Cell Technologies in Biology and Medicine, Vol. 1, No. 4, November, 2005

Monoaminergic Regulation of the Pool of Erythropoietic Stem Cells in Active and Passive Mouse in Experimental Neuroses

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Translated from *Kletochnye Tekhnologii v Biologii i Meditsine*, No. 4, pp. 247-253, November, 2005
Original article submitted March 1, 2005

We studied monoaminergic mechanisms of regulation of the pool of erythropoietic precursors in active and passive mice under conditions of conflict situation and during paradoxical sleep deprivation. It was found that in animals with different behavior subjected to experimental neuroses the regulatory effects of CNS monoamines on proliferation and differentiation are mediated via adrenergic and erythropoietin-sensitive structures on erythroid stem cells. The revealed intergroup differences in precursor proliferation and differentiation rates (and erythrokaryocyte content in the bone marrow) are related to peculiarities in the activity of monoaminergic system and their interaction with peripheral structures on erythropoietic precursors.

Key Words: *erythropoietic stem cells; monoamines; individual reaction; and experimental neurosis*

The pool of erythroid precursor cells in CBA/CaLac mice of different behavioral types demonstrates different reaction to experimental neurotic sates [8]: the intensity of proliferation and differentiation of erythropoietic precursors increase in active animals, while in passive mice we observe dysregulation of these processes.

According to modern views, monoamines play an important role in the genesis of neurotic disorders and especially in their somatization [1-4]. Peculiarities in animals behavior during emotional stress are related to differences in the content of norepinephrine, dopamine, and serotonin in brain structures [9,10,2,13].

Here we studied monoaminergic mechanisms of regulation of the pool of erythropoietic stem cells in active and passive mice subjected to experimental neuroses.

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MATERIALS AND METHODS

Experiments were carried out on 2-2.5-month-old CBA/CaLac male mice (n=420). The animals (certified animal strain) were obtained from the collection of Laboratory of Experimental Biological Models (Institute of Pharmacology, Tomsk Research Center).

Neurotic states were modeled using 10-min conflict situation [4,7] and 48-h paradoxical sleep deprivation (PSD) [4,15]. Ten days before neurosis modeling the animals were divided into passive and active by the type of their behavior in a 3-component T-maze [8]. The animals were sacrificed by cervical dislocation under ether narcosis on days 1, 2, 4, and 5 after neurosis modeling and the number of erythrokaryocytes in the bone marrow was determined [5]. The content of erythroid CFU (CFU-E) and BFU (BFU-E) was studied by *in vitro* culturing in methylcellulose medium [5]. Proliferative activity of erythropoietic precursors was evaluated by the method of hydroxyurea-induced cell suicide, while

0007-4888/05/1405-0648

the intensity of their differentiation was determined by the index of maturation (ratio of the number of bursts to the number of colonies in the same well) [5].

Sympatholytic reserpine (2 mg/kg, Polfa) was injected intraperitoneally 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 using standard methods of variation statistics. Significance of differences was evaluated using parametric Student *t* test and nonparametric Wilcoxon—Mann—Whitney *U* tests.

RESULTS

Under conditions of conflict situation the content of erythrokaryocytes in the bone marrow increased in active mice (days 2, 4, and 5) and decreased in passive animals (days 2 and 5). At the same time, in mice subjected to PSD the cellularity of the erythroid stem in the bone marrow decreased in both active (days 1, 2, 4, and 5) and passive (days 2 and 4) animals.

Thus, conflict situation induced hyperplasia of the bone marrow erythropoiesis in active mice. In passive animals, conflict situation induced depression of the erythroid hemopoietic stem. PSD suppressed erythropoiesis in both active and passive mice.

Stem cell factor (c-kit-ligand, Steel factor), erythropoietin, and other cytokines participate in the regulation of erythropoiesis [14]. Erythropoietin affects primarily CFU-E, proerythroblasts, and basophilic erythroblasts. At the same time, erythropoietic precursors are the targets for catecholamines [4,6]. In light of this, it was interesting to evaluate the involvement of adrenergic and erythropoietic-sensitive receptors into proliferation and differentiation of precursor cells in active and passive mice under conditions of experimental neurosis.

Under conditions of conflict situation erythropoietin (to a greater extent) and mesaton stimulated the yield of erythroid precursors in bone marrow culture from active mice throughout the observation period (Table 1). After addition of erythropoietin proliferative activity of BFU-E and CFU-E and precursor differentiation rate increased on days 1, 4, and 5. Mesaton increased proliferation of BFU-E on days 1, 2, 4, and 5, proliferation of CFU-E on days 4 and 5, and precursor differentiation on days 1 and 4 (Fig. 1). In passive animals the intensity of erythropoietic precursor growth also increased (although to a lesser extent than in active animals). At the same time, proliferation and differentiation processes underwent phasic changes: when myelokaryocytes were cultured in the presence of mesaton, inhibition of mitotic activity of BFU-E (day 1) and CFU-E (days 1, 2, 4) change into stimulation (days 2, 4, and 5 and day 5, respectively). The BFU-E/CFU-E rate increased on days 2, 4, and 5 of the experiment. Addition of erythropoietin to methylcellulose medium inhibited proliferation of BFU and CFU on day 4 and days 1, 2, 5, respectively, and increased these values on days 1 and 5 and on day 4, respectively. Precursor differentiation increased on day 1 and decreased on days 4 and 5.

In mice subjected to PSD mesaton *in vitro* increased the number of CFU-E (day 5) and BFU-E (days 1 and 2) in active animals and the number of bursts in passive animals (days 1 and 2, Table 1). In passive mice we observed stimulation of proliferation (CFU-E on 2, 4, and 5 and BFU-E on days 4 and 5) and differentiation (days 1, 2, and 5) of erythroid precursors (Fig. 2). In active mice we observed not only stimulation of CFU-E division (days 2, 4, and 5) and precursor maturation (days 1 and 2), but also inhibition BFU-E division (days 2 and 4) and differentiation (days 4 and 5). Erythropoietin stimulated the yield of BFU-E and CFU-E in methylcellulose medium in both groups: in active mice on days 1, 2, and 5 and on day 2, respectively, and in passive mice on days 1 and 2 (Table 1). Under these conditions we observed both stimulation and inhibition of cell division and maturation (Fig. 2).

Thus, α-adrenergic and erythropoietin-sensitive structures on erythropoietic stem cells not only participate in the regulation of proliferation and differentiation, but also determine intergroup differences in the intensity of these processes in active and passive mice. For instance, *in vitro* stimulation of division and maturation of BFU-E and CFU-E by erythropoietin and mesaton can explain the capacity of the bone marrow tissue (at least, at the level of precursors) to ensure enhanced production of mature cells of the erythron lineage in active mice under conditions of conflict situation. In passive mice under conditions of conflict situation and in mice subjected to PSD the interaction of erythropoietin and adrenoceptor agonist with the corresponding receptors leads to suppression and dysregulation of proliferation and differentiation of precursor cells (at terms specific for the used neurosis models) causing depression of the erythroid hemopoietic stem.

It is accepted that differences in the content of monoamines in the brain are responsible for peculiar behavioral reactions [9,10,13] and specific responses of some visceral systems [12], therefore we studied monoaminergic mechanisms of regulation

TABLE 1. Dynamics of the Content of Erythroid Precursor Cells ($\times 10^5$ Cells) in Bone Marrow of Active and Passive CBA/CaLac Mice after Experimental Neurotic Influences ($X \pm m$)

Experimental conditions		Active mice				Passive mice			
		erythropoietin		mesaton		erythropoietin		mesaton	
		CFU-E	BFU-E	CFU-E	BFU-E	CFU-E	BFU-E	CFU-E	BFU-E
Intact control		0.2±0.1	0.25±0.20	0.50±0.29	0.50±0.29	0.2±0.1	0.25±0.20	0.40±0.29	0.50±0.29
Conflict situation									
Day 1	saline	4.5±0.5*	12.00±0.93*	1.60±0.33*o	4.00±0.25*o	1.8±0.2*+	3.96±0.50*+	1.60±0.25*	1.60±0.25*+o
	reserpine	1.80±0.25*x	3.96±0.25*x	4.00±0.83*x	12.0±1.0*x	1.5±0.5*	3.5±0.5*	1.50±0.25*	4.00±0.35*x
Day 2	saline	1.50±0.25*	1.50±0.25*	1.50±0.25*	1.50±0.25*	1.50±0.25*	1.50±0.25*	1.50±0.25*	3.75±0.50*+o
	reserpine	1.88±0.31*	1.88±0.25*	3.75±0.62*x	3.75±0.50*x	1.50±0.25*	1.50±0.25*	1.50±0.25	1.0±0.1x
Day 4	saline	3.33±0.33*	5.17±0.31*	1.75±0.25*o	6.89±0.41*	2.5±0.5*	0.75±0.25+	0.75±0.25+o	1.80±0.25*+o
	reserpine	3.00±0.25*	5.0±0.5*	5.25±0.75*x	5.17±0.50*	1.45±0.25*x	4.5±0.5*x	5.63±0.50*x	14.00±0.75*x
Day 5	saline	5.15±0.48*	9.67±0.50*	1.61±0.50*o	1.61±0.25*o	2.07±0.30*+	1.64±0.30*+	1.61±0.25*	4.83±0.50*+o
	reserpine	10.34±0.90*x	19.35±1.50*x	19.32±2.50*+	19.32±3.00*+	8.25±0.75*+	13.12±1.00*+	8.86±0.75*+	14.40±1.25*x
PSD									
Day 1	saline	0.50±0.22	3.11±0.32*	0.33±0.21	2.00±0.01*o	1.00±0.17*	1.67±0.21*+	0.50±0.22	1.33±0.21
	reserpine	2.14±0.17*x	3.11±0.19*	1.33±0.21*x	3.33±0.21*x	0.75±0.17	0.75±0.10x	0.33±0.21	0.25±0.12x
Day 2	saline	1.50±0.22*	3.89±0.38*	0.17±0.17o	1.33±0.15*o	1.17±0.17*	1.50±0.22*+	0.67±0.21	2.33±0.21*+
	reserpine	0.33±0.21x	1.17±0.17*x	0.50±0.22	2.75±0.48*x	0.25±0.21x	1.17±0.17*	0.33±0.21	0.50±0.22x
Day 4	saline	0.25±0.20	0.2±0.2	1.00±0.01o	0.50±0.29	0.2±0.2	0.2±0.2	0.25±0.25	0.25±0.25
	reserpine	0.25±0.25	0.50±0.29	0.25±0.25	0.2±0.2*	0.2±0.1	0.2±0.1	0.2±0.1	0.25±0.25
Day 5	saline	0.50±0.29	1.50±0.29*	1.50±0.15*o	1.00±0.01	0.25±0.25	0.75±0.25+	0.25±0.25+	0.50±0.29
	reserpine	0.25±0.25	0.50±0.29x	1.50±0.29*	1.50±0.29*	0.50±0.25	0.75±0.25	0.2±0.1	0.75±0.25

Note. $p<0.05$ compared to: *intact control, +active animals, oerythropoietin, xphysiological saline.

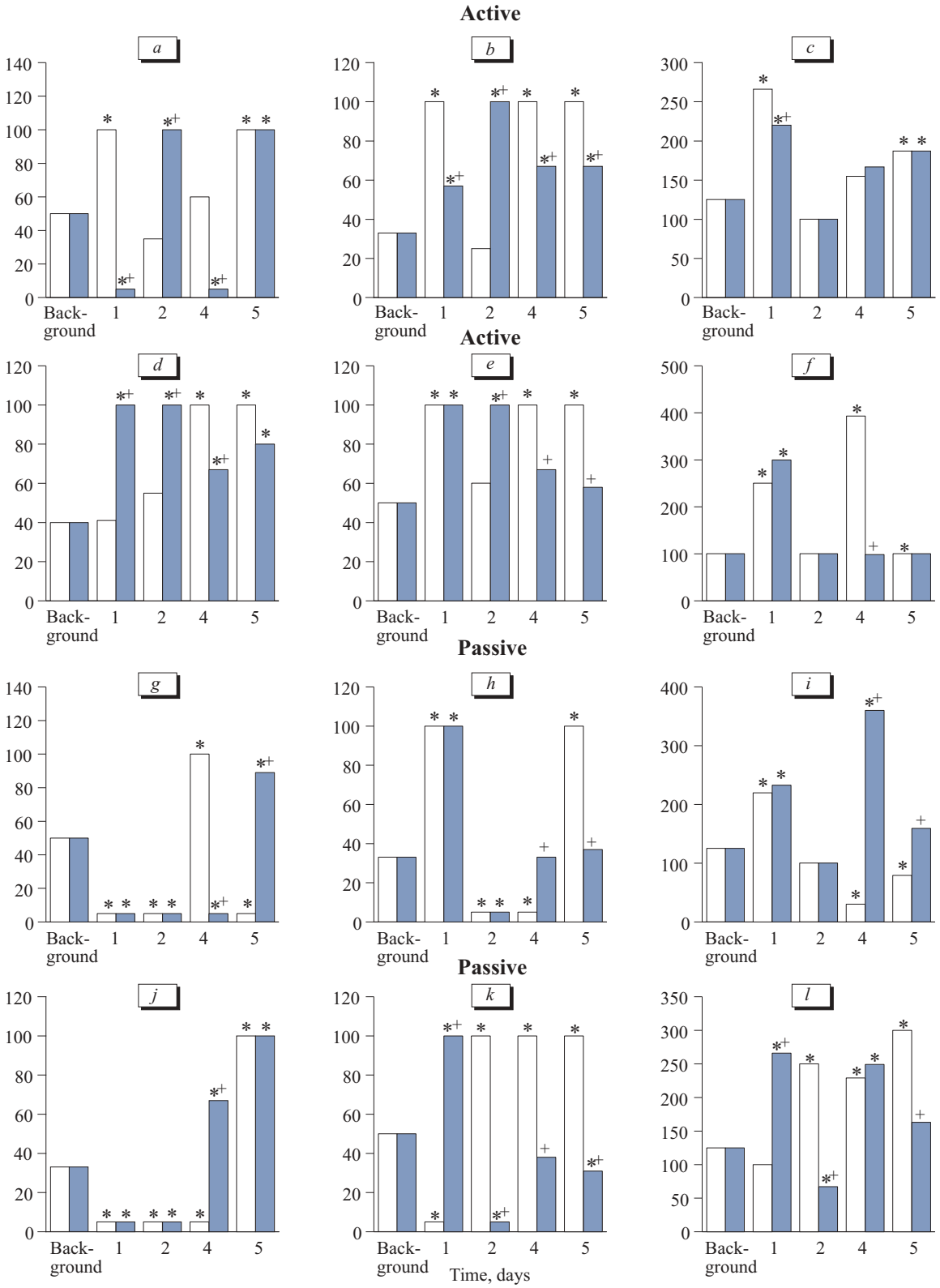

Fig. 1. Changes in the percentage of S-phase CFU-E (*a*, *d*, *g*, *j*) and BFU-E (*b*, *e*, *h*, *k*) and intensity of maturation of erythroid precursors (*c*, *f*, *i*, *l*) in the bone marrow of active and passive CBA/CaLac mice subjected to conflict situation. Erythropoietin (*a-c*, *g-i*) and mesaton (*d-f*, *j-l*) were used as growth factors *in vitro*. Conflict situation was modeled against the background of physiological saline treatment (open bars) and reserpine (dark bars). Here and on Fig. 2: ordinate — percentage of S-phase precursors (%, *a*, *b*, *d*, *e*, *g*, *h*, *j*, *k*) and index of maturation, *i.e.* ratio of number of bursts to number of colonies in the same well (%, *c*, *f*, *i*, *l*). $p<0.05$ compared to *baseline, +physiological saline.

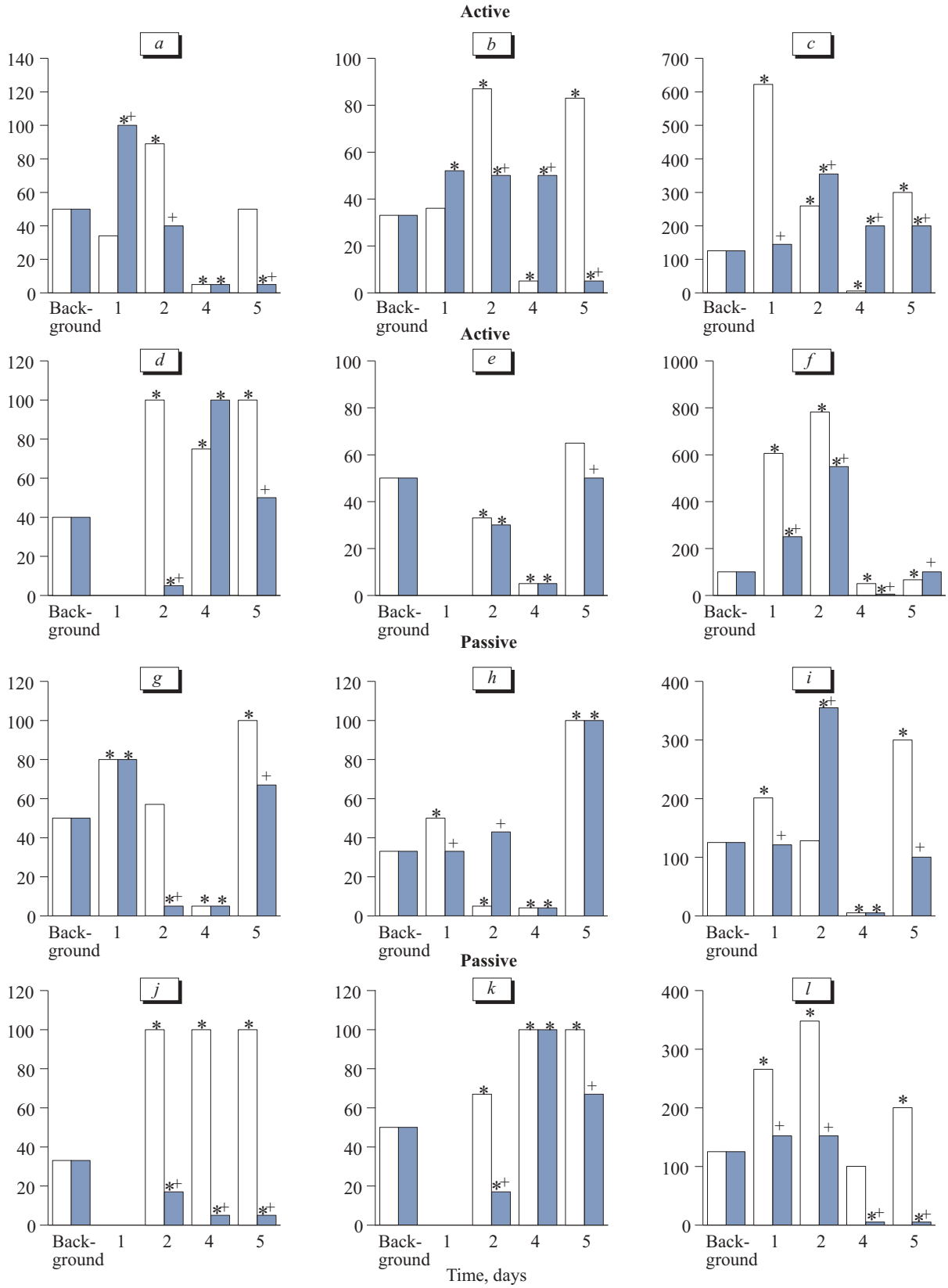

Fig. 2. Changes in the percentage of S-phase CFU-E (*a*, *d*, *g*, *j*) and BFU-E (*b*, *e*, *h*, *k*) and intensity of maturation of erythroid precursors (*c*, *f*, *i*, *l*) in the bone marrow of active and passive CBA/CaLac mice subjected to paradoxical sleep deprivation. Erythropoietin (*a-c*, *g-i*) and mesaton (*d-f*, *j-l*) were used as growth factors *in vitro*. Paradoxical sleep deprivation was modeled against the background of physiological saline treatment (open bars) and reserpine (dark bars).

of erythropoietic precursor pool in mice with different behavior.

Reserpine-induced exhaustion of catecholamine depots in the CNS prevented hyperplasia of the bone marrow erythropoiesis in active mice (days 2, 4, and 5) and aggravated depression of the erythron in passive mice (days 1 and 5) under conditions of conflict situation. Erythropoietin *in vitro* prevented the increase in the content of erythroid precursors in active mice (day 1) and CFU-E in passive animals (day 4; Table 1). At later terms we observed enhanced production of BFU-E and CFU-E in active (days 4 and 5) and passive (day 5) animals. Erythropoietin produced different effects on proliferation and differentiation of erythropoietic precursor cells. In active mice it decreased division rate of BFU-E (days 1, 4, and 5) and CFU-E (days 1 and 4) and the intensity of precursor maturation (day 1; Fig. 1). However, on day 2 of the experiment proliferative activity of bursts and colonies increased. At the same time, reserpine suppressed proliferation of precursors, but increased BFU-E/CFU-E rate (days 4 and 5).

Under conditions of monoaminergic system inhibition and conflict modeling, stimulation of α-adrenoceptors with mesaton *in vitro* promoted the increase in the content of erythropoietic precursor cells in bone marrow culture from mice of different behavioral types (the effect was more pronounced in active animals; Table 1). In active mice enhancement of BFU-E and CFU-E proliferation (on day 2 and days 1 and 2, respectively) was followed by a decrease in the intensity of precursor division and maturation (days 4 and 5; Fig. 1). Reserpine produced a modulating effect on functional activity of erythroid precursors in passive mice: at terms corresponding to inhibition of their proliferation and differentiation we observed activation of those processes (day 1), while inhibition of burst and colony division and maturation were observed instead of their stimulation (days 2 and 5).

Reserpine aggravated depression of bone marrow erythropoiesis in active (days 4 and 5) and passive (day 5) mice subjected to PSD. Under these conditions erythropoietin *in vitro* inhibited production of CFU-E and BFU-E in active (day 2 and days 2 and 4, respectively) and passive (day 2 and day 1, respectively) animals (Table 1). However, on day 1 of the experiment, the number of colonies in bone marrow culture from active animals increased. In animals of both groups, the sympatholytic reduced (or delayed) activation of erythroid precursor proliferation and differentiation (Fig. 2). In contrast, in active animals erythropoietin stimulated the intensity of CFU-E division on day 1 of the experiment.

In animals with sleep structure disorders against the background of sympatholytic treatment, mesaton *in vitro* increased the number of BFU-E (days 1, 2, and 4) and induced earlier enhancement of CFU-E production (on day 1 *vs*. day 4 in the control group) in bone marrow culture from active mice, while in passive animals the growth of BFU-E was suppressed (days 1 and 2; Table 1). In both groups, functional activity of erythropoietic precursor pool was decreased (predominant inhibition of proliferation and differentiation in passive and active animals, respectively; Fig. 2).

Thus, in mice of different behavioral types subjected to neurotic influences the regulatory effects of monoamines of CNS on proliferation and differentiation are mediated via adrenergic and erythropoietic-sensitive structures on erythroid precursors. The observed specific effects of the sympatholytic on the pool of erythropoietic stem cells in the used experimental models of neurotic states attest to peculiar activity of monoaminergic systems and their interactions with peripheral receptors in active and passive animals. Our data on stimulation of precursor division and maturation with reserpine are at controversy with the data that this drug sharply decreased the number of erythrokaryocytes in the bone marrow of mice with experimental neuroses. This can be explained by indirect (mediated via hemopoiesis-inducing microenvironment) influence of CNS on erythropoiesis. On the other hand, synaptotropic (transmitter) agents can modulate functional activity of elements of hemopoiesis-inducing microenvironment [4,11]. However, peculiarities of the state of distant mechanisms are not confined to monoamines. For instance, acethylcholine, neurokinins, and substance P can affect not only hemopoietic precursors, but also bone marrow stromal cells and blood vessels via the corresponding receptors on the plasma membranes [4,6].

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