Mechanisms of Regulation of Erythropoiesis during Experimental Neuroses

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We studied the mechanisms of erythropoiesis regulation in a conflict situation and during paradoxical sleep deprivation. Plastic reconstruction of the erythroid hemopoietic stem during experimental neuroses is regulated by a complex multicomponent and multilevel system. This system consists of central adrenergic structures, sympathetic part of the autonomic nervous system, and α- and β-adrenoceptors on erythroid precursors and cells of the hemopoietic microenvironment. Erythroid cells receive instructive information from the central nervous system via adrenoceptors on cells of the hemopoiesis-inducing microenvironment and erythroid precursors. Hyperplasia (conflict situation) and suppression of erythropoiesis (paradoxical sleep deprivation) are associated with specificity of central adrenergic regulatory mechanisms.

Key Words: *adrenergic system; hemopoiesis-inducing microenvironment; erythropoiesis; regulation*

Neuroses and neurotic states contribute to the development of somatic disturbances in various functional systems of the organism. Experimental neuroses are always accompanied by changes in the erythron system [4]. Individual components of the system responsible for local regulation of the erythron underwent changes in a conflict situation (CS) and during paradoxical sleep deprivation (PSD) [3]. Published data show that the central nervous system (CNS) plays a role in the pathogenesis of hyperplasia or suppression of the erythroid hemopoietic stem [8]. Little is known about the mechanisms of regulation of proliferation and differentiation of erythroid precursors during experimental neuroses.

Here we studied the role of the adrenergic system and hemopoiesis-inducing microenvironment (HIM) in the regulation of erythropoiesis during experimental neuroses.

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MATERIALS AND METHODS

Experiments were performed on 892 CBA/CaLac mice (class I conventional mouse strain) aging 2.0-2.5 months and obtained from the collection of the Laboratory of Experimental Biological Modeling (Institute of Pharmacology, Tomsk Research Center).

CS (10 min) [6] and PSD (48 h) [10] served as the models of experimental neurosis. Peripheral blood reticulocytes were counted on days 1-7 after CS and PSD. The mice were euthanized by cervical dislocation under ether anesthesia. The count of bone marrow erythrokaryocytes was estimated [2]. The number of erythroid colony-forming (CFU-E) and cluster-forming units (ClFU-E) in the bone marrow was determined *in vitro* by cloning of myelokaryocytes in a methylcellulose culture medium [2]. Proliferative activity of erythroid precursors was assayed by the method of cell suicide using hydroxyurea [1]. The rate of differentiation of hemopoietic precursors was estimated by the index of maturation (ratio between the counts of clusters and colonies in a well) [2]. Structural and

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functional characteristics of the bone marrow were determined by enzymatic isolation of hemopoietic islets and study of their quantitative and qualitative characteristics [9]. We counted colonies formed by bone marrow cells from intact mice before and after 2-h coincubation with adherent myelokaryocytes of neurotic animals [5]. The ability of adherent myelokaryocytes from neurotic mice to bind intact CFU-E was determined by the difference between these parameters. Erythropoietic activity of conditioned media from adherent and nonadherent cells of HIM and blood plasma was measured in a semisolid medium on intact mouse myelokaryocytes [2].

α-Adrenoceptor agonist mesatone (5 mg/kg), β-adrenoceptor agonist orciprenaline sulfate (2 mg/kg), α-adrenoceptor antagonist dihydroergotamine (3.9 mg/kg), and β-adrenoceptor antagonist propranolol (5 mg/kg) were injected subcutaneously 3-5 min before and 5-6 h after neurosis. Ganglionic blocker pentamine (6 mg/kg) was infused intravenously in the same periods. Sympatholytic reserpine in a single dose of 2 mg/kg was injected intraperitoneally 5-7 min before treatment. Myelokaryocytes were *in vitro* treated with mesatone and orciprenaline sulfate in a concentration of 10^{-8} M.

The results were analyzed by standard methods of variational statistics. The significance of differences was evaluated by parametric Student's *t* test and nonparametric Wilcoxon—Mann—Whitney *U* test.

RESULTS

CS was accompanied by stimulation of the erythroid hemopoietic stem. We revealed an increase in the number of bone marrow erythrokaryocytes (days 1-6) and peripheral blood reticulocytosis (days 1-7, Tables 1 and 2, Fig. 1). These changes were accompanied by an increase in the yield of erythroid precursors in methylcellulose culture medium (days 1-4, 6, and 7), proliferative activity of ClFU-E (days 1-7) and CFU-E (days 1, 2, 5, and 7), and rate of erythroid precursor maturation (days 3, 4, 5, and 7).

The number of bone erythrokaryocytes on days 3-6 after PSD was much lower than in the control. Peripheral blood reticulocytosis (days 1-2) was followed by reticulocytopenia (days 4-7, Tables 1 and 2, Fig. 1). The pool of erythroid precursors underwent different changes. The decrease in the count of ClFU-E and CFU-E in the bone marrow (days 5-7) was preceded by an increase in the number of erythroid precursors

TABLE 1. Effects of Reserpine and Pentamine on the Number of Bone Marrow Erythroid Cells in CBA/CaLac Mice with Neurosis ($\times 10^6$ cells/femur, $X \pm m$, $n=7$)

Period, days	Series I		Series II	
	physiological saline	reserpine	physiological saline	pentamine
CS				
Intact control	0.58±0.07		0.64±0.04	
1	1.14±0.18*	0.13±0.06*+	0.96±0.08*	1.08±0.15*
2	0.80±0.06*	0.33±0.06+	0.88±0.07*	0.32±0.04*+
3	0.62±0.08	0.21±0.05*+	1.41±0.13*	0.48±0.06+
4	0.68±0.10	0.19±0.05*+	0.44±0.20	0.44±0.09
5	0.72±0.07	0.34±0.03+	0.78±0.16	0.96±0.08*
6	1.02±0.14*	0.38±0.08+	0.88±0.08*	2.28±0.25*+
7	1.16±0.21*	0.17±0.07*+	1.28±0.22*	0.96±0.09*
PSD				
Intact control	0.58±0.07		0.78±0.06	
1	0.23±0.04*	0.13±0.04*	0.57±0.09	0.24±0.03*+
2	0.73±0.48	0.11±0.03*+	0.48±0.06*	0.21±0.03*+
3	0.19±0.05*	0.45±0.08	0.45±0.06*	0.15±0.02*+
4	0.13±0.02*	0.15±0.09*	0.15±0.03*	0.24±0.03*
5	0.57±0.04	0.37±0.04*+	0.33±0.06*	0.15±0.02*+
6	0.35±0.03*	0.21±0.03*+	0.48±0.03*	0.15±0.03*+
7	0.41±0.03	0.36±0.04*	0.27±0.03*	0.36±0.06*

Note. Here and in Table 2: $p<0.05$: *compared to intact controls; +compared to physiological saline.

(days 1, 3, and 4). Proliferative activity of ClFU-E decreased on day 5. The rate of CFU-E division significantly exceeded the baseline level (days 1, 3, 5, and 7). The intensity of erythroid precursor maturation underwent phasic changes. Differentiation of precursors increased on days 2 and 4, but decreased on days 5 and 7.

These data indicate that simultaneous increase in proliferative activity and rate of erythroid precursor differentiation (*i.e.*, synchronization of processes) is a mechanism of activation of erythropoiesis in CS. Desynchronization and blockade of proliferation and differentiation of erythroid precursors during PSD inhibit the formation of erythroid colonies and clusters and lead to suppression of erythropoiesis.

The study of structural and functional organization of the bone marrow showed that the number of macrophage-associated cell complexes increases on days 1-5 and 7 after CS. The study of their qualitative composition revealed increased count of erythroid hemopoietic islets on days 1, 2, 5, and 7 after treatment (Fig. 1). Moreover, the ability of adherent cells from the bone marrow of experimental mice to bind intact CFU-E increased on days 2, 5, and 7.

PSD decreased the number of macrophage-positive cell associations (days 1 and 3) and erythroid hemopoietic islets (days 1-3, Fig. 1). The ability of macrophages from experimental mice to bind erythroid precursors from intact animals decreased significantly on days 1-3, 6, and 7.

Analysis of erythropoietic activity of blood plasma and myelokaryocyte-conditioned media showed that CS was associated with increased production of cytokines by nonadherent (days 1-4 and 7) and adherent bone marrow cells (days 1, 2, 4, and 5) and increased erythropoietic activity of blood plasma on days 1-6 (Fig. 1). Secretion of these factors by nonadherent nucleated cells increased 1-7 days after PSD. Under these conditions erythropoietic activity of conditioned media of adherent myelokaryocytes was lower than in intact controls (days 2 and 4). Treatment with blood plasma was followed by activation (days 1 and 3) and inhibition of erythroid colony formation in methylcellulose medium (days 2, 6, and 7).

Our results indicate that the increase in proliferative activity and rate of erythroid precursor maturation in CS is associated with increased formation of erythroid hemopoietic islets and high secretory activity of HIM (high concentration of plasma hemopoietins). The inhibition of proliferation and differentiation of erythroid precursors during PSD is directly related to impaired formation of cell complexes with central macrophage, suppressed production of humoral erythropoiesis stimulators by adherent HIM cells, and decreased blood erythropoietic activity.

TABLE 2. Effects of Dihydroergotamine, Propranolol, Mesatone, and Orciprenaline Sulfate on the Number of Bone Marrow Erythroid Cells in CBA/CaLac Mice with Neurosis ($\times 10^6$ cells/femur, $X \pm m$, $n=7$)

Period, days	Physiological saline	Dihydroergotamine	Propranolol	Mesatone	Orciprenaline sulfate
CS					
Intact control	0.78±0.02				
1	1.36±0.09*	1.19±0.16*	0.76±0.03+	1.50±0.21*	1.61±0.08*
2	1.46±0.07*	0.42±0.09*+	0.58±0.02+	1.12±0.14*	1.56±0.07*
3	1.19±0.09*	0.23±0.04*+	0.44±0.02*+	1.11±0.09*	1.68±0.11*+
4	1.11±0.11*	0.69±0.08+	0.87±0.03	1.71±0.24*+	1.32±0.09*
5	2.61±0.12*	0.35±0.04*+	1.03±0.24*+	1.65±0.08*+	1.91±0.21*
6	1.08±0.08*	0.94±0.09	1.23±0.28*	0.97±0.04	—
7	0.99±0.21	1.59±0.43*+	0.51±0.06*+	1.06±0.03*	—
PSD					
Intact control	0.78±0.02				
1	0.74±0.04	0.82±0.02	1.63±0.21*+	1.04±0.25*+	0.68±0.08
2	0.71±0.07	0.65±0.09	1.64±0.23*+	1.61±0.33*+	1.13±0.11*+
3	0.37±0.04*	1.18±0.35*+	1.53±0.11*+	1.81±0.47*+	0.70±0.05+
4	0.45±0.01*	1.67±0.35*+	1.18±0.57*+	1.37±0.44*+	0.58±0.06
5	0.44±0.03*	0.99±0.05+	2.26±0.71*+	0.95±0.08+	0.84±0.09+
6	0.42±0.02*	2.06±0.47*+	1.03±0.29*+	0.85±0.09+	1.09±0.12*+
7	0.64±0.05	1.86±0.14*+	2.06±0.53*+	0.80±0.07	1.22±0.14*+

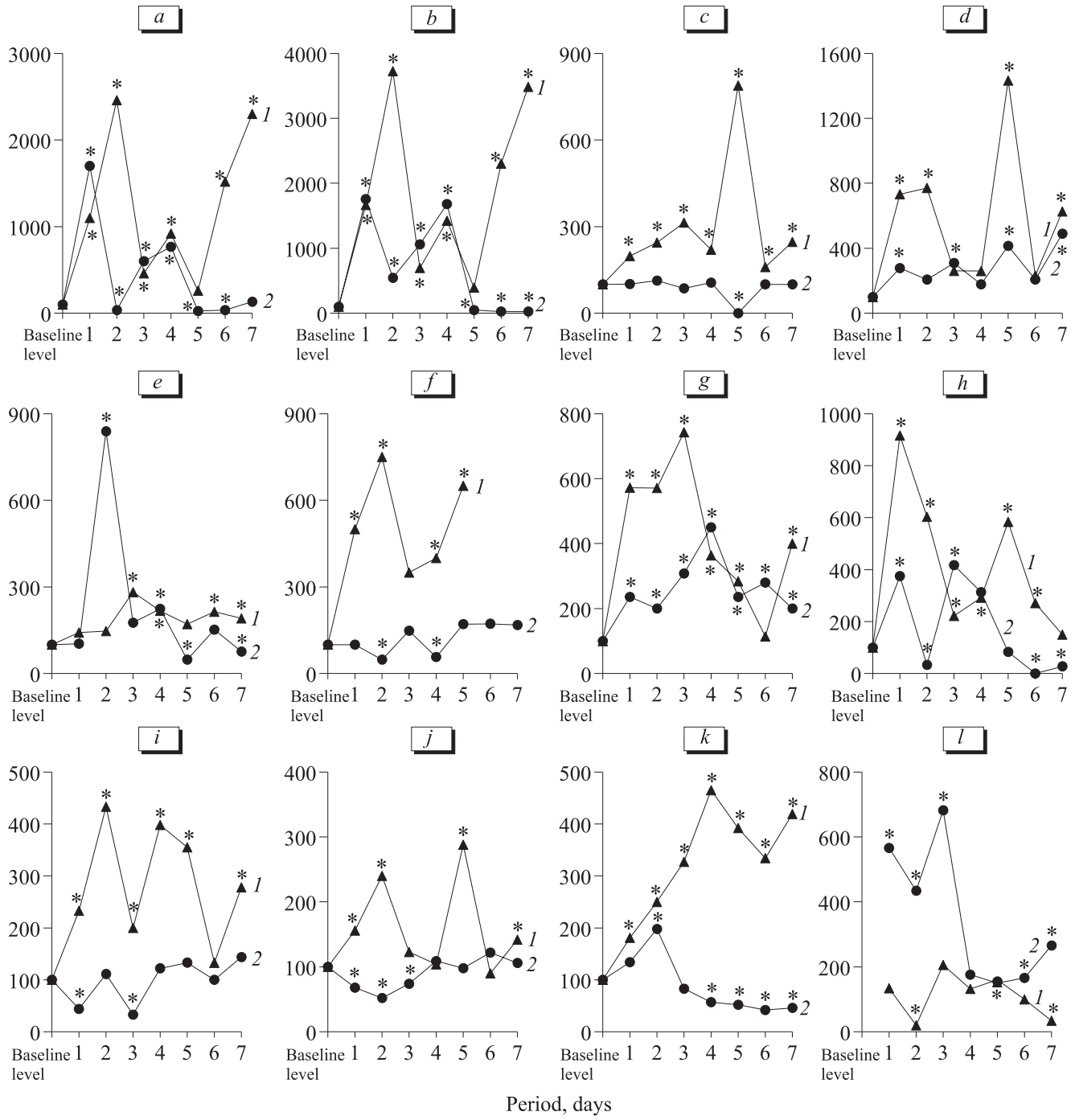

Fig. 1. Number of erythroid CFU (*a*) and erythroid cluster-forming units (*b*), ratio of erythroid CFU (*c*) and cluster-forming units (*d*) in S-phase of the cell cycle, maturation of erythroid precursors (*e*), erythropoietic activity in supernatants of adherent (*f*) and nonadherent (*g*) myelokaryocytes and blood plasma (*h*), and count of bone marrow macrophage-positive (*i*) and erythroid hemopoietic (*j*) islets and peripheral blood reticulocytes (*k*) in CBA/CaLac mice with neurosis. Ability of adherent myelokaryocytes from neurotic animals to bind erythroid CFU (*l*). Ordinate: % of the baseline level. Conflict situation (*1*) and paradoxical sleep deprivation (*2*). *$p<0.05$ compared to the control (100%).

In series I we studied the role of the adrenergic system in the regulation of the erythroid hemopoietic stem during neuroses mediated by the monoaminergic mechanisms. Depletion of catecholamine depots in CNS caused by sympatholytic reserpine abolished accumulation of erythroid precursors in methylcellulose medium (days 5 and 6) and prevented hyperplasia of the bone marrow erythropoiesis in CS (days 1, 2, 6, and 7, Table 1, Fig. 3). The decrease in the number of CFU-E (days 5 and 6) and deficiency of bone marrow erythrokaryocytes (days 2, 5, and 6) under conditions of PSD were more pronounced after treatment with reserpine.

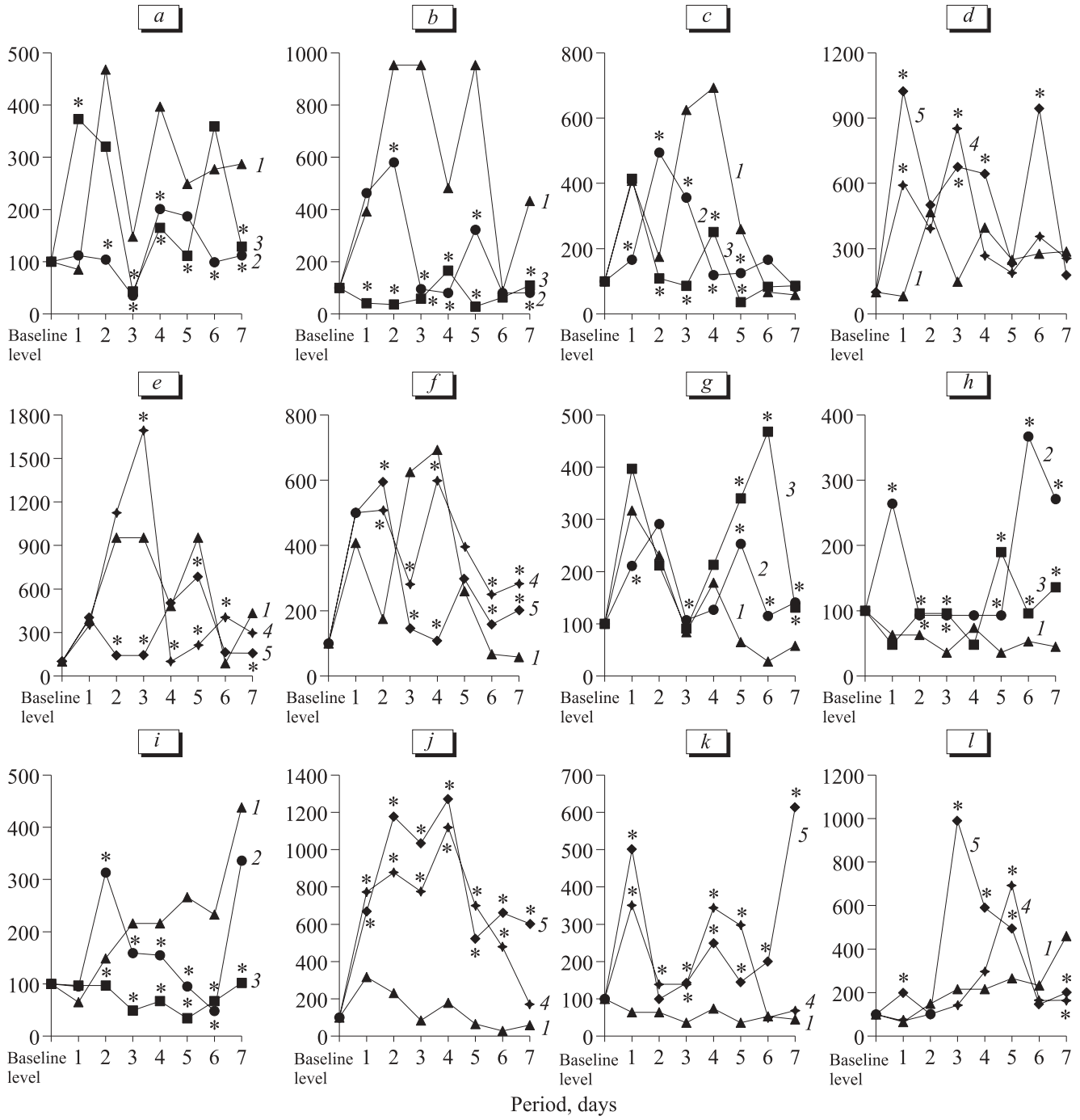

Fig. 2. Number of erythroid CFU (*a*, *d*, *g*, *j*) and erythropoietic activity of supernatants from adherent (*b*, *e*, *h*, *k*) and nonadherent (*c*, *f*, *i*, *l*) myelokaryocytes in the bone marrow of CBA/CaLac mice exposed to a conflict situation (*a-f*) or paradoxical sleep deprivation (*g-l*) and receiving physiological saline (*1*), dihydroergotamine (*2*), propranolol (*3*), mesatone (*4*), and orciprenaline sulfate (*5*). Ordinate: number of erythroid precursors in the bone marrow, $\times 10^5$ cells.

In series II pharmacological blockade of autonomic ganglia with pentamine abolished the increase in the number of bone marrow CFU-E (days 5 and 6) and delayed hyperplasia of bone marrow erythropoiesis in CS. Pentamine prevented inhibition of erythroid colony formation (days 5 and 6) and aggravated suppression of the erythroid hemopoietic stem during PSD (Table 1, Fig. 3).

In series III adrenoceptor blockade with dihydroergotamine and propranolol abolished activation of the erythroid hemopoietic stem in CS (days 2-5; and days 1-3 and 5, respectively) and its suppression during PSD (days 3-7, Table 2). The increase in the number of CFU-E during CS was blocked with dihydroergotamine (days 2-4, 6, and 7) and propranolol (days 3-5

and 7, Fig. 2). Five-seven days after PSD the number of erythroid precursors returned to the level observed in CS without administration of adrenoceptor antagonists.

Adrenoceptor antagonists significantly decreased erythropoietic activity of myelokaryocyte-conditioned media in CS (Fig. 2). In animals exposed to PSD dihydroergotamine and propranolol prevented the increase in the production of erythropoietic activity in nonadherent nuclear cells (days 5-6 and 3-7, respectively) and abolished inhibition of secretory activity in adherent myelokaryocytes (days 1 and 5-7; and 3 and 5-7, respectively).

Treatment with adrenoceptors agonists simulates the increase in catecholamine concentration in the organism [7]. In our experiments mesatone (days 3-6) and orciprenaline sulfate (days 3 and 5-7) normalized PSD-suppressed erythropoiesis (Table 2). Agonists of α- and β-adrenoceptors stimulated CFU-E growth (days 1-6 and 1-7, respectively), activated production of erythropoietic activity in adherent myelokaryocytes (days 1-5; and 1 and 3-7, respectively), and increased secretion of factors by nonadherent nuclear cells (days 5; and 1 and 3-5, respectively) (Fig. 2). Further activation of adrenergic structures in CS was followed by a greater stimulation of bone marrow erythropoiesis. Moreover, mesatone and orciprenaline sulfate *in vitro* stimulated erythroid colony formation in cultured bone marrow tissue from neurotic mice and animals exposed to a neurotic situation, depletion of catecholamines, and pharmacological blockade of autonomic ganglia and adrenergic receptors (Fig. 3).

Our findings suggest that monoamines of CNS, autonomic ganglia, and adrenoceptors are involved in the progression of changes in the erythroid hemopoietic stem during neuroses. Their effects are associated with changes in functional activity of precursors and HIM cells. Central adrenergic structures, sympathetic nervous system, α- and β-adrenoceptors on erythroid precursors and HIM cells, and hemopoietic microenvironment constitute a complex system regulating

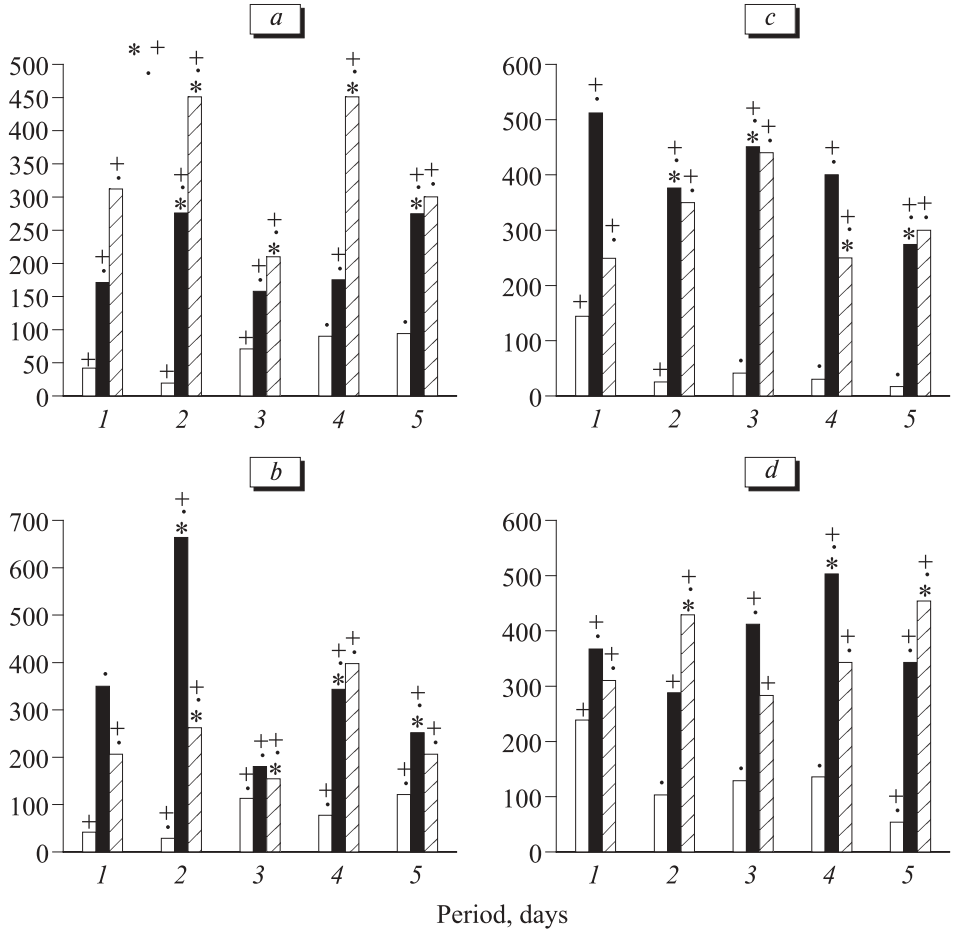

Fig. 3. Number of erythroid CFU in the bone marrow of CBA/CaLac mice on days 5 (*a*, *c*) and 6 (*b*, *d*) after paradoxical sleep deprivation (*a*, *b*) and conflict situation (*c*, *d*). Light bars: neurotic situation (NS). Dark bars: NS and treatment of myelokaryocytes with mesatone. Shaded bars: NS and treatment of myelokaryocytes with orciprenaline sulfate. Administration of physiological saline (*1*), reserpine (*2*), pentamine (*3*), dihydroergotamine (*4*), and propranolol (*5*). $p<0.05$: +compared to the baseline level (100%); °compared to *1*; *compared to NS without treatment of myelokaryocytes.

plastic reconstruction of the erythron in CS and PSD. Erythroid cells receive instructive information from CNS via adrenergic structures on HIM cells and precursors. Administration of sympatholytic, ganglionic blocker, and adrenoceptor antagonist produces the same effect under conditions of CS and PSD. Adrenoceptor agonists abolish suppression of the erythron during sleep disorders. These data attest to the exictence of two different pathways of plastic reconstruction of the hemopoietic system during neuroses, which can be explained with specificity of the adrenergic regulatory mechanisms.

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