PROTECTIVE EFFECT OF SEROTONIN AGAINST THE INHIBITORY ACTION OF CHLORPROMAZINE ON MEMBRANE POTENTIAL OF TWO-BLASTOMERE MOUSE EMBRYOS IN VITRO

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The depolarizing effect of chlorpromazine (in a concentration of $1 \cdot 10^{-5}-2 \cdot 10^{-5}$ g/ml nutrient medium) on the membrane potential of mouse embryonic cells (at the two-blastomere stage) and also the effect of serotonin and prostaglandin $F_{2\alpha}$ in concentrations of $1 \cdot 10^{-4}$ and $4 \cdot 10^{-6}-8 \cdot 10^{-6}$ g/ml, respectively, on this depolarization were investigated. Preliminary administration of serotonin reduced the depolarizing effect of chlorpromazine, but prostaglandin had no such action.

KEY WORDS: cell membrane potential; chlorpromazine; serotonin; prostaglandin $F_{2\alpha}$.

There is evidence [1, 4, 5] to suggest that serotonin may play an important role in the regulation of early development.

The mechanisms of the biological action of the prostaglandins and, in particular, of prostaglandin $F_{2\alpha}$ as a substance with very great promise of a wide field of application in obstetric practice are at present being actively studied [8, 9].

It was decided to study the effect of chlorpromazine, serotonin, and prostaglandin $F_{2\alpha}$ on the membrane potential (MP) in the period of early embryonic development in order to examine the mechanisms of their action on embryonic cells.

EXPERIMENTAL METHOD

The MP was measured in cells of mouse embryos flushed from the oviducts of noninbred albino mice [8] on the 2nd day of pregnancy and at the two-blastometer stage, using a special constant-temperature cell filled with Brinster's nutrient medium [10] for this purpose. The diameter of the tips of the glass microelectrodes filled with 3 M KCl solution did not exceed 0.5 μ and their resistance was below 200 mΩ. The microelectrode was introduced into the embryonic cell by means of the MM-1 micromanipulator.

The MP was recorded by the pH-340 millivoltmeter. The tromethamine salt of prostaglandin was used. The calculation was based on the actual content of prostaglandin $F_{2\alpha}$ itself.

EXPERIMENTAL RESULTS

Addition of chlorpromazine to the nutrient medium in concentrations of $5 \cdot 10^{-7}-4 \cdot 10^{-6}$ g/ml caused no change in MP. However, an increase in the concentration to $1 \cdot 10^{-5}-2 \cdot 10^{-5}$ g/ml was accompanied by a significant decrease in MP (Table 1). Addition of chlorpromazine to the nutrient medium in a concentration of $2 \cdot 10^{-5}$ g/ml caused the MP to fall by about 50% of its initial level in the course of 10-20 min.

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TABLE 1. Effect of Chlorpromazine, Serotonin, and Prostaglandin $F_{2\alpha}$ on MP of Mouse Embryos at the Two-Blastomere Stage in Vitro

Embryo No.	Inhibition of MP by chlorpromazine, $1 \cdot 10^{-5}$ g/ml (% of initial level)	Embryo No.	Protective effect of serotinin, $1 \cdot 10^{-4}$ g/ml, against inhibitory action of chlorpromazine, $2 \cdot 10^{-5}$ g/ml on MP (% of initial level)	Embryo No.	Effect of chlorpromazine, $2 \cdot 10^{-5}$ g/ml in the presence of prostaglandin $F_{2\alpha}$, $4 \cdot 10^{-6}$–$8 \cdot 10^{-6}$ g/ml on MP (% of initial level)
1	57,0	1	7,0	1	68,0
2	28,0	2	46,0	2	50,0
3	72,5	3	32,0	3	27,0
4	33,0	4	—14,0*	4	55,0
5	37,0	5	37,0	—	—
6	50,0	—	—	—	—
M ± m	51,4±11,2	M ± m	21,6±22,0	M ± m	50,0±14,8

*No inhibition, MP increased by 14%.

The absolute value of the MP in this series of experiments varied on the average from 26.6 (21, 22, 16, 40, 25, 16) to 10.5 mV (9, 11, 10, 13, 13, 9 mV, respectively). The decrease in MP began 2-3 min after addition of the chlorpromazine. The MP did not fall to zero under the influence of chlorpromazine. The data showing the degree of the depolarizing effect of chlorpromazine are given in Table 1.

If serotonin was added to the nutrient medium (in a concentration of $1 \cdot 10^{-4}$ g/ml) before chlorpromazine, the decrease in MP was smaller and sometimes it was completely prevented. This effect of weakening of the depolarizing action of chloropromazine by serotonin was significant ($P < 0.05$).

The presence of serotonin alone in the nutrient medium in the same concentration had no effect on the MP of the embryonic cells, at least during the first 10-15 min after its addition.

Prostaglandin $F_{2\alpha}$ (in concentrations of $4 \cdot 10^{-6} - 8 \cdot 10^{-6}$ g/ml) had no action either on the magnitude of the MP or the effect of chlorpromazine on it.

It has been postulated that serotonin may reduce the permeability of the nuclear membranes in embryonic cells to mRNA and tRNA and also to amino acids, thereby modifying protein synthesis in the cells as a result of changes in the transport of these macromolecules and amino acids [2]. The results suggest that serotonin may also act at the level of the outer membranes, and that it may participate in this way in the regulation of the functions of the embryonic cells.

Prostaglandin $F_{2\alpha}$ has been shown by the writers to increase the percentage of embryos that reach the blastocyst stage when grown in culture in vitro. In the present investigation no effect of prostaglandin on the MP was found. This may indicate some slowly developing response of the embryonic cells to prostaglandin.

In subtoxic concentrations (about $1 \cdot 10^{-4}$ g ml) prostaglandin $F_{2\alpha}$ weakened the effect of toxic concentrations of chlorpromazine, as the writers have shown on sea urchin embryos in pregastrulation stages [3, 6]. The possibility of interaction between prostaglandin and embryonic cells at the level of the outer membranes cannot therefore be completely ruled out at the present level of our knowledge.

LITERATURE CITED

1. G. A. Buznikov, Low-Molecular-Weight Regulators of Embryonic Development [in Russian], Moscow (1967).
2. G. A. Buznikov, G. G. Gauze, and N. D. Zvezdina, Ontogenez, No. 5, 539 (1971).
3. G. A. Buznikov, N. D. Zvezdina, N. V. Prokazova, et al., Dokl. Akad. Nauk SSSR, No. 6, 1457 (1973).
4. B. V. Leonov and A. Yu. Budantsev, Dokl. Akad. Nauk SSSR, No. 3, 734 (1971).
5. B. V. Leonov, A. P. Kamakhin, and G. A. Buznikov, Dokl. Akad. Nauk SSSR, No. 4, 958 (1969).
6. L. S. Persianinov, B. V. Leonov, L. M. Massal'skaya, et al., Byull. Éksperim. Biol. i Med., No. 10, 105 (1973).
7. L. S. Persianinov, A. V. Limantsev, and B. V. Leonov, Byull. Éksperim. Biol. i Med., No. 9, 10 (1973).
8. L. S. Persianinov, I. A. Manuilova, and E. A. Chernukha, Akush. i Gin., No. 7, 3 (1972).
9. S. Bergström, L. B. Carlson, and J. R. Weeks, Pharmacol. Rev., 20, 1 (1968).
10. R. L. Brinster, Advances Biosci., 4, 199 (1970).