GENERAL PATHOLOGY AND PATHOLOGICAL PHYSIOLOGY

Adrenergic and Cholinergic Mechanisms of Hemopoiesis Regulation during Experimental Neuroses

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 129, No. 4, pp. 381-385, April, 2000
Original article submitted November 17, 1999

Scopolamine abolished hyperplasia of erythropoiesis caused by conflict situation and its inhibition after paradoxical sleep deprivation, but did not affect granulomonocytopoiesis. The modulatory effects of this muscarinic receptor blocker on the erythron indicated involvement of the cholinergic system in the formation of neurotransmitter interrelations. *In vitro* restoration of the colony-forming ability of myelokaryocytes under the effect of sympathomimetics during *in vivo* blockade of the central or peripheral part of the adrenergic system indicated that adrenoceptors play the major role in the information transfer from the higher regulatory centers to hemopoietic cells.

Key Words: *experimental neuroses; granulomonocytopoiesis; erythropoiesis; scopolamine; adrenergic structures*

The autonomic sympathetic nervous system [2] and brain catecholamines [7] are involved in the regulation of hemopoiesis under conditions of experimental neuroses (EN), in particular those caused by conflict situations (CS) and paradoxical sleep deprivation (PSD). At the same time, it is known that various neurotransmitters [6], in particular acetylcholine [8,9], are involved in the formation of specific neurotransmitter interrelations in CS and PSD. However, the role of the cholinergic system in the regulation of hemopoiesis and its interaction with other neurotransmitter systems, including the adrenergic system, are unclear.

Here we studied the role of the cholinergic system and adrenoceptors in the adaptive response of the blood system to CS and PSD.

MATERIALS AND METHODS

Experiments were performed on 300 CBA/CaLac mice (class I conventional mouse strain) aging 2-2.5 months (Laboratory of Experimental Biological Modeling, Institute of Pharmacology, Tomsk Research Center). To modulate blood changes in EN, the following preparations were injected: muscarinic receptor blocker scopolamine in a dose of 2 mg/kg intraperitoneally 20 min before EN; sympatholytic reserpine in a dose of 2 mg/kg intraperitoneally 4 h before EN; and α-adrenoceptor blocker dihydroergotamine in a dose of 3.9 mg/kg and β-adrenoceptor blocker propranolol in a dose of 5 mg/kg subcutaneously 3-5 min before EN and 5 h after the onset of EN [6]. Control mice received an equivalent volume of isotonic NaCl. On day 1-7 of observations, the count of segmented neutrophils, lymphocytes, and reticulocytes in the peripheral blood was determined. After blood tests, the animals were euthanized by cervical dislocation, and the contents of immature and mature neutrophilic granulocytes, erythrokaryocytes, and lymphoid elements in the bone marrow were estimated. On days 5 and 6, the content of committed precursors of erythro- and granulomonocytopoiesis was determined [1]. The effects of α-sympathomimetic mesatone and β-sympathomimetic salbutamol on the growth of committed erythro- and

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0007-4888/00/0004-323$25.00

granulomonocytopoiesis precursors in the control and during EN were determined by adding test drugs (10^{-8} M) or physiological saline to tissue methyl cellulose culture. 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 content of bone marrow erythroid precursors in CS increased compared to the control. *In vivo* administration of reserpine, dihydroergotamine, and propranolol reduced (day 5) or normalized (day 6) this parameter (Table 1). The addition of salbutamol and mesatone to bone marrow culture from animals subjected to CS and injected with β-adrenoceptor antagonists increased the number of erythroid precursors to the level typical of CS. It should be emphasized that the content of erythroid precursors was below that observed in CS without these preparations (Table 1).

Reserpine, dihydroergotamine, and propranolol *in vivo* suppressed the growth of granulomonocytic precursors on days 5 and 6 of CS (Table 1). Mesatone and salbutamol stimulated the growth of granulocyte-macrophage colonies against the background of exhaustion of catecholamine stores and blockade of adrenoceptors.

Hence, the content of erythroid and granulocyte-macrophage precursors increased in CS, while adrenoceptor antagonists inhibited colony formation. *In vitro* administration of adrenoceptor agonists restored the number of hemopoietic precursors to a level observed in CS.

In mice subjected to PSD, the count of erythroid precursors decreased on day 6 of observations (Table 1). This suppression was potentiated by sympatholytic and abolished by adrenoceptor blocker. Sympathomimetics considerably increased the number of erythroid precursors on days 5 and 6 of PSD compared to the initial level. The content of granulomonocytopoiesis precursors in the bone marrow increased on days 5 and 6 of PSD (as in CS, Table 1). This decrease was abolished by mesatone and salbutamol.

Thus, the reaction of hemopoietic precursors to PDS was ambiguous: the content of granulomonocytic precursors in the bone marrow increased, while the number of erythroid precursors decreased. Dihydroergotamine and propranolol normalized the content of committed precursors, while reserpine potentiated suppression of erythroid colony formation and decreased the concentration of granulocyte-macrophage precursors to the initial level. Mesatone and salbutamol abolished the effects of adrenoceptor antagonists during PSD and elevated the count of erythro- and granulomonocytopoiesis precursors to the level recorded during CS.

These results and previous data [2-4,6,7] suggest that catecholamines regulate hemopoiesis during EN via the sympathetic nervous system. The signals to hemopoietic target cells are transferred via adrenoceptors on hemopoietic microenvironmental cells and committed precursors of myelopoiesis.

It is known that activity of CNS under various conditions is characterized by complex interaction between various neurotransmitters [6]. Some authors reported that activation of the adrenergic system in PSD is preceded by activation of cholinergic structures

TABLE 1. Content of Erythroid and Granulocyte-Macrophage Precursors in Cultured Bone Marrow after CS and PSD

Experimental conditions		Number of precursors			
		erythroid		granulocyte-macrophage	
		CS	PSD	CS	PSD
NaCl		575*/351*	109/50*	452*/258*+	149*/358*
Reserpine		400*+/150*+	75+/6*+	149+/72+	45*+/215*+
	+mesatone	300*+/250*o	275*+o/100*+o	525*o/646*+o	225*+o/574*+o
	+salbutamol	350*/250*o	450*+o/401*+o	345*o/718*+o	164o/287*
Dihydroergotamine		250*+/200+	60/80+	119+/72+	37*+/215*+
	+mesatone	400o/200+	175*+o/502*+o	225*+o/287*o	149*o/431*o
	+salbutamol	250+/200+	450*+o/512*+o	239*+o/358*o	225*+o/358*o
Propranolol		250*+/50*+	124/100+	149+/72+	64+/215*+
	+mesatone	225+/401*o	275*+o/301*+o	300*o/216*+o	74+/574*+o
	+salbutamol	300+/201*o	300*+o/301*+o	149*+/71+	149*/287*

Note. $p<0.05$ compared with: *intact animals, +neurosis against the background of NaCl administration, owithout mesatone and salbutamol. Numerator: day 5; denominator: day 6.

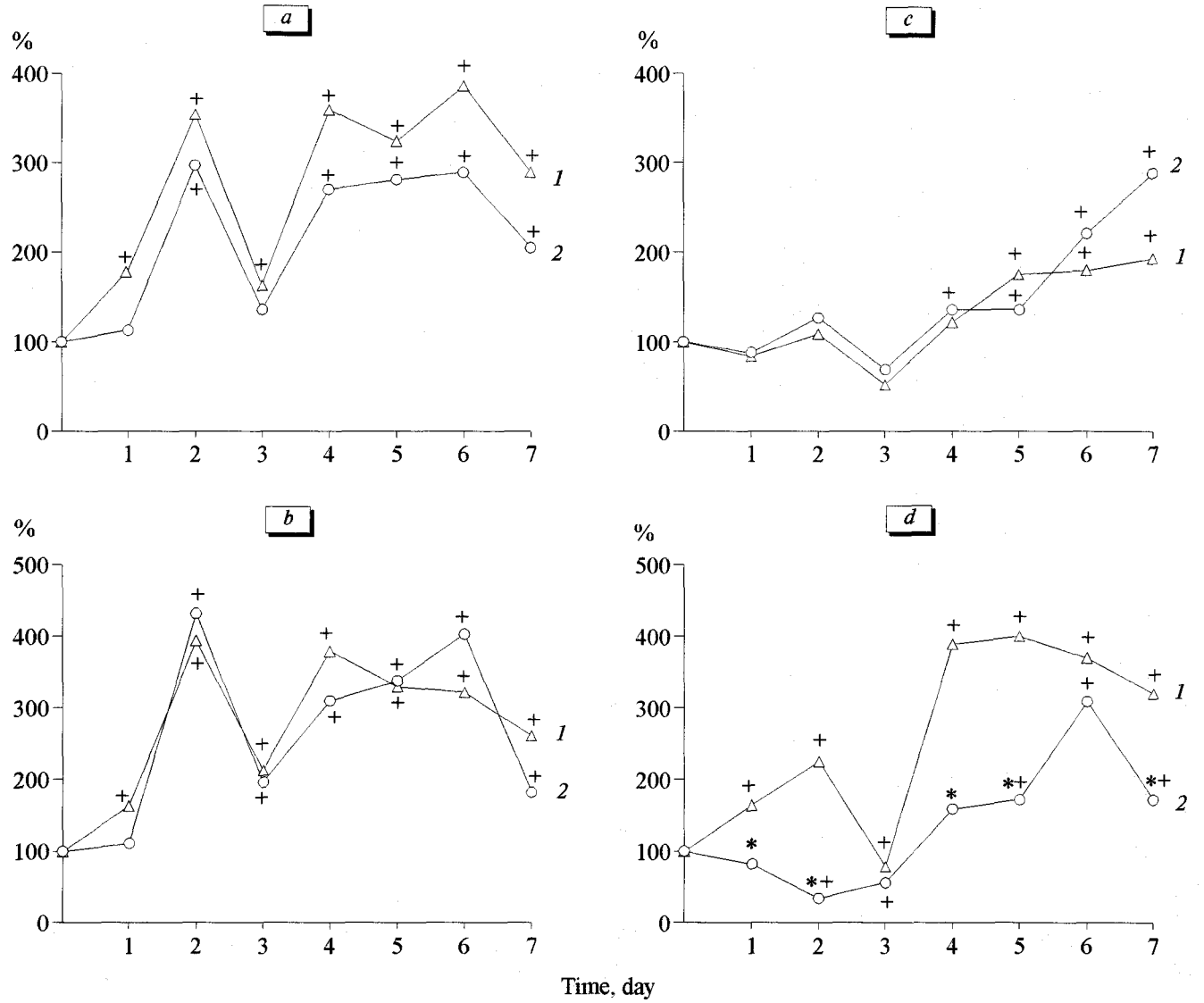

Fig. 1. Content of bone marrow cells in mice subjected to conflict situation. Here and in Fig. 2: contents of immature (*a*) and mature neutrophilic granulocytes (*b*), lymphoid elements (*c*), and erythrokaryocytes (*d*) after *in vivo* administration of isotonic NaCl (*1*) and scopolamine (*2*). $p<0.05$ compared with: *NaCl administration, +intact control. Ordinate: cell count.

[8], and that phase I disturbances in the higher nervous activity during EN depends on the cholinergic system [5,9]. Therefore, the involvement of cholinergic structures in the adaptation of hemopoietic tissues during EN can not be excluded.

Scopolamine practically did not affect the content of neutrophilic granulocytes and lymphoid elements in the bone marrow during EN (Figs. 1 and 2) and the distribution of lymphocytes and neutrophilic leukocytes in the peripheral blood in CS and PSD. At the same time, scopolamine abolished accumulation of bone marrow erythroid elements in CS (Fig. 1). Scopolamine abolished suppression of erythropoiesis caused by PSD and induced moderate hyperplasia of the bone marrow erythropoiesis associated with accumulation of erythrokaryocytes (Fig. 2). Moderate reticulocytosis was revealed in the peripheral blood. Thus, scopolamine produced modulatory effects on the erythron system, but did not affect white blood cell count during EN.

Our findings indicate that the acetylcholine system plays an important role in the formation of neurotransmitter integration in brain structures determining hematologic disturbances during EN. We assume that activity of catecholamines depends on the cholinergic system, but further autonomic changes are directly related to the adrenergic system. The cholinergic system probably plays the major role in the regulation of erythropoiesis. The observed changes in hemopoiesis indirectly confirm our assumption that shifts in the granulocytic and erythroid hemopoietic stems during EN reflect activity of two functional systems [6].

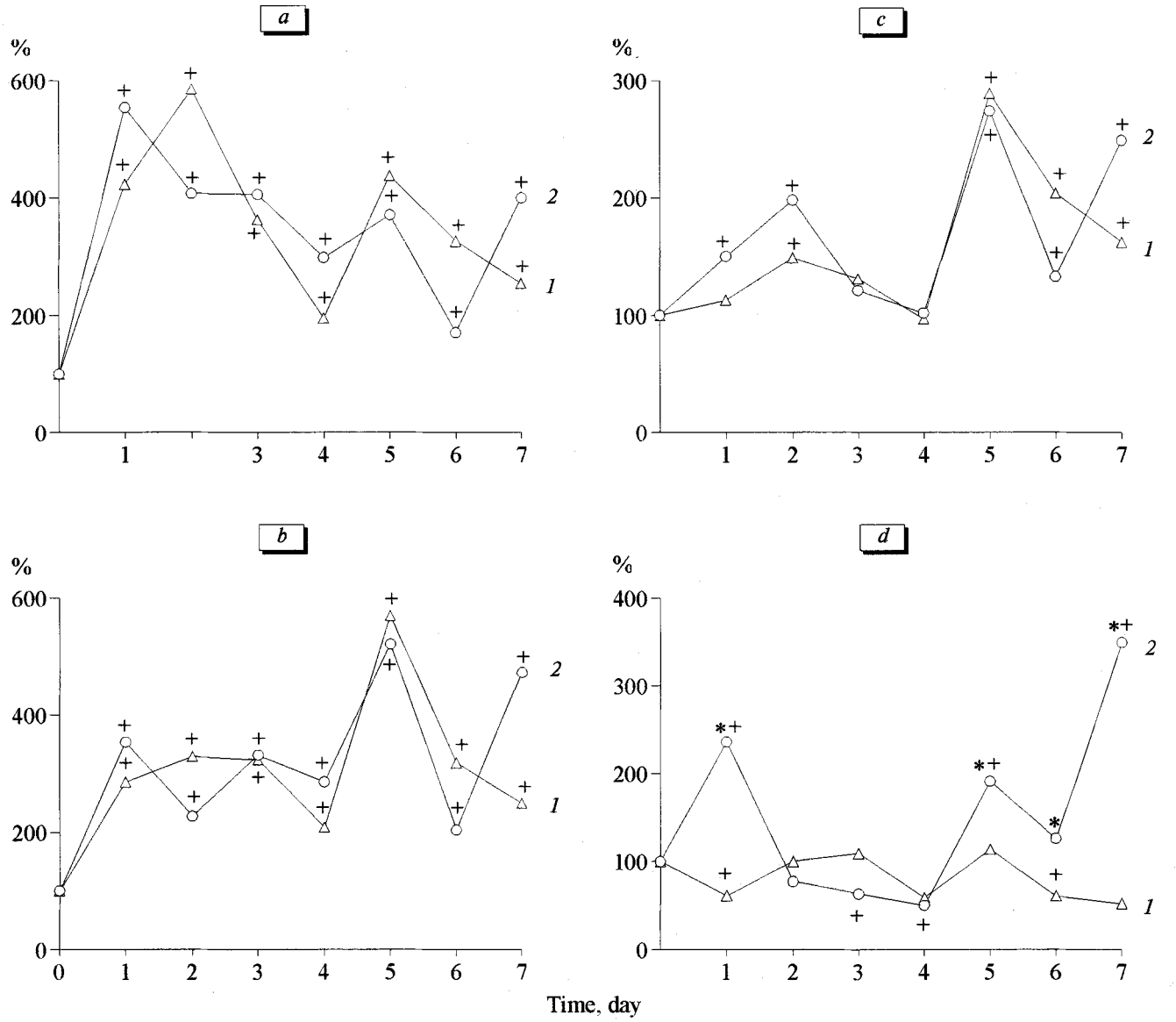

Fig. 2. Content of bone marrow cells in mice after paradoxical sleep deprivation.

REFERENCES

1. E. D. Gol'dberg, A. M. Dygai, and V. P. Shakhov, *Tissue Cultures in Hematology* [in Russian], Tomsk (1992).
2. E. D. Gol'dberg, A. M. Dygai, N. I. Suslov, *et al.*, *Byull. Sib. Otd. Ros. Akad. Med. Nauk*, No. 1, 129-134 (1998).
3. A. M. Dygai, N. I. Suslov, E. G. Skurikhin, and A. A. Churin, *Byull. Eksp. Biol. Med.*, **123**, No. 2, 158-161 (1997).
4. A. M. Dygai, E. G. Skurikhin, N. I. Suslov, *et al.*, *Ibid.*, **126**, No. 12, 628-631 (1998).
5. R. Yu. Il'yuchenok, *Zh. Vyssh. Nervn. Deyat.*, **23**, No. 2, 315-322 (1973).
6. N. V. Provalova, *Role of Adrenergic Nervous Structures in the Regulation of Hemopoiesis during Experimental Neuroses*, Abstract of Cand. Med. Sci. Dissertation, Tomsk (1990).
7. E. G. Skurikhin, N. I. Suslov, N. V. Provalova, *et al.*, *Byull. Eksp. Biol. Med.*, **127**, Suppl. 1, 7-11 (1999).
8. B. B. Mrsulja and L. M. Rakic, *J. Neurochem.*, **17**, 455-456 (1970).
9. G. Pepeu, G. Spignoli, M. G. Giovannini, and M. Magnani, *Pharmacopsychiatry*, **22**, No. 2, 116-119 (1989).