Effect of adrenotropic substances on the growth and maturation of oocytes of the sea urchin, *Strongylocentrotus nudus*

Yu. S. Khotimchenko

Institute of Marine Biology, Far East Science Center, Academy of Sciences of the USSR, Vladivostok 690022 (USSR),
26 February 1981

Summary. Growth and maturation of oocytes in sea urchins is inhibited under the influence of catecholamines. A monoaminergic system is assumed to participate in the regulation of oogenesis.

Radial nerves of echinoderms contain certain low-molecular-weight polypeptide hormones which cause 1-methyladenine formation in ovaria[1-3]. The latter stimulates oocyte maturation in starfishes and sea urchins[4-6]. We have made an assumption that the growth and maturation of sea urchin oocytes is doubly controlled, by peptidergic and monoaminergic neurones. The present investigation studies the effect of adrenomimetics and adrenolytics on growth and maturation of oocytes.

Materials and methods. The study was conducted on mature sea urchin females *S. nudus* from the Sea of Japan. The influence of drugs on oocyte growth was studied in vivo. For this purpose animals at the beginning of the growth stage of the reproductive cycle were placed in an aquarium with aerated sea water with pH 7.9–8.1, an oxygen content of 9.9–12.4 mg/l, salinity 33.0–34.9‰ and a temperature of 0–6 °C. Gonad development was stimulated by increasing the water temperature up to 14–16 °C during 16–30 days[7]. The drugs to be studied were introduced into the coelomic cavity of the tested animals once a day. These were adrenomimetics-noradrenaline (3 mg/kg), dopamine (10 mg/kg), ephedrine (15 mg/kg) and adrenolytics-propranolol (20 mg/kg), oxprenolol (20 mg/kg) and dihydroergotamine (10 mg/kg). Control animals were injected with an equivalent amount of solvent. When the tests were finished, gonads were isolated and fixed in Bouin liquid.

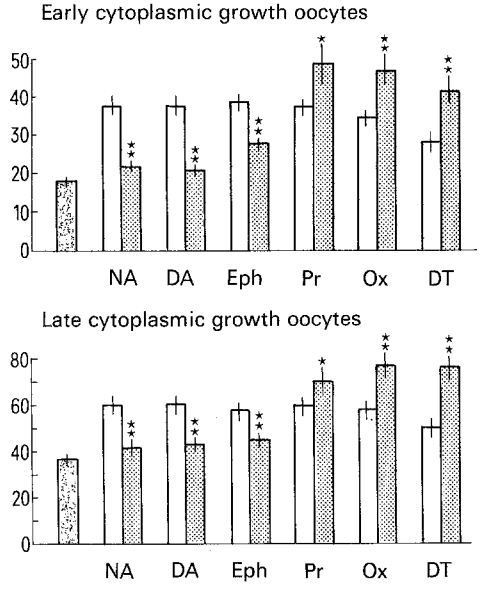

Figure 1. Influence of noradrenaline (NA, 3 mg/kg; the drug was introduced into coelomic cavity of tested animals once a day during 28 days), dopamine (DA, 10 mg/kg, 28 days); ephedrine (Eph, 15 mg/kg, 30 days); propranolol (Pr, 20 mg/kg, 26 days); oxprenolol (Ox, 20 mg/kg, 24 days) and dihydroergotamine (DT, 10 mg/kg, 16 days) on the growth of sea urchin oocytes. Vertically, volume of oocytes (thousands μm^3); $^*p < 0{,}01$; $^{**}p < 0{,}001$; ▨ volume of oocytes before thermal stimulation; □ control group (thermal stimulation); ▩ test group (thermal stimulation plus a drug).

Sections 3 µm thick were colored with hematoxylin. Sizes of early and late cytoplasmic growth oocytes were measured and cell volumes were calculated. To investigate the influence of the drugs on oocyte maturation the experiments were performed in vitro in the pre-spawning season. We used those females in whose acini the greater part of the cells were essentially trophoplasm growth oocytes (50–80%) and matured eggs (20–50%). Gonad fragments were incubated in artificial sea water supplemented with the substances under study at 16–18 °C. After 5 h of incubation the gonads were fixed. The number of trophoplasm growth oocytes and matured eggs was calculated and the correlation between them was determined before and after the test[6].

Results and discussion. In vitro experiments were conducted to evaluate the effects of adrenotropic drugs on growth oocytes. Figure 1 illustrates that noradrenaline, dopamine and ephedrine introduction resulted in oocyte sizes becoming significantly less than control values. Nucleus and nucleolus volumes were also decreased (data not shown). Also, the average amount of matured oocytes per acinus was 4.8–5.2% in the control groups, whereas these oocytes were absent in all test groups. After introduction of propranolol, the amount of mature eggs was 13.9% until the end of the test in comparison with 4.8% in control animals, and values for oocyte volumes exceeded control data. Oxprenolol and dihydroergotamine also stimulated oocyte growth.

In one of the in vitro experiments before the test 74% of oocytes in acini were essentially trophoplasm growth oocytes and 26% were matured eggs. After 5 h of gonad incubation in artificial sea water the amount of matured oocytes rose to 51% in control fragments. After the same incubation with noradrenaline (10^{-5} M), dopamine (10^{-5} M) and euphylline (10^{-6} M) it changed to 31, 34 and 32% respectively ($p < 0.001$). Adrenolytics in doses of 10^{-8}–10^{-4} M had no visible effect on oocyte maturation (fig. 2).

The experiments described show that catecholamines found in radial nerve cords of sea urchins[8] are capable of inhibit-

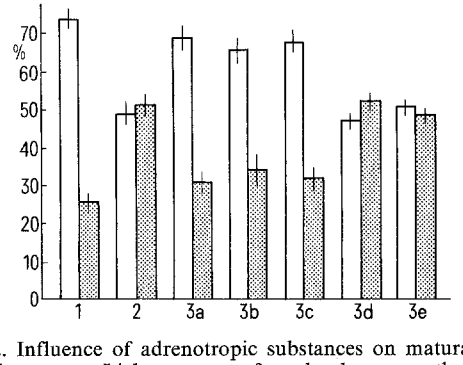

Figure 2. Influence of adrenotropic substances on maturation of sea urchin oocytes. Light, amount of trophoplasm growth oocytes; dark amount of matured eggs in acini before the experiment (1), after 5 h of incubation without drugs (2) and with noradrenaline (3a, 10^{-5} M); dopamine (3b, 10^{-5} M); euphylline (3c, 10^{-6} M); propranolol (3d, 10^{-4} M) and dihydroergotamine (3e, 10^{-4} M), %.

ing the growth and maturation of oocytes. Previously we found an inhibiting effect of noradrenaline, dopamine and euphylline in relation to the levels of labelled uridine and leucine uptake into growing oocytes of the sea urchin, *S. nudus*[9,10]. Evidently inhibition of oocyte growth under the influence of catecholamines is connected with a depression of RNA and protein biosynthesis. Hypothetically the above data may by explained by catecholamine acting on the cell membranes and activating adenyl cyclase, which promotes the formation of adenosine 3′:5′-cyclic monophosphate (cyclic AMP) from ATP. At present this mechanism has been described both for mammals and invertebrates[11,12]. The effects of cyclic AMP, dibutyryl cyclic AMP and inhibitors of cyclic nucleotide phosphodiesterase are compensated or complete inhibition of oocyte maturation in mice and rats[13–15]. It has also been found that cAMP participates in the regulation of some metabolic processes in sea urchin eggs[16,17]. Proceeding from the data obtained we have come to the conclusion that the monoaminergic system does participate in the regulation of oocyte growth and maturation in sea urchins. As no adrenergic nerve fibers have been found in sea urchin gonads[18] it might be proposed that catecholamines control the functions of sea urchin sexual glands by neurohumoral means.

1 A.B. Chaet, Symp. Zool. Soc. Lond. *20,* 13 (1967).
2 H. Kanatani, S. Ikegami, H. Shirai, H. Oide and S. Tamura, Devl Growth Differ. *13,* 151 (1971).
3 R.C. Cochran and F. Engelmann, Gen. Comp. Endocr. *30,* 189 (1976).
4 A.W. Schuetz, Gen. Comp. Endocr. *12,* 209 (1969).
5 H. Kanatani and H. Shirai, Devl Growth Differ. *13,* 53 (1971).
6 H. Kanatani, Devl Growth Differ. *16,* 159 (1974).
7 P.A. Motavkin and V.V. Evdokimov, Biol. morya (Russ.) *1,* 58 (1975).
8 G.A. Cottrell, Br. J. Pharmac. Chemother. *29,* 63 (1967).
9 Yu.S. Khotimchenko, Tsitologiya (Russ.) *21,* 972 (1979).
10 Yu.S. Khotimchenko, Zh. evolyuts, biokhim. fisiol. (Russ.) *16,* 80 (1980).
11 S. Eden, K. Albertsson-Wiland, S. Rosberg and O. Isaksson, Acta endocr. *85,* 806 (1977).
12 M.S. Wollemann and K. Rozsa, Comp. Biochem. Physiol. *51C,* 63 (1975).
13 S. Stern and P.M. Wassarman, J. Cell Biol. *59,* 335a (1973).
14 W.K. Cho, S. Stern and J.D. Biggers, J. exp. Zool. *187,* 383 (1974).
15 C. Magnusson and T. Hillensjo, J. exp. Zool. *201,* 139 (1977).
16 C.M. Amy and L.G. Rebhun, J. Cell Biol. *70,* 337a (1976).
17 I. Yasumasu, A. Hino and K. Asami, Cell Struct. Funct. *2,* 11 (1977).
18 J.L.S. Cobb, Comp. Biochem. Physiol. *28,* 967 (1969).

Plasma transcortin concentration in thyroidectomized chick embryos

C. Martin and B. Martin

Institut d'Embryologie du C.N.R.S. et du Collège de France, 49bis avenue de la Belle Gabrielle, F-94130 Nogent-sur-Marne (France), and Laboratoire de Physiologie de la Reproduction (Groupe Stéroïdes) ERA C.N.R.S. 694, Université Pierre et Marie Curie, 9, Quai Saint Bernard, F-75005 Paris (France), 1 July 1981

Summary. Concentrations of transcortin binding sites and apparent dissociation constants for corticosterone have been measured in the blood of control and thyroidectomized chick embryos. The levels of corticosterone binding are quantitatively similar in normal and thyroidectomized embryos and vary in parallel during development.

In the blood of the chick embryo a protein has been detected that binds progesterone and corticosteroids with high affinity and limited capacity[1]. This embryonic transcortin-type protein, homologous with the adult chick transcortin, was found to be synthesized by the liver[2,3]. Transcortin concentration in embryonic chicken plasma increased up to day 15 of incubation and then sharply decreased until day 20[1]. The evident consequence of this drop in C_{21}-steroid binding protein is an increase of unbound C_{21} steroids in blood during the last days of incubation. Thus free steroids, the active form of hormones, are available to receptor sites in steroid target cells[4]. More recently transcortin contents of embryonic plasma have been measured in partially decapitated (i.e. hypophysectomized) chick embryos[5]. A specific effect of partial decapitation, i.e. the inhibition of the drop in transcortin level, was observed after day 15 of incubation. Thus the transcortin level in the blood appears to be controlled at the pituitary level but it is not known whether this is a direct effect of the hypophysis or if other organs and indirect pathways are involved. The thyroid, which is affected by decapitation[6,7] could be a candidate for transcortin regulation. The aim of the present study was to analyze in ovo the effects of thyroid on transcortin activity in the plasma of the chick embryo. We have devised a microsurgical procedure by which the thyroid primordium is removed at day 5 or 6 of incubation.

Material and methods. Outbred white Leghorn chick embryos were incubated at 38 °C in a humid environment. Experimental embryos were obtained through a microsurgical procedure. Eggs at 5 or 6 days of incubation were opened at the blunt pole above the air chamber. Shell membrane, chorion and amnion were torn open. The torn amnion was used as a cord one end of which remained connected to the umbilicus, while the other was pulled over to the egg shell where it adhered. In order to extend the neck, the head of the embryo was pulled with a hairloop in the opposite direction to that of the umbilical cord (fig. 1). After skin incision, the median bilobed thyroid primordium (5-day-old embryos) or the 2 separate thyroid glands (6-day-old embryos) were dissected with a small hairloop and

Corticosterone binding capacities in the plasma of chick embryos

Days of incubation	Number of determinations*	Corticosterone binding capacity (10^{-9} M)
13, Control	5	164±54**
13, Thyroidectomy	3	188±97; NS***
17, Control	3	141±17
17, Thyroidectomy	4	178±111; NS
20, Control	9	50±28
20, Thyroidectomy	7	59±29; NS

* Each determination was made on a sample of blood obtained from one embryo. ** Mean± SD. *** Not significantly different (NS) from the preceding value ($p > 0.05$) (Student's t-test).