# EFFECT OF CYCLIC AMP ON THE DEVELOPMENT OF SEA URCHIN EMBRYOS

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The effect of cyclic adenosine-3',5'-phosphate (cyclic AMP) on cleavage of early embryos of the sea urchin Strongylocentrotus intermedius was studied. A nontoxic concentration of cyclic AMP was found to have a protective action against the embryotoxic effect of serotonin antagonists NK-122 and chlorpromazine, as well as a protective action against the effect of a toxic concentration of prostaglandin $F_{2\alpha}$; prostaglandin $F_{2\alpha}$ also had a protective action against the effect of a toxic concentration of cyclic AMP.

KEY WORDS: cyclic AMP; embryotoxic action; prostaglandin $F_{2\alpha}$.

The object of the present investigation was to study the possibility of protecting developing embryos of the sea urchin Strongylocentrotus intermedius by means of cyclic adenosine-3',5'-phosphate (cyclic AMP) against the embryotoxic action of two serotonin antagonists – $\beta$-2-indolyl-$\alpha$-dimethylethylamine hydrochloride (NK-122)* and chlorpromazine – and also of the hormone-like compound prostaglandin $F_{2\alpha}$.

The investigation was carried out along these lines for two reasons: first, the writers had previously isolated endogenous prostaglandin-like compounds from the fertilized oocytes of this species of sea urchin [3] and demonstrated the ability of exogenous prostaglandin $F_{2\alpha}$† to protect the developing sea urchin embryos against the toxic action of the above-mentioned serotonin antagonists [4], and second, prostaglandins and cyclic AMP are mutually related in a definite manner in cell metabolism, for prostaglandins control the synthesis of cyclic AMP from ATP [6].

## EXPERIMENTAL METHOD

Experiments were carried out on embryos of the sea urchin S. intermedius. The method of obtaining the oocytes and of their fertilization and incubation to the mesenchymal blastula stage was described previously [1, 4]. The fertilized oocytes were placed in the test solutions not later than 5 min after fertilization and they were incubated for 20-22°C in watchglasses kept in wet chambers.

In the experiments of series I the effect of cyclic AMP in concentrations of 1 to $4 \cdot 10^{-1}$ mg/ml on the development of early embryos was studied. To investigate the protective effect of cyclic AMP against the embryotoxic action of NK-122 ($3 \cdot 10^{-2}$ mg/ml) and chlorpromazine ($3 \cdot 10^{-3}$ mg/ml) cyclic AMP was used in a nontoxic concentration ($3 \cdot 10^{-1}$ mg/ml).

The embryo toxic action of prostaglandin in a concentration of $4 \cdot 10^{-1}$ mg/ml was abolished by cyclic AMP in a concentration of $3 \cdot 10^{-1}$ mg/ml and the embryotoxic action of cyclic AMP in a concentration of $8 \cdot 10^{-1}$ mg/ml was abolished by a nontoxic concentration of prostaglandin $F_{2\alpha}$ of $1 \cdot 10^{-2}$ mg/ml.

In all the control series the embryos developed in seawater.

* The preparation was synthesized by N. F. Kucherova at the Institute of Pharmacology, Academy of Medical Sciences of the USSR, who generously provided it for the investigation.
† The preparation Dinoprost, the tromethamine salt of prostaglandin $F_{2\alpha}$.

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TABLE 1. Effect of Cyclic AMP on Cleavage of Sea Urchin Embryos (in % of control)

| Concentration (mg/ml) | Stage of development | | | | |
|---|---|---|---|---|---|
| | 2 blastomeres | 4 blastomeres | 8 blastomeres | morula | mesenchymal blastula |
| 1 | 0 | 0 | 0 | 0 | 0 |
| $9.10^{-1}$ | 48 | 41 | 31 | 0 | 0 |
| $8.10^{-1}$ | 86 | 64 | 57 | 30 | 27 |
| $7.10^{-1}$ | 100 | 96 | 90 | 58 | 52 |
| $6.10^{-1}$ | 100 | 100 | 98 | 86 | 78 |
| $5.10^{-1}$ | 100 | 100 | 100 | 94 | 91 |
| $4.10^{-1}$ | 100 | 100 | 100 | 100 | 100 |
| Control | 100 | 100 | 100 | 100 | 100 |

Development of the embryos was assessed at the stages of 2, 4, 8, and 16 blastomeres and at the mesenchymal blastula stage. The percentage of embryos in the experimental period was calculated, starting from the time when 100% of embryos of the control series had reached the above-mentioned stages of development.

## EXPERIMENTAL RESULTS

Cyclic AMP in a concentration of 1 mg/ml is absolutely toxic, for in 100% of cases the development of the embryo was arrested at the zygote stage. The percentage of normally developing embryos increased with a decrease in the concentration of cyclic AMP from $9 \cdot 10^{-1}$ to $4 \cdot 10^{-1}$ mg/ml (Table 1). In a concentration of $4 \cdot 10^{-1}$ mg/ml cyclic AMP was not toxic, for all the embryos developed just as in the control series.

Administration of NK-122 in a concentration of $3 \cdot 10^{-2}$ mg/ml was followed by development of only 3% of the embryos into abnormal half-blastulas. Meanwhile, 83.5% of embryos protected with cyclic AMP reached the mesenchymal blastula stage (Fig. 1A).

In the presence of chlorpromazine alone, 71.5% of the embryos reached the two blastomere stage, 57.0% of the four blastomere stage, and 13.5% the 8 blastomere stage; development ceased at the 8 blastomere stage. On the addition of cyclic AMP, 96%, 90%, and 65.5% of embryos respectively reached these stages of development. The morula stage was reached by 47% of embryos (Fig. 1B).

Prostaglandin in a concentration of $4 \cdot 10^{-1}$ mg/ml, inhibited the onset of cleavage: 31% of embryos passed through the first cleavage division, when embryos of the control series were already at the 8 blastomere stage. Abnormal half-blastulas were formed by the cleaving embryos. The addition of cyclic AMP led 100% of embryos to reach the mesenchymal blastula stage. No delay in cleavage of the embryos of the experimental series was observed (Fig. 1C).

In a nontoxic concentration of $1 \cdot 10^{-2}$ mg/ml, prostaglandin abolished the embryotoxic effect of cyclic AMP. Under the influence of cyclic AMP, 12.5% of the embryos developed to the 16 blastomere stage. The two blastomere stage was reached by 31% of the embryos at a time when all the control embryos were at the 8 blastomere stage. After the addition of prostaglandin, 90% of embryos reached the two blastomere stage, 89% the morula stage, and 49% the mesenchymal blastula stage (Fig. 1D).

The view is held at the present time that cyclic AMP plays an important role in the regulation of biosynthesis in animal cells of various species [6, 7]. Hormones are considered to act as "primary regulators" in this situation, and cyclic AMP as a "secondary regulator" [6]. A certain hormone, behaving as the "primary regulator," reacts with the cell to activate the enzyme adenylcyclase, which converts ATP into cyclic AMP, the "secondary regulator." Later, with the aid of the universal regulator cyclic AMP-dependent protein kinase, cyclic AMP transfers a phosphate residue from ATP to various enzymes, thereby bringing about, in particular, a phosphorylation reaction. Enzymes activated in this manner modify the course of various physiological processes in the cell. The essential level of cyclic AMP in the cell is maintained, first, by the action of adenylcyclase (activation of synthesis from ATP) and, second, through the action of the enzyme cyclic nucleotide phosphodiesterase, which catalyzes the breakdown of cyclic AMP into 5'-AMP.

Meanwhile, it has been shown that adenylcyclase activity can be modified by prostaglandins [5].

In connection with the view of the role of cyclic AMP and prostaglandins in the regulation of biosynthesis described above, it was most interesting to study whether cyclic AMP can protect against the embryotoxic action of serotonin antagonists, namely NK-122 and chlorpromazine. The study of this problem was interesting also in connection with earlier findings showing the presence of prostaglandin-like compounds in sea urchin embryos [3] and the protective action of prostaglandin $F_{2\alpha}$ against the embryotoxic effects of NK-122 and chlorpromazine.

In the writers' view, there are very interesting data in the literature which show that chlorpromazine, acting as serotonin antagonist during the early embryogenesis of sea urchins [1] and mammals [2], can block the activity of the adenylcyclase system.

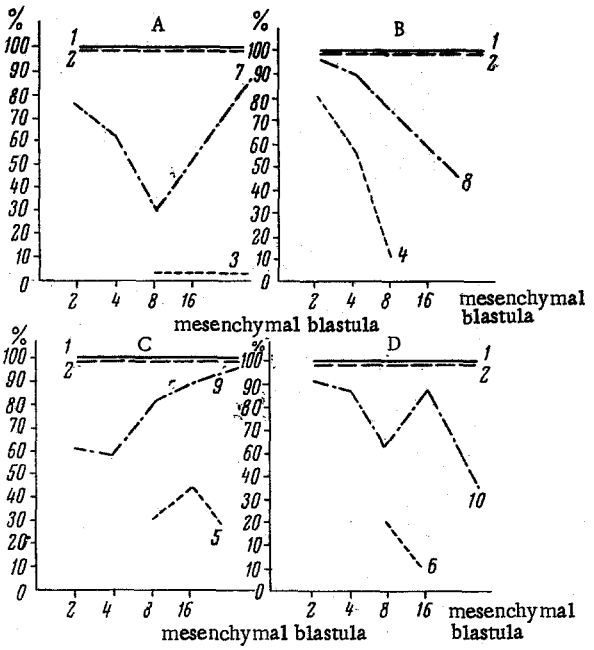


Fig. 1. Protective action of cyclic AMP (A, B, C) and prostaglandin $F_{2\alpha}$ (D) against embryotoxic effect of certain neuropharmacological agents (concentrations given in mg/ml): 1) development of embryos in sea water; 2) in nontoxic concentrations of cyclic AMP ($4 \cdot 10^{-1}$; A, B, C) and prostaglandin ($1 \cdot 10^{-2}$; D); 3,7) in NK-122 ($3 \cdot 10^{-2}$) and NK-122 ($3 \cdot 10^{-2}$) + cyclic AMP ($4 \cdot 10^{-1}$) respectively; 4,8) in chlorpromazine ($3 \cdot 10^{-3}$) and chlorpromazine ($3 \cdot 10^{-3}$) + cyclic AMP ($4 \cdot 10^{-1}$) respectively; 5,9) in prostaglandin ($4 \cdot 10^{-1}$) and prostaglandin ($4 \cdot 10^{-1}$) + cyclic AMP ($4 \cdot 10^{-1}$) respectively; 6,10) in cyclic AMP ($8 \cdot 10^{-1}$) and cyclic AMP ($8 \cdot 10^{-1}$) + prostaglandin ($1 \cdot 10^{-2}$) respectively. Abscissa, stage of development of embryos; ordinate, percentage of surviving embryos.

The protective effect of cyclic AMP against the embryotoxic action of NK-122 and chlorpromazine on developing sea urchin embryos discovered in these experiments may be associated with the fact that exogenous cyclic AMP can restore the level of endogenous cyclic AMP when disturbed by the possible blockade of adenylcyclase by serotonin antagonists.

It can also be postulated that the protective effect of prostaglandin $F_{2\alpha}$ discovered previously, although only weak in character, may be explained on the grounds that, by blocking the adenylcyclase system, they disturbed normal regulatory processes in the cell, and the exogenous prostaglandin abolished these disturbances.

The hypotheses expressed above regarding the role of cyclic AMP and prostaglandin in the regulation of early embryogenesis require experimental confirmation.

So far as the mechanism of the mutual protective effect of cyclic AMP and prostaglandin against their embryotoxic action is concerned, this is difficult at present to explain.

## LITERATURE CITED

1. G. A. Buznikov, Low-Molecular-Weight Regulators of Embryonic Development [in Russian], Moscow (1967).
2. B. V. Leonov, A. P. Kamakhin, and G. A. Buznikov, Dokl. Akad. Nauk SSSR, 188, 734 (1969).
3. M. A. Lomova, L. S. Persianinov, B. V. Leonov, et al., Akush. i Gin., No. 11, 73 (1973).
4. L. S. Persianinov, B. V. Leonov, L. M. Massal'skaya, et al., Byull. Éksperim. Biol. i Med., No. 10, 105 (1973).
5. R. W. Butcher, Advan. Enzyme Reg., 6, 357 (1968).
6. E. W. Sutherland, J. Am. Med. Assn., 214, 1281 (1970).
7. E. W. Sutherland, Science, 177, 401 (1972).