PROTECTIVE EFFECT OF THYMIDINE AGAINST CYTOSTATIC ACTION OF CYCLIC-AMP IN MAMMALIAN CELL CULTURES

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Under the influence of 1 mM cyclic-adenosine-3',5'-monophosphate (cyclic-AMP) the degree of survival and rate of reproduction of Chinese hamster cells in culture were reduced to 27 and 42% of the control level, respectively. Addition of 0.02 mM thymidine along with the cyclic-AMP almost completely abolished the cytostatic effect of the latter. Thymidine also prevented the cytostatic effect of noncyclic 5'-AMP, but did not affect death of the cells due to the action of dibutyryl cyclic-AMP and theophylline. Thymidine did not prevent the inhibitory action of cyclic-AMP on a mutant line of mouse cells deficient in thymidine kinase. It is concluded that, in the concentrations used, the cytostatic action of exogenous cyclic-AMP on mammalian cells is the result of its splitting to 5'-AMP in the culture medium, and that it acts by blocking one of the stages of TMP biosynthesis.

KEY WORDS: Cell culture; cyclic-AMP; thymidine; cytostatic effect.

An important aspect of the regulatory function of cyclic-adenosine-3',5'-monophosphate (cyclic-AMP) in the cell is its presumptive role in controlling the rate of cell proliferation [1-4]. Among the arguments confirming this hypothesis, investigators cite, in particular, the results of experiments to show the inhibitory effect of exogenous cyclic-AMP on cell division in culture [5, 6]. However, there is evidence that the use of preparations of cyclic-AMP, especially in cell culture, does not reflect the mechanism of action of intracellular cyclic-AMP, the level of which in the cell can be increased by the use of agents such as dibutyryl

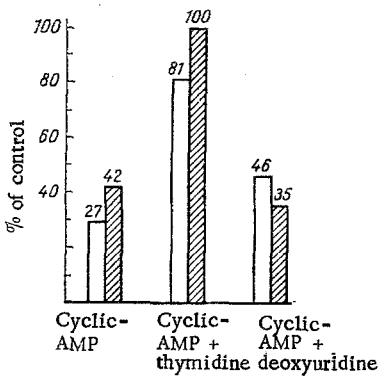

Fig. 1

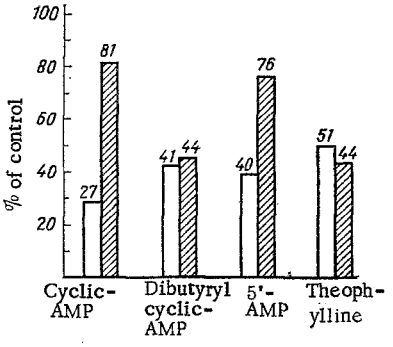

Fig. 2

Fig. 1. Effect of thymidine (0.02 mM) and deoxyuridine (0.02 mM) on cytostatic effect of 1 mM cyclic-AMP. Unshaded columns – survival rate, shaded columns – rate of cell division.

Fig. 2. Effect of thymidine (0.02 mM on cytostatic effect of 1 mM cyclic-AMP, 0.1 mM dibutyryl cyclic-AMP, 1 mM 5'-AMP, and 0.35 mM theophylline. Unshaded column – inhibitor, shaded column – inhibitor + thymidine.

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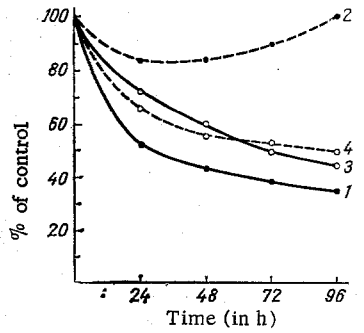


Fig. 3. Effect of thymidine (0.02 mM) on inhibitory action of 0.75 mM cyclic-AMP on growth of mouse cells differing in their thymidine kinase activity. Number of cells counted after incubation for various times with cyclic-AMP in presence of thymidine (control) or in its absence. 1) L-cells (TK^+) treated with cyclic-AMP; 2) L-cells (TK^+) treated with cyclic-AMP and thymidine; 3) 3T3-4E cells (TK^-) treated with cyclic AMP; 4) 3T3-4E cells (TK^-) treated with cyclic-AMP and thymidine.

cyclic-AMP, theophylline, certain hormones, and so on [7]. It is suggested that this fact may be connected with extracellular enzymic breakdown of cyclic-AMP to noncyclic 5'-AMP. There is thus some doubt about the specificity of cyclic-AMP as an inhibitor of cell proliferation.

This paper describes the results of the study of the effect of thymidine on the cytostatic effect of cyclic-AMP, dibutyryl cyclic-AMP, 5'-AMP, and theophylline, on the basis of which the inhibition of cell growth by exogenous cyclic-AMP can be interpreted in a new light.

EXPERIMENTAL METHOD

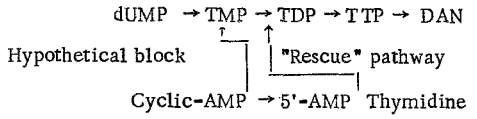
Chinese hamster fibroblasts of transplantable line BII d-ii-FAF--28, clone 237, mouse cells of line L(TK^+) with normal thymidine kinase activity, and cells of line 3T3(TK^-), deficient in thymidine kinase, were used. The survival rate was studied by counting the number of colonies after seeding 200-300 cells on a Petri dish (diameter 5 cm) after 10 days of growth on Eagle's medium with 10% bovine serum in an atmosphere containing 5% CO_2. The rate of cell division was determined as the ratio between the total number of cells per dish and the number of colonies on that dish (the mean number of cells per colony) or by counting the number of cells per dish at different times after seeding 50,000 cells on the dish. Each point of the graph and the figures on the diagrams reflect the mean value of counts on four dishes in three repetitions of the experiment.

EXPERIMENTAL RESULTS

To study the cytostatic effect the concentrations of the inhibitors were chosen so that, following their use, the rate of survival and of division of the cells was 25-50% of the control. In particular, the presence of 1 mM cyclic-AMP in the medium reduced the survival rate to 27% and reduced the mean number of cells per colony to 42% of the control. If thymidine (0.02 mM) was present together with cyclic AMP in the medium, the survival rate and rate of division of the cells were restored almost to the control level. Deoxyuridine in a similar concentration had a much less marked protective action (Fig. 1). The results showing the effect of thymidine on the cytostatic action of cyclic-AMP, dibutyryl cyclic-AMP, 5'-AMP, and theophiline are given in Fig. 2. Thymidine had a marked protective action, when added to the medium together with cyclic-AMP and 5'-AMP, on spatial organization, but it did not affect the cytostatic action of dibytyryl cyclic-AMP and theophylline. Cyclic-AMP and 5'-AMP thus have a similar mechanism of inhibition of cell growth, and one that differs from the mechanism of action of dibutyryl cyclic-AMP and theophylline. Some conclusions regarding this mechanism can be drawn from the data in Fig. 3. The curves in Fig. 3 reflect changes in the number of cells after different times of growth in the presence of cyclic-AMP and thymidine in two cultures of mouse cells differing in their level of activity of thymidine kinase, the enzyme responsible for phosphorylation of exogenous thymidine. L(TK^+) cells, with normal activity of the enzyme, can be protected against the cytostatic action of cyclic-AMP by the addition of thymidine. Cells of the mutant line 3T3-4E(TK^-), deficient in thymidine kinase, die if exposed to the action of cyclic-AMP despite the presence of thymidine in the medium. The initial cell concentration in both cases was 50,000 per dish. Phosphorylation of exogenous thymidine is thus evidently an essential condition for depression of the inhibitory effect of cyclic-AMP on cell division. Presumably cyclic-AMP, like 5'-AMP, if present in the nutrient medium, induces TMP deficiency in the cells by blocking at least one stage of its biosynthesis de novo. Since deoxyuridine gives only a negligible protective effect, this block possibly takes place in the transition from dUMP to TMP. In cells with normal thymidine kinase activity TMP is formed through bypassing of the block by phosphorylation of exogenous thymidine; for that reason the viability of the cells is restored by the addition of thymidine to the medium.

On the basis of these results the following mechanism of the cytostatic action of exogenous cyclic-AMP in cell culture can be postulated. 1) Under the influence of factors in the nutrient medium (probably

serum phosphodiesterase) cyclic-AMP breaks down to 5'-AMP. 2) 5'-AMP or its metabolic products block the formation of TMP from dUMP, leading to inhibition of DNA synthesis and of cell division. The mechanism of the cytostatic effect of cyclic-AMP and of the "rescue" of the cells by thymidine can be represented schematically as follows:



Inhibition of growth of cells in culture in the presence of cyclic-AMP is thus not specific, and it cannot be interpreted in the light of the regulatory function of intracellular cyclic-AMP. The mechanism of blocking of TMP synthesis remains obscure and requires special investigation.

LITERATURE CITED

1. J. Otten, G. S. Johnson, and I. Pastan, J. Biol. Chem., 247, 7082 (1972).
2. C. W. Abell and T. M. Monahan, J. Cell Biol., 59, 549 (1973).
3. R. V. Teel and R. G. Rall, Exp. Cell Res., 76, 390 (1973).
4. J. E. Froehlich and M. Rachmeler, J. Cell Biol., 60, 240 (1974).
5. B. M. Bombic and M. M. Burger, Exp. Cell Res., 80, 88 (1973).
6. J. W. Kreider, M. Rosenthal, and N. Lengle, J. Nat. Cancer Inst., 50, 555 (1973).
7. H. Hiltz and E. Kaukel, Molec. Cell. Biochem., 1, 229 (1973).