EFFECT OF CYCLIC AMP ON CELLS OF EXPERIMENTAL LYMPHATIC LEUKEMIA L-5718

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The effect of cyclic AMP (cAMP), dibutyryl-cAMP, and theophylline (an inhibitor of phosphodiesterase, the enzyme converting adenosine-3',5'-monophosphate into adenosine-5'-monophosphate) on the intensity of proliferation (as reflected in the increase in the nucleic acid content in the culture), DNA synthesis (thymidine-H^3 incorporation), and transplantation properties (ability to repopulate *in vivo*) of leukemic cells of strain L-5178 was studied. The experiments showed that cAMP in a concentration of 0.8 mM inhibits thymidine-H^3 incorporation considerably, retards proliferation, and reduces the transplantability of the leukemic cells. Theophylline and dibutyryl-cAMP have comparatively weak ability to inhibit DNA synthesis and the proliferative activity and transplantation properties of the cells.

KEY WORDS: *cyclic nucleotides; proliferation.*

Cyclic AMP (cAMP), like its antagonist cyclic GMP, is a universal mediator in the regulation of cell proliferation and differentiation [4, 7, 10, 11]. As a rule the content of cAMP in tumor tissues is low, one possible cause of the constant proliferation of the cells which form them [1].

The antitumor activity of cAMP and its derivatives has been demonstrated in many experimental systems but the mechanism of its action itself has received little study. To begin with, it is not sufficiently clear to what extent tumor growth is due to the extracellular and intracellular content of cAMP, or how cAMP affects DNA synthesis and the proliferative activity of tumor cells.

In this investigation the effect of various doses of cAMP, dibutyryl-cAMP, and theophylline (an inhibitor of phosphodiesterase, the enzyme converting adenosine-3',5'-monophosphate into adenosine-5'-monophosphate) on the intensity of proliferation (reflected in the increase in the nucleic acid content in the culture), DNA synthesis (thymidine-H^3 incorporation), and transplantation properties (ability to repopulate *in vivo* in healthy animals) of leukemic cells of strain L-5178 was studied.

EXPERIMENTAL METHOD

Experiments were carried out with a primary suspended culture of cells of transplantable lymphatic leukemia L-5178. The cells of the strain were adapted to a system *in vitro* at the Institute for the Study of New Antibiotics, Academy of Medical Sciences of the USSR, by L. P. Ivanitskaya [2]. For the experiments the cells were transferred to medium No. 199 containing 20% native bovine serum, in a concentration of 400,000 cells to 1 ml nutrient medium. The cell suspension thus prepared was poured in volumes of 2 ml into tubes and

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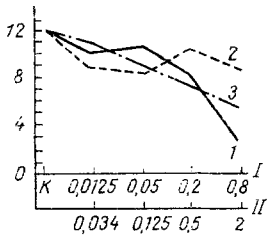

Fig. 1

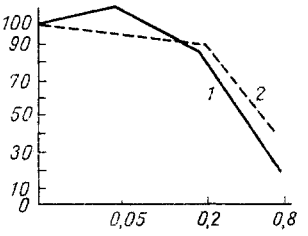

Fig. 2

Fig. 1. Effect of cAMP (1), theophylline (2), and cAMP + theophylline (3) on synthesis of nucleic acids (RNA + DNA) in L-5178 cells. Ordinate, increase in nucleic acids (in μg/ml); abscissa: I) cAMP concentration (in mM), II) theophylline concentration (in mM).

Fig. 2. Action of cAMP (1) and dibutyryl-cAMP (2) on incorporation of thymidine-H^3 into L-5178 cells. Ordinate, number of counts per minute (in % of control); abscissa, concentration of compounds (in mM).

incubated at a constant temperature of 37°C. After incubation for 3 h, the test agents were added to the tubes: cAMP, dibuytryl-cAMP, and theophylline in 0.1 ml physiological saline. The concentration of the compounds in the nutrient medium were expressed in molar units. Tubes containing the cell suspension to which 0.1 ml of solvent was added served as the control.

The biological action of the compounds on the leukemic cells was estimated 24 h after the beginning of incubation from the increase in nucleic acids determined by Spirin's method [3] and incorporation of thymidine-H^3 into the cells of the experimental tubes compared with the control. Radioactivity was measured on the Nuclear Chicago Mark II scintillation counter.

The rate of development of the leukemic process also was studied in mice after injection of L-5178 cells previously incubated at 37°C in nutrient medium with cAMP, dibutyryl-cAMP, theophylline, and cAMP + theophylline, in doses found to be active in the previous experiments. The leukemic cells were transplanted intraperitoneally into noninbred mice 48 h after the beginning of incubation. Animals with transplanted leukemic cells previously incubated for the same time but without the compounds acted as the control. The mean lifespan of the experimental mice was determined and its increase over the control calculated.

EXPERIMENTAL RESULTS

Proliferation of cells and a rapid increase in the total nuclei acid (DNA + RNA) content were observed in the control cultures. Before incubation of the cells the nucleic acid content in the culture was 6.1 μg/ml. After incubation for 24 h the nucleic acid content in the culture increased to 17.9 μg/ml, 193% more than initially.

Data on the effect of cAMP and theophylline on the increase in the nucleic acid concentration in the cultures are shown in Fig. 1. Cyclic AMP, in a concentration of 0.8 mM, reduced the increase in nucleic acids in the cultures by 78%, and in a concentration of 0.2 mM by 31% compare with the control. Lower cAMP concentrations (0.05 and 0.0125 mM) inhibited the increase of nucleic acids in the cultures by 12%. The phosphodiesterase inhibitor theophylline inhibited the increase in the nucleic acids in the cultures by a much lesser degree, and no dependence of effect on dose could be found during its use. For instance, with theophylline in concentrations of 0.5, 0.125, and 0.034 mM, inhibition of the increase in nucleic acids in the cultures was 14, 30, and 24% respectively. When theophylline was given together with cAMP, the effect on the increase in nucleic acids in the cultures was the same as when cAMP was given alone: During incubation in medium containing

0.8 mM cAMP and 2 mM theophylline inhibition of growth was 63%, whereas cAMP alone, in a concentration of 0.8 mM, inhibited the increase in the nucleic acids by 78%.

The results of the study of the effect of cAMP and dibutyryl-cAMP on incorporation of thymidine-H^3 into L-5178 cells showed that after incubation for 24 h the mean intensity of thymidine-H^3 incorporation in the control samples was 4160 counts/min. Cyclic AMP, in a concentration of 0.2 mM, inhibited incorporation by 15%, and in a concentration of 0.8 mM by 75%. When dibutyryl-cAMP was used, the maximal inhibition of thymidine-H^3 incorporation into the cells in culture was 48% (Fig. 2).

On transplantation of L-5178 cells, previously incubated for 48 h under ordinary conditions, into mice the mean lifespan of the animals was 18.3±2.3 days. If the transplanted leukemic cells were previously incubated in a 0.8 mM solution of cAMP, the mean lifespan of the mice was 27±1.7 days, or 47.5% longer than the lifespan of the control group of mice. Dibutyryl-cAMP, if used to treat the leukemic cells in an equimolar dose, and also theophylline (5 mM) did not alter the transplantation properties of the cells, for no significant increase was observed in the mean lifespan of the mice: After transplantation of cells treated with dibutyryl-cAMP it was 20±1.95 days, and after treatment with theophylline 19.7±2.3 days.

In a concentration of 0.8 mM, cyclic AMP thus considerably inhibited the incorporation of thymidine-H^3 and delayed proliferation of the leukemic cells. Incubation of the cells for 48 h with cAMP depressed their malignant properties, as shown by the longer lifespan of the experimental animals than of the controls. Cyclic AMP is known not to pass through the cell membrane; the antileukemic action observed can thus be regarded as the result of its interaction with the appropriate receptors on the cell surface. The complement of these receptors is determined by differentiation of the cells. In this connection the data of Gallo [5] can be cited: According to this worker exogenous cAMP, in concentrations of 10^{-7}-10^{-6} M in a culture with phytohemagglutinin, inhibits DNA synthesis in the lymphocytes of patients with chronic lymphatic leukemia although it stimulates DNA synthesis in the lymphocytes of healthy blood donors.

Unlike cAMP, theophylline and dibutyryl-cAMP had comparatively little ability to inhibit DNA synthesis, the proliferative activity, or the transplantation properties of the cells. Since theophylline inhibits hydrolysis of cAMP, its action can be compared with that of intracellular cAMP. Dibutyryl-cAMP, which also passes through the cell membrane, essentially simulates the effects of intracellular cAMP. On this basis it can be concluded that the ability of cells of strain L-5178 to proliferate is dependent only a little on the intracellular content of cAMP.

It is most likely that the low activity of theophylline is due to a change in the isozyme spectrum of the phosphodiesterases of this leukemic strain. Evidence has been obtained in several investigations of the existence of phosphodiesterases with different levels of sensitivity of substrates and inhibitors [8, 9]. The lower activity of dibutyryl-cAMP should most probably be regarded as the result of a decrease in the affinity of the cells of this form of leukemia for CAMP-dependent enzymes. The low effective level of cAMP in tumor cells in also supported by the observations of Goldberg et al. [6].

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