EFFECT OF PROSTAGLANDIN $F_{2\alpha}$ ON SEA URCHIN EMBRYOS AT DIFFERENT STAGES OF DEVELOPMENT AND ON THEIR SENSITIVITY TO NEUROPHARMACOLOGICAL AGENTS

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The effect of prostaglandin $F_{2\alpha}$ on early cleavage of sea urchin (Stronglyocentrotus intermedius) embryos was studied. Prostaglandin $F_{2\alpha}$ has an embryotoxic action on the developing oocytes. Adrenalin has a protective action against this embryotoxic action of prostaglandin $F_{2\alpha}$ and prostaglandin $F_{2\alpha}$ itself has a protective action against the toxic effects of chloropromazine and NK-122.

In the period of early embryogenesis of the sea urchin and certain other marine invertebrates physiologically active substances, including noradrenalin, adrenalin, and serotonin, are synthesized. Some phenothiazine (chlorpromazine) and indole derivatives (β-2-indolyl-α-dimethylethylamine hydrochloride – NK-122) have an embryotoxic action. This action is abolished by injection of exogenous catecholamines or serotonin, respectively [1].

The relations between these monoamines are not antagonistic. At the same time it has been postulated that postaglandins may be identical with the endogenous factors which reduce the sensitivity of embryos to certain neuropharmacological agents [2].

The presence of prostaglandin-like compounds in sea urchin oocytes was demonstrated by the writers previously [3]. There is evidence in the literature of antagonistic relations between prostaglandins and adrenalin [6]. The existence of antagonism has accordingly been postulated between prostaglandins and adrenalin in the period of early embryogenesis.

Chlorpromazine and NK-122 have an embryotoxic action, evidently through competition with adrenalin and serotonin. Chlorpromazine and NK-122 are regarded as mimetics of adrenalin and serotonin which, by their action on the embryo, induce irreversible changes [1]. Since adrenalin and serotonin do not disturb the normal process of embryogenesis in the sea urchin, even in very high concentrations, chlorpromazine and NK-122 were chosen for the study of this postulated antagonism.

The direct effect of prostaglandins on early embryogenesis has not yet been studied, and only isolated details are known of the action of prostaglandins on implantation processes in some mammals [3-5].

The effect of prostaglandin $F_{2\alpha}$ on the development of sea urchin embryos and on their sensitivity to chlorpromazine and to NK-122 was studied in the present investigation.

EXPERIMENTAL METHOD

Embryos of the sea urchin Strongylocentrotus intermedius were used. Ripe oocytes were removed from the gonads by means of 0.5M KCl solution and fertilized with freshly obtained sperm. The fertilized oocytes were incubated in watch glasses (25 oocytes in each glass) at 20-22°C.

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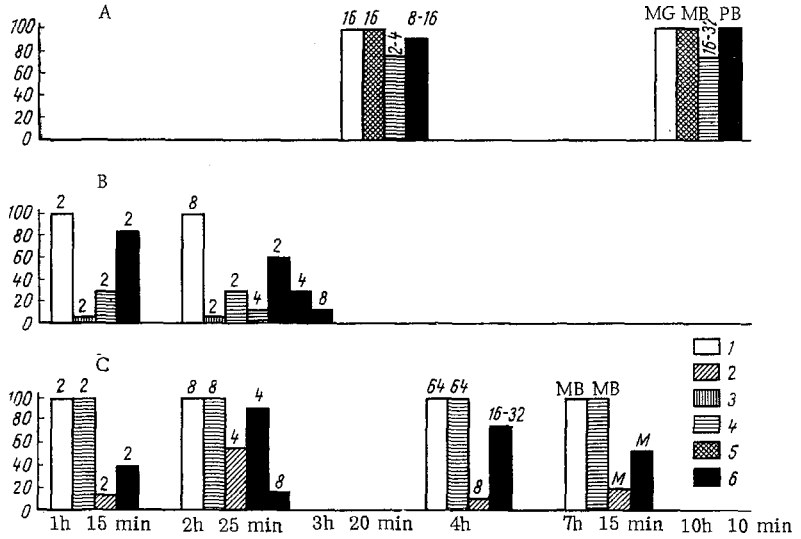

Fig. 1. Effect of prostaglandin $F_{2\alpha}$ on developing sea urchin oocytes and on their sensitivity to some neuropharmacological agents: A) protective action of adrenalin against the toxic action of prostaglandin $F_{2\alpha}$ on fertilized oocytes; B, C) protective action of prostaglandin $F_{2\alpha}$ on toxic effects of chlorpromazine and NK-122, respectively. Stages of development: 1) not passed through first clevage division; 2, 4, 8, 16, 32, 64) number of blastomeres in developing oocytes. M) morula; PB) parvocellular blastula; MB) mesenchymal blastula. 1) Development of oocytes in sea water (control); 2) in adrenalin (5×10^{-2} mg/ml); 3) in chlorpromazine (8×10^{-3} mg/ml); 4) in prostaglandin (A and B, 1×10^{-1} mg/ml; C, 1×10^{-2} mg/ml); 5) in NK-122 (5×10^{-2} mg/ml); 6) in A – prostaglandin + adrenalin, in B – chlorpromazine + prostaglandin, in C – NK-122 + prostaglandin. Abscissa, time of development; ordinate, percentage of oocytes.

To study the embryotoxic effect of prostaglandin $F_{2\alpha}$* on cleavage of the sea urchin embryos, concentrations of the prostaglandin in sea water of between 2×10^{-1} and 4×10^{-2} mg/ml were used.

To study the protective action of adrenalin against the embryotoxic action of prostaglandin $F_{2\alpha}$ and the protective action of prostaglandin $F_{2\alpha}$ against the embryotoxic effect of chlorpromazine and NK-122, the oocytes were incubated in watch glasses up to the mesenchymal blastula stage in 0.75 ml sea water, containing in one case prostaglandin with adrenalin hydrochloride and in the other case prostaglandin with chlorpromazine or NK-122. A concentration of prostaglandin of 1×10^{-1} mg/ml was taken as toxic and a concentration of adrenalin of 5×10^{-2} mg/ml as protective. To protect against the toxic action of chlorpromazine (8×10^{-3} mg/ml) and NK-122 (5×10^{-2} mg/ml) prostaglandin was used in concentrations of 1×10^{-1} and 1×10^{-2} mg/ml, respectively. When 100% of the oocytes of the control series had completed the stages of fractionation (the stages of 2, 4, 8, and 16 blastomeres and the mesenchymal blastula) the development of the oocytes in the experimental series was analyzed.

A 10% level of significance was adopted for the statistical analysis (Student's criterion).

EXPERIMENTAL RESULTS

The investigations showed that prostaglandin $F_{2\alpha}$, depending on its concentration, produced various degrees of disturbance of development of the sea urchin embryos ranging to complete arrest of development at the zygote stage. In concentrations of 2×10^{-1} and 1×10^{-1} mg/ml it arrested development at the zygote stage although some oocytes passed through several of the first divisions. The blastomeres of these embryos were extremely abnormal. Prostaglandin in a concentration of 9×10^{-2} mg/ml or below blocked development at later stages, while in concentrations of 5×10^{-2} and 4×10^{-2} mg/ml it caused no appreciable disturbances in development as far as the gastrula stage.

* The prostaglandin $F_{2\alpha}$ was obtained from Professor S. Bergstrom's laboratory in Sweden.

Adrenalin, injected in a dose of 5×10^{-2} mg/ml simultaneously with prostaglandin in the toxic concentration of 1×10^{-1} mg/ml, was found to protect against the embryotoxic action of the prostaglandin (Fig. 1A). It is clear from Fig. 1 that 3 h 20 min after fertilization, when 100% of the oocytes of the control series had reached the 16-blastomere stage, 76% of the embryos developing in prostaglandin had reached the stage of 2-4 blastomeres. The remaining 24% had not passed through the first cleavage division. However, in the presence of adrenalin 90% of the oocytes reached the 8-16 blastomere stage. After development for 10 h 10 min, when the control oocytes had reached the mesenchymal blastula stage, 76% of the oocytes treated with prostaglandin reached the 16-32-blastula stage. As a rule the blastomeres in these ooctyes were unequal in size. Embryos protected against the action of prostaglandin by adrenalin reached the parvocellular blastula stage in 100% of cases and were not more than 2 h behind the control embryos in their development. No visible morphological changes were found in these embryos.

In a concentration of 1×10^{-1} mg/ml prostaglandin $F_{2\alpha}$ had a protective action against the toxic effect of chlorpromazine in a concentration of 8×10^{-3} mg/ml (Fig. 1B). In the control series 100% of the oocytes had reached the two blastomere stage 1 h 15 min after fertilization. Oocytes treated with chlorpromazine reached the 2-blastomere stage in only 4% of cases. Treatment with prostaglandin led to 28% of oocytes reaching the 2-blastomere stage. Addition of prostaglandin simultaneously with chlorpromazine to the incubation medium had a protective action and 83% of the oocytes reached the 2-blastomere stage. After 2 h 25 min 100% of the control oocytes had reached the 8-blastomere stage. The protective effect of prostaglandin by this time was less strong. Only 10% of oocytes reached the 8-blastomere stage, 30% the 4-blastomere, and 60% the 2-blastomere stage. As before, in 96% of oocytes treated with chlorpromazine the first cleavage division did not take place. Signs of degeneration were observed in them. Hence, despite the fact that prostaglandin $F_{2\alpha}$ in a concentration of 1×10^{-1} mg/ml itself has a toxic action, in this concentration it protects against the embryotoxic action of chlorpromazine.

In the study of the protective action of prostaglandin $F_{2\alpha}$ against the embryotoxic effect of NK-122, a protective effect of a nontoxic concentration (1×10^{-2} mg/ml) of the former was observed (Fig. 1C). Oocytes treated with NK-122 reached the 2-blastomere stage after 1 h 15 min in only 14% of cases. Simultaneous injection of prostaglandins increased the number of these oocytes to 37%. After 2 h 25 min, when 100% of the oocytes in the control were at the 8-blastomere stage, 86% of oocytes protected by prostaglandin reached the 4-blastomere stage, and 14% the 8-blastomere stage. The protective effect still remained after 4 h and 7 h 15 min of development.

Adrenalin hydrochloride, which was used to protect against the toxic action of prostaglandin $F_{2\alpha}$, is a salt of a strong acid (HCl) and a weak base. The functional groups of adrenalin (amino groups) are blocked by a strong acid. Accordingly, a weak acid (prostaglandin $F_{2\alpha}$) cannot compete with it. The probability of neutralization by adrenalin at the moment of mixing these compounds is thus nonexistent. It can only be assumed that the protective effect of adrenalin against toxic doses of prostaglandin is accomplished at the level of cellular structures. It is difficult at present to make any categorical statement on the concrete mechanism of the protective action of prostaglandin $F_{2\alpha}$.

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