GENERAL PATHOLOGY AND PATHOPHYSIOLOGY

Monoaminergic Regulation of Proliferation and Differentiation of Granulomonocytopoietic Precursors during Neuroses

E. G. Skurikhin, O. V. Pershina, M. Yu. Minakova, A. M. Dygai, and E. D. Gol'dberg

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 141, No. 6, pp. 616-621, June, 2006
Original article submitted March 1, 2006

We studied the role of monoamines in the regulation of functional activity of granulocyte-macrophage precursors during neuroses. Under conditions of conflict situation and paradoxical sleep deprivation, monoamines of the central nervous system regulate proliferation and differentiation via α-adrenergic structures on granulomonocytopoietic precursors and cells of the hemopoiesis-inducing microenvironment.

Key Words: *granulocyte-macrophage precursors; hemopoiesis-inducing microenvironment; monoamines*

Monoamines regulate activity of erythroid precursors under conditions of experimental neuroses [7]. The regulatory influence of monoaminergic systems is realized via adrenergic and erythropoietin-sensitive receptors on erythroid precursors and cells of the hemopoiesis-inducing microenvironment. The granulocytic hemopoietic stem is also involved in the compensatory and adaptive response in a conflict situation and during paradoxical sleep deprivation [1]. However, the monoaminergic regulation of proliferation and differentiation of granulomonocytopoietic precursors during neuroses is poorly understood.

Here we studied the role of monoamines in the regulation of functional activity of granulocyte-macrophage precursors during neuroses.

MATERIALS AND METHODS

Experiments were performed on 520 male CBA/CaLac mice (class I conventional mouse strain) aging 2-2.5 months and obtained from the collection of the Laboratory for Experimental Biological Modeling (Institute of Pharmacology, Tomsk Research Center). Conflict situation (10 min) [1,5] and paradoxical sleep deprivation (48 h) [1,6,9] served as the models of experimental neurosis. The animals were anesthetized with ether and euthanized by cervical dislocation on days 1, 2, 3, 4, 5, and 6 after neurosis modeling. The number of granulocyte-macrophage colony-forming (CFU-GM) and cluster-forming units (ClFU-GM) in the bone marrow was estimated by *in vitro* cloning of myelokaryocytes in a methylcellulose culture medium [3]. Proliferative activity of granulocytic precursors was assayed by the method of hydroxyurea cell suicide. The intensity of cell differentiation was determined by the index of maturation (cluster/colony ratio in a well) [3]. Colony-stimulating activity of conditioned media from adherent and nonadherent cells of the hemopoiesis-inducing microenvironment was studied on intact mouse myelokaryocytes [3]. Sympatholytic agent reserpine (Polfa) in a single dose of 2 mg/kg was injected intraperitoneally 5-7 min before neurosis. The final concentration of α-adre-

Institute of Pharmacology, Tomsk Research Center, Siberian Division of the Russian Academy of Medical Sciences

0007-4888/06/1416-0669

nergic agonist mesatone (State Research Center of Medicines, Kharkov) in the culture medium was 10^{-8} M. The final concentration of recombinant granulocyte colony-stimulating factor (CSF, Sigma) was 5 ng/ml.

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

The number of neutrophilic granulocytes in the bone marrow initially decreased (day 1), but then increased (days 4-5) under conditions of conflict situation. Neutrophilic leukocytosis in the peripheral blood was observed in different periods of the study. The number of neutrophilic leukocytes in the peripheral blood reached maximum on days 4-6 (Table 1). However, the number of cells in the granulocytic hemopoietic stem increased on days 1 and 2 after paradoxical sleep deprivation.

Therefore, experimental neuroses are accompanied by hyperplasia of bone marrow granulomonocytopoiesis. These changes are most pronounced in conflict situation. In animals exposed to conflict situation, the decrease in the number of neutrophilic granulocytes in the bone marrow and development of neutrophilic leukocytosis in the peripheral blood contribute to cell redistribution.

Granulocyte CSF is the major regulator of granulocytopoiesis in the organism [8]. This cytokine plays a role in the formation of granulocyte colonies *in vitro* [10]. However, granulocytic precursors serve as the target for catecholamines [2,4]. It is important to study the role of adrenergic structures in proliferation and differentiation of granulocyte-macrophage precursors during neuroses.

Conflict situation increased the number of CFU-GM (day 1) and ClFU-GM in the bone marrow (days 1, 4, and 5). Acceleration of division and maturation of granulocyte-macrophage precursors was observed on days 1, 2, 4, and 5 (Fig. 1). Under these conditions α-adrenoceptor agonist mesatone *in vitro* increased the number of granulomonocytopoietic precursors in a methylcellulose culture medium (CFU-GM, days 1 and 4; ClFU-GM, days 1, 4, and 5). We revealed an increase in proliferative activity of CFU-GM (days 4 and 5) and ClFU-GM (days 1, 2, 4, and 5) and acceleration of precursor differentiation (days 1, 2, 4, and 5).

Paradoxical sleep deprivation increased the number of CFU-GM (days 2 and 5) and ClFU-GM (days 1 and 2). The intensity of CFU-GM division increased on days 1, 2, and 5. Maturation of precursors increased on days 1, 2, and 5. α-Adrenoceptor agonist *in vitro* increased the number of CFU-GM (day 2) and ClFU-GM in a methylcellulose culture medium (days 2 and 4, Fig. 2). Activation of ClFU-GM proliferation (days 4 and 5) and granulocyte-macrophage precursor differentiation (days 1 and

TABLE 1. Effect of Reserpine on the Number of Neutrophilic Granulocytes in the Bone Marrow ($\times 10^6$ cells per femur) in CBA/CaLac Mice with Neuroses ($X \pm m$)

Period, days	*In vivo* agent	Conflict situation		Paradoxical sleep deprivation	
		immature neutrophilic granulocytes	mature neutrophilic granulocytes	immature neutrophilic granulocytes	mature neutrophilic granulocytes
Intact control	Physiological saline (n=7)	1.49±0.16	5.99±0.57	1.49±0.16	5.99±0.57
1	Physiological saline (n=7)	0.77±0.07*	3.08±0.31*	2.04±0.18*	5.07±0.59
	Reserpine (n=7)	1.03±0.09*+	3.48±0.33 *	1.75±0.16	5.66±0.58
2	Physiological saline (n=7)	1.12±0.13	4.92±0.45	1.37±0.14	7.81±0.66*
	Reserpine (n=7)	1.05±0.09*	3.59±0.36*+	1.89±0.17	5.54±0.53+
3	Physiological saline (n=7)	1.43±0.15	4.81±0.44	1.33±0.12	6.23±0.61
4	Physiological saline (n=7)	1.92±0.18*	7.86±0.79*	1.24±0.13	5.84±0.59
	Reserpine (n=7)	1.42±0.13+	6.03±0.58+	1.59±0.16	5.42±0.55
5	Physiological saline (n=7)	1.48±0.15	7.44±0.61*	1.47±0.14	5.3±0.49
	Reserpine (n=7)	0.99±0.08*+	4.2±0.4+	0.82±0.08*+	3.35±0.32*+
6	Physiological saline (n=7)	1.37±0.14	5.91±0.61	1.49±0.16	6.23±0.57

Note. n, number of animals. $p<0.05$: *compared to intact control animals; +compared to animals not receiving reserpine (immediately after neurotizing exposure).

4) was accompanied by a decrease in the number of S-phase CFU-GM (day 2).

Peripheral α-adrenergic structures on granulocyte-macrophage precursors are involved in the regulation of cell proliferation and differentiation during experimental neuroses. Mesatone *in vitro* stimulates division and maturation of CFU-GM and ClFU-GM, which contributes to enhanced production of mature neutrophils in hemopoietic tissue under these conditions.

Monoamines of the central nervous system (CNS) determine the number of neutrophils in hemopoietic tissue during neuroses [1]. We studied the mechanisms for monoaminergic regulation of functional activity of granulomonocytopoietic precursors.

Catecholamine depletion in CNS with reserpine abolished hyperplasia of bone marrow granulocytopoiesis on days 4 and 5 after conflict situation (Table 1). Sympatholytic treatment suppressed the growth of CFU-GM and ClFU-GM in methylcellulose culture medium 1 day after conflict situation. The number of precursor cells increased on days 4 and 5 (Fig. 1). Activation of precursor proliferation and differentiation was less pronounced or delayed under these conditions. After *in vitro* mesatone treatment, reserpine suppressed the growth of granulomonocytopoietic precursors and inhibited division of these cells. The rate of cell maturation decreased on day 2, but increased on days 4 and 5.

Sympatholytic treatment suppressed bone marrow granulocytopoiesis on day 5 after paradoxical sleep deprivation (Table 1). Suppression of CFU-GM and ClFU-GM growth in the bone marrow culture was accompanied by a decrease in functional activity of granulocyte-macrophage precursors. We observed a decrease in proliferation of CFU-GM (days 1 and 2) and ClFU-GM (days 5) and inhibition of cell differentiation (day 2, Fig. 2). α-Adrenergic stimulation with mesatone had different effects on granulomonocytopoietic precursors. This treatment was accompanied by suppression of colony formation and cluster formation (days 2 and 4, respectively), decrease in proliferative activity of CFU-GM and ClFU-GM (days 5 and 4, respectively), and inhibition of precursor differentiation (day 4). However, on days 1 and 2 after treatment the number of ClFU-GM and rate of precursor cell division and maturation were higher compared to the control.

Our results indicate that under conditions of neuroses, monoamine of CNS regulate proliferation and differentiation of granulocyte-macrophage precursors via α-adrenergic structures. However, *in vitro* experiments with mesatone showed that function of precursor cells increases in various periods after reserpine treatment. These data seem to contradict the hypothesis that sympatholytic agent decreases the number of neutrophilic granulocytes in the bone marrow. Probably, the mechanisms of local regulation of granulocytopoiesis are modified under these conditions. Adrenoceptor antagonists and agonists modulate production of colony-stimulating activity by cells of the hemopoietic microenvironment [1]. The regulation of erythroid precursors by monoamines is realized via α-adrenoceptors on cells of the hemopoiesis-inducing microenvironment [7]. We studied whether reserpine can affect granulocyte-macrophage colony formation by modulating secretion of colony-stimulating activity in myelokaryocytes.

Sympatholytic agent abolished the increase in the production of colony-stimulating activity by adherent (days 4 and 5) and nonadherent cells of the hemopoiesis-inducing microenvironment during conflict situation (days 1, 2, and 5; Fig. 1). Under conditions of paradoxical sleep deprivation, this agent inhibited secretory activity of nucleated cells in the adherent (days 2 and 5) and nonadherent bone marrow fraction (day 4, Fig. 2). However, media conditioned by nonadherent myelokaryocytes obtained on day 1 of paradoxical sleep deprivation stimulated granulocyte-macrophage colony formation.

Hence, the inhibitory effect of reserpine on the granulocytic hemopoietic stem during neuroses results from the decrease in functional activity of cells in the hemopoiesis-inducing microenvironment.

We conclude that under conditions of experimental neuroses the state of granulomonocytopoietic precursors and secretory activity of cells in the hemopoiesis-inducing microenvironment are regulated by monoamines of CNS. Instructive information from CNS is transmitted via α-adrenergic structures on cells of the hemopoietic microenvironment and granulocyte-macrophage precursors. Probably, the role of distant mechanisms in the regulation of the blood system under these experimental conditions is not limited to the combined effect of norepinephrine, dopamine, and serotonin. For example, dopamine, serotonin, and norepinephrine may exert the effector effect on hemopoietic precursors, stromal cells of the bone marrow, and nutrient blood vessels via specific receptors on the plasma membrane [1,4]. Neurotransmitter systems modulate the balance between hemopoietic microenvironmental cells and functional activity of cells (secretion of hemopoietic stimulators and inhibitors, cell-cell interaction, *etc*.). These specific features contribute to the increase in the production of colony-stimulating activity by nonadherent myelo-

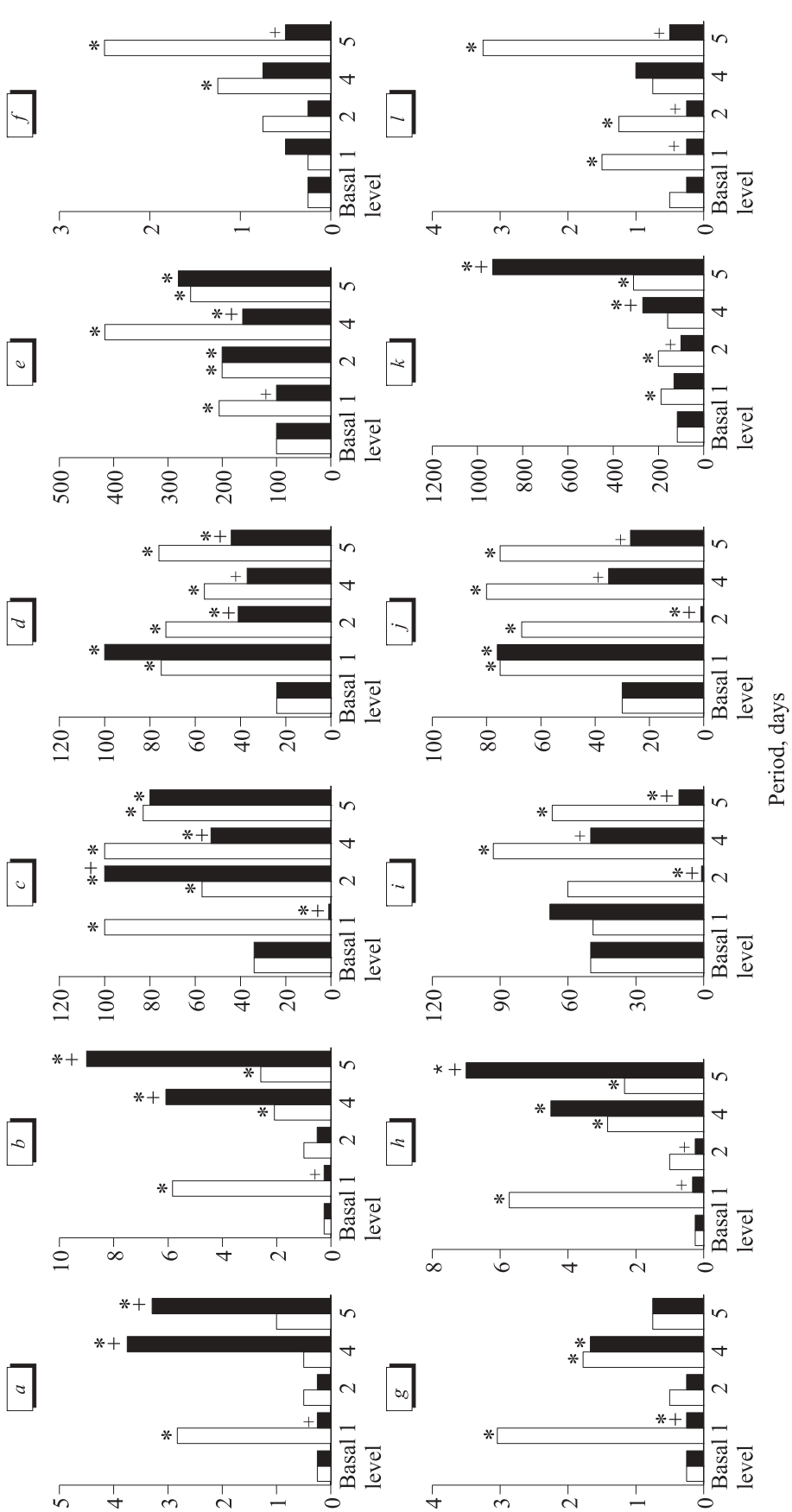

Fig. 1. Number of CFU-GM (*a*, *g*) and CIFU-GM (*b*, *h*), ratio of CFU-GM (*c*, *i*) and CIFU-GM (*d*, *j*) in S-phase of the mitotic cycle, maturation of granulocyte-macrophage precursors (*e*, *k*), and colony-stimulating activity in supernatants of adherent (*f*) and nonadherent myelokaryocytes (*l*) from the bone marrow of CBA/CaLac mice after conflict situation. *In vitro* growth factors: granulocyte CSF (*a*, *b*, *c*, *d*, *e*) and mesatone (*g*, *h*, *i*, *j*, *k*). Ordinate: number of granulocyte-macrophage precursors in the bone marrow ($\times 10^5$ cells; *a*, *b*, *g*, *h*, *f*, *l*), ratio of precursors in S-phase of the mitotic cycle (%; *c*, *d*, *i*, *j*), and maturation index (cluster/colony ratio in a well, %; *e*, *k*). Light bars: administration of physiological saline during conflict situation. Dark bars: administration of reserpine during conflict situation. $p<0.05$: *compared to intact animals; +significant differences between animals receiving *in vivo* reserpine and physiological saline during conflict situation.

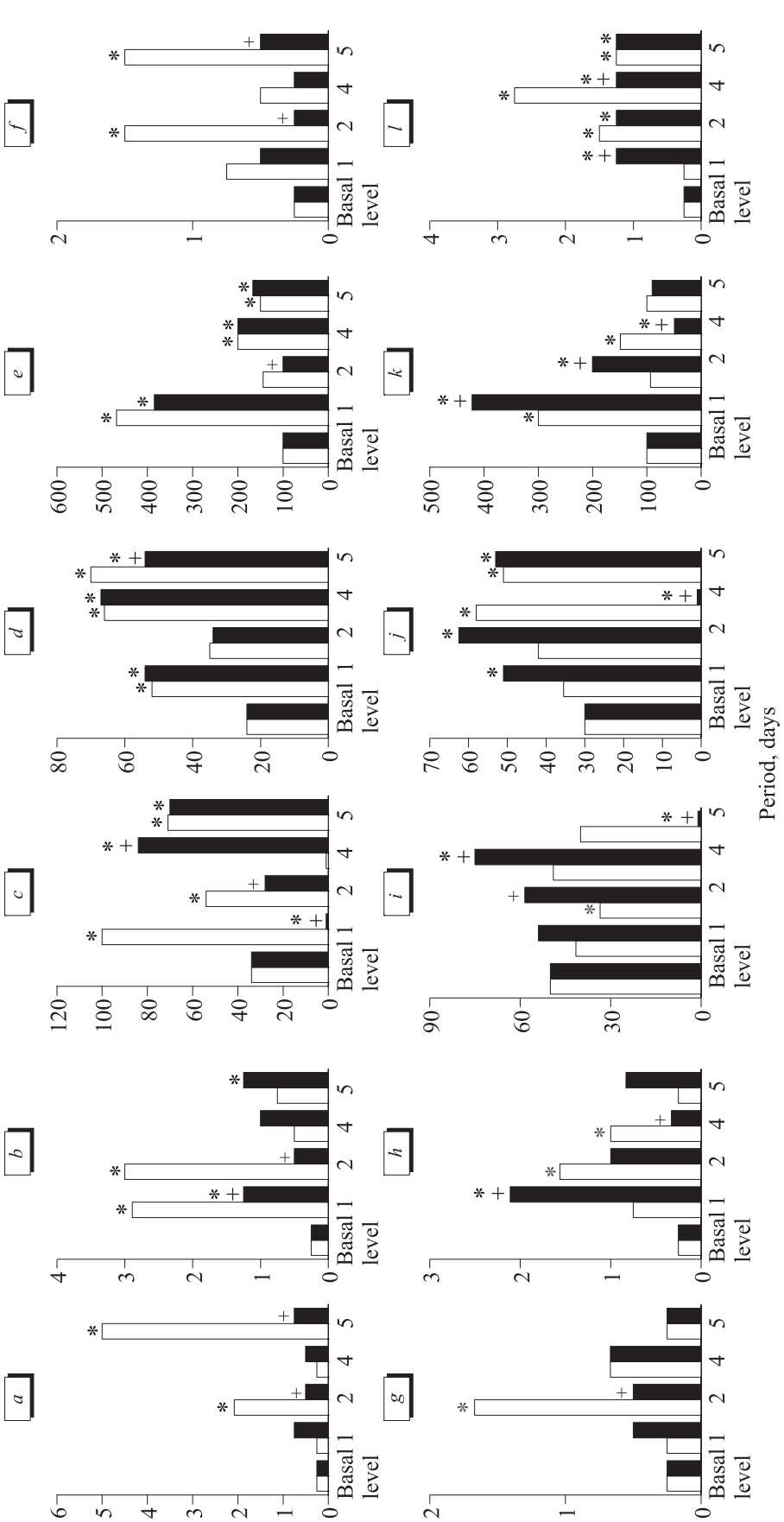

Fig. 2. Number of CFU-GM (*a*, *g*) and CIFU-GM (*b*, *h*), ratio of CFU-GM (*c*, *i*) and CIFU-GM (*d*, *j*) in S-phase of the mitotic cycle, maturation of granulocyte-macrophage precursors (*e*, *k*), and colony-stimulating activity in supernatants of adherent (*f*) and nonadherent myelokaryocytes (*l*) from the bone marrow of CBA/CaLac mice after paradoxical sleep deprivation. *In vitro* growth factors: granulocyte CSF (*a*, *b*, *c*, *d*, *e*) and mesatone (*g*, *h*, *i*, *j*, *k*). Ordinate: number of granulocyte-macrophage precursors in the bone marrow ($\times 10^5$ cells; *a*, *b*, *g*, *h*, *f*, *l*), ratio of precursors in S-phase of the mitotic cycle (%; *c*, *d*, *i*, *j*), and maturation index (cluster/colony ratio in a well, %; *e*, *k*). Light bars: administration of physiological saline during paradoxical sleep deprivation. Dark bars: administration of reserpine during paradoxical sleep deprivation. $p<0.05$: *compared to intact animals; +significant differences between animals receiving *in vivo* reserpine and physiological saline during paradoxical sleep deprivation.

karyocytes under conditions of reserpine treatment during paradoxical sleep deprivation.

REFERENCES

1. E. D. Gol'dberg, A. M. Dygai, N. V. Provalova, *et al.*, *Role of the Nervous System in Regulation of Hemopoiesis* [in Russian], Tomsk (2004).
2. E. D. Gol'berg, A. M. Dygai, and I. A. Khlusov, *Role of the Autonomic Nervous System in Regulation of Hemopoiesis* [in Russian], Tomsk (1997).
3. E. D. Gol'berg, A. M. Dygai, and V. P. Shakhov, *Tissue Culture Methods in Hematology* [in Russian], Tomsk (1992).
4. Yu. M. Zakharov, *Ros. Fiziol. Zh.*, **90**, No. 8, 987-1000 (2004).
5. T. A. Klygul' and N. A. Krivopalov, *Farmakol. Toksikol.*, No. 2, 241-244 (1966).
6. V. M. Koval'zon, *Vestn. Biol. Psikhiatr.*, No. 3, 3-6 (2003).
7. E. G. Skurikhin, N. V. Provalova, O. V. Pershina, and M. Yu. Minakova, *Kletochn. Tekhnol. Biol. Med.*, No. 4, 220-225 (2005).
8. O. I. Epshtein, M. B. Shtark, A. M. Dygai, *et al.*, *Pharmacology of Antibodies to Endogenous Functional Regulators in Ultralow Doses* [in Russian], Moscow (2005).
9. D. Jouvet, P. Vimont, F. Delorme, *et al.*, *Compt. Rend. Soc. Biol.*, **158**, 756-759 (1964).
10. Y. Sonoda, Y.-C. Yang, G. G. Wong, *et al.*, *Proc. Natl. Acad. Sci. USA.*, **85**, 4360-4364 (1988).