would make possible the introduction of convenient methods for assessment of differences in molecular parameters of several hormones.

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EFFECT OF ALKYLATING DERIVATIVES OF CYCLIC AMP ON PROLIFERATION OF MOUSE BONE MARROW STEM CELLS

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It has been frequently demonstrated that cyclic AMP can restore normal differentiation of many tumor cells, can inhibit their proliferation, and can exert a cytostatic action [1]. It was accordingly decided to study the antileukemic action of various new alkylating derivatives of cyclic AMP synthesized in Professor E. S. Severin's laboratory. Nearly all of the alkylating derivatives of cyclic AMP studied inhibited proliferation of P-388A mouse leukemic cells in culture [2]. However, the antileukemic action of a compound largely depends on its side effects on normal hematopoiesis and, in particular, on proliferation of bone marrow stem cells (colony-forming units — CFU_c). If alkylating derivatives of cyclic AMP, such as dibutyryl-cyclic AMP, stimulate proliferation of CFU_c, this gives them an undoubted advantage over all antitumor preparations so far known, which inhibit hematopoiesis or even bring about its aplasia.

EXPERIMENTAL METHOD

The effect of cyclic AMP analogs of alkylating type — 1-(N-chloroacetylaminoethoxy)-cyclic AMP, 8-(N-chloroacetylaminoethylamino)-cyclic AMP, and 1-[N-(p-fluorosulfonyl)-benzoyl-aminoethoxy]-cyclic AMP — generously provided by Professor E. S. Severin, on proliferation of stem cells was studied. The action of cyclic AMP (from FERAK, Berlin) was studied as the control. Hydroxyurea (from Serva) was used to determine the number of CFU_c.

Tests were carried out on female (CBA × C57BL)F_1 mice weighing 18-20 g obtained from "Stolbovaya" Nursery, Academy of Sciences of the USSR. The number of CFU_c was determined by the method of Till and McCulloch [5]. Bone marrow was flushed from the femora with medium No. 199 with the addition of Hepes (10 mM), penicillin (50 units/ml), and streptomycin (50 μg/ml), and was forced through a needle to produce disaggregation of the cells. The medullary cells were washed and resuspended in medium No. 199. A cell suspension with a density of 4 million 400 thousand cells/ml was poured into flasks, allowing for the volume of solution of the reagents, each of which was added in a volume of 0.1 ml, and of cyclic AMP and its analogs (final concentration of the sample 10^{-8} M) and of hydroxyurea (10^{-3} M). The volume of the mixture thus prepared did not exceed 1 ml. The incubation time of the samples

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TABLE 1. Action of Alkylating Derivatives of Cyclic AMP on Proliferation of CFU_c (M ± m)

Substance used	Without hydroxyurea	With hydroxyurea	Reduction in No. of CFU_c, %	P
Control (no treatment)	17,5±0,76 (8)	16,2±1,4 (8)	7,4	—
1-(N-chloroacetylaminoethoxy)-cyclic AMP	17,9±0,9 (9)	12,1±1,1 (9)	32,4	<0,01
8-(N-chloroacetylaminoethylamino)-cyclic AMP	18,1±1,1 (10)	11,0±0,8 (10)	39,2	<0,01
1-[N-(p-fluorosulfonyl)-benzoylamino-ethoxy]-cyclic AMP	13,7±0,9 (10)	10,7±1,0 (8)	21,9	<0,05
Cyclic AMP	17,3±0,7 (19)	6,9±0,8 (18)	60,0	<0,01

Legend. Number of spleens given in parentheses.

was 3 h at 37°C. A solution of hydroxyurea, which causes death of cells synthesizing DNA, was added to the experimental samples 1 h before the end of incubation. At the end of incubation 4×10^4 living cells were injected intravenously into lethally irradiated mice (dose 1050 rads from a ^{137}Cs source). The number of colonies in the spleen was determined after 8 days. The number of CFU_c in mice of the control group and in animals from which the material taken was treated with hydroxyurea was compared. The decrease in the number of CFU_c was expressed as a percentage.

EXPERIMENTAL RESULTS

The experimental results (Table 1) show that treating bone marrow cells with hydroxyurea reduced the number of CFU_c by only 7.4%. This value, reflecting low proliferative activity of the stem cells, is in good agreement with the results obtained by other workers [3]. Preliminary incubation of mouse bone marrow cells with cyclic AMP and its three analogs stimulated proliferation of CFU_c, for subsequent treatment with hydroxyurea, leading to destruction of cells in the S-phase, increased the percentage loss of CFU_c. The stimulating effect of cyclic AMP was greatest and amounted to 60%. The increase in proliferation of CFU_c under the influence of the three alkylating derivatives of cyclic AMP tested was 39.2, 32.4, and 21.9% respectively. The substance 1-[N-(p-fluorosulfonyl)-benzoylamino-ethoxy]-cyclic AMP produced the least effect, evidently because of the complexity of configuration of the substituent group.

The problem of membrane permeability for exogenous cyclic AMP and its derivatives must be solved in a concrete manner, as it applies to cells of a given type. The possibility cannot be ruled out that for some mammalian cells exogenous cyclic AMP is a trigger signal for proliferation through activation of protein kinase, bound with the outer surface of the cytoplasmic membrane [4], by further phosphorylation of cytoplasmic membrane proteins, and by an increase in permeability of the membrane for Ca^{++}. Different inducers of cell division, including the mitogenic ionophore Ca^{++}, are known to cause accumulation of Ca^{++} in cells.

From our point of view it is an important fact that all three alkylating derivatives of cyclic AMP retain the property of stimulating proliferation of CFU_c. The abundant literature on the use of antitumor preparations of alkylating type shows that they have a radiomimetic action on the body, i.e., they always suppress hematopoiesis. It is the radiomimetic effect of alkylating compounds which does not allow them to be used for a long period of time or in higher doses. In this respect alkylating derivatives of cyclic AMP are the exception and, consequently, they are a new and more promising class of antitumor compounds.

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