of which was not described and which may have undergone acrosome reaction. Perhaps, too, the sperm-produced AMP can be transferred to, and act on, the egg. These and other interesting possibilities are subjects for further investigation.

References

Butcher, R. W., Ho, R. J., Meng, H. C. and Sutherland, E. W., J. Biol. Chem., 240, 4515 (1965)
Honda, F. and Imamura, H., Biochim. Biophys. Acta, 161, 267 (1968)
Konijn, T. M., Barkley, D. S., Chang, Y. Y. and Bonner, J. T., Am. Naturalist, 102, 225 (1968)
Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., J. Biol. Chem., 193, 265 (1951)
Metz, C. B., in C. B. Metz and A. Monroy (Eds.), Fertilization, Academic Press, New York, 1, 163 (1967)
Rasmussen, H. and Tenenhouse, A., Proc. Nat. Acad. Sci. U. S., 59, 1364 (1968)
Robison, G. S., Butcher, R. W. and Sutherland, E. S., in P. D. Boyer (Ed.), Ann. Rev. of Biochem., 37, 149 (1968)

Rosen, O. M. and Rosen, S. M., Biochem. Biophys. Res. Commun., 31, 82 (1968)
Streeto, J. M. and Reddy, W. J., Anal. Biochem., 21, 416 (1967)
Tyler, A., Biol. Rev., 16, 291 (1941)
Tyler, A., Am. Naturalist, 99, 309 (1965)
Tyler, A. and Tyler, B. S., in R. A. Boolootian (Ed.), Physiology of Echinodermata, Wiley, New York, 639 (1966)
Weiss, B. and Costa, E., Science, 156, 1750 (1967)
Williams, R. H., Walsh, S. A. and Enzinck, J. W., Proc. Soc. Exptl. Biol. and Med., 128, 279 (1968)