Adrenergic Mechanisms for Controlling the Proliferation and Differentiation of Hemopoietic Precursors in Immobilization Stress

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The study of the neurohumoral regulation of the proliferation, differentiation, and functional activity of different cell populations in the blood system in the context of psychoneuroimmunological achievements [1] shows considerable promise for explaining the mechanisms of vital activity of hemopoietic tissue. The wide distribution of specific receptors for neurotransmitters and hormones on the membranes of hemopoietic cells [1,7,9,15] is beyond question. Neurotransmitters realize a direct receptor effect and a indirect effect on the processes of cell division (via changes of hemodynamics, of hemopoietin production, and of the function of the hemopoiesis-inducing microenvironment) and maturation of hemopoietic precursors [9]. The exact mechanisms of neurotransmitter supply of the plastic processes developing in hemopoietic tissue under normal and extreme conditions remain unclear in many respects. We showed previously that the functional integrity of the peripheral sympathetic structures is a necessary condition for intensive production of hemopoietins by the cells of the hemopoiesis-inducing microenvironment in stress [2,5].

The aim of the present investigation was to study the role of the adrenergic structures in the proliferation and differentiation of hemopoietic precursors under conditions of immobilization stress.

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MATERIALS AND METHODS

Experiments were carried out on 180 male F_1 (CBA×C57Bl/6) mice weighing 18-20 g (Rassvet nursery, Tomsk) in the fall-winter season in the morning. For 10 h animals were immobilized on their backs with a soft bandage. The α-adrenergic blocker dihydroergotamine (3.9 mg/kg) or the β-adrenoblocker propranolol (5 mg/kg) was injected s.c. twice (3-5 min before and 5 h after the onset of immobilization). Control stressed animals received an equal volume of saline (0.2 ml). The mice were killed by dislocation of the neck at different times (1-8 days after treatment). Hemopoietic islets (HI) were isolated using 0.05% collagenase (Sigma, USA), as described elsewhere [3].

For a study of the direct action of the pharmacological adrenergic agonists on the colony-forming capacity of bone marrow cells *in vitro*, nonadhesive viable myelokaryocytes were isolated from the bone marrow of intact mice, of control stressed animals, and of stressed mice treated with α- or β-adrenergic blockers. The cells from the last two groups were taken on the 4th day from the onset of stress. The cell suspension was diluted to a concentration of 10^8 nuclears per liter of all-purpose culture medium of the following composition: 1% methylcellulose (Sigma, USA), 15% fetal calf serum (Flow, Great Britain), 10% FGA-M conditioned medium (Serva, Germany) of stimu-

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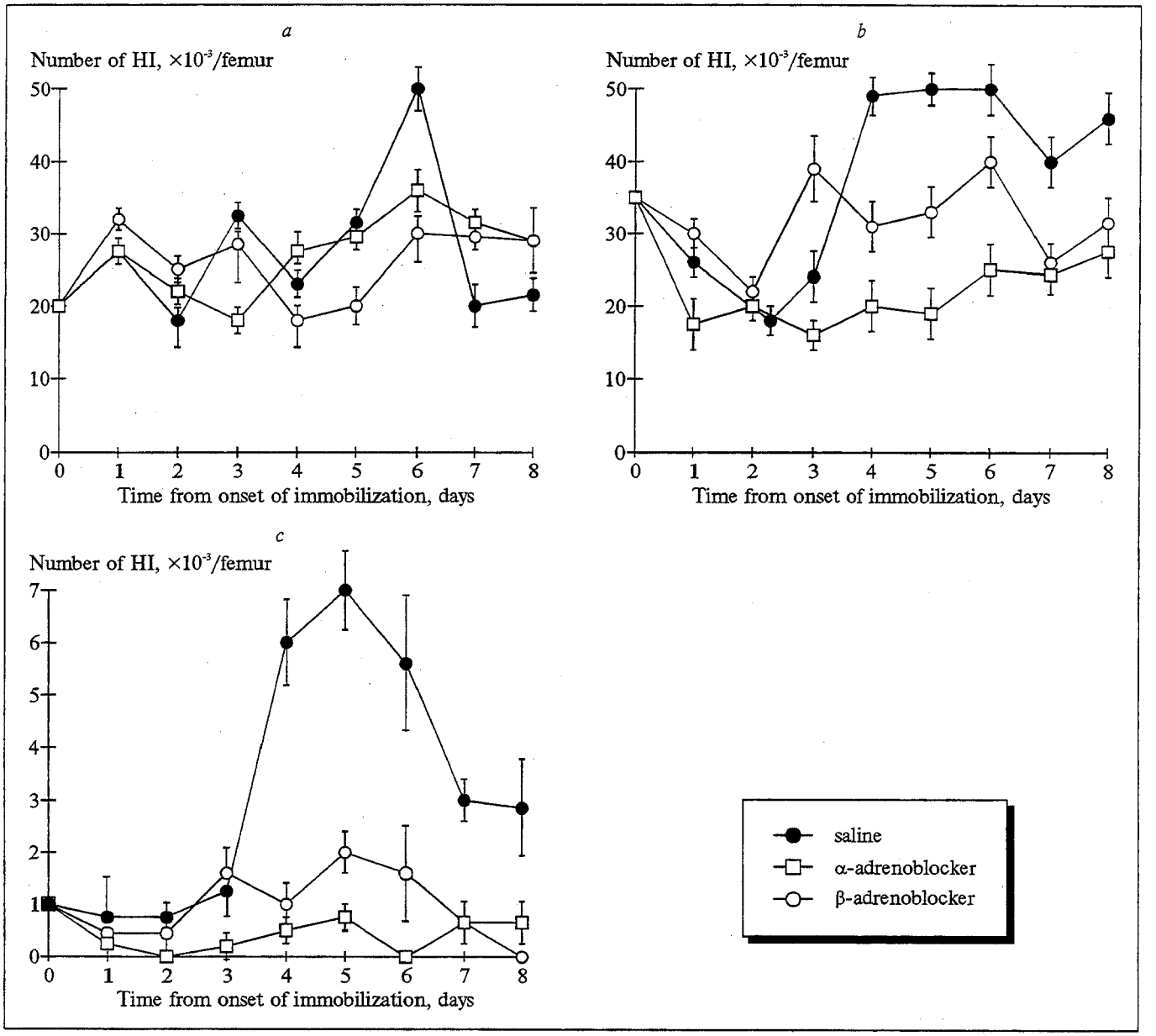

Fig. 1. Dynamics of granulocytic (*a*), erythrogranulocytic (*b*), and erythroid (*c*) types of HI in the bone marrow of F_1 (CBA×C57Bl/6) subjected to 10-h immobilization for administration of pharmacological adrenergic antagonists. Abscissa: time from onset of immobilization (days); ordinate: number of HI (×10³/femur).

lated splenocytes, 23% HAM's F-10 medium (Serva, Germany), 50% Fisher medium (Flow, Great Britain), 5×10^{-5} M 2-mercaptoethanol (Sigma, USA), 0.25 IU/ml recombinant erythropoietin (Sigma, USA), 40 mg/liter gentamicin (Serva, Germany), 300 mg/liter L-glutamine, 10 mg/liter 2-deoxyadenosine, and 10 mg/liter 2-deoxyuridine (Sigma, USA). Culturing was performed in 96-well plates (Cel-Cult, Great Britain), and the erythrocytic and granulocyte-monocytic colonies formed were counted as reported elsewhere [3]. Phenylephrine hydrochloride (α-adrenomimetic) and orciprenaline sulfate (β-adrenomimetic) in concentrations of 10^{-5}, 10^{-7}, and 10^{-9} M were added to myelokaryocytes from intact mice and from stressed animals treated with the corresponding adrenergic antagonist. The data were processed statistically using the Student *t* test.

RESULTS

Hemopoietic islets are structural-fuctional associations of resident macrophages or mechanocytes of bone marrow stroma of high lability [10]. They are able to respond rapidly to different extreme influences, including immobilization stress [12]. In our experiments a significant increase in the total number of HI was found in the bone marrow of mice subjected to a 10-h immobilization on the

5th-6th day of the study (up to 162-195% of the baseline level), due to the boosted formation of both macrophage-positive types (more that a two-fold increase as compared to the baseline) and macrophage-negative forms (to 176% of the baseline value). The analysis of the qualitative composition of the HI showed a stimulated of release of all types of structural-functional units (Fig. 1), namely, the erythroid (to 629%, 5th day), the erythrogranulocytic (to 149%, 5th day), and the granulocytic (to 259% of the baseline level, 6th day). It is to be pointed that an intensified formation of HI is one of the principal mechanisms of hyperplasia of erythro- and granulocytopoiesis on the 4th-8th day after the onset of immobilization [12].

The treatment of stressed mice with a pharmacological antagonist of α- or β-adrenergic structures resulted in a pronounced drop of HI production in the bone marrow. A decrease of the total content of HI to the baseline level, induced by antiadrenergic agents, was noted due to the macrophage-negative and, to a greater degree, to the positive forms of cell associations. The capacity to decrease significantly (to the baseline level and below) the release of erythroid and erythrogranulocytic types of HI was characteristic for the β-adrenoblocker. On the other hand, the use of the α-adrenoblocker was accompanied by a more pronounced (as compared to the β-agent) suppression of the formation of granulocytic structural-functional units of the bone marrow (Fig. 1). It has been shown that there is proliferation and differentiation of hemopoietic elements from committed to mature forms in the composition of HI [10]. Impeded formation of HI induced by administration of adrenoblockers in stress, accompanied by an

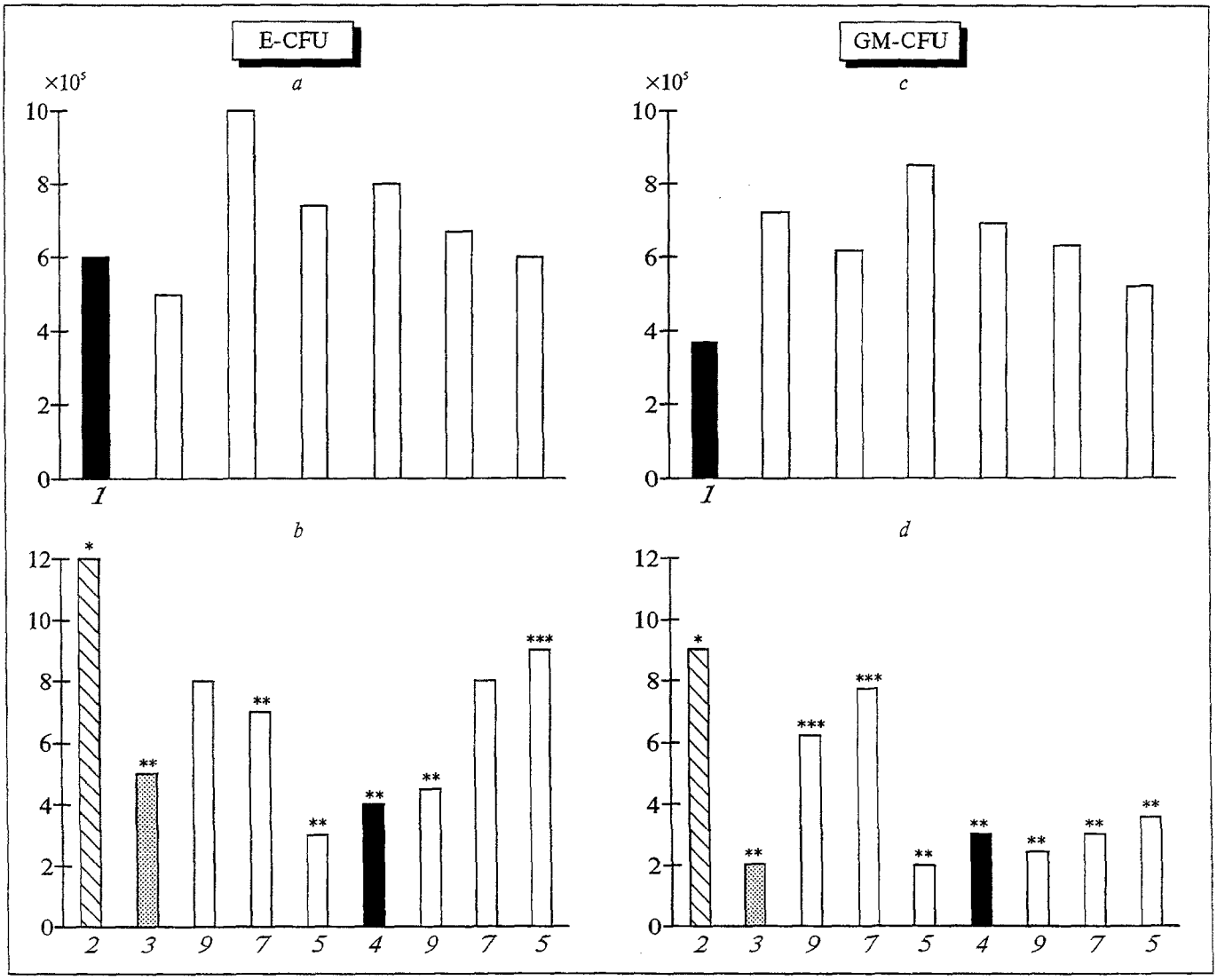

Fig. 2. Effect of adrenergic agonists on E−CFU and GM−CFU contents in culture of intact bone marrow cells (*a*, *c*) and myelokaryocytes taken on the 4th day after adrenoblocker administration to immobilized mice (*b*, *d*). Abscissa: molar concentration (log M); ordinate: number of colonies (×10⁻⁵ cells); *1*) baseline level; *2*) stress control (saline); *3*) α−adrenoblocker; 4) β−adrenoblocker. Asterisks mean significance of differences, * with 1; ** with 2, *** with 3 or 4.

activation of the sympatheticoadrenal system [8], attests to one of the mechanisms of the mediated stimulatory effect of catecholamines on the functional state of hemopoietic precursors. Moreover, an inhibitory effect of adrenoblockers on the growth and maturation of erythroid and granulocyte-macrophagal colonies and on the increase of the number of morphologically identifiable erythroid and granulocytic elements of the bone marrow was established previously in an analogous experiment [2,5].

It was found in experiments *in vitro* that the content of erythroid (E-CFU) and granulomonocytic (GM-CFU) colony-forming units (Fig.2) markedly (more than twofold) increases in the nonadhesive fraction of myelokaryocytes on the 4th day of immobilization stress. Administration of the α- or β-adrenoblocker according to the above scheme prevented the enhanced growth of both erythroid and granulomonocytic colonies in stressed mice. However, the addition of the a-adrenergic agonist to the culture of nonadhesive bone marrow cells taken on the 4th day after administration of the α-adrenoblocker in stressed animals in doses of 10^{-9}-10^{-7} M increased the release of granulomonocytic precursor cells. The content of the latter in the culture more or less reached the level of the stress control. In addition, the α-adrenomimetic in a concentration of 10^{-5} M markedly (to 254% of the baseline level) increased the number of GM-CFU in the culture of intact nonadhesive myelokaryocytes (Fig. 2). The β-adrenomimetic under analogous conditions did not exhibit a growth-stimulating effect on either GM-CFU or E-CFU in any of the dilutions used. At the same time, the number of erythroid colonies rose steeply when the β-adrenergic agonist (10^{-5} M) was added to nonadhesive bone marrow nuclears of rats immobilized against the background of the β-adrenoblocker (Fig. 2).

Thus, it may be concluded that the α-adrenoreceptor agonist has a direct activating effect on the proliferation and differentiation of FM-CFU, but not on E-CFU both under normal and immobilization stress conditions. The β-adrenergic agonist selectively enhances the growth and maturation of E-CFU, but not of GM-CFU, and under extreme conditions only. It should be noted that the interpretation of *in vitro* data is rather difficult. Nevertheless, the selectivity found in the α- and β-adrenergic stimuli in relation to the granulomonocytic and erythroid precursors, respectively, may be related, on the one hand, to the specificity of distribution of the adrenoreceptors in different populations of cell precursors. (Specifically, β-adrenoreceptors are detected on the erythroid precursors [14] but are not found on the committed precursors of granulocytopoiesis [6].) On the other hand, more likely is a dissimilar effect of α- and β-adrenergic stimuli on the levels of intracellular messengers (cAMP and cGMP) [4,7], as well as a putative effect of cAMP and cGMP on the choice of the erythroid or granulocyte-macrophagal pathway of differentiation. The erythropoietin-dependent formation of erythroid colonies is potentiated by β-adrenergic agonists via a rise of the intracellular level of cAMP [13,14]. There is no such evidence for cGMP [13]. Moreover, the antiproliferative potential of cAMP agonists has been shown in relation to colony-stimulating growth factors (multi-, G- and GM-CSF) [11].

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