Glucocorticoid Interaction with Bone Marrow Lymphoblast Receptors in Acute Leukemia

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Preliminary evaluation of tumor cell *in vitro* sensitivity to drugs is a pressing problem in the treatment of acute leukemia [10]. One of the prevalent tests of individual sensitivity to corticosteroids is radioassay (radioligand analysis) of the number of glucocorticoid receptor systems in tissues and target cells [1,8]. Some recent publications report an absence of correlation between the number of glucocorticoid receptors in bone marrow lymphoblasts and the efficacy of hormonal therapy in acute lymphoblastic leukemia [14]. An error in determination of the true concentration of glucocorticoid intracellular receptors may be one of the causes of this discrepancy. Besides the intracellular receptor system, target cells have been shown to contain membrane sites for steroid hormone binding that appreciably contribute to the total specific steroid binding [3,4,15]. Still, current radioassay schemes do not take into account glucocorticoid membrane binding sites.

The present research was aimed at investigating the specific binding of various labeled glucocorticoids by bone marrow lymphoblasts of patients with acute lymphoblastic leukemia in order to detect the membrane stage of this interaction and its role in glucocorticoid cytoreception.

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MATERIALS AND METHODS

A previously described approach was used to identify glucocorticoid binding membrane sites [6]. Labeled glucocorticoids immobilized on a high-molecular (mol. wt. 25,000-30,000) hydrophilic carrier, polyvinyl pyrrolidone (PVP) [5]. Glucocorticoids covalently bound to polymer do not penetrate inside the cell; only the external surface of the plasma membrane is accessible to them. Twelve patients with newly diagnosed acute lymphoblastic leukemia were examined. Bone marrow puncture biopsy specimens (0.5-1.5 ml) were obtained before drug therapy. Lymphoblastic intracellular (cytosol and nuclear) glucocorticoid receptors were assayed after cytoplasmic membrane destruction by hypoosmotic shock [16]. The final concentrations of ^{3}H-dexamethasone (Amersham), ^{3}H-prednisolone (Amersham), and ^{3}H-hydrocortisone (Izotop) in the incubation media were 2 to 30 nM. In whole cell experiments 0.2 ml of a lymphoblast suspension ($10\text{-}30 \cdot 10^6$ per ml) was incubated at 37°C for 25 min in the presence of various labeled polymers (PVP-dexamethasone, PVP-prednisolone, PVP-hydrocortisone). The cells were then precipitated on GF/C (Whatman) filters and washed twice in Hanks solution at 4°C. After drying, the fil-

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ters were placed in scintillation flasks for radiometry. The numerical value of glucocorticoid binding by the lymphoblasts was determined using Scatchard's transformations with Microstat software for Epson J2 computers.

RESULTS

Dexamethasone, prednisolone, and hydrocortisone specific binding parameters by lymphoblastic intracellular and membrane glucocorticoid receptors determined by radioassay are presented in Table 1. (K_d = equilibrium dissociation constant, N = binding sites concentration). It is evident from the table that the intracellular glucocorticoid binding sites are characterized by a high affinity K^{ic}_d does not exceed 15 nm) and a relatively low binding capacity (0.2-0.5 pmol/mg protein or $5 \cdot 10^3$ binding sites per cell on average). The glucocorticoid receptor affinities may be ranked as follows: dexamethasone > prednisolone > hydrocortisone.

The lymphoblastic membrane glucocorticoid receptors are characterized by a higher concentration of binding sites (3-5 pmol/mg protein or $50 \cdot 10^3$ per cell) and a far less marked affinity (K^m_d equal to approximately 0.3 μM). Glucocorticoid tropy to the membrane receptor system falls into the following range: hydrocortisone > prednisolone > dexamethasone; hence, the interaction of various glucocorticoids with the lymphoblastic receptor system is characterized by some specific features. Synthetic dexamethasone and prednisolone in the examined range of concentrations (2-30 nM) were selectively bound by the intracellular glucocorticoid receptors.

The K^m_d/K^{ic}_d ratio reflecting the glucocorticoid distribution between the intracellular and membrane receptors is equal to 56.6 for dexamethasone and to 40.8 for prednisolone; for hydrocortisone this ratio is 10.3, indicating a predominant binding of the natural hormone by the lymphoblastic plasma membrane. Therefore, labeled synthetic corticosteroid analogs

TABLE 1. Parameters of Bone Marrow Lymphoblast Specific Binding of Glucocorticoids ($M \pm m$)

Receptor	Dexamethasone	Prednisolone	Hydrocortisone
Intracellular glucocorticoid receptor:			
K^{ic}_d, nM	6.9±1.2	9.4±2.0	14.1±3.3
N^{ic}, pmol/mg protein	0.32±0.04	0.35±0.06	0.27±0.03
Membrane glucocorticoid receptor:			
K^m_d, μM	0.39±0.07	0.38±0.04	0.15±0.02
N^m, pmol/mg protein	3.2±0.6	3.6±0.5	4.2±0.7

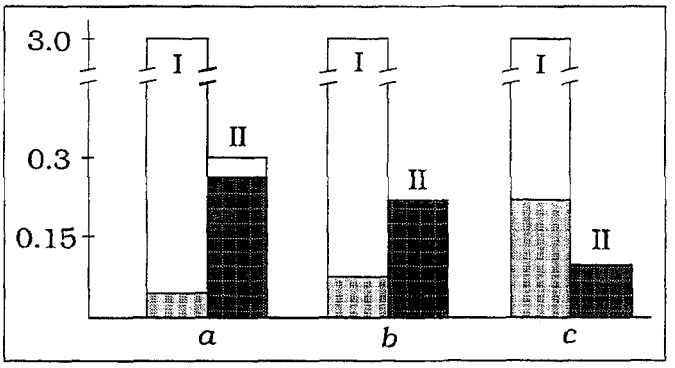

Fig. 1. Dexamethasone binding (10 nM) by membrane (I) and intracellular (II) lymphoblastic receptors as a function of receptor system capacity (mathematical model). Receptor capacity (N) in pmol per mg protein is plotted on the ordinate. *a*) number of intracellular receptors 0.3 pmol/mg protein or $5 \cdot 10^3$ binding sites per cell; *b*) 0.18 pmol/mg protein or $3 \cdot 10^3$ binding sites per cell; *c*) 0.09 pmol/mg protein or $1.5 \cdot 10^3$ binding sites per cell. Hatching shows the number of formed glucocorticoid receptor complexes.

should be preferred for radioassay of intracellular receptor content determining glucocorticoid cytolytic effect and clinical efficacy [7, 11]. Moreover, the possibility of synthetic glucocorticoid interactions with the lymphoblastic membrane receptors cannot be completely ruled out, as well as an error in the assessment of the true concentration of intracellular glucocorticoid receptors.

On the basis of the mathematical model previously proposed [2], we analyzed the lymphoblastic distribution of 10 nM of ^{3}H-dexamethasone for a changed receptor capacity of the intracellular system (Fig. 1). The dexamethasone concentration was selected in due consideration of the available published data. Under the usual conditions (Fig. 1, *a*), when the number of receptors is 4 to $6 \cdot 10^3$ per cell, the contribution of the membrane sites to total dexamethasone binding by the lymphoblasts is not more than 10-15%. When the intracellular receptor content is lowered due to prolonged glucocorticoid therapy [9] or in patients with a relapse of acute lymphoblastic leukemia or with its hormone-resistant form [12,13], the contribution of the membrane level to total specific dexamethasone binding by lymphoblasts is much higher (20-60%). In such cases (Fig. 1, *b, c*) additional evaluation of dexamethasone binding with the membrane receptors is needed for a more precise assessment of the true concentration of intracellular glucocorticoid receptors.

The experimental findings demonstrate the presence of membrane and intracellular sites of glucocorticoid binding in bone marrow lymphoblasts. The specific features of glucocorticoid interaction with various receptor systems, whose parameters may change depending on the microenvironment, should be taken into consideration in a radioassay of lymphoblastic glucocorticoid receptor content.

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Antiamnesic Properties of the Sesquiterpene Lactone Azerin

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Terpene compounds include the so-called sesquiterpene lactones, isolated from plants. These substances have a broad spectrum of biological and pharmacological activity, including an effect on the brain [1,6,8,10]. In the present investigation the effect of the sesquiterpene lactone azerin was estimated during the conditioning and performance of the passive avoidance response after a short period of memory training.

Azerin (empirical formula $C_{20}H_{24}O_5$) was obtained at the Institute of Botanics of the Azerbaidzhan Academy of Sciences [3] from plants of a species of *Ferula*.

The antiamnesic properties of azerin were compared with those of the nootropic drug nootropyl (piracetam), which is widely used in clinics for the improvement of mnestic-intellectual abilities [5].

MATERIALS AND METHODS

The experiments were carried out on 70 noninbred male rats, weighing 250-300 g, which were trained in the passive avoidance response (PAR) by a method described previously [4]. All the animals were divided into three groups. The first two groups comprised

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