Possible Mechanisms Underlying the Effect of Natural Preparations on Erythropoiesis Under Conditions of Conflict Situation

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We studied the effects of various natural preparations, including extracts of Siberian ginseng, *Rhodiola rosea*, bergenia, and ginseng and pantohematogen on erythropoiesis under conditions of conflict situation. The test preparations were divided into 2 groups depending on their modulatory effect on intensified erythropoiesis under conditions of conflict situation. Some of them reduced (extracts of ginseng, bergenia, and *Rhodiola rosea*), while others increased the degree of hyperplasia in the erythropoietic stem (extract of Siberian ginseng and pantohematogen). The regulatory effect of preparations depended on activity of the corresponding neurotransmitter systems in the brain and local regulatory mechanisms of hemopoiesis.

Key Words: *conflict situation; erythropoiesis; natural preparations; neurotransmitters*

Neurotic disorders are polyetiological diseases with various somatic manifestations [1]. Our previous studies showed that the adaptive response of the blood system and reactivity of the neurotransmitter systems in the brain differ in two models of experimental neuroses (EN, conflict situation and paradoxical sleep deprivation) [5]. Extracts of Siberian ginseng, *Rhodiola rosea*, bergenia, and ginseng and pantohematogen normalize erythropoiesis suppressed during paradoxical sleep deprivation. Here we studied whether these preparations can be used for the correction of changes in erythropoiesis during the conflict situation. The mechanisms underlying the influence of these preparations were evaluated.

MATERIALS AND METHODS

Experiments were performed on 290 CBA/CaLac mice (class I conventional mouse strain) aging 2-2.5 months and obtained from the collection of the Laboratory of Experimental Biological Modeling (Institute of Pharmacology, Tomsk Research Center). Conflict situation (10 min) served as a model of EN [8]. The extract of ginseng (80 mg/kg, G115, Phermaton), officinal *Rhodiola rosea* extract (1 ml/kg), Siberian ginseng extract (1 ml/kg), bergenia extract (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 intragastrically through a tube for 5 days before EN (1 time a day). Control mice received an equivalent volume of the solvent. The α-adrenoceptor antagonist dihydroergotamine (3.9 mg/kg), β-adrenoceptor antagonist propranolol (5 mg/kg), sympatholytic reserpine (2 mg/kg), neuroleptic haloperidol (3 mg/kg), antiserotonin preparation cyproheptadine (30 μg/kg), muscarinic receptor antagonist scopolamine (2 mg/kg), GABAergic system stimulator piracetam (400 mg/kg), and ganglioblocker pentamine (6 mg/kg) served as neurotransmitters. The test preparations were administered 2 times 5 min before and 5-6 h after EN.

The count of peripheral blood reticulocytes was estimated on days 1-7 [7]. The mice were euthanized

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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 media of adherent and nonadherent cells from the hemopoiesis-inducing microenvironment in the bone marrow [7]. The intensity of hemopoietic precursor differentiation was estimated by the index of maturation [7]. Proliferative activity of precursors was evaluated by the method of cell self-destruction with hydroxyurea [7]. The integral parameter (IP) characterizing the effect of pharmacological preparations on the counts of erythrokaryocytes and reticulocytes on days 1-2 (IP_{1-2}) and 3-7 (IP_{3-7}) was calculated by the formula [6]:

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

where n is the number of measurements, Mij is parameter j in period i of measurements, and $Mj(0)$ is the initial value. Numerically, IP is the standardized mean value. The initial value is taken as 100%. The general inhibitory effect of preparations is characterized by IP of less than 100%. The stimulatory effect corresponds to IP of more than 100% [6].

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

The count of bone marrow erythroid precursors CFU-E and ClFU-E significantly increased on days 2-5 and 1-5 of conflict, respectively (Fig. 1). Proliferative activity erythroid precursors in mice increased at these terms. The index of maturation for erythroid precursors increased on days 1, 2, 4, and 5. Adherent and nonadherent cells of the hemopoietic microenvironment intensively secreted humoral factors regulating erythropoiesis (Fig. 1). These results indicate that hyperplasia of the bone marrow erythropoiesis under conditions of conflict situation is associated with activa-

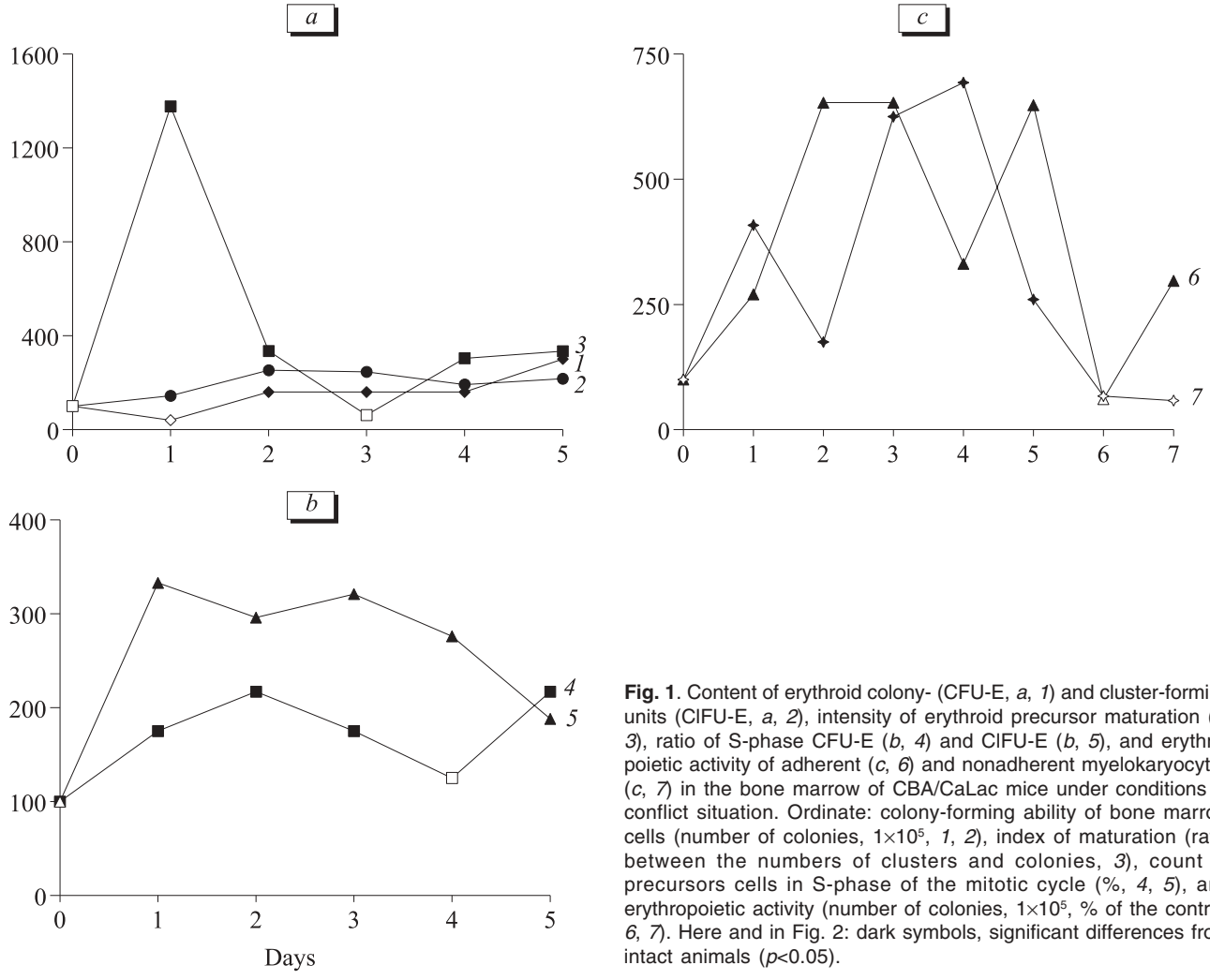

Fig. 1. Content of erythroid colony- (CFU-E, *a*, *1*) and cluster-forming units (ClFU-E, *a*, *2*), intensity of erythroid precursor maturation (*a*, *3*), ratio of S-phase CFU-E (*b*, *4*) and ClFU-E (*b*, *5*), and erythropoietic activity of adherent (*c*, *6*) and nonadherent myelokaryocytes (*c*, *7*) in the bone marrow of CBA/CaLac mice under conditions of conflict situation. Ordinate: colony-forming ability of bone marrow cells (number of colonies, 1×10^5, *1*, *2*), index of maturation (ratio between the numbers of clusters and colonies, *3*), count of precursors cells in S-phase of the mitotic cycle (%, *4*, *5*), and erythropoietic activity (number of colonies, 1×10^5, % of the control, *6*, *7*). Here and in Fig. 2: dark symbols, significant differences from intact animals ($p<0.05$).

tion of proliferation and differentiation of hemopoietic precursors due to the increase in secretory activity of cells in the hemopoiesis-inducing microenvironment.

Treatment with ginseng, bergenia, and *Rhodiola rosea* extracts under conditions of conflict situation significantly decreased the count of bone marrow erythrokaryocytes on days 2, 2 and 4, and 1-2, respectively (Fig. 2, *c*). These preparations decreased the number of peripheral blood reticulocytes on days 3, 2-4, and 1 and 5, respectively (Fig. 2, *d*). It should be emphasized that *Rhodiola rosea* extract increased the count of erythroid cells in the bone marrow (day 5) and peripheral blood (days 1 and 6, Fig. 2, *c*, *d*).

Siberian ginseng extract and pantohematogen promoted accumulation of erythrokaryocytes on days 2 and 3 after treatment. At later stages the number of bone marrow erythroid cells decreased in animals receiving Siberian ginseng extract (days 5 and 7) and pantohematogen (day 4, Fig. 2, *a*). The count of peripheral blood reticulocytes decreased under conditions of conflict situation after administration of the Siberian ginseng extract (days 2-6) and pantohematogen (days 5 and 6, Fig. 2, *b*).

Our results indicate that natural preparations were potent in modulating erythropoiesis activated under conditions of conflict situation. By the influence on the erythropoietic stem, the test preparations were divided into 2 groups. Some of them suppressed (ginseng, bergenia, and *Rhodiola rosea* extracts), while others stimulated the erythropoietic stem (Siberian ginseng extract and pantohematogen).

Previous studied showed that Baikal skullcap stimulates regeneration of hemopoiesis during cytostatic-induced myelosuppression and normalizes erythro-

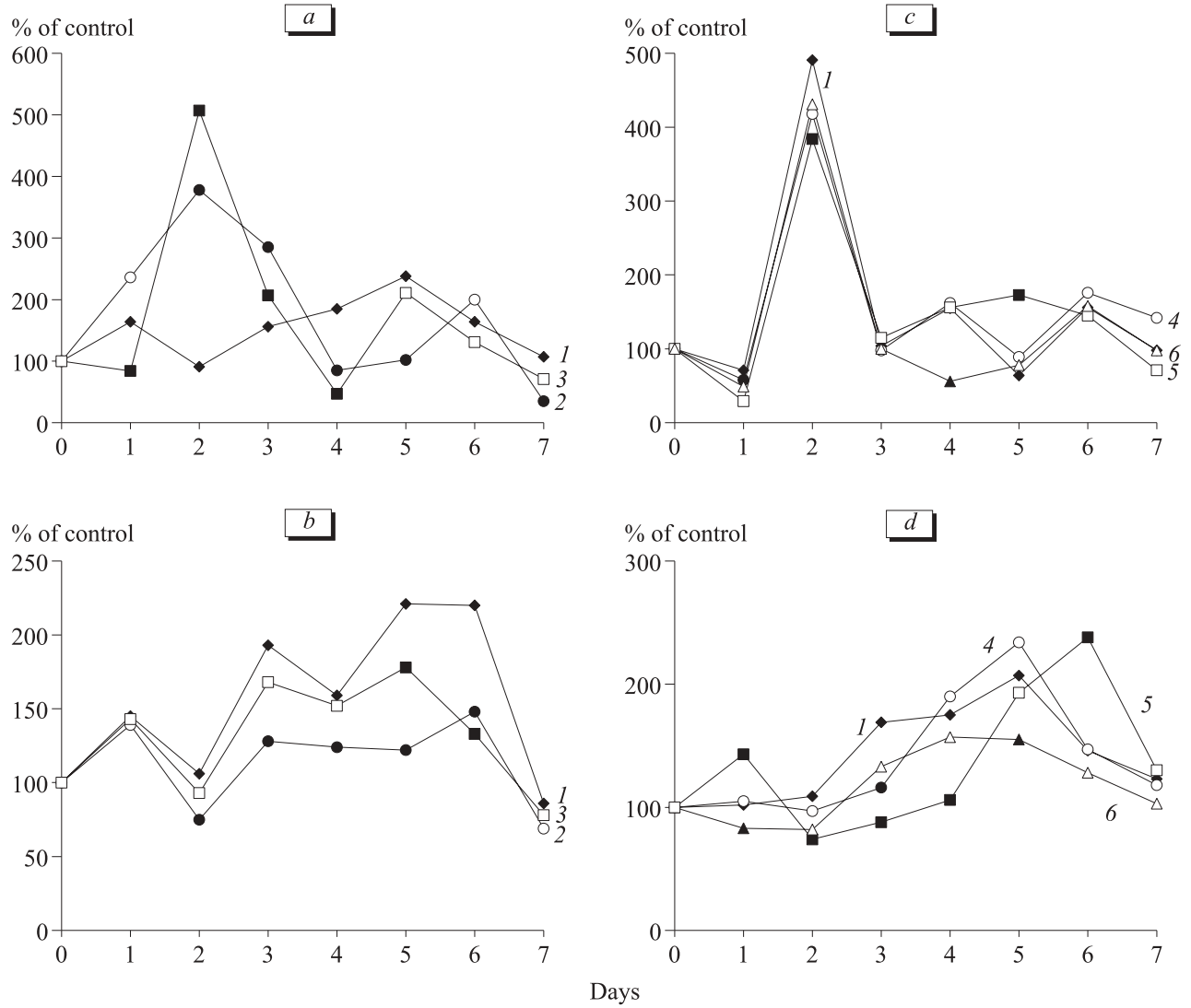

Fig. 2. Contents of bone marrow erythrokaryocytes (*a*, *c*) and peripheral blood reticulocytes (*b*, *d*) in CBA/CaLac mice receiving physiological saline (*1*), Siberian ginseng extract (*2*), pantohematogen (*3*), G115 (*4*), and extracts of *Rhodiola rosea* (*5*) and bergenia (*6*) under conditions of conflict situation. Ordinate: count (% of the control) of bone marrow erythrokaryocytes (*a*, *c*) and peripheral blood reticulocytes (*b*, *d*).

TABLE 1. Integral Parameter Characterizing the Effect of Pharmacological Preparations on Erythroid Cell Count in CBA/CaLac Mice under Conditions of Conflict Situation (% of Initial Value)

Series		IP_{1-2}		IP_{3-7}	
		erythrokaryocytes	reticulocytes	erythrokaryocytes	reticulocytes
1	physiological saline	181	203	179	388
	dihydroergotamine	103	95	97	148
	propranolol	86	89	105	123
2	physiological saline	167	81	149	133
	reserpine	40	85	44	101
3	physiological saline	150	106	149	148
	pentamine	114	132	164	176
4	physiological saline	195	116	310	126
	scopolamine	58	119	174	126
5	physiological saline	147	101	97	133
	haloperidol	169	72	79	151
	cyproheptadine	101	46	81	136
	piracetam	137	112	92	117
6	physiological saline	281	105	115	164
	ginseng extract	238	100	133	161
	Rhodiola rosea extract	206	109	132	151
	bergenia extract	240	82	67	135
7	physiological saline	127	126	170	176
	Siberian ginseng extract	307	107	141	119
	pantohematogen	295	118	133	142

poiesis and granulocytopoiesis in EN [4]. Experimental and clinical observations indicate that pantohematogen promotes the recovery of hemopoiesis suppressed by cytostatic treatment [2]. These preparations stimulate regeneration of hemopoiesis by increasing secretory activity of cells in the hemopoiesis-inducing microenvironment. Probably, the regulatory effect of natural preparations under conditions of conflict situation is mediated by their influence on local mechanisms of erythropoiesis.

According to current concept, the hemopoiesis-inducing microenvironment includes not only cells and their products, but also nerve fibers [3]. Receptors for norepinephrine, acetylcholine, and other neurotransmitters were found on committed precursor cells [3,6]. We evaluated the role of CNS in the realization of changes produced by natural preparations. Pharmacological preparations modulated erythropoiesis that was activated in the early post-conflict period (days 1-2). It should be emphasized that propranolol (86 *vs*. 181% in the control, *n*=2), reserpine (40 *vs*. 167% in the control, *n*=2), and scopolamine (58 *vs*. 195% in the control, *n*=2) markedly suppressed the erythropoietic stem of hemopoiesis. However, dihydroergotamine (103 *vs*. 181% in the control, *n*=2), pentamine (114 *vs*. 150% in the control, *n*=2), and cyproheptadine (101 *vs*. 147% in the control, *n*=2) only slightly reduced the degree of erythroid hyperplasia (Table 1).

We compared IP in mice receiving neurotransmitters and natural preparations. Reduction of erythroid hyperplasia produced by ginseng, *Rhodiola rosea*, and bergenia extracts was similar to that observed after treatment with dihydroergotamine, pentamine, and cyproheptadine. It should be noted that the stimulatory effect of pantohematogen and Siberian ginseng extract did not differ from that of haloperidol (Table 1). It can be hypothesized that the regulatory effect of group 1 preparations is related to changes in activity of noradrenergic and serotoninergic systems. Our results agree with published data that the regulation of erythropoiesis primarily depends on activity of these transmitters [5]. Group 2 preparations probably modulate activity of the dopaminergic system and, therefore, stimulate erythropoiesis under conditions of conflict situation.

Studies performed in the 1980s showed that cerebral hypoxia plays a role in the development of terminal mental disorders [9]. Preparations with antihypoxic properties were proposed to be used for the therapy of patients with neuroses (*e.g.*, piracetam, Aminalon, and vitamin E). Extract of Baikal skullcap pos-

sesses antihypoxic properties and counteracts deenergization of brain mitochondria and cascade pathological reactions due to optimization of functional activity in the respiratory chain. This substance improved the state of hemopoiesis disturbed in EN. The extract of bergenia leaves normalized energy metabolism in the brain under hypoxic conditions. Probably, the regulation of hemopoiesis by plant preparations during EN depends on their direct membranotropic and cerebroprotective effects.

Our findings indicate that neurotransmitters systems of the brain and local mechanisms regulating hemopoiesis play an important role in the modulatory effect of natural preparations on erythropoiesis during neuroses.

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