Effect of Purine Antagonists on the cAMP Level in Lymphocytes and Bone Marrow Lymphoblasts

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The effect of the purine receptor ligands N-ethylcarboxamide adenine and adenosine and of the purine antagonists mercaptopurine and azathioprine on the intracellular cAMP content in human peripheral blood lymphocytes and bone marrow lymphoblasts was studied. All preparations tested induced an increase in the cAMP level in peripheral blood lymphocytes. The selective immunosuppressive effect of adenosine antagonists may be due to their ability to modulate the activity of adenylate cyclase in lymphoid cells.

Key Words: *adenosine; azathioprine; mercaptopurine; cAMP*

One of the universal mechanisms regulating the physiological activity of target cells by hormones, neurotransmitters, and drugs is the triggering of a cascade of reactions proceeding in the membrane-bound adenylate cyclase system. In cells cAMP is synthesized from adenosine triphosphate in the presence of adenylate cyclase. Hydrolysis of cAMP is implemented by cytosolic phosphodiesterase. By recording the changes in the cellular cAMP level caused by various pharmacological agents it is possible to elucidate the molecular mechanisms of their action. The sensitivity of adenylate cyclase to hormones which enhance or inhibit its activity as well as the magnitude of the cell's functional response to the hormone signal depend not only on the affinity of the hormone receptors, but also on the ratio of different subtypes of hormone receptors [10].

The purine nucleotide adenosine is an intermediate product of adenylnucleotide metabolism. The complex of adenosine properties permits it to be regarded as a regulatory metabolite. The biological action of adenosine is mediated by its interaction with purine receptors residing on the outer surface of the target cell plasma membranes. The A_1 and A_2 subtypes of purine receptors mediate, respectively, an inhibitory and a stimulating effect of adenosine on adenylate cyclase activity. The role of adenosine as an intracellular metabolite consists in the fact that it is a substrate for DNA and RNA synthesis [1].

It is also known that the immunosuppressive and cytostatic action of purine antagonist-type immunodepressants, such as mercaptopurine (MP) and azathioprine (AZT), can be connected with their interaction with A_2 purine receptors of human peripheral blood lymphocytes and bone marrow lymphoblasts [6].

In the present work we studied the effect of the ligands of adenosine-binding purine receptors, MP, and AZT on the content of intracellular cAMP in lymphocytes and bone marrow lymphoblasts.

MATERIALS AND METHODS

Bone marrow blast cells were obtained from patients with acute lymphoblastic leukemia by means of sternal puncture. Punctate (0.5-1 ml) taken under sterile conditions was placed in a test tube and washed twice in HEPES buffer (pH 7.35) [5].

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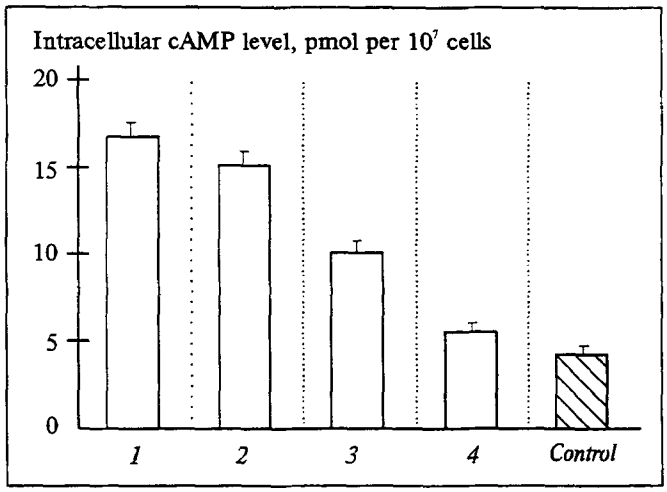

Fig. 1. Effect of immunodepressants on cAMP content in peripheral blood lymphocytes. Concentration of NECA (*1*) and adenosine (*2*) 10^{-6} M, and that of AZT (*3*) and MP (*4*) is 10^{-5} M. Incubation with preparations lasted 10 min at 37°C.

Lymphocytes were isolated from the peripheral blood of healthy donors. Thirty milliliters of blood was taken from the ulnar vein into a plastic tube containing 6 ml of anticoagulant (25 g dihydrated Na-citrate). Five milliliters of blood were layered onto 3 ml of Ficoll gradient solution using a pasteur pipette and centrifuged at 1500 g for 15 min at room temperature. The mononuclear cell-containing transparent layer above the gradient was aspirated, resuspended in Hanks solution, and washed by centrifugation [3]. The suspension of peripheral blood mononuclear cells separated after Boyum (1968) consists of 90% lymphocytes and 10% monocytes. The percentage of T cells is about 70%; thus, these represent the main population.

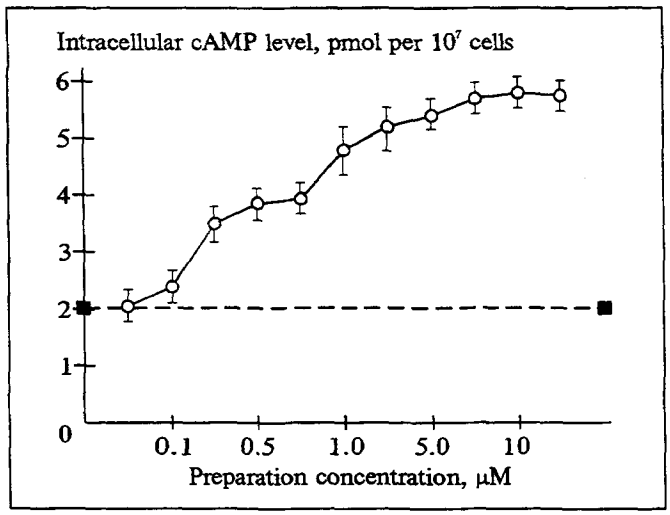

Fig. 2. Effect of NECA on cAMP content in bone marrow lymphoblasts. Dotted line depicts the background lymphoblastic cAMP level. The experimental conditions are the same as in Fig. 1.

Samples containing 1 ml cell suspension per tube (cell concentration 5 mln/ml) were incubated for 40 min at 37°C, after which 20 ml of the test substances per tube were added to the experimental tubes and an equal volume of Hanks solution to the control. Hanks solution (pH 7.4) served as a working medium. The substances were administered in the following concentrations: adenosine - 2.5×10^{-7}, 5×10^{-7}, 10^{-6}, and 2×10^{-6} M; N-ethylcarboxamide adenine (NECA) - 10^{-7} to 3×10^{-5} M; AZT - 10^{-5} and 2×10^{-5} M; MP - 2.5×10^{-6}, 5×10^{-6}, 10^{-5}, and 2×10^{-5} M. Lymphocyte suspensions were incubated with these compounds for 10 min, followed by cell disruption in ice-cold Tris-EDTA buffer (pH 7.5), after which samples were immediately placed in a water bath for 3 min at 90°C. Following centrifugation (10 min, 800 g) the supernatants were tested for cAMP concentration using standard Amersham kits.

RESULTS

The data concerning the effect of NECA, adenosine, and purine antagonists on the cAMP content in human peripheral blood lymphocytes are summarized in Fig. 1. The background cAMP level varied within limits of 3.9-4.5 pmol/10^7 cells. All ligands under study induced an increase of the cAMP intracellular level. The inductive potential decreased in the following order: NECA>adenosine>AZT>>MP. Introduction of adenosine (1 μM) and AZT (10 μM) led to 3.6-fold and 2.4-fold rise of the cAMP intracellular level, respectively. These results make it possible to assume that the recorded intralymphocyte cAMP increment caused by adenosine, NECA, and AZT is determined by the presence of the A_2 subtype purine receptors on the lymphocyte membrane. Purine receptors are known to be broadly represented on all lymphocyte subpopulations [7,8] and, as shown earlier, one of the mechanisms of the immunosuppressive effect of purine antagonists lies in their interaction with A_2-subtype purine receptors on the surface of target cells [5].

The dynamics of the cAMP level in bone marrow lymphoblasts shows a biphasic pattern. A diagram reflecting the dependence of the intracellular cAMP level on the NECA concentration is presented in Fig. 2. NECA in a concentration range of 10^{-7}-10^{-6} M causes a rise of the cAMP level up to 3.2-3.8 pmol per 10^7 cells. The background cAMP level in lymphoblasts is reliably lower than in lymphocytes and is about 2.0-2.3 pmol per 10^7 cells (in the diagram the background level is plotted as a dotted line). A further increase in the NECA concentration (10^{-6}-10^{-5} M) is accompanied

by a parallel rise in the cAMP level to 4.9-5.7 pmol per 10^7 cells, i.e., on average 2.5-fold.

A study of the influence of immunodepressants on the cAMP level in bone marrow lymphoblasts showed that the effect of MP is dose-dependent; the maximal cAMP rise is attained at a dose of 10^{-5} M, a concentration which corresponds to the therapeutic dose of this preparation [2,4,8]. Under these conditions the cAMP level reached 4.3 ± 0.3 pmol per 10^7 cells. The AZT-induced increment in cAMP is reliably lower (3.3 ± 0.2 pmol per 10^7 cells).

The increase in the intracellular cAMP level induced by purine receptor agonists leads to an inhibition of the functional activity and finally to lysis of lymphoid cells. Thus, the selective immunosuppressive activity of adenosine analogs, including MP and AZT, may be mediated by their ability to modulate adenylate cyclase activity in lymphoid cells.

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