CHARACTERISTICS OF β-ADRENORECEPTORS OF CELLS OF LEUKEMIA L1210 AND ITS SARCOLYSIN-RESISTANT VARIANT

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One of the main problems in tumor chemotherapy is the appearance of resistance of the tumors to drugs. The causes of resistance are as a rule multiple in character. For instance, resistance of tumors to bifunctional alkylating agents is connected with a disturbance of transport of the substances into the cell [9], with intensification of DNA repair processes [8], with a high level of SH compounds in the plasma and tumor [12] and with the particular features of the mitochondrial membranes of tumor cells [5]. These agents also inhibit cyclic AMP phosphodiesterase with a low K_m value (PDE_2), only in tumors sensitive to them [13]. The content of cyclic AMP (cAMP) in the cells is increased [13], nonhistone proteins in the chromatin are phosphorylated [10], and mitosis of the cells is blocked in the G_2 phase [4]. These effects are not observed in resistant tumors. The writers also have shown that sarcolysin [p-di-(2-chloroethyl)amino-D,L-phenylalanine] inhibits membrane PDE_2 in cells of ascites sarcoma 37, which is sensitive to it [2]. Since data showing that PDE is linked with β-adrenoreceptors have been published in the literature [7], A. K. Belousova has suggested that sarcolysin acts indirectly on the membrane PDE of tumor cells through β-adrenoreceptors [1]. Experimental testing of this hypothesis showed that sarcoma 37 cells contain functionally active β-adrenoreceptors and that sarcolysin interacts with them [2]. In this investigation an attempt was made to discover possible correlation between the sensitivity of tumor cells to sarcolysin and the characteristic features of the β-adrenoreceptors of these cells. The aim of the present investigation was accordingly to study the effect of β-adrenoreceptors and their characteristics (K_d, number of receptors, degree of coupling with adenylate cyclase) in cells of leukemia L1210 sensitive and resistant to sarcolysin.

EXPERIMENTAL METHOD

Mouse leukemia L1210 cells and those of its variant resistant to sarcolysin constituted the test object. Leukemia L1210 was obtained in the Laboratory of Tumor Strains, Research Institute of Experimental Diagnosis and Treatment of Tumors, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR.

The variant resistant to sarcolysin, obtained by Z. P. Sof'ina and T. L. Efremova, was obtained from the Laboratory of Experimental Chemotherapy. On the 6th-7th day after intraperitoneal transplantation of about $5 \cdot 10^5$ tumor cells into BDF_1 mice the animals were killed, the ascites fluid was isolated, and tumor cells were freed from erythrocytes by centrifugation in a Ficoll gradient for 20 min at 600 g [6]. The cells were then washed several times with physiological saline and medium 199 with 0.05 M Na_2HPO_4 (pH 7.2), followed by centrifugation for 2 min at 600g. All operations involved in isolation and washing of the cells were done at 20-22°C. The cells washed free from erythrocytes were suspended in the same medium and used in the experiment. The number of damaged cells, determined after the experiment by staining with trypan blue, did not exceed 5-10%.

The tumor cell suspension (10^6-$1.5 \cdot 10^6$ cells in 0.5 ml of medium) was incubated for 15 min at 20-22°C with increasing concentrations (0.05-30 nM) of the β-adrenoblocker L-^{3}H-dihydroalprenolol (^{3}H-DHA, from Amersham International, England, specific activity 60 Ci/mmole) in the presence or absence of 0.5 μM L-propranolol. The reaction was stopped by addition

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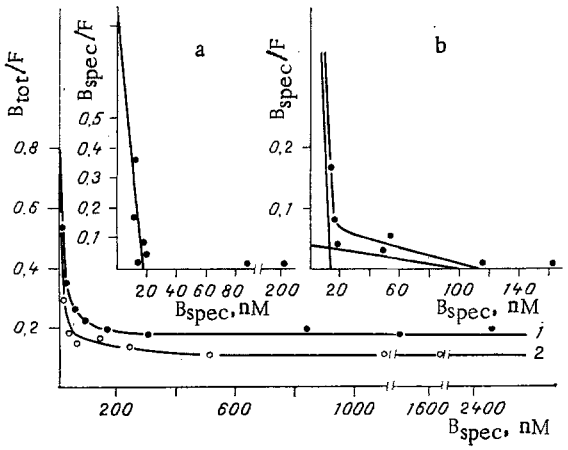

Fig. 1

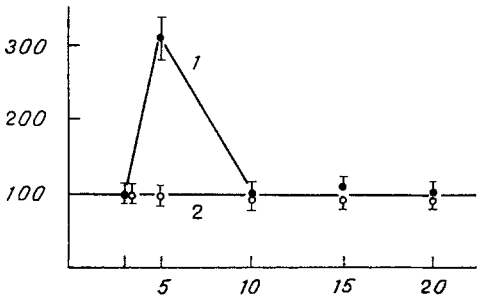

Fig. 2

Fig. 1. Discovery of β-adrenoreceptors on surface of intact mouse leukemia L1210 cells. a, 1) Leukemia L1210; b, 2) variant of L1210 resistant to sarcolysin. B_{tot}) Total concentrations of labeled ligand (^{3}H-DHA) bound with cells; F and B_{spec}) concentrations of free and specifically bound ligand. Numbers shown are mean value of 4-6 (1) or 3 (2) experiments with three parallel measurements ± the standard error.

Fig. 2. Degree of binding of β-adrenoreceptor with adenylate cyclase. Abscissa, time (in min); ordinate, cAMP level (% of control). 1) Leukemia L-1210, 2) variant of L1210 resistant to sarcolysin. Numbers shown are mean results of three experiments ± the standard error.

of 10 volumes of physiological saline (pH 7.2) and the cells were sedimented by centrifugation for 1 min at 1000g. This procedure was repeated once more. The washed cells were lysed in 0.25 ml of 0.1 M NaOH with 0.5% sodium deoxycholate, 10 ml of ZhS-8 scintillation fluid was added, and radioactivity was measured in a Mark III scintillation counter. The results were analyzed by Scatchard's method in Rosenthal's modification [3, 11].

Coupling was tested as the ability of the powerful β-agonist L-isoproterenol to activate adenylate cyclase and cause an increase in the cAMP concentration in the cells. A suspension of tumor cells [$(50-150)\cdot10^6$ cells in 25 ml of medium] was incubated at 37°C with 10^{-6} M L-isoproterenol (from Sigma, USA) for 3-20 min. The cells were then sedimented by centrifugation at 2000g for 1 min and 1 ml of 5% TCA was added immediately to the residue. After freezing (—20°C) and thawing (20-40°C) the samples were centrifuged, the supernatants were pooled, and the TCA was removed from the pooled product by fivefold extraction with 4-6 volumes of water-saturated ether. The cAMP concentration in the supernatant was determined by means of a kit of reagents (Amersham International).

EXPERIMENTAL RESULTS

The results are given in Figs. 1 and 2. They show that β-adrenoreceptors are present on the surface of leukemia L1210 cells, both sensitive and resistant to sarcolysin. In the sensitive strain, one type of β-adrenoreceptor was found, with an apparent K_d for ^{3}H-DHA of about 0.02 nM. The number of receptors was small, namely 360 per cell (Fig. 1a). In the resistant variant of L1210 cells two types of β-adrenoreceptors were found (Fig. 1b), with K_d of about 0.02 nM (420 receptors per cell) and K_d about 2.5 nM (3000 receptors per cell), respectively.

The presence of functionally active receptors linked with adenylate cyclase was demonstrated only for the strain of L1210 sensitive to sarcolysin. L-isoproterenol induced a temporary threefold increase in the cAMP concentration in these cells (Fig. 2). No such effect of isoproterenol was observed in the resistant strain of L1210, i.e., its β-adrenoceptors were not coupled with adenylate cyclase. Thus significant differences between β-adrenoreceptors in affinity for ^{3}H-DHA, the number of types of receptors, and the degree of their coupling with adenylate cyclase were found in cells of leukemia L1210 and its variant resistant to sarcolysin. These differences and, in particular, the absence of functionally active receptors, bound with adenylate cyclase, are evidently one reason for the resistance of tumor cells to sarcolysin. The results of this investigation confirmed the view that β-adrenoreceptors of tumor cells take part in the realization of the antitumor action of sarcolysin.

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