Responses of Erythroid Cells to Different Neurotic Influences

A. M. Dygai, N. I. Suslov, E. G. Skurikhin, and A. A. Churin

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Cells of the erythrocytic series develop hyperplasia in mice exposed to immobilization stress, conflict situation, or conflict situation while being deprived of REM sleep. These mice contain increased numbers of morphologically recognizable erythroid cells and erythroid cell precursors in the bone marrow, and culture media conditioned by their nonadherent or adherent karyocytes shows elevated erythropoietic activity. In contrast, bone marrow erythropoiesis is depressed for a long time in mice deprived of REM sleep and mice with and without experiencing a conflict situation. Probably, enhancement of erythropoiesis is due to activation of adrenergic mechanisms regulating hematopoiesis, while its depression is due to impaired operation of the central adrenergic mechanisms.

Key Words: *stress; conflict; sleep deprivation; erythropoiesis; adrenergic regulation*

Somatization of psychoemotional tension makes an important contribution to the clinical picture of mental disorders and borderline states [8]. A substantial role in the development of somatic response to any emotional influence is played by the blood system [1,4-6]. However, the reactions of hematopoietic tissues to various influences leading to neurotic disorders remain largely unexplored as well as the mechanisms underlying the changes occurring in the blood. In the present study we examined the responses of erythroid cells and erythroid cell precursors to several neurotic influences and compared them with the reactions of hematopoietic tissue after immobilization stress.

MATERIALS AND METHODS

The study was performed during fall and winter months on 250 male CBA mice weighing 18-20 g. The mice were immobilized in supine position for 10 h, experienced a conflict situation for 5 min, or deprived of REM sleep for 48 h as described previously [7]. Two combinations of neurotic influences — a 5-min conflict situation during 48-h REM sleep deprivation (first model of exposure to combined stimuli) or 48-h REM sleep deprivation following a 5-min conflict situation (second model of exposure to combined stimuli) were used. The mice were killed by cervical dislocation at different times after stress, and erythroid cells and their precursors were counted in the bone marrow; erythropoietic activity of culture media conditioned by adherent or nonadherent cells was measured [3].

The results were analyzed using standard statistical methods (Student's parametric t test and Wilcoxon's nonparametric rank test) and Statgraphics-3.0 software.

RESULTS

This study revealed several consistent patterns of reactions developed by the blood system in response to the immobilization stress, conflict situation, and REM sleep deprivation. The number of bone marrow erythroid cells (erythrokaryocytes) was increased throughout a 7-day observation period in the three groups exposed, respectively, to the immobilization

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

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stress, conflict situation, and conflict situation during REM sleep deprivation. In contrast, these cells were present in decreased numbers on days 3-6 of the observation period in mice deprived of REM sleep for 48 h as well as in mice deprived of REM sleep for 48h after a conflict situation (Fig. 1).

In all groups, the number of erythroid cell precursors in the bone marrow (CFU-E) changed with time. A biphasic increase in the number of erythroid cell precursors (days 1-3 and 5-6) was observed in mice exposed to immobilization stress, conflict situation, or conflict situation during REM sleep deprivation, while the number of these cells fell below baseline in two other groups (Fig. 1).

A consistent observation made for the first three groups was an elevated erythropoietic activity of culture media conditioned by adherent or nonadherent cells (karyocytes) from these mice. In two other groups (mice deprived of REM sleep for 48 h or exposed to conflict situation during REM sleep deprivation), the erythropoietic activity of culture medium conditioned by adherent and nonadherent cells decreased, while that of supernatants from these media increased (Fig. 2).

Our results indicate that psychoemotional tensions of various origins leads to marked changes in hematopoiesis. Thus, hyperplasia of bone marrow erythropoiesis developed in mice exposed to a conflict situation with or without REM sleep deprivation. The finding that such a hyperplasia occurs after immobilization stress suggests a universal nature of this phenomenon. Subsequent activation of the sympathetic nervous system is probably accompanied by migration of T lymphocytes to the bone marrow, where they cooperate with resistant macrophages and

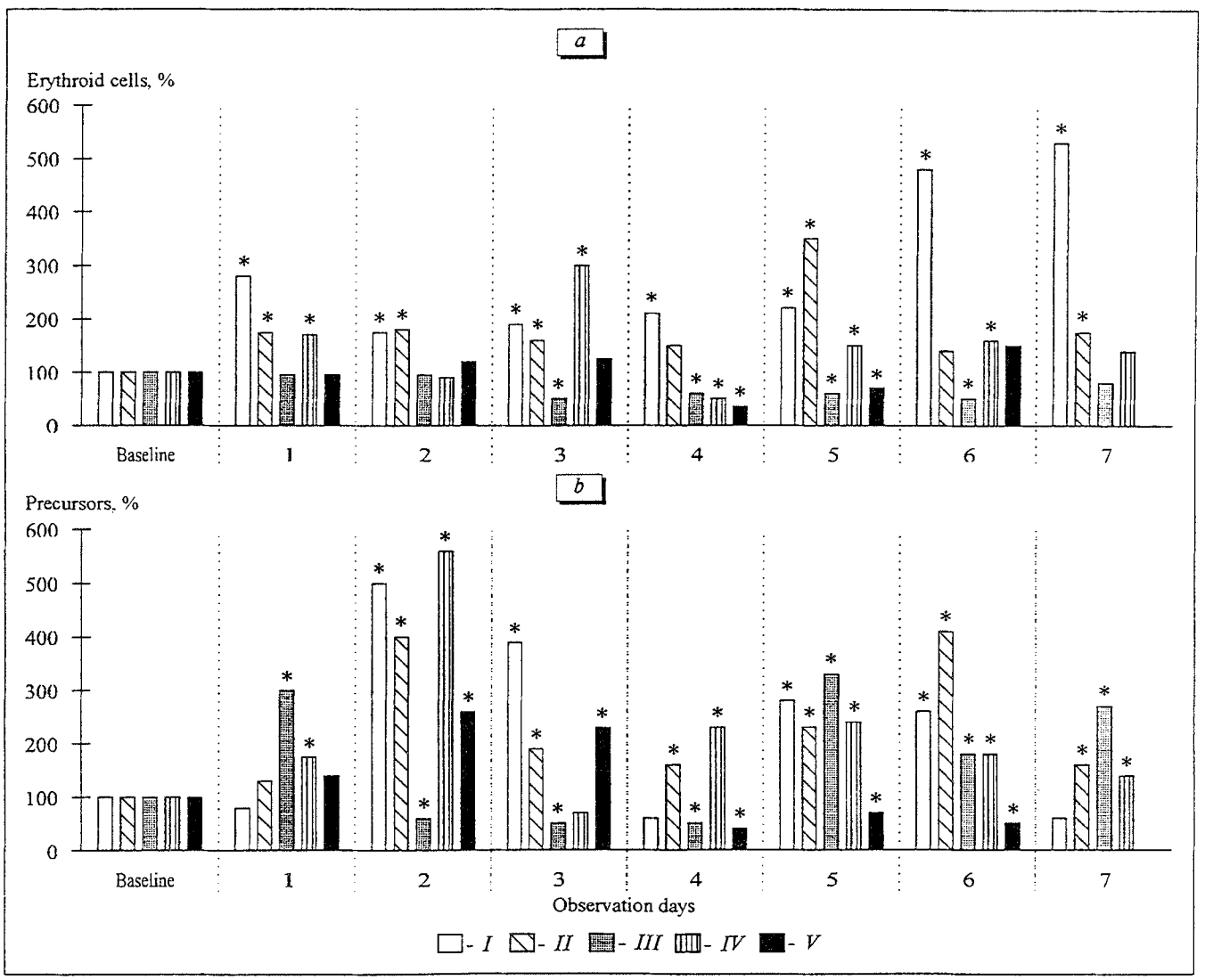

Fig. 1. Temporal variations in the content of erythroid cells (*a*) and erythroid cell precursors (*b*) in the bone marrow of CBA mice. Here and in Fig. 2: *I*) after immobilization stress; *II*) after conflict situation; *III*) after REM sleep deprivation; *IV*) after conflict situation during REM sleep deprivation; *V*) after REM sleep deprivation following conflict situation. *$p<0.05$ in comparison with baseline.

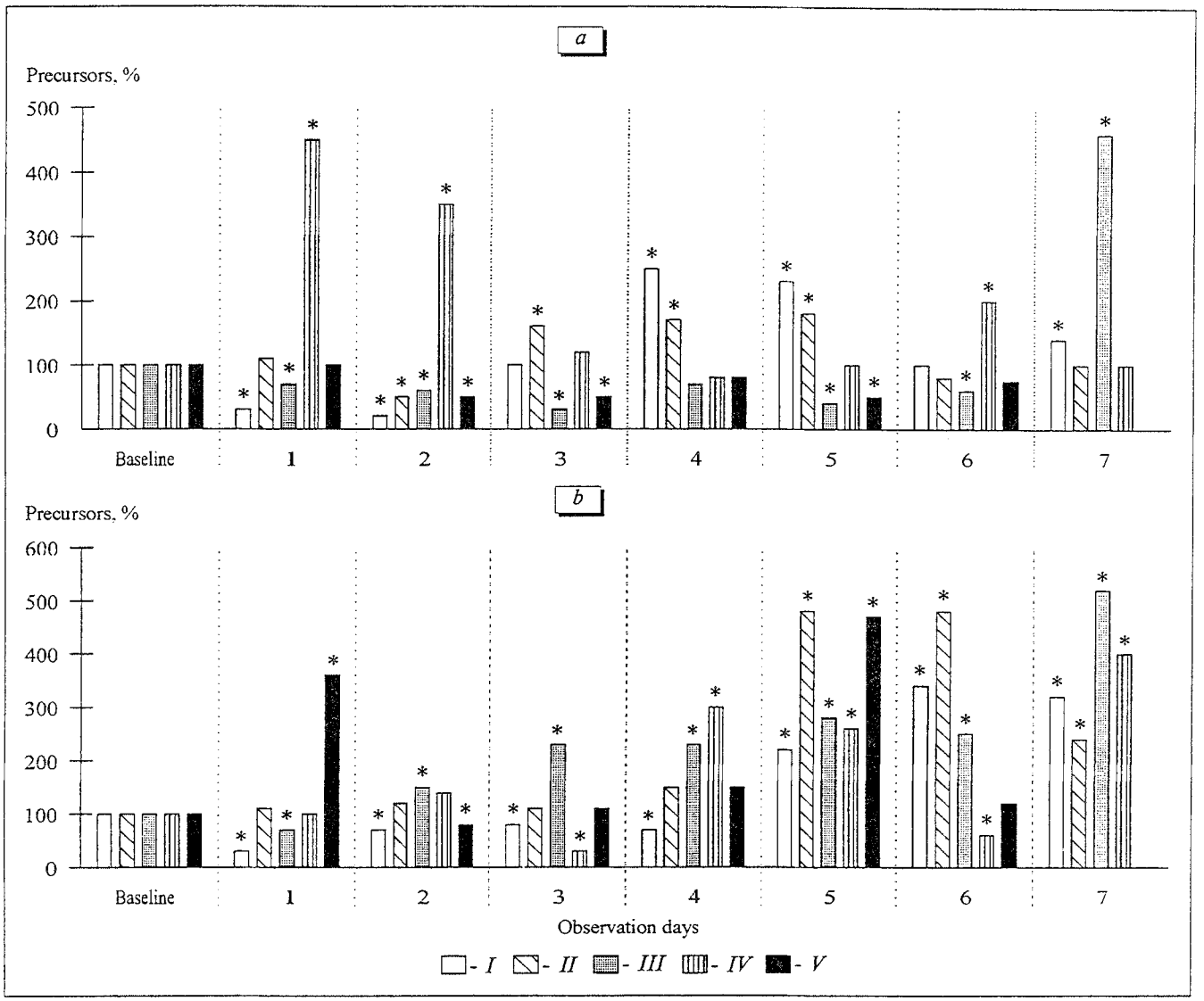

Fig. 2. Erythropoietic activity of culture medium conditioned by adherent (*a*) and nonadherent (*b*) bone marrow cells from CBA mice.

activate proliferation and differentiation of committed erythroid cell precursors [2,6]. A long-lasting depression of the erythrocytic series observed in REM sleep-deprived mice and in mice deprived of REM sleep after a conflict situation indicates that the regulation of hematopoiesis was impaired.

Our results can be explained by the specific role of the α-adrenergic system in the regulation of stress reactions, sleep, conflict situations, and hematopoiesis. Deprivation of fast (REM) sleep diminishes the catecholamine stores, and the duration of this sleep can be prolonged by administering antagonists of α-adrenergic receptors [9]. Under conditions of stress or a conflict situation with or without REM sleep deprivation, the activity of cerebral α-adrenergic system is increased. In stress, proliferation and differentiation of hematopoietic cells can be increased by adrenomimetics and inhibited by antagonists of adrenergic structures [6].

Probably, REM sleep deprivation with or without exposure to a conflict situation leads to inhibition of erythropoiesis because of inadequate operation of central adrenergic mechanisms, whereas immobilization stress and a conflict situation with or without REM sleep deprivation stimulate erythropoiesis by activating these mechanisms.

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Toxic Effect of Glutamate on Granular Cells of the Cerebellum Reduces Cell ATP Content. The Role of Calcium Ions

V. G. Pinelis, L. P. Bykova, A. P. Bogachev, N. K. Isaev,* I. V. Viktorov,* and B. I. Khodorov**

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The content of ATP in cultured cerebellar granular cells during and after glutamate intoxication is determined using the luciferin-luciferase method. A 15-min exposure to glutamate (100 μM in a Mg^{2+}-free medium) reduces the content of ATP to 63.8±3.01% of the initial value, followed by further drop of ATP during the postglutamate period. In a Ca^{2+}-free medium, the content of ATP during incubation with glutamate and in the postglutamate period drops to 89.2±4.8 and 74.7±3.6% of the initial level, respectively, while subsequent washout with a medium containing 1.8 mM Ca^{2+} results in a further decrease in the ATP content.

Key Words: *ATP; cerebellar granular cells; calcium; glutamate; toxicity*

It is known that hyperstimulation of glutamate receptors by a long-term (15-30 min) exposure of nerve cells to glutamate (GLU) results in a steady elevation of cell Ca^{2+} concentration ($[Ca^{2+}]_i$), leading to cell death [5,10,12]. However, despite extensive studies the mechanism of this phenomenon remains unclear. Until recently, the steady elevation of $[Ca^{2+}]_i$ in nerve cells after GLU exposure has been supposed to result from irreversible increase in Ca^{2+} permeability of the neuronal membrane [5,10]. Our studies demonstrated that Ca^{2+} overload of cells in the postglutamate period arises from inhibition of the processes responsible for the elimination of excessive Ca^{2+} from the cytoplasm, in particular, Na^+- and Ca^{2+}-exchange. The same conclusion has been recently made by L. Kiedrowski *et al.* [7].

Since Na^+- and Ca^{2+}-exchange and Ca^{2+}-pump largely depend on cell ATP concentration, the present study explored changes in cell ATP content during and after exposure of cerebellar granular cells to GLU.

MATERIALS AND METHODS

The study was performed on cerebellar granular cells from 7-8-day-old Wistar rats obtained as described previously [3]. The cell suspension was transferred to poly-L-lysine-coated coverslips and placed into 35-mm Petri plastic dishes. The cultures were placed

Institute of Pediatrics, Russian Academy of Medical Sciences; *Institute of the Brain, Russian Academy of Medical Sciences; **Institute of General Pathology and Pathological Physiology, Russian Academy of Medical Sciences, Moscow

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