Parmacological Regulation of Erythropoietic Precursor Pool in Experimental Neuroses

E. G. Skurikhin, N. V. Provalova, O. V. Pershina, and M. Yu. Minakova

Translated from *Kletochnye Tekhnologii v Biologii i Meditsine*, No. 4, pp. 220-225, November, 2005
Original article submitted March 1, 2005

We studied the effect of bone marrow Thy $1,2^+$ cells on the growth of erythroid colonies and the role of monoamine in the regulation of proliferation and differentiation of erythroid precursor cells in experimental neuroses. Under conditions of conflict situation Thy $1,2^+$ cells stimulated the growth of erythropoietic precursors (directly and via interaction with adherent cells of the hemopoiesis-inducing environment). Under conditions of paradoxical sleep deprivation the absence of interaction between Thy $1,2^+$ cells and adherent bone marrow fraction disturbs erythroid colony formation. In experimental neuroses the state of erythropoietic precursors and secretory activity of elements of the hemopoiesis-inducing microenvironment are controlled by monoamines. The regulatory effect of monoamines is realized via adrenergic and erythropoietin-sensitive receptors on erythroid precursors and cells of the hemopoiesis-inducing microenvironment.

Key Words: *erythroid precursors; Thy $1,2^+$ cells; adherent cells of hemopoiesis-inducing environment, monoamines*

The role of the pool of committed hemopoietic precursor cells and adherent and nonadherent elements of the hemopoiesis-inducing microenvironment (HIM) in plastic reconstruction of the blood system in experimental neuroses is now proven [1]. The involvement of α- and β-adrenoceptors on erythro- and granulomonocytopoiesis precursors and HIM cells in the realization of the regulatory effects of CNS on the blood system is beyond doubts. At the same time the role of cooperative interactions of HIM cells in the maintenance of hemopoiesis during neurotic states remains unclear. Monoamine regulation of proliferation and differentiation of hemopoietic precursors and functional activity of HIM in experimental neurosis is little studied.

Here we studied the interactions of Thy $1,2^+$ cells with adherent HIM cells and erythropoietic precursors and evaluated the role of monoamines in the regulation of proliferation and differentiation of erythroid precursor cells in experimental neuroses.

MATERIALS AND METHODS

Experiments were carried out on 2-2.5-month-old male CBA/CaLac mice (n=520, certified animals obtained from the Collection Stock of Experimental Biological Modeling Laboratory, Institute of Pharmacology). For modeling neurotic states we used 10-min conflict situation [1,6] and 48-h paradoxical sleep deprivation (PSD) [1,7,12]. The mice were sacrificed by cervical dislocation under ether narcosis on days 1, 2, 4, and 5 after modeling of neurotic states. The content of erythroid CFU (CFU-E) and BFU-E in the bone marrow was evaluated by *in vitro* cloning of myelocaryocytes in methylcellulose [3]. Proliferative activity of erythropoietic precursors was evaluated by the method of hydroxyurea-induced cell suicide; the intensity of differentiation was determined by the index of maturation

Institute of Pharmacology, Tomsk Research Center, Siberian Division of Russian Academy of Medical Sciences

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(the ratio of the number of bursts to the number of colonies in the same well) [3]. The content of Thy $1,2^+$ cells in the bone marrow and their role in the regulation of erythroid colony formation was studied using anti-Thy $1,2^+$ monoclonal antibodies (clone 5A-8 Cl 8600A, Cedarlane) [4]. Erythropoietic activity in media conditioned by adherent and nonadherent HIM elements and in serum was measured using intact mouse myelokaryocytes [3]. Sympatholytic reserpine (Polfa) was injected intraperitoneally in a single dose of 2 mg/kg 5-7 min before neurosis modeling. The final concentrations of α-adrenoceptor agonist mesaton (phenylephrine) and recombinant erythropoietin (Sigma) in the culture medium were 10^{-8} M and 0.5 U/ml, respectively.

The data were processed statistically using standard methods of variation statistics. Significance of differences was evaluated using parametric Student's *t* test and nonparametric Wilcoxon—Mann—Whitney *U* test.

RESULTS

Under conditions of conflict situation we observed a long-term (days 1-5) and pronounced (to 596, 442, 389, 491, 301% from the initial level, respectively, $p<0.05$) increase in the content of bone marrow Thy $1,2^+$ cells. In parallel, the capacity of nuclears of the specified phenotype to stimulate erythroid colony formation in methylcellulose culture increased on days 2-3 (Table 1). Feeder properties of T cells for CFU-E (on days 2 and 3) were most pronounced, if they were realized in combination with adherent bone marrow cells.

The content of bone marrow Thy $1,2^+$ cells in PSD increased on days 2 and 5 of the experiment (to 315 and 163% from the initial level, respectively), but decreased on days 3 and 6 (to 30 and 15% from the initial level, respectively). The absence of T cells did not change the efficiency of the formation of erythroid colonies by bone marrow cells at terms corresponding to their accumulation (day 2) and decrease in their count (day 3, Table 1). The absence of adherent bone marrow feeder considerably decreased the intensity of erythroid colony formation (day 2).

Thus, the function of erythroid precursors during neurotic states largely depends on bone marrow T cells expressing surface Thy $1,2^+$ antigen. Under conditions of conflict situation we observed direct and indirect (via interaction with adherent HIM elements) stimulation of the growth of CFU-E from erythropoietic precursors by regulatory T cells. We believe that cooperation of Thy $1,2^+$ cells with macrophages and increased affinity of microenvironment elements for CFU-E [8] induce cascade changes in the local regulation of bone marrow erythropoiesis: activation of the formation of hemopoietic islets and cytokine production by HIM elements, which, in turn, determines stimulation of proliferation and differentiation of erythroid precursors [1,8]. In animals subjected to PSD the interaction between Thy $1,2^+$ cells and adherent bone marrow elements and binding of CFU-E with stromal cells are limited [8]. Disturbed cell-cell interactions lead to suppression of the formation of additional focuses of erythroid hemopoiesis, inhibition of the production of humoral erythropoiesis stimulators by adherent HIM fraction, and suppression of mitotic activity and maturation of erythroid cells.

Regulation of erythropoiesis in the body is effected by two major cytokines: stem cell factor (c-kit ligand, Steel factor) and erythropoietin [10,11, 14,15]. Erythropoietin acts primarily on CFU-E, proerythroblasts, and basophilic erythroblasts [13, 16,17]. At the sate time, hemopoiesis precursors are the target for catecholamines [2,5]. In light of

TABLE 1. Effect of Thy $1,2^+$ Cells on Growth of Erythroid Colonies ($\times 10^5$ Cells) from Nonadherent Myelokaryocytes of CBA/CaLac Mice with Experimental Neuroses (X±*m*)

Time, days		Intensity of erythroid colony formation on adherent bone marrow feeder		Intensity of erythroid colony formation without adherent bone marrow feeder	
		without Thy$1,2^+$ cells	with Thy$1,2^+$ cells	without Thy$1,2^+$ cells	with Thy1.2^+ cells
Intact control		0.33±0.21	0.33±0.21	0.33±0.21	0.33±0.21
Conflict situation	2	1.50±0.42	4.17±0.60*+	0.50±0.22	2.00±0.25*+o
	3	0.83±0.31	2.67±0.31*+	0.17±0.17	1.25±0.18+o
PSD	2	1.50±0.42*	1.67±0.20*	0.50±0.22	0.25±0.17o
	3	0.33±0.21	0.33±0.21	0.17±0.17	0.17±0.17

Note. $p<0.05$ compared to *intact control; +corresponding parameter without Thy $1,2^+$ cells, ocorresponding parameter on adherent feeder.

this it was interesting to evaluate the possibility of participation of peripheral adrenergic structures in proliferation and differentiation of erythropoiesis precursor cells during experimental neuroses.

In our experiments α-adrenoceptor agonist mesaton *in vitro* stimulated the yield of erythroid precursors (days 1, 2, 4, 5), their mitotic activity (BFU-E on days 1, 2, 4, 5 and CFU-E on days 4 and 5) and intensity of differentiation (days 4 and 5) in bone marrow culture from animals subjected to conflict situation (Fig. 1). However, on days 1 and 2 proliferative activity of BFU-E decreased. In animals subjected to PSD mesaton increased the content of ClFU-E (days 1 and 2) and enhanced mitotic activity of erythroid precursors (days 4 and 5, Fig. 2). The intensity of maturation of erythroid precursors underwent phasic changes: increased on days 1 and 2 and decreased on day 4.

Thus, adrenergic receptors participate in the regulation of proliferation and differentiation of erythropoietic precursors under conditions of experimental neuroses. In animals subjected to conflict situation we observed simultaneous activation of cell division and stimulation of precursor differentiation. PSD leads to dysregulation of these processes. Our findings suggest that *in vitro* effect of erythropoietin on erythroid cells is similar to that of mesaton (Fig. 1, 2)

Since monoamines of CNS to a great extent determine the content of mature erythroid cells in the hemopoietic tissue during neurotic states [1], we studied monoaminergic mechanisms of regulation of functional activity of erythropoietic precursors.

In cultures from animals treated with reserpine and subjected to conflict situation erythropoietin *in vitro* prevented the increase in the number of erythroid bursts on day 1 of the experiment (Fig. 1), but then we observed increased yield of BFU-E (days 2 and 5) and CFU-E (day 5). The intensity of cell proliferation and differentiation underwent wave-like changes. For instance, mitotic activity of bursts and colonies increased on day 2 and decreased on days 4 and 5; the intensity of differentiation of erythroid precursors decreased on day 1 and increased on days 2 and 4. At the same time, the cytokine under conditions of exhausted catecholamine depots and PSD prevented stimulation of erythroid colony and bursts formation (day 2) and acceleration of precursor proliferation (days 2 and 5) and decreased the intensity of cell differentiation (days 1, 4, and 5; Fig. 2). Stimulation of CFU-E proliferation on day 1 of the experiment increased the number of colonies in methylcellulose cultures.

Mesaton increased the yield of BFU-E (days 1 and 5) and CFU-E (days 4 and 5), intensity of CFU-E proliferation (days 1 and 2) and differentiation of erythroid cells (day 1) in bone marrow culture from mice subjected to conflict situation under conditions of exhausted catecholamine depots (Fig. 1). On days 4 and 5 mitotic activity of CFU-E and intensity of maturation of erythroid precursors decreased. In animals treated with reserpine and subjected to PSD, α-adrenoceptor agonist had no effect on the dynamics of the content of erythropoietic precursors, but considerably inhibited their proliferation (day 5) and prevented accelerated differentiation of precursors (days 1 and 4; Fig. 2). The number of S-phase BFU-E increased on day 4 of the experiment.

Thus, monoamines of CNS affect proliferation and differentiation processes via adrenergic and erythropoietin-sensitive receptors on erythroid precursors in experimental neurotic states. However, the data that reserpine stimulates precursor division and maturation in methylcellulose culture are at controversy with the fact that this sympatholytic sharply decreases the content of erythrokaryocytes in the bone marrow during neurotic states [1]. This can be explained by local mechanisms of erythropoiesis regulation (adrenergic receptor agonists and antagonists modulate functional activity of HIM cells) [1,8]. In light of this, for evaluation of the mechanisms underlying the effect of sympatholytic on bone marrow erythropoiesis we measured erythropoietic activity of myelokaryocyte supernatants.

Under conditions of conflict situation reserpin abolished enhanced production of erythropoietic activity by adherent (days 1 and 2) and nonadherent (days 2 and 5) HIM elements (Fig. 1). Administration of the sympatholytic against the background of PSD suppressed secretory activity of nuclears of the adherent fraction of the bone marrow (day 2, Fig. 2). These findings suggest that the inhibitory effect of reserpine on the erythroid hemopoietic stem in experimental neurotic states is realized via an indirect pathway consisting in suppression of functional activity of HIM cells.

Thus, the pool of erythropoietic precursors and secretory activity of HIM elements is regulated by CNS-derived monoamines. The instructive information from CNS is transmitted via adrenergic structures on cells of the hemopoietic microenvironment and precursors and via erythropoietic receptors on erythropoietic precursors. At the same time the influence of distant mechanisms on the blood system under these model situations is not confined to epinephrine. There is a possibility of effector influence of dopamine, serotonin, acetylcholine, and neurokinins on hemopoietic precursor cells, bone marrow stromal cells and blood vessels via the

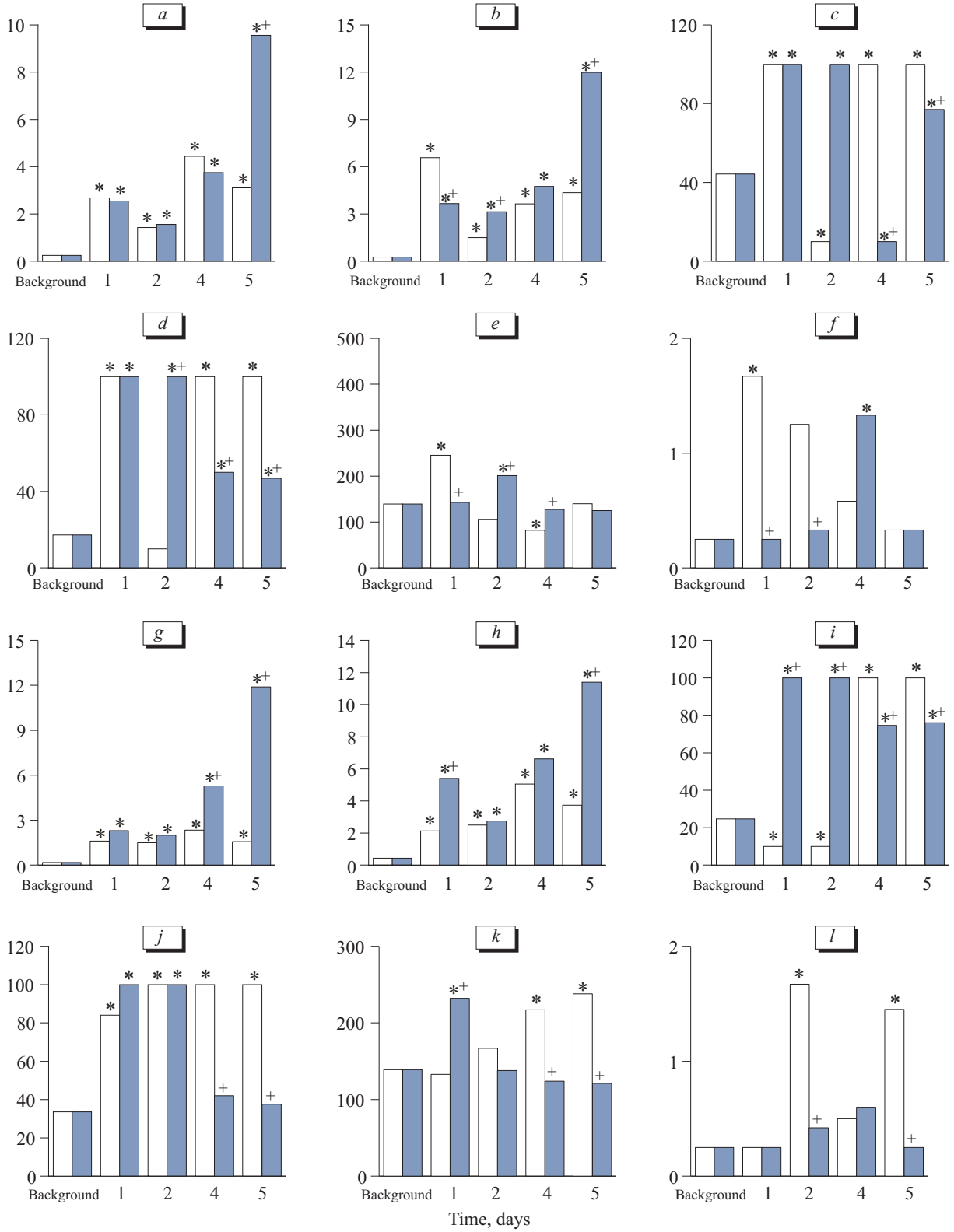

Fig. 1. Dynamics of the content of CFU-E (*a*, *g*), BFU-E (*b*, *h*), changes in percentage of S-phase CFU-E (*c*, *i*) and BFU-E (*d*, *j*), intensity of maturation of erythroid precursors (*e*, *k*), erythropoietic activity in supernatants of adherent (*f*) and nonadherent (*l*) myelokaryocytes in the bone marrow of CBA/CaLac mice subjected to conflict situation. Erythropoietin (*a-e*) and mesaton (*g-k*) were used *in vitro* as growth factors. Here and on Fig. 2: ordinate: content of erythroid precursors in the bone marrow ($\times 10^5$ cells, *a*, *b*, *f*, *g*, *h*, *l*), percent of S-phase precursors (%, *c*, *d*, *i*, *j*), index of maturation (ratio of number of clusters to number of colonies in the same well, %; *e*, *k*). Open bars: conflict situation+saline; dark bars: conflict situation+reserpine. $p<0.05$: compared to *baseline, +saline.

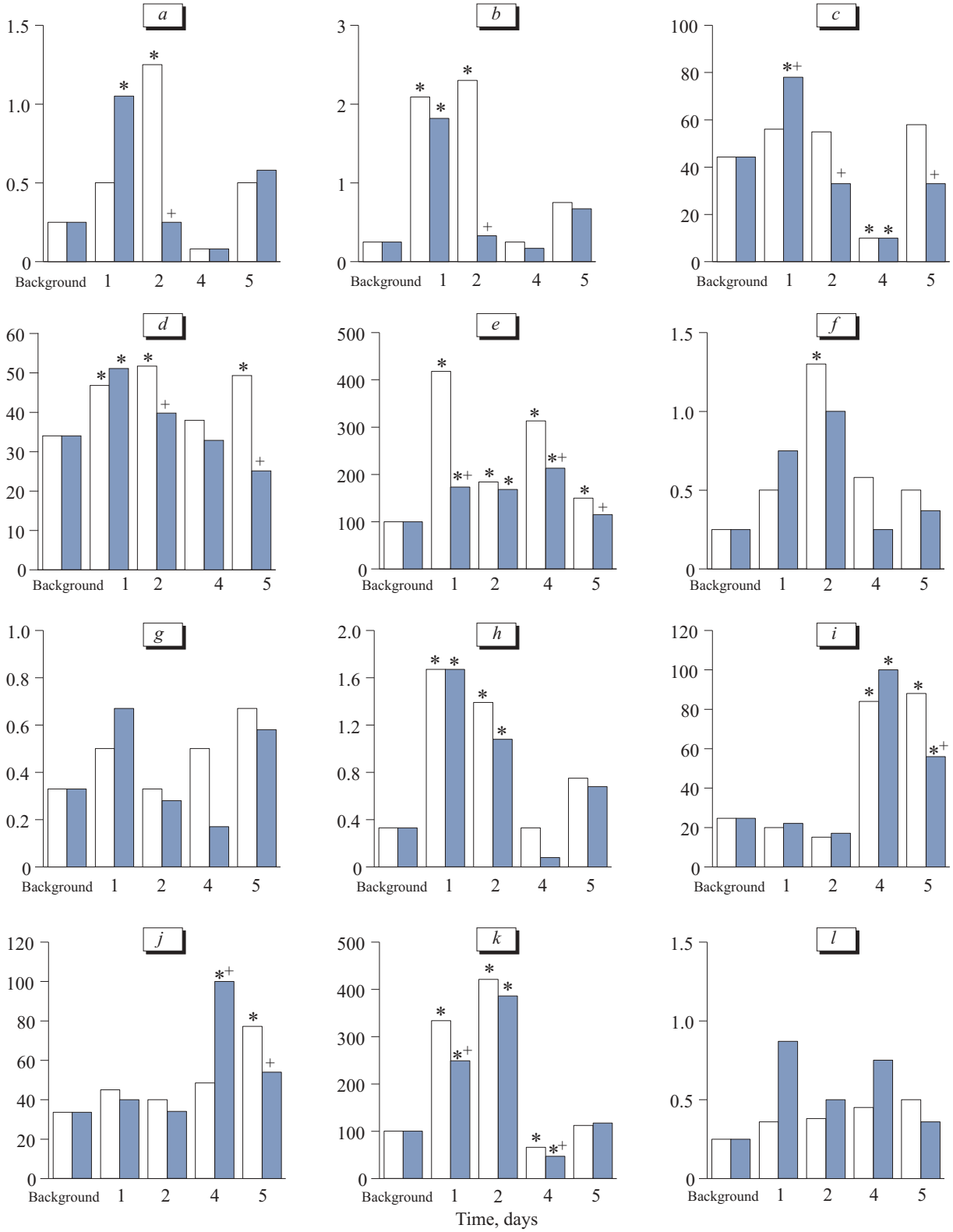

Fig. 2. Dynamics of the content of CFU-E (*a*, *g*), BFU-E (*b*, *h*), changes in percentage of S-phase CFU-E (*c*, *i*) and BFU-E (*d*, *j*), intensity of maturation of erythroid precursors (*e*, *k*), erythropoietic activity in supernatants of adherent (*f*) and nonadherent (*l*) myelokaryocytes in the bone marrow of CBA/CaLac mice subjected to paradoxical sleep deprivation. Erythropoietin (*a*-*e*) and mesaton (*g*-*k*) were used *in vitro* as growth factors. Open bars: paradoxal sleep deprivation+saline; dark bars: paradoxical sleep deprivation+reserpine. $^{+}p<0.05$ compared to the corresponding parameter without the test drug.

corresponding membrane receptors [1,5]. Moreover, the neurotransmitter systems regulate the blood flow in the hemopoietic tissue, modulate the balance of cell elements of HIM and their function (secretion of factors stimulating and inhibiting hemopoiesis, cell-cell interactions, *etc*.).

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