Effects of Adaptogens on Granulocytopoiesis during Paradoxical Sleep Deprivation

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We studied the effects of extracts from Siberian ginseng, *Rhodiola rosea*, bergenia, and ginseng (G115) and pantohematogen on granulocytopoiesis after paradoxical sleep deprivation. The effects of adaptogens on the blood system were most pronounced during hyperplasia of granulocytopoiesis. Natural preparations were divided into groups depending on their activity. Extracts of Siberian ginseng and *Rhodiola rosea* did not modulate granulocytopoiesis. Ginseng G115 extract suppressed granulocytopoiesis. Bergenia extract and pantohematogen produced ambiguous effects on the granulocytic hemopoietic stem.

Key Words: *paradoxical sleep deprivation; granulocytopoiesis; adaptogens*

Insidiousness of neuroses and neurotic disorders associated with their somatization at all levels of various functional systems is a well-established fact [1,6,10]. Pharmacological correction of neuroses and dysadaptation includes tranquilizers, antidepressants, neuroleptics, and nootropic preparations. However, these drugs do not provide complex therapy (*e.g.*, correction of disturbances in higher nervous activity and somatic disorders) and cause various side effects [2,7,8]. The search for new preparations that produce systemic effects, promote rapid adaptation, and do not cause side effects attract much recent attention. According to current views, natural adaptogens possess these properties [12]. Various adaptogens, including extracts of Baikal skullcap, Siberian ginseng, *Rhodiola rosea*, and bergenia, as well as Pantovit, and pantohematogen improve learning and memory. The positive effect of adaptogens on cognitive activity in animals is realized via inhibition of neurotic reactions. It should be emphasized that extract of Baikal skullcap possesses not only nootropic properties during experimental neuroses, but also normalizes blood parameters [11]. Pantohematogen stimulates regeneration of granulomonocytopoiesis under conditions of cytostatic-induced myelosuppression [4].

The aim of the present study was to evaluate the possibility of using plant preparations and pantohematogen for correction of granulocytic hemopoietic stem during experimental neurosis induced by paradoxical sleep deprivation (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 [13]. The extract of ginseng (80 mg/kg, G115, Farmaton), 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 hemopoiesis. These preparations were *ex tempore* dissolved in distilled water and administered to experimental animals for 5 days before PSD (1 time a day through a tube). Control mice received an equivalent volume of distilled water. The count of peripheral blood segmented neutrophils was

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estimated on days 1-7. The animals were sacrificed by cervical dislocation under ether anesthesia. The counts of immature and mature neutrophilic granulocytes in the bone marrow were determined [3]. Cloning of colony- (CFU) and cluster-forming units (ClFU) of granulomonocytopoiesis (GM) was performed by culturing unfractionated bone marrow cells (2×10^5 nuclears/ml) for 7 days in a methylcellulose tissue culture [3]. The intensity of hemopoietic precursor differentiation was estimated by the index of maturation (ratio between the counts of clusters and colonies in the same well). Proliferative activity of precursors was evaluated by the method of cell suicide using hydroxyurea [3]. Colony-stimulating activity in conditioned slices of adherent and nonadherent cells from the hemopoiesis-inducing microenvironment (HIM) was measured in a semisolid culture medium on intact mouse myelokaryocytes [3].

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 activated bone marrow granulocytopoiesis. We observed an increase in the counts of bone marrow CFU-GM (days 1-5), ClFU-GM (days 1, 2, 5, and 6), and immature (days 1 and 2) and mature neutrophilic granulocytes (days 1, 2, and 3) and development of

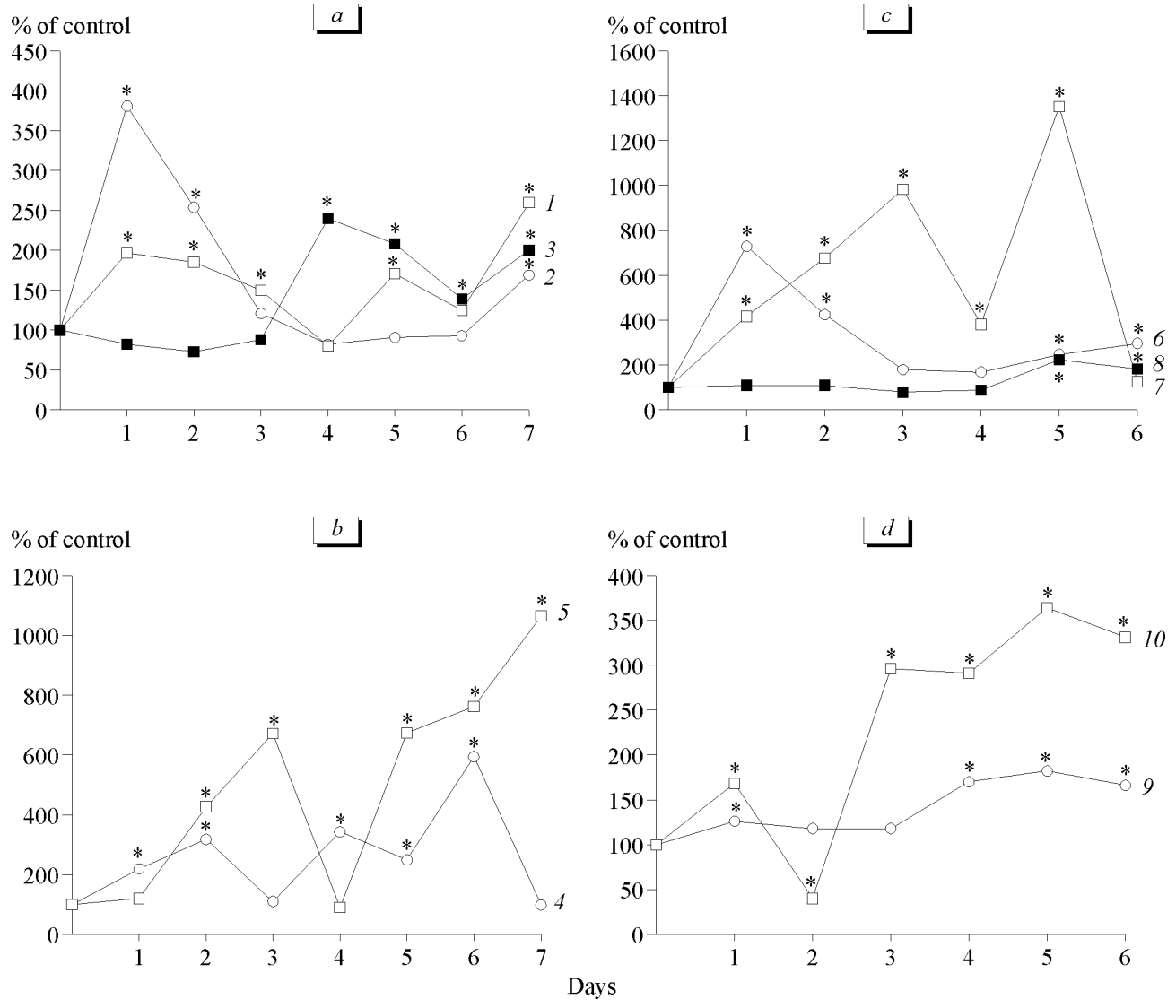

Fig. 1. Effects of paradoxical sleep deprivation on the granulocytic hemopoietic stem in CBA/CaLac mice. *a*) Contents of immature (*1*) and mature (*2*) neutrophilic granulocytes in the bone marrow and segmented neutrophils (*3*) in the peripheral blood (*b*) colony-stimulating activity of adherent (*4*) and nonadherent (*5*) myelokaryocytes; *c*) contents of CFU-GM (*6*) and ClFU-GM (*7*) and intensity of CFU-GM maturation (*8*); *d*) counts of CFU-GM (*9*) and ClFU-GM (*10*) in S-phase of the mitotic cycle. Here and in Fig. 2: **p*<0.05 compared to intact animals.

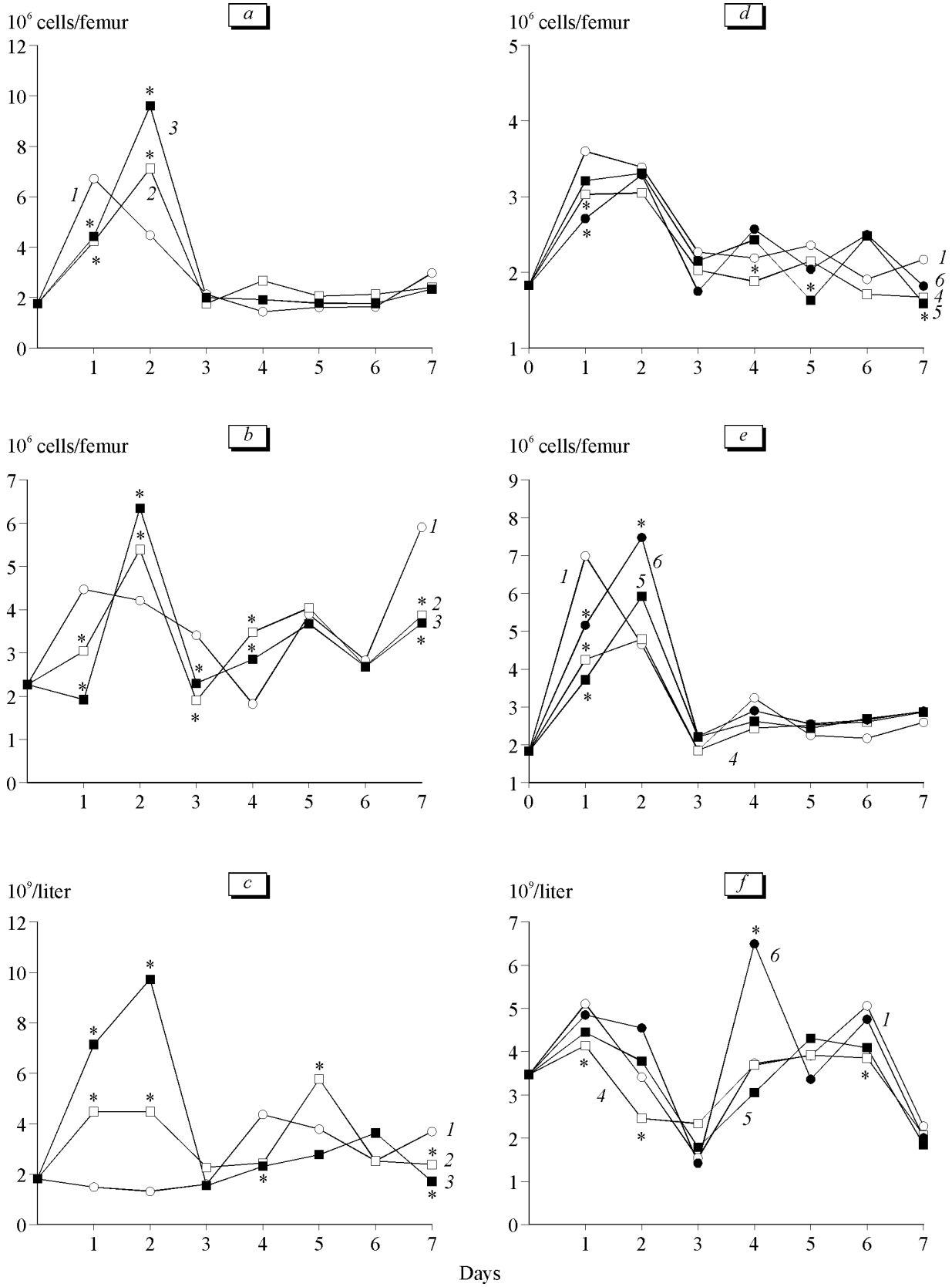

Fig. 2. Contents of immature (*a*, *d*) and mature (*b*, *e*) neutrophilic granulocytes in the bone marrow and segmented neutrophils (*c*, *f*) in the peripheral blood in CBA/CaLac mice after paradoxical sleep deprivation and treatment with physiological saline (*1*), Siberian ginseng extract (*2*),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

neutrophilic leukocytosis in the peripheral blood (days 4-7, Fig. 1, *a*, *c*). Hyperplasia of granulocytopoiesis was associated with changes in proliferation of granulocyte-macrophage precursors. This was confirmed by an increase in the counts of CFU-GM (days 1 and 4-6) and ClFU-GM in S-phase of the mitotic cycle (days 1 and 3-6, Fig. 1, *d*). The intensity of precursor differentiation increased on days 5 and 6 (Fig. 1, *c*). Proliferation and differentiation of precursors are regulated by humoral factors produced by HIM cells [6]. It should be emphasized that PSD was accompanied by a sharp increase in colony-stimulating activity in supernatants of adherent (days 1, 2, and 4-7) and nonadherent myelokaryocytes (days 2, 3, and 5-7, Fig. 1, *b*).

Adaptogens produced different effects on hyperplasia of granulocytopoiesis during PSD. Extracts of *Rhodiola rosea* and Siberian ginseng did not modulate changes in the content of bone marrow neutrophilic granulocytes (Figs. 2, *a*, *b*, *d*, *e*). Siberian ginseng extract stimulated migration of mature neutrophils from the bone marrow into the peripheral blood. The count of peripheral blood segmented neutrophils underwent biphasic changes (*i.e.*, increase on days 1-2 and 5, Fig. 2, *c*). G115 decreased the number of immature (days 1, 4, and 7) and mature neutrophilic granulocytes (day 1) in the bone marrow (Figs. 2, *d*, *e*). The count of peripheral blood neutrophils decreased on days 1, 2, and 6 (Fig. 2, *f*).

Pantohematogen and bergenia extract produced ambiguous effects on granulocytopoiesis. Bergenia extract markedly decreased the count of immature neutrophilic granulocytes, but increased the number of mature neutrophilic granulocytes in the bone marrow (days 1 and 2, respectively, Fig. 2, *d*, *e*). Pantohematogen promoted accumulation of immature neutrophils (day 2, Fig. 2, *a*), but decreased the count of mature neutrophilic granulocytes (days 1, 3, and 7, Fig. 2, *b*). Bergenia extract and pantohematogen increased the count of peripheral blood neutrophils on days 4 (Fig. 2, *f*) and 1-2 (Fig. 2, *c*), respectively.

Therefore, adaptogens were divided into 3 groups depending on their effects on the granulocytic hemopoietic stem under conditions of PSD. There were preparations that inhibited (G115), did not modulate (extracts of Siberian ginseng and *Rhodiola rosea*), or produced ambiguous effects on granulocytopoiesis (extract of bergenia and pantohematogen).

The effects of adaptogens can be mediated by several mechanisms. The normalizing effect of Baikal skullcap extract on hemopoiesis during experimental neuroses is associated with its direct influence on committed precursors and inhibition of secretory activity in HIM cells [11]. Pantohematogen normalizes proliferation and differentiation of granulomonocytic precursors during cytostatic-induced myelosuppression and stimulates secretory activity of hemopoietic microenvironment [4]. These data suggest that various effects of adaptogens on hemopoiesis during PSD are associated with different changes in local mechanisms regulating granulocytopoiesis.

Previous studies showed that catecholamines and acetylcholine play an important role in adaptive reconstruction of the hemopoietic tissue during PSD [5,7, 9,10]. Extracts of Baikal skullcap, Siberian ginseng, *Rhodiola rosea*, bergenia, and G115, Pantovit, and pantohematogen possess psychotropic activity. These data indicate that the regulatory effects of these preparations on granulomonocytopoiesis are realized via neurotransmitter systems.

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