USE OF BIOLOGICAL METHODS TO ANALYZE PURINERGIC ACTIVITY OF ETHIMIZOLE

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A culture of cleaving sea urchin oocytes is used as a biological object with which to test the action of drugs and toxic compounds [5, 10]. Already in the initial stages of development, sea urchins have virtually all the preneural mediator systems: cholinergic, adrenergic, and serotoninergic [4]. The active extracellular action of purine compounds (ATP, AMP, adenosine) also has been demonstrated on this model [4].

We have analyzed the effect of ethimizole [1], a compound of purine nature with activity relative to P_1-purinergic cultures [6], on the development of a culture of the sea urchin *Strongylocentrotus intermedius*. Another biological method used to analyze the purinergic activity of ethimizole was recording motor and electrical activity of isolated smooth-muscle strips of the guinea pig cecum by the sucrose gap method.

EXPERIMENTAL METHOD

Experiments to test the effect of ethimizole on the development of sea urchin embryos were carried out at the "Vityaz'" marine aquarium base, Far Eastern Scientific Center, Academy of Sciences of the USSR, in July and August, 1985. The method described in [5, 7] was used in order to culture the sea urchins.

Incubation of cleaving sea urchin oocytes was carried out in filtered sea water (control) and after the addition of ethimizole in a concentration of 10^{-13}-10^{-8} M, and also of theophylline, which blocks T_1 purine receptors, in a concentration of 10^{-12}-10^{-9} M. The development of sea urchin embryos was observed for 3 days (72 h) until the beginning of the middle pluteus stage.

The preparations were added 40 min after fertilization, at the stages of 2 and 16 blastomeres and the early blastula. The number of embryos reaching the middle pluteus stage was counted in 100 individuals and expressed in per cent.

The action of ethimizole on activity of a circular preparation of the guinea pig cecum was analyzed by the sucrose gap method [2, 9]. Nerve-muscle preparations of the myenteral plexus and a circular layer of smooth muscle of the cecum were placed in an experimental chamber in Krebs' solution (37°C, pH 7.4), saturated with a mixture of 95% O_2 and 5% CO_2, of the following composition (in mmoles/g): NaCl — 120.4, KCl — 5.9, $NaHCO_3$ — 15.5, $MgCl_2$ — 1.2, $CaCl_2$ — 2.5, glucose — 11.5.

The action of ethimizole in a concentration of 10^{-7}-10^{-4} M, and also of theophylline, and of a combination of ethimizole and theophylline, on the muscle potential of smooth-muscle cells, synaptic potentials, and spontaneous electrical and mechanical activity was studied by simultaneous recording of contractions of the preparation by means of a mechanotron (6 × 1B). The initial tension of the strip was 0.3 g. Transmural stimulation of nerve structures was carried out with square pulses (0.1 msec, frequency in the burst 10 and 30 Hz, duration of burst 1 sec). The strength of stimulation was the threshold for evoking a contractile response. To inhibit adrenergic and cholinergic effects, propanolol 10^{-6} g/ml and atropine 10^{-5} g/ml were added to the solution.

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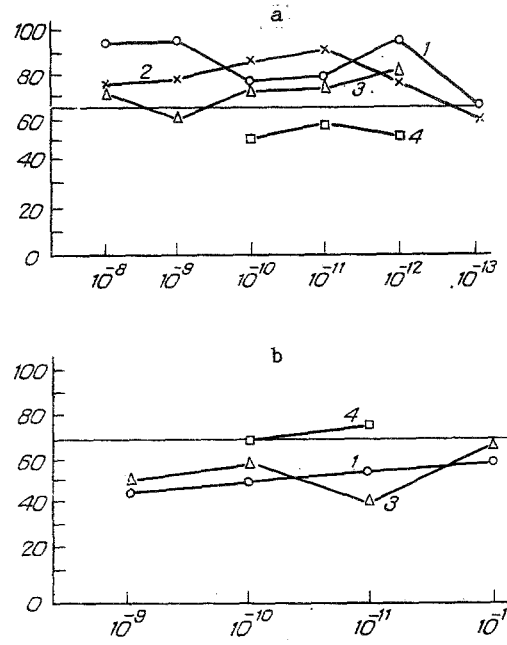

Fig. 1

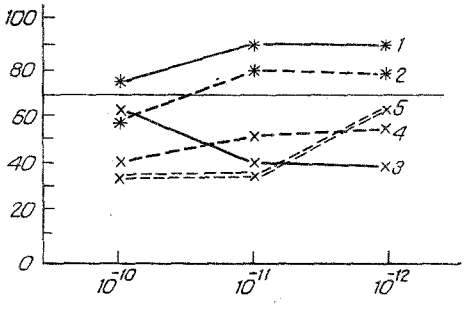

Fig. 2

Fig. 1. Number of sea urchin embryos in the middle pluteus stage 72 h after fertilization (in per cent of the total number of embryos) on incubation with ethimizole (a) and theophylline (b), added at the following stages: before cleavage (1), and 2 blastomeres (3). Here and in Fig. 2: 2 blastomeres (2), 16 blastomeres (3), blastula (4). Horizontal line indicates control. Abscissa, concentration (in M).

Fig. 2. Number of sea urchin embryos in the middle pluteus stage 72 h after fertilization (in per cent of total number of embryos) on incubation in medium containing ethimizole and theophylline, added at the 2-blastomere stage. 1) Ethimizole, 2) ethimizole + theophylline 1 h later, 3) theophylline, 4) theophylline + ethimizole 1 h later, 5) theophylline + ethimizole simultaneouly. Abscissa, concentration (in M).

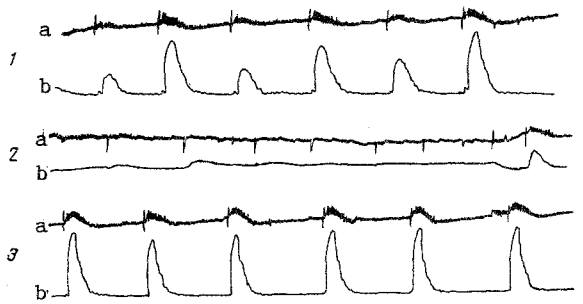

Fig. 3. Changes in electrical (a) and motor (b) activity of smooth-muscle preparation of guinea pig cecum before (1) and after (2) addition of 5×10^{-7} M ethimizole, and after washing out and combined addition of theophylline and ethimizole in concentrations of 5×10^{-7} M (3).

EXPERIMENTAL RESULTS

Addition of ethimizole in the early stages of development of the sea urchin embryos increased their rate of growth. Under control conditions — when the embryos were incubated in filtered sea water — the number of mature forms reaching the middle pluteus stage averaged 65-70%. On incubation of the embryos with ethimizole — after addition of the preparation before the onset of cleavage in concentrations of 10^{-12} and 10^{-9}-10^{-8} M, this figure increased to 90%. The effect of ethimizole was somewhat weaker if it was added at the 2 and 16-blastomere stages, and it was absent altogether if added at the blastula stage (Fig. 1a).

The results of similar tests using theophylline, a blocker of P_1-purinergic adenosine receptors, are given in Fig. 1b. In this case development of the sea urchin embryos was depressed by addition of the preparation in the early stages of development, but it showed little change if it was added at the blastula stage.

To analyze the mechanism of the stimulating action of ethimizole on the development of sea urchin embryos, observations were made during the combined action of ethimizole, which has purinergic activity [6], and theophylline, a blocker of purine receptors. The experiments were carried out with the preparations in equimolar concentrations (10^{-12}-10^{-10} M): 1) with simultaneous addition of the drugs to the incubation medium containing embryos at the 2-blastomere stage, 2) addition of one drug 1 h after the other, i.e., at the 4-blastomere stage, after the first drug added has exerted its effect during cleavage. The preparations in this series of investigations were washed out at the blastula stage.

The investigations showed that on addition of ethimizole, both simultaneously with and after theophylline, the stimulating effect of ethimizole was absent (Fig. 2). If ethimizole was added to the incubation medium before theophylline, an effect of stimulation of embryonic development was observed, but it was somewhat weaker than during the action of ethimizole alone.

Thus when embryonic cultures of the sea urchin *Strongylocentrotus intermedius* were used to test the activity of ethimizole, conclusive evidence was obtained that the drug stimulates embryogenesis even if used in extremely low concentrations (10^{-12}-10^{-22} M), and that this action is abolished by theophylline, a blocker of P_1-purinergic receptors. It can be concluded from these results that if this biological model is used, competition takes place between the drugs for the same receptor systems, and it can be tentatively suggested that these are in fact purinergic mediator systems. The appearance of purinergic mediation has been established previously in the very earliest stages of evolution, long before the formation of other types of neuroreception [3, 8].

Competitive interaction between ethimizole and theophylline was demonstrated by the use of a circular preparation of the guinea pig cecum. These experiments showed that ethimizole, within the concentration range from 10^{-7} to 10^{-4} M inhibits contractility of the smooth-muscle cells, as shown by a fall in the amplitude of contractions of the preparation or even their cessation. The motor activity of the smooth-muscle preparations was restored after removal of the ethimizole by rinsing.

When evoked activity was recorded in response to an electrical stimulus, the frequency of the action potentials was found to be reduced by ethimozole. Under the influence of ethimozole the following effects, which can be interpreted on the whole as inhibitory, were observed: shortening of the latent period of the inhibitory synaptic potential, an increase in its duration and amplitude (Fig. 3). Combined administration of ethimizole and theophylline, a competitive blocker of P_1 purine receptors, in equimolar concentrations (5×10^{-7} M) after rinsing out of the ethimizole was accompanied by spontaneous activity typical of the smooth-muscle preparation, evidence of abolition of the effects of ethimizole.

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