GENERAL PATHOLOGY AND PATHOPHYSIOLOGY

Role of Adrenergic Mechanisms of Erythropoiesis Regulation during Severe Hypoxia

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We studied the role of β-adrenergic mechanisms of regulation of erythropoiesis in the formation of the erythron system reaction during severe hypoxia. Blockade of β-adrenoceptors after the incidence of hypoxic encephalopathy of different genesis was followed by an increase in the number of committed precursors in the bone marrow, hyperplasia of the erythroid hemopoietic stem, and rise in the count of peripheral blood erythrocytes. These changes were accompanied by decreased formation of abnormal erythrocytes.

Key Words: *hypoxia; erythropoiesis; adrenergic mechanisms; encephalopathy*

Hypoxia is a general pathological process that accompanies progression of various diseases. The blood system plays a key role in the maintenance of homeostasis and development of compensatory and adaptive reactions during oxygen deficiency [3]. Published data show that catecholamines are involved in the regulation of hemopoiesis under extreme conditions [7]. Hyperactivation of the adrenergic system during antimetabolite-produced myelosuppression is followed by damage to hemopoietic precursors and impairs the recovery of erythropoiesis after cytostatic treatment. The development of these disturbances mainly depends on activity of the β-adrenergic system [4,7].

Our previous studies demonstrated that severe hypoxia accompanied by encephalopathy is followed by disadaptation of the erythron system. These changes are manifested in a decrease in hyperplasia of the erythroid hemopoietic stem due to reduction of committed precursor count in the bone marrow and formation of abnormal erythrocytes [5,6,10,11]. Hyperactivation of the sympathoadrenal system during the posthypoxic period is associated with decompensation of the inhibitory transmitter mechanisms highly sensitive to oxygen deficiency [1,3,13].

Here we studied the role of adrenergic mechanisms of hemopoiesis regulation in the formation of hematological shifts during severe hypoxia of different genesis.

MATERIALS AND METHODS

Experiments were performed on 366 CBA/CaLac mice (class I conventional mouse strain) weighing 18-20 g and obtained from the nursery of the Department of Experimental Biomedical Modeling (Institute of Pharmacology, Tomsk Research Center). Hypoxic hypoxia and 2 regimens of hemic hypoxia served as the experimental models. To produce hypoxic hypoxia the animals were repeatedly placed in a 500-ml sealed chamber with a 10-min interval. The mice were removed from the chamber after termination of generalized convulsions and/or visual respiratory arrest for 10-15 sec.

Hemic hypoxia was produced by single intraperitoneal injection of phenylhydrazine hydrochloride (150 mg/kg) or blood loss. The retroorbital sinus was punctured, and 70% circulating blood volume (CBV) were withdrawn through a graduated Pasteur pipette

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washed with heparin solution over 2-3 h (3 procedures). The volume of withdrawn blood was estimated taking into account that CBV in rodents corresponds to $^1/_{13}$ of their body weight. Oxygen deficiency was accompanied by the development of encephalopathy. It was estimated by amnesia of a conditioned passive avoidance response [2] and disturbances in orientation and exploratory activity in the open field [2,14]. Propranolol (Arzneimittelwerk) in a single dose of 5 mg/kg was injected subcutaneously 2 days after hypoxia. Control mice received an equivalent volume of physiological saline (0.2 ml). The peripheral blood from the control (days 1-10) and experimental animals (days 3-10) was studied on an Abacus automatic blood analyzer (Diatron) under veterinary conditions. The intensity of bone marrow hemopoiesis was estimated by routine blood tests [12]. We determined the number of bone marrow erythroid precursors (CFU-E), proliferative and differentiation activity of cells, production of erythropoietic compounds by individual fractions of the hemopoiesis-inducing microenvironment (HIM), erythropoietic activity (EPA) of blood plasma, and structural and functional organization of the bone marrow [8]. The results were analyzed by Student's *t* test and nonparametric Wilcoxon—Mann—Whitney *U* test.

RESULTS

Anemia was observed in animals of various groups. It was related to the specific effect of treatment with

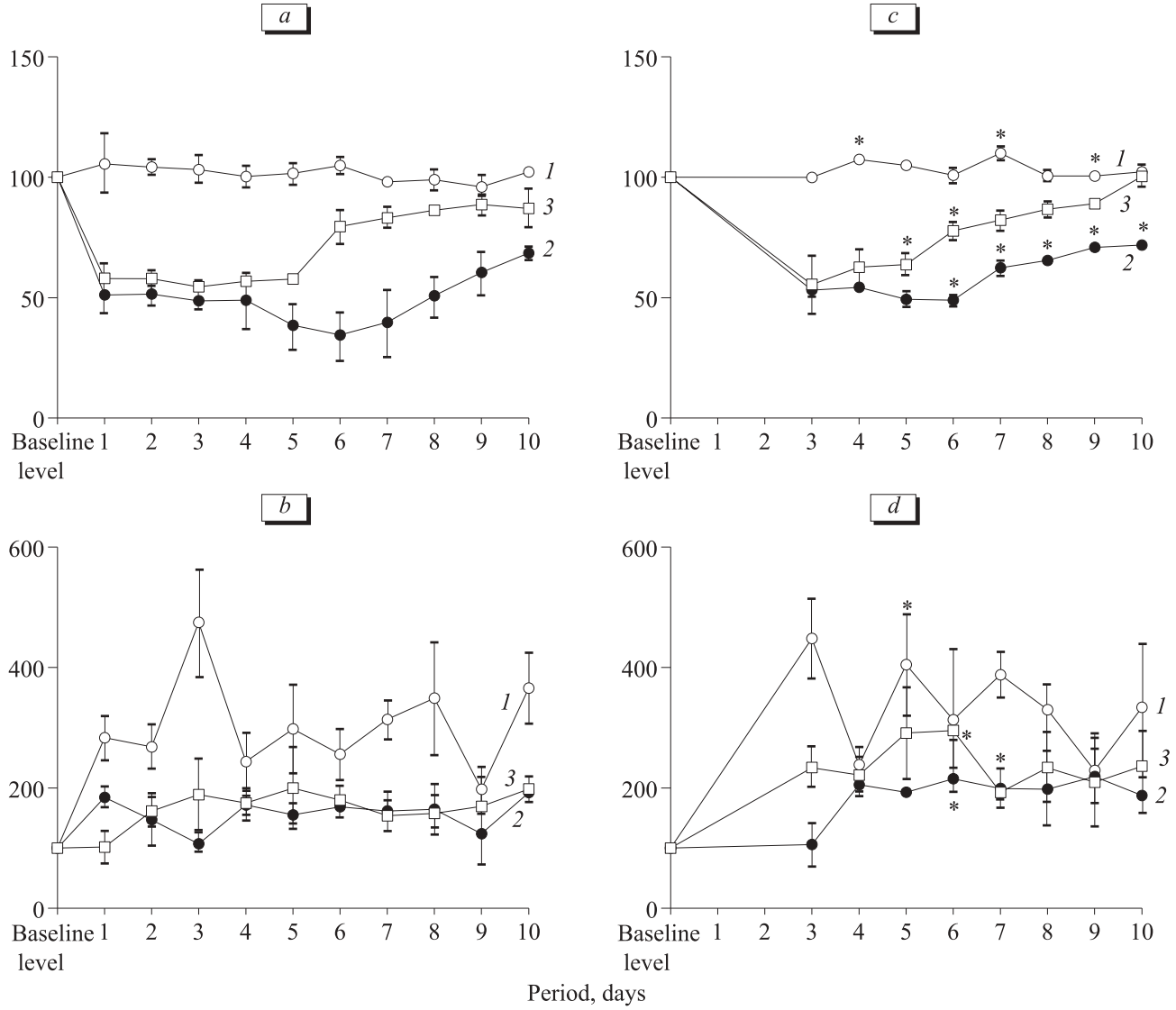

Fig. 1. Number of peripheral blood erythrocytes (*a*, *c*) and bone marrow erythrokaryocytes (*b*, *d*) in CBA/CaLac mice after hypoxic hypoxia (*1*), administration of 150 mg/kg phenylhydrazine hydrochloride (*2*), and loss of 70% circulating blood volume (*3*). Absence of preparation (*a*, *b*); administration of propranolol 2 days after treatment (*c*, *d*). Here and in Figs. 2 and 3: ordinate, study parameter relative to intact control animals (%). **p*<0.05 compared to animals not receiving the preparation.

phenylhydrazine hydrochloride in high dose and massive blood loss. Anemia was most pronounced on days 6 (31.4%) and 1 (57.9%), respectively. These changes were also associated with impaired recovery of erythrocyte number due to macrocytosis in animals with hemolytic anemia (days 5-10) and blood loss (days 3-8 and 10). Moreover, anemia could result from rapid dieresis of newly formed large erythrocytes (Fig. 1, *a*). Hypochromic anemia developed in the late period after the incidence of hypoxic hypoxia-induced brain injury. Hemoglobin concentration decreased on days 4, 5, and 9. The observed changes were probably related to excessive increase in the size of mature red blood cells (day 4) and decrease in hemoglobin concentration under conditions of intensive erythropoiesis. The mean corpuscular concentration of hemoglobin decreased on days 5, 7, and 9. The number of erythroid karyocytes in the bone marrow increased

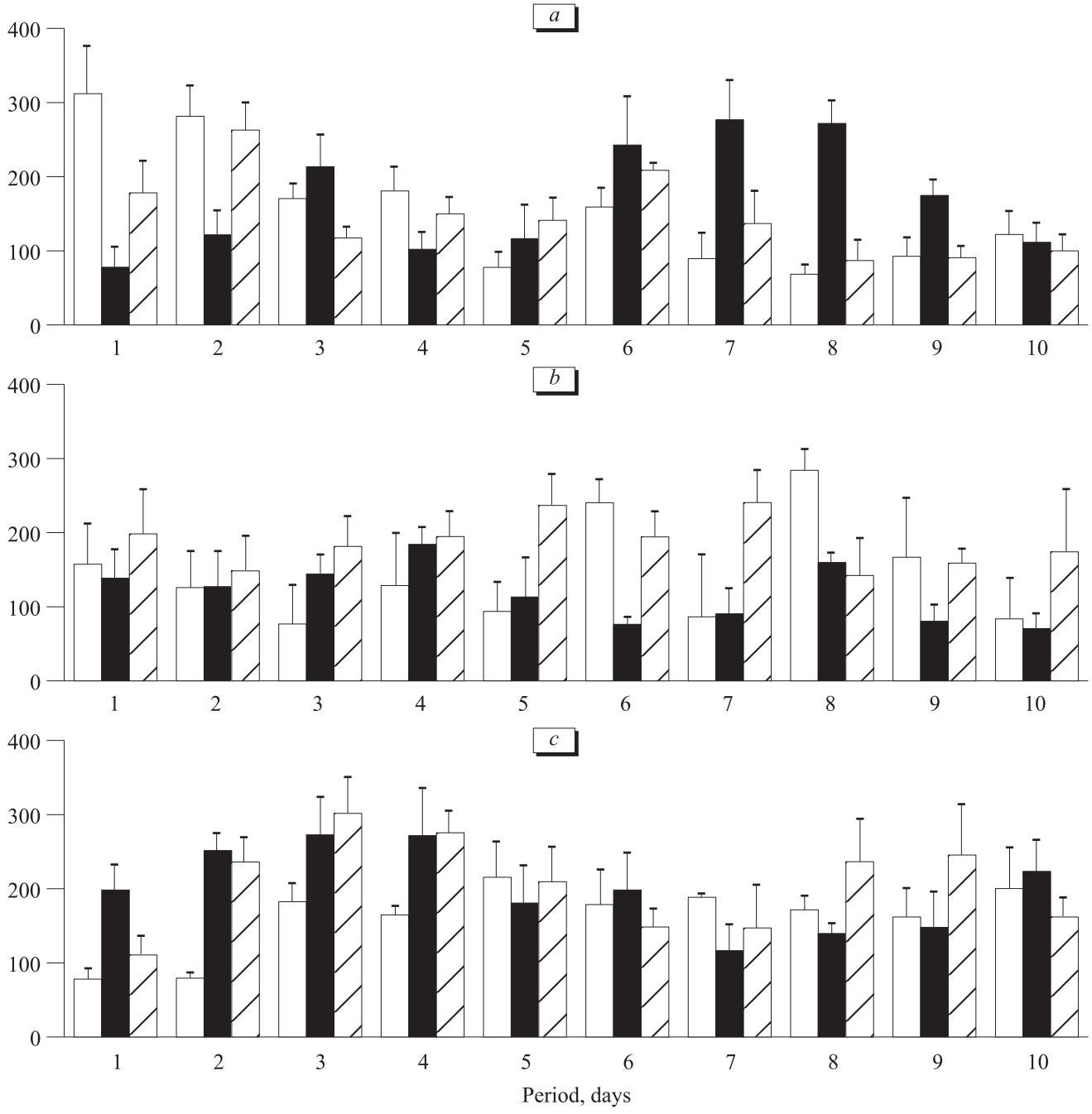

Fig. 2. Content of bone marrow CFU-E (*a*), ratio of cells in S-phase of the mitotic cycle (*b*), and maturation of cells in CBA/CaLac mice (*c*) after hypoxic hypoxia (light bars), administration of 150 mg/kg phenylhydrazine hydrochloride (dark bars), and loss of 70% circulating blood volume (shaded bars).

(Fig. 1, *b*) due to increase in the count and functional activity of CFU-E in hemopoietic tissue (Fig. 2). These changes were associated with the increase in blood plasma EPA and secretory function of adherent myelokaryocytes at various stages after treatment. Moreover, production of EPA by nonadherent karyocytes increased after administration of the hemolytic poison (days 3, 5, 6, and 10) and blood loss (days 3 and 6).

The reaction of hemopoietic tissue is consistent with changes in hemopoiesis observed during oxygen deficiency [3,9]. Our previous studies demonstrated that the development of encephalopathy is accompanied by disadaptation of the erythron system (erythropoietic distress) [5,6,10,11].

We studied the role of adrenergic structures in the regulation of hemopoiesis during encephalopathy.

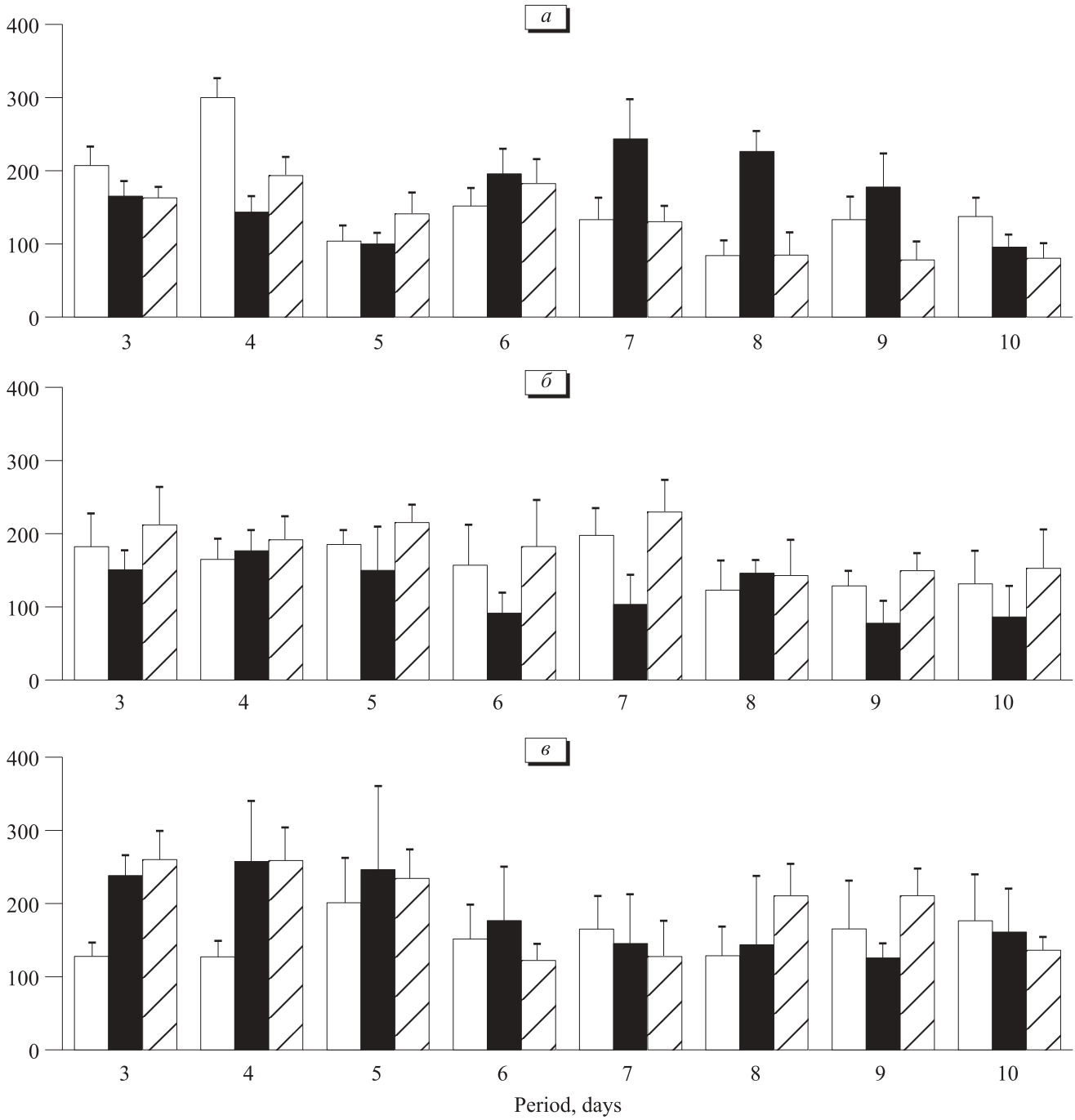

Fig. 3. Content of bone marrow CFU-E (*a*), ratio of cells in S-phase of the mitotic cycle (*b*), and maturation of cells (*c*) in CBA/CaLac mice receiving propranolol 2 days after hypoxic hypoxia (light bars), administration of 150 mg/kg phenylhydrazine hydrochloride (dark bars), and loss of 70% circulating blood volume (shaded bars).

Blockade of β-adrenoceptors on day 2 after hypoxic hypoxia increased the number of peripheral blood erythrocytes (days 4, 7, and 9) and concentration of hemoglobin (day 5). This treatment abolished the development of hypochromic anemia. Administration of propranolol contributed to recovery of red blood parameters in animals with hemolytic and posthemorrhagic anemia: we observed an increase in the number of erythrocytes (days 6-9; and 5, 6, and 9, respectively) and hematocrit (days 6-8 and 4, respectively, Fig. 1, *c*). The size of erythrocytes in mice receiving propranolol was much lower compared to control animals (days 4, 7-10, and 5-10 after hypoxia in a sealed chamber, phenylhydrazine administration, and blood loss, respectively). These changes in the peripheral blood reflected variations in bone marrow erythropoiesis. The number of bone marrow erythrokaryocytes increased in propranolol-treated mice exposed to repeated hypoxia in the sealed chamber (day 5), treatment with 150 mg/kg phenylhydrazine hydrochloride (days 6 and 9), and loss of 70% CBV (days 6 and 7) compared to animals without correction of the adrenergic system (Fig. 1, *d*).

Experiments with cell cultures showed that changes in the blood system depend on the state of progenitor cells in hemopoietic tissue. Administration of propranolol significantly increased the number of erythroid precursors in the bone marrow on day 4 after hypoxic hypoxia and massive hemolysis (by 65.85 and 57.14%, respectively). In the posthemorrhagic period the count of these cells increased by 38.9 and 29% (days 3 and 4, respectively). We revealed a increase in proliferative activity of erythroid precursors. These changes were most pronounced 3 days after hypoxia in the sealed chamber (Fig. 3). Despite decreased production of erythropoietic substances by adherent bone marrow cells 4 days after hypoxia in the sealed chamber and blood loss, the rate of CFU-E differentiation was similar in animals of different groups. Administration of β-adrenoceptor antagonist had no effect on secretory function of nonadherent HIM cells and blood plasma EPA.

Our results show that administration of propranolol after severe oxygen deficiency of different genesis is followed by the increase in the number of CFU-E in hemopoietic tissue, hyperplasia of the erythroid hemopoietic stem, and rise in the count of peripheral blood erythrocytes. The observed changes were accompanied by decreased formation of abnormal erythrocytes. These data indicate that the adrenergic system suppresses erythropoiesis under conditions of severe hypoxia. The "unnatural" effect of excess catecholamines is probably associated with damage to extremely modified erythroid precursors and realized via membrane β-adrenoceptors [4,7].

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