GENERAL PATHOLOGY AND PATHOPHYSIOLOGY

Humoral Mechanisms of Regulation of Erhythropoiesis during Hypoxia

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We studied the dynamics of erythropoietin content and erythropoietic activity of the serum during hypoxia of different genesis and severity. Our results show that humoral factors play an important role in the regulation of erythropoiesis during oxygen deficiency. Serum erythropoietic activity underwent similar changes in different types of hypoxia associated with similar hematological shifts. A discrepancy was observed between erythropoietic activity and serum erythropoietin concentration.

Key Words: *hypoxia; erythropoiesis; erythropoietic activity; erythropoietin*

Practically all diseases are accompanied by impairment of aerobic oxidation. Hypoxic factors produce a strong effect on the oxygen transporting system. Functional reconstruction of structures involved in oxygen supply in the organism is directed at the maintenance of energy metabolism [2]. The blood system contributes to the formation of a qualitatively new pattern of functional activity of organs during oxygen deficiency and adaptation to hypoxia. Erythropoietin-producing systems play a key role in the regulation of hemopoiesis during oxygen deficiency of different genesis [2,12]. Apart from erythropoietin (EPO), a variety of other humoral factors are capable of modulating erythropoiesis [3,7]. The *in vivo* effect of these factors on hyperplasia of the erythroid stem induced by erythropoiesis-stimulating exposures remains unclear.

Our previous experiments showed that hypoxia of different genesis sharply stimulated erythropoiesis [4, 5,8]. It manifested in an increase in the number of bone marrow erythroid nuclear cells and peripheral blood reticulocytes. Blood changes were associated with an increase in the number, proliferative activity, and rate of differentiation of erythroid precursors in the bone marrow. The development of encephalopathy during severe oxygen deficiency (2-fold hypoxia in a sealed chamber; loss of 70% circulating blood volume, CBV) was accompanied by delayed injury to committed precursors and less pronounced hyperplasia of the erythroid hemopoietic stem. These changes developed despite compensatory activation of erythroid precursor maturation.

Here we studied changes in EPO concentration and erythropoietic activity (EPA) of the serum during hypoxia of different genesis and severity.

MATERIALS AND METHODS

Experiments were performed on 642 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 placed 1 or 2 times (10-min interval) in a 500-ml sealed chamber. 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 intraperitoneal in-

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jection of phenylhydrazine hydrochloride (single doses 30 and 150 mg/kg) or blood letting. Blood loss was induced by puncture of the retroorbital sinus and withdrawal of 30% CBV through a graduated Pasteur pipette washed with heparin solution (series I). In series II, 70% CBV were repeatedly withdrawn for 2-3 h (3 procedures). The volume of withdrawn blood was estimated taking into account that CBV in rodents corresponds to $^1/_3$ of body weight. Single hypoxic exposure in the sealed chamber, administration of hemolytic

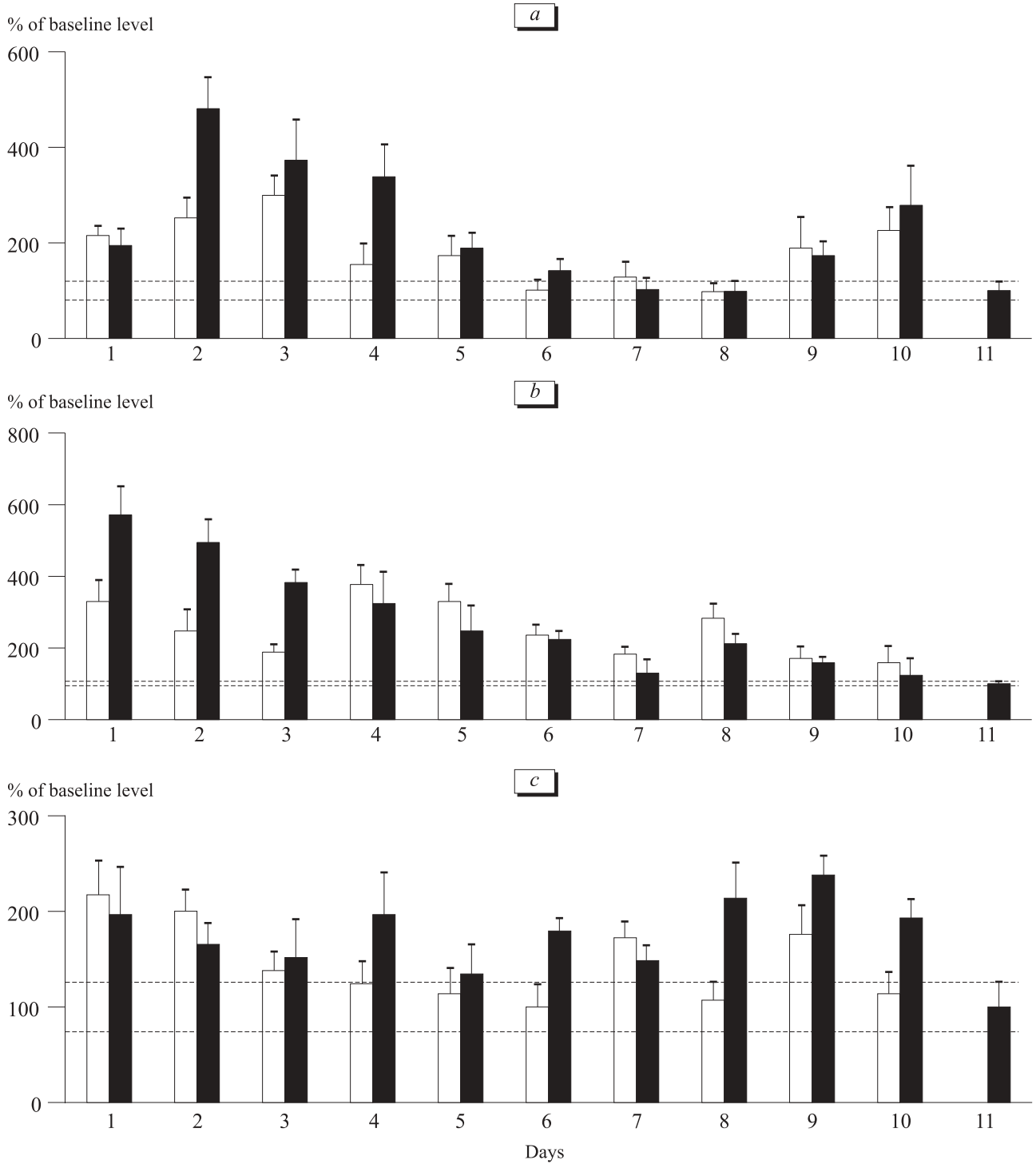

Fig. 1. Serum erythropoietic activity in CBA/CaLac mice during hypoxia in a sealed chamber (*a*), hemolytic anemia (*b*), and acute blood loss (*c*) not producing injury in CNS (light bars) or accompanied by the development of encephalopathy (dark bars). Here and in Fig. 2: dotted lines: confidence interval of the parameter in intact mice ($p \leq 0.05$).

poison in a dose of 30 mg/kg, and effusion of 30% CBV produced no significant changes in the psychoneurological status. Serious oxygen deficiency (2-fold hypoxia in a sealed chamber, administration of hemolytic poison in a dose of 150 mg/kg, and effusion of 70% CBV) was followed by the development of encephalopathy. It was evaluated by amnesia during passive avoidance performance [1] and disturbances in orientation and exploratory activity in the open field [1,13]. EPA was estimated on days 1-10. Serum EPO concentration was measured 12 h and 1-9 days after treatment. EPA was determined by the intensity of colony formation in 3-day-old cultures of the test systems using peripheral blood sera from experimental

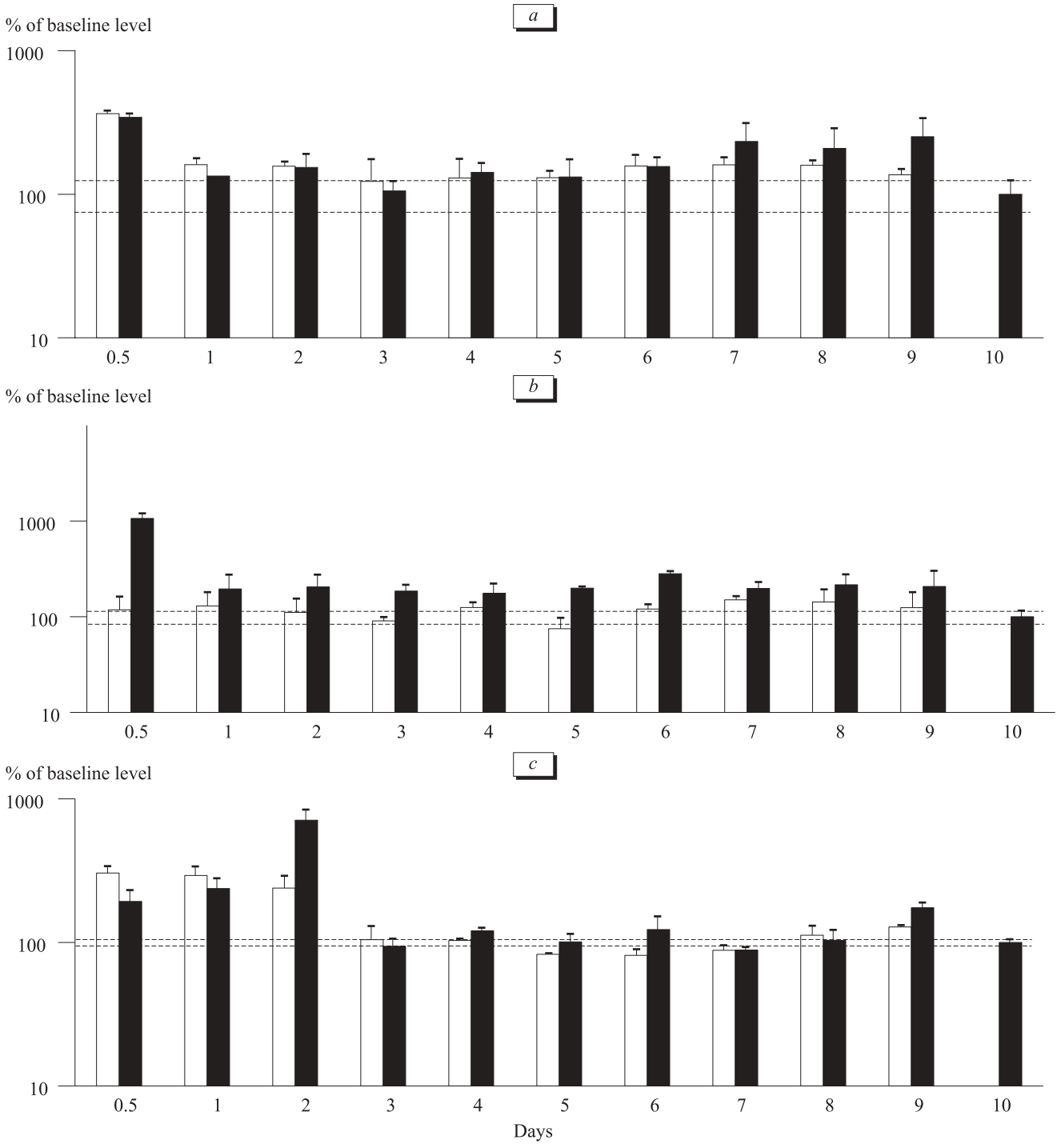

Fig. 2. Serum erythropoietin concentration in CBA/CaLac mice during hypoxia in a sealed chamber (*a*), hemolytic anemia (*b*), and acute blood loss (*c*) not producing injury in CNS (light bars) or accompanied by the development of encephalopathy (dark bars). Ordinate: logarithmic scale.

animals [6]. EPO concentration was measured by enzyme immunoassay with Biomerica Inc. kits. The results were analyzed by methods of variational statistics (Student's *t* test, nonparametric Wilcoxon—Mann—Whitney *U* test).

RESULTS

The intensity of colony formation in a test system (EPA) increased on days 1-5, 9, and 10 after hypoxic hypoxia not producing damage to the central nervous system (CNS). EPO concentration increased 12 h and 1, 2, and 6-9 days after treatment. In the early period after hypoxic hypoxia accompanied by encephalopathy, serum EPA increased more significantly than EPO concentration (days 2-4). However, an opposite relationship was revealed between changes in the study parameters during the follow-up period (Figs. 1, *a*, 2, *a*).

The yield of erythroid colonies in methylcellulose supplemented with serum (EPA) significantly increased in various periods after hemolytic anemia induced by phenylhydrazine in a dose of 30 mg/kg. Serum EPO concentration significantly increased only on day 7. Administration of the hemolytic poison in a dose of 150 mg/kg produced psychoneurological dysfunction, increased serum EPO concentration (various periods of observations), and decreased total EPA of the serum (day 8, Figs. 1, *b*, 2, *b*).

EPA and EPO concentration underwent various changes during hypoxia produced by blood loss. Serum EPA increased more significantly than EPO concentration after effusion of 30% CBV. Serum EPO concentration surpassed the baseline level only 12 h and 1-3 and 9 days after treatment. Effusion of 70% CBV was followed by the development of brain dysfunction in the posthemorrhagic period. EPO concentration increased less significantly 12 h and 1-2 days after treatment. However, changes in blood EPA were similar under these conditions. The observed changes were probably associated with microcirculatory disturbances in the kidneys due to hemorrhagic shock and impairment of hemopoietin release into the vascular bed. Serum EPA and EPO concentration in animals with encephalopathy significantly increased in the follow-up period, which was related to severe oxygen deficiency. It should be emphasized that EPA increased more significantly than EPO concentration (Figs. 1, *c*, 2, *c*).

Changes in study parameters were compared on different models of hypoxia with similar blood changes [4,5,8]. Variations in serum EPA were similar, while changes in serum EPO concentration differed under various conditions of hypoxia.

Published data show that erythrocyte degradation products stimulate the erythroid hemopoietic stem [3], while proinflammatory cytokines inhibit erythropoiesis [7]. Hypoxic exposure is followed by a significant increase in serum level of phlogogenic substances (including proinflammatory cytokines) [9,11] and activation of erythrodieresis [2,10]. The discrepancy between serum EPA and EPO concentration during oxygen deficiency of different genesis and severity was probably associated with variations in the intensity of hemolysis and inflammatory reaction [9,11].

Our results suggest that EPO concentration and EPA determine adaptive response of the erythron to oxygen deficiency.

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