GENERAL PATHOLOGY AND PATHOLOGICAL PHYSIOLOGY

Mechanisms Underling the Effects of Adaptogens on Erythropoiesis during Paradoxical Sleep Deprivation

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We studied the effects of adaptogens extracts of Siberian ginseng, *Rhodiola rosea*, bergenia, and ginseng and pantohematogen, on erythropoiesis after paradoxical sleep deprivation. Adaptogens stimulated bone marrow erythropoiesis in the early stage, but decreased the count of bone marrow erythrokaryocytes 3-7 days after treatment. The effect of adaptogens on erythropoiesis is associated with modulation of the state of brain neurotransmitter systems followed by changes in functional activity of cells in the hemopoiesis-inducing microenvironment.

Key Words: *paradoxical sleep deprivation; erythropoiesis; adaptogens; neurotransmitters*

Sleep disorders are the major manifestation of neuroses (depressive state). Paradoxical sleep deprivation (PSD) in animals is the most adequate model for human insomnia [14]. PSD is accompanied by various changes in neurotransmitter metabolism. Excitation of cholinergic structures during sleep disorders contributes to activation of catecholamines and γ-aminobutyric acid [12]. D_2 receptors in the brain are involved in sleep regulation. However, this process primarily depends on the interaction between norepinephrine- and dopaminergic systems [11,13]. Some authors reported that serotoninergic structures play the major role in sleep deprivation [10].

PSD is followed not only by behavioral disorders and disturbances in higher nervous activity [7], but also by profound changes in hemopoiesis [3,9]. Published data show that the extract of Baikal skullcap improves cognitive functions and the state of the blood system during neuroses [2,8].

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Here we studied whether various adaptogens, including extracts of Siberian ginseng, *Rhodiola rosea*, bergenia, and ginseng and pantohematogen, can be used for the correction of changes in erythropoiesis during PSD.

MATERIALS AND METHODS

Experiments were performed on 290 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). PSD for 48 h served as the model of experimental neurosis [14]. Ginseng extract (80 mg/kg, G115, Pharmaton), officinal extracts of *Rhodiola rosea* (1 ml/kg), Siberian ginseng (1 ml/kg), and bergenia (50 mg/kg), and pantohematogen (50 mg/kg, Pantoproekt) were used for the correction of blood changes. These preparations were *ex tempore* dissolved in distilled water and administered through a gastric tube for 5 days before PSD (1 time a day). Control mice received an equivalent volume of distilled water. Blockers of α-adre-

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noceptors (3.9 mg/kg dihydroergotamine), β-adrenoceptors (5 mg/kg propranolol), serotonin receptors (30 mg/kg cyproheptadine), dopamine receptors (3 mg/kg haloperidol), and cholinoceptors (2 mg/kg scopolamine) were administered 5 min before experimental neurosis. The count of peripheral blood reticulocytes was estimated on days 1-7 [5]. The animals were sacrificed by cervical dislocation under ether anesthesia.

We estimated the contents of erythrokaryocytes and colony- (CFU-E) and cluster-forming units of erythropoiesis (ClFU-E) and erythropoietic activity in conditioned slices of adherent and nonadherent cells from the hemopoiesis-inducing microenvironment in the bone marrow [5]. The intensity of hemopoietic precursor differentiation was estimated by the index of maturation [5]. Proliferative activity of precursors was evaluated by the method of cell suicide with hydroxyurea [5]. The integral parameter (IP) characterizing the effects of pharmacological preparations on erythrokaryocyte count was calculated by the formula [4]:

$$IP=\frac{\sum_{i=1}^{n} Mji}{n\times Mj(0)}\times 100\%,$$

where n is the number of series for measurements, Mij is the value of parameter j in series i of measurements, and $Mj(0)$ is the initial value. Numerically, IP is equal to the standardized averaged value of the parameter. The initial value is taken as 100%. The total inhibitory effect of preparations is characterized by IP below 100%. The stimulatory effect corresponds to IP above 100% [4].

The results were analyzed by standard methods of variational statistics. The significance of differences was evaluated by Student's t test and Wilcoxon nonparametric rank test.

RESULTS

PSD suppressed bone marrow erythropoiesis (Figs. 1 and 2). The count of bone marrow erythroid cells decreased on days 1-3, and peripheral blood reticulocytopenia developed on days 1-2 (Fig. 2). We observed a decrease in the counts of bone marrow CFU-E (days 1-3, 5, and 6) and ClFU-E (days 1-3). The number of DNA-synthesizing CFU-E in the bone marrow decreased, while the count of proliferating erythroid clusters increased. The rate of erythroid precursor differentiation significantly decreased on days 1 and 6, but increased on days 3-5 (Fig. 1). After PSD erythropoietic activity increased in nonadherent cells (days 2-7), but decreased in adherent myelokaryocytes (days 1-3 and 5-7, Fig. 1).

Adaptogens abolished suppression of bone marrow erythropoiesis in mice after PSD. The count of bone marrow erythrokaryocytes increased on days 1 and 2 (Fig. 2). It should be emphasized that the extract of Siberian ginseng increased the number of erythrokaryocytes not only in the early, but also in the late stage after treatment (day 4). However, extracts of *Rhodiola rosea*, bergenia, and G115 and pantohematogen inhibited accumulation of erythroid cells in the bone marrow on days 4-7 (Fig. 2).

Adaptogens produced various effects on the count of peripheral blood reticulocytes in mice after PSD. Pantohematogen produced more severe and long-lasting reticulocytopenia than physiological saline (Fig. 2). Treatment with plant preparations was also followed by the development of peripheral blood reticulocytopenia. Extracts of Siberian ginseng, *Rhodiola rosea*, and bergenia produced reticulocytopenia on days 1-3, 1-2, and 1, respectively (Fig. 2). It should be emphasized that plant preparations increased the count of peripheral blood reticulocytes in the late stage after treatment. These changes were observed after treatment with extracts of Siberian ginseng (day 5), G115 and *Rhodiola rosea* (days 4, 5, and 7), and bergenia (days 3-4, Fig. 2).

TABLE 1. IP (% of Initial Value) of Bone Marrow Erytrokaryocytes in CBA/CaLac Mice after Paradoxical Sleep Deprivation

Experiments	Days 1-2	Days 3-7
1st		
Physiological saline	87	107
Haloperidol	140	117
Cyproheptadine	140	138
2nd		
Physiological saline	93	59
Dihydroergotamine	94	199
Propranolol	210	207
3rd		
Physiological saline	80	78
Scopolamine	155	155
4th		
Physiological saline	61	71
Extract of Siberian ginseng	114	52
Pantohematogen	148	39
5th		
Physiological saline	62	92
Extract of *Rhodiola rosea*	90	38
Extract of bergenia	112	28
Extract of ginseng (G115)	109	57

Note. All experiments were in different years in autumn-winter.

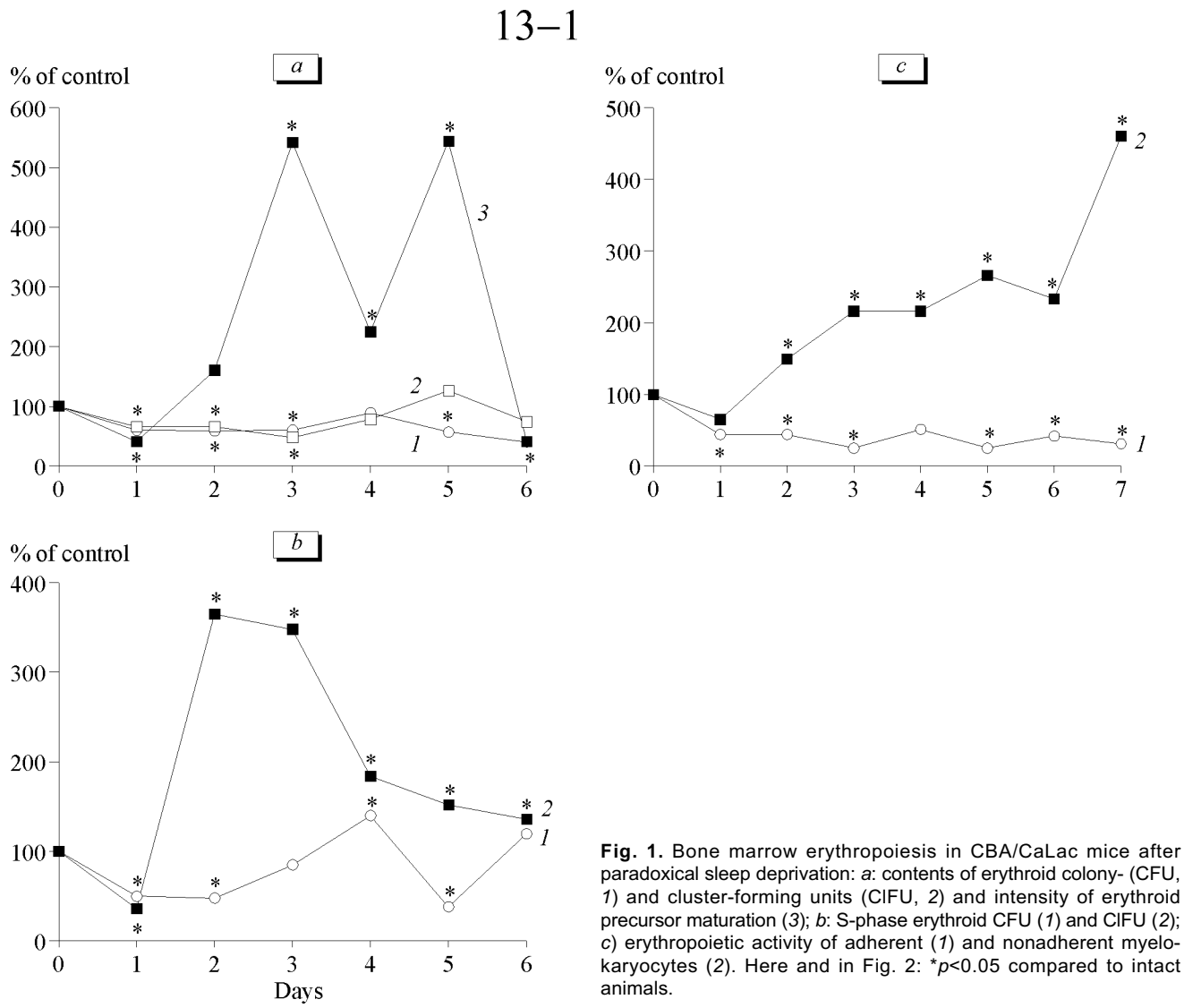

Fig. 1. Bone marrow erythropoiesis in CBA/CaLac mice after paradoxical sleep deprivation: *a*: contents of erythroid colony- (CFU, *1*) and cluster-forming units (ClFU, *2*) and intensity of erythroid precursor maturation (*3*); *b*: S-phase erythroid CFU (*1*) and ClFU (*2*); *c*) erythropoietic activity of adherent (*1*) and nonadherent myelokaryocytes (*2*). Here and in Fig. 2: **p*<0.05 compared to intact animals.

These results indicate that adaptogens abolished suppression of bone marrow erythropoiesis after PSD, but produced peripheral blood reticulocytopenia. In the late stage adaptogens decreased the count of bone marrow erythroid cells (except Siberian ginseng extract) and increased the number of peripheral blood reticulocytes (except pantohematogen).

Published data show that Baikal skullcap extract improves learning and memory under conditions of psychoemotional strain and during disturbances in integrative and mnesic functions produced by various extreme factors [2]. Moreover, the preparation stimulates regeneration of hemopoiesis after cytostatic therapy [2] and normalizes erythro- and granulocytopoiesis during experimental neuroses [2,8]. Pantohematogen stimulates granulocytopoiesis during cytostatic-induced myelosuppression [1]. The extract of Baikal skullcap and pantohematogen normalize hemopoiesis via stimulation of secretory activity of the hemopoiesis-inducing microenvironment. Therefore, the regulatory effect of adaptogens during PSD is associated with their influence on local mechanisms controlling erythropoiesis.

It was shown that the effects of adaptogens are related to modulation of functional activity in the nervous system. We analyzed IP to evaluate the contribution of the central nervous system into adaptogen-produced changes.

We compared IP for erythrokaryocytes after treatment with adaptogens and pharmacological antagonists of neurotransmitter systems under conditions of PSD. Administration of haloperidol, cyproheptadine, propranolol, and scopolamine was accompanied by accumulation of erythrokaryocytes in the bone marrow on days 1-2 (Table 1). Stimulation of bone marrow erythropoiesis by these pharmacological agents was

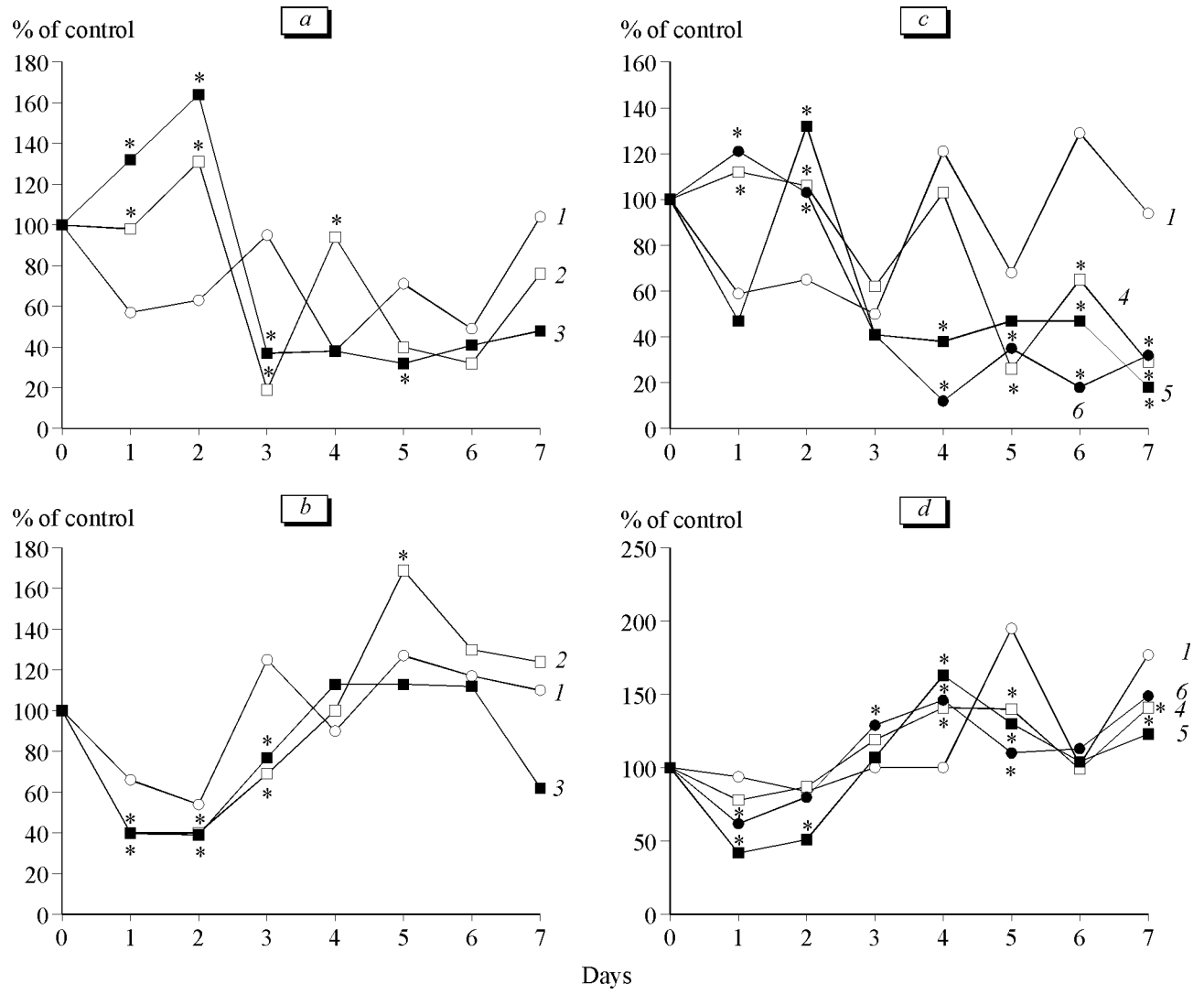

Fig. 2. Contents of bone marrow erythrokaryocytes (*a*, *c*) and peripheral blood reticulocytes (*b*, *d*) in CBA/CaLac mice after paradoxical sleep deprivation and treatment with physiological saline (*1*), Siberian ginseng extract (*2*), pantohematogen (*3*), G115 (*4*), and extracts of *Rhodiola rosea* (*5*) and bergenia (*6*).

also observed in the late stage after treatment (days 3-7). α-Adrenoblocker dihydroergotamine produced less pronounced changes in erythropoiesis, which is consistent with published data that α-adrenergic structures are primarily involved in the regulation of bone marrow granulocytopoiesis [4]. In the early stage after treatment with adaptogens (except of *Rhodiola rosea* extract) under conditions of PSD, IP surpassed 100%. These data indicate that natural preparations stimulate bone marrow erythropoiesis (Table 1). The potency of adaptogens increased in the following order: G115—extract of bergenia—extract of Siberian ginseng—pantohematogen (Table 1). It should be emphasized that IP markedly decreased on days 3-7, which indicates that these preparations produce inhibitory effect at the late stage after treatment (Table 1).

Our previous studies showed that the regulatory effects of brain catecholamines on hemopoiesis during experimental neuroses are realized via the sympathetic part of the autonomic nervous system. Target cells receive information via adrenoceptors on elements of the hemopoiesis-inducing microenvironment [9]. Moreover, erythropoiesis is primarily regulated by serotonin, norepinephrine, and acetylcholine [3,9]. Serotonin plays an important role in the regulation of sleep, aggression, and secretion of neurotransmitters [6,7]. Activation of serotoninergic structures is followed by immune suppression [4] and depression of bone marrow erythropoiesis during sleep disorders [9]. However, the cholinergic system is first activated during PSD. This is followed by activation of adrenergic structures [9]. The data suggest that natural preparations stimulate bone marrow erythropoiesis during PSD via changes in brain neurotransmitter systems.

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