CHANGES IN SENSITIVITY OF DEVELOPING SEA URCHIN EMBRYOS TO PROSTAGLANDIN $F_{2\alpha}$ AND TO CYCLIC AMP

L. S. Persianinov and L. M. Massal'skaya

UDC 612.646.014.46 : 615.357.637

The developing ova of the sea urchin Strongylocentrotus intermedius, at different stages of the first mitotic cycles, vary in their sensitivity to toxic concentrations of prostaglandin $F_{2\alpha}$ and cyclic 3',5'-AMP. Differences in sensitivity to each of these substances follow a similar and regular pattern and depend on the time elapsing after the preceding division. Two periods of increased sensitivity of the ova were found: the first, 20 min after completion of each division, to both substances and the second, after 40 min to cyclic AMP and after 50 min to prostaglandin.

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

The early development of sea urchins is characterized by regular changes in their content of certain endogenous biologically active substances such as serotonin, adrenalin, etc., not only in the course of cleavage divisions, but within each mitotic cycle [1, 2]. In previous papers the writers described the isolation of endogenous prostaglandin-like compounds from embryos of the sea urchin Strongylocentrotus intermedius [5], the protective role of exogenous prostaglandin $F_{2\alpha}$ against the embryotoxic action of certain antiserotonin agents [6], and also interaction in early sea urchin embryos at the level of cell receptors of prostaglandin $F_{2\alpha}$ and cyclic AMP [7]. Other workers have described interaction between prostaglandins and cyclic AMP at the level of cellular structures in the tissues of adult organisms [8, 9].

It was therefore decided to study changes in the sensitivity of cleaving sea urchin ova to prostaglandin and to cyclic AMP during the first mitotic cycles and to discover any regular patterns in the response of the embryos.

EXPERIMENTAL METHOD

Experiments were carried out on ova of the sea urchin Strongylocentrotus intermedius. The ova were obtained, fertilized, and incubated to the mesenchymal blastula stage as described previously [6]. In order to detect differences in the sensitivity of the developing embryos to prostaglandin $F_{2\alpha}$ and cyclic AMP, toxic concentrations of these substances were used – $1 \cdot 10^{-1}$ and 1 mg/ml, respectively. The fertilized ova, 100 at a time, were placed in these substances after every 5 min throughout the period of the first mitotic cycle (75 min), starting from 5 min after fertilization (15 points) and incubated in these solutions as far as the mesenchymal blastula stage. Ova of the control series developed in sea water.

Differences in the embryotoxic action of prostaglandin and cyclic AMP during the mitotic cycles were analyzed, also at 5-min intervls, by counting the number of embryos reaching the control stages of development, starting from the time when the corresponding stage of development in the control series was 100%. For example, if the corresponding batch of ova was placed in the above-mentioned solutions 5 min after fertilization, the developing embryos in this batch were analyzed in every case 5 min after completion of the corresponding division in the control embryos.

All-Union Research Institute of Obstetrics and Gynecology, Ministry of Health of the USSR, Moscow.
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 80, No. 11, pp. 87-90, November, 1975. Original article submitted January 8, 1975.

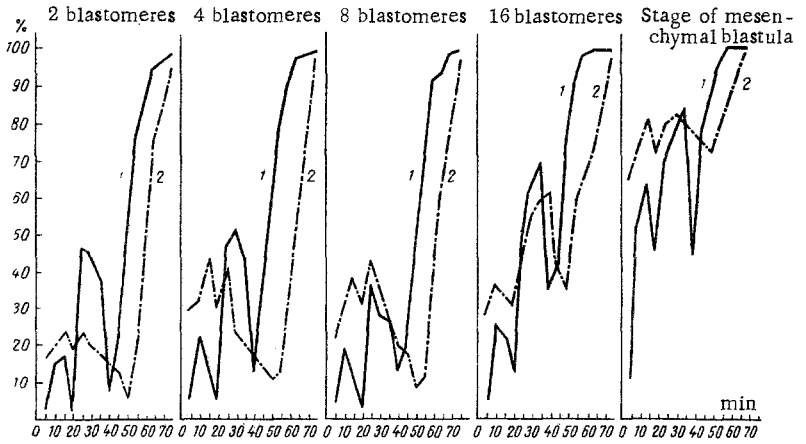

Fig. 1. Changes in sensitivity of cleaving sea urchin ova to prostaglandin $F_{2\alpha}$ and cyclic AMP: 1) cleavage of ova on immersion in cyclic AMP solution ($1 \cdot 10^{-3}$ g/ml); 2) cleavage of ova on immersion in prostaglandin $F_{2\alpha}$ solution ($1 \cdot 10^{-4}$ g/ml). Abscissa, time from beginning of mitotic cycle to immersion in solution (in min); ordinate, number of ova reaching the corresponding stage of development (in %).

The significance of differences in the sensitivity of the embryos was investigated by means of Wilcoxon's nonparametric criterion. Series of indices were compared at the 15th and 25th minutes for periods at the 20th minute, series at the 35th and 45th minutes for periods at the 40th minute, and series at the 45th and 55th minutes for periods at the 50th minute. Differences for which $\Phi_{(t)} \leq \beta$ were regarded as significant. In all series of experiments the value of β did not exceed 5%.

EXPERIMENTAL RESULTS

The results showed that the developing sea urchin ova in the various stages of the mitotic cycles varied in their sensitivity to toxic concentrations of prostaglandin and cyclic AMP. The differences in the sensitivity to these substances were perfectly regular and identical in character (Fig. 1) and they depended on the time elapsing after completion of each corresponding division. The investigations revealed two periods of increased sensitivity of the developing ova. Maximal sensitivity of the ova to both substances was first found 20 min after passage through the first cleavage division. Of the ova taken at this time, 19.3% in the prostaglandin solution and 3.3% in the cyclic AMP solution reached the stage of two blastomeres, whereas of those taken at the 15th and 25th minutes, the percentages reaching this stage were 23.4 and 17, and 23.3 and 47%, respectively. Increased sensitivity of the ova was repeated regularly 20 min after passage through the cleavage division in each subsequent mitotic cycle: at the stages of 2, 4, 8, and 16 blastomeres and the mesenchymal blastula stage. The second period of increased sensitivity was found 40 min after completion of each division in cyclic AMP solution and at the 50th minute in prostaglandin solution. In prostaglandin, only 4.7% of ova reached the stage of two blastomeres at the 50th minute, compared with 13% at the 45th and 23% at the 55th minute. In cyclic AMP, 8% of ova reached the stage of two blastomeres at the 40th minute and 39.7 and 26.3% at the 35th and 45th minutes, respectively. The absolute sensitivity of the ova started to fall at the 16-blastomere stage. For instance, whereas in prostaglandin, 19.3, 31.3, and 32.3% of ova reached the stages of two, four, and eight blastomeres, respectively, at the 20th minute, 36 and 73.7% reached the stages of 16 blastomeres and the mesenchymal blastula, respectively. A similar pattern also was characteristic of ova developing in cyclic AMP; 3.3, 4.7, and 3% of ova reached the stages of two, four, and eight blastomeres, respectively, whereas 13 and 45.7% reached the stages of 16 blastomeres and the mesenchymal blastula. This pattern was more marked still for the second period of increased sensitivity. For instance, 4.7, 10.7, and 8.7% of ova reached the stages of two, four, and eight blastomeres, respectively, at the 50th minute in prostaglandin compared with 36.7 and 74.3% at the stages of 16 blastomeres and the mesenchymal blastula. In cyclic AMP, 8, 13, and 13.7% of ova reached the stages of two, four, and eight blastomeres, respectively, at the 40th minute, whereas 36 and 45.3% reached the stages of 16 blastomeres and the mesenchymal blastula, respectively.

The periods of increased sensitivity and also their alternation with periods of relative resistance were more clearly defined in the ova developing in the presence of cyclic AMP.

The unequal sensitivity of sea urchin ova to the action of toxic concentrations of prostaglandin and cyclic AMP could thus be clearly traced in all mitotic cycles studied. The two periods of increased sensitivity of the developing ova within each cycle to the two substances indicate not only that prostaglandin and cyclic AMP participate at this stage of ontogeny in as yet unknown intracellular processes, but also a regular and successive contribution by prostaglandin after cyclic AMP to processes that are repeated in each mitotic cycle.

Although periods of increased sensitivity coincide in time in all the cycles studied, the degree of the increase diminished as the ova developed (starting from the stage of 16 blastomeres), most probably in connection with the onset of desynchronization in cleavage.

It is relevant at this stage to mention results obtained by Buznikov et al. [3]. In an investigation of the effect of serotonin on the activation of protein synthesis in fertilized sea urchin ova they showed that the endogenous serotonin concentration falls to a minimum in sea urchins of the species Strongylocentrotus intermedius at the 21st minute after fertilization. In the same investigation they found that marked activation of protein biosynthesis, which they connected with the minimal level of endogenous serotonin, first occurred at the 20th minute after fertilization. It is not difficult to suggest that, at least for the period of synchronous cleavage (the first four divisions), the drop in the serotonin level and the beginning of activation of protein biosynthesis is repeated regularly at the 20th-21st minute after completion of each successive division. It is therefore perfectly possible that the maximal delay of each successive division in the present experiments at the 20th minute is explained by the minimal endogenous serotonin concentration at that time, in connection with the rhythm of activation of protein biosynthesis immediately before division. To this it must be added that an excess of exogenous serotonin in the medium reliably protects developing sea urchin embryos [1] and mouse embryos developing in vitro [4] from a whole range of neuropharmacological agents.

The coincidence of the periods of increased sensitivity of the embryos at the 20th minute and their desynchronization at the 40th and 50th minutes are evidence of differences in the nature of the damaged mechanisms and, possibly, of the presence of two independent groups of reactive structures in the ovum, each of which has the functional responsibility for the pharmacological effect of prostaglandin and cyclic AMP separately. Whereas the increased sensitivity of the ova at the 20th minute can be explained by the minimal endogenous serotonin level, the mechanism of the increased sensitivity at the 40th and 50th minutes cannot yet be explained.

The participation of prostaglandin and cyclic AMP in processes of biosynthesis in sea urchin embryos has thus been demonstrated indirectly and the temporal parameters of the action of the hypothetical mechanism determined.

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