Mechanisms of Changes in the Hemoglobin Profile during Acute Adaptation to Extreme Conditions

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We studied changes in rat hemoglobin isoforms during acute adaptation to various hypoxic conditions. Erythrocytes were heterogeneous in the content of various hemoglobin isoforms. Changes in the hemoglobin profile during hypoxia are related to variations in the ratio between populations of erythrocytes differing in the content of individual hemoglobin isoforms.

Key Words: *hemoglobin isoforms; adaptation; erythrocyte heterogeneity*

Much attention was given to adaptive reactions of the erythroid hemopoietic stem to extreme factors. However, the mechanisms of changes in the erythron are poorly understood. Changes in the ratio between individual hemoglobin isoforms under extreme conditions remain unknown.

In most mammals hemoglobin is presented by several isoforms [5]. Hemoglobins A, A_2, and F are present in humans. Rats, goats, and cattle contain 6, 3, and 4 types of hemoglobin, respectively [5]. Isoforms of this protein differ in electrophoretic mobility, resistance to acids and alkalies, time for maximum synthesis during ontogeny, and affinity for oxygen [1,5]. Changes in the oxygen conditions are accompanied by a shift in the ratio between hemoglobin fractions [5-9]. Taking into account various functional characteristics of isoforms [1], these changes can be considered as compensatory. The mechanisms of these reactions are unknown. The data regarding heterogeneity of hemoglobin under the influence of extreme factors concern the conditions accompanied by marked stimulation of erythropoiesis. To evaluate the mechanism of changes in hemoglobin, it is important to determine variations in the ratio between its isoforms over the first hours of exposure to extreme conditions. In this period the intensity of erythropoiesis remains practically unchanged, and erythron reactions are associated with redistribution of cells.

Here we studied the mechanisms of changes in the hemoglobin system over the first hours of adaptation to acute massive blood loss (AMBL) and hypoxic hypoxia (HH).

MATERIALS AND METHODS

Experiments were performed on 120 male outbred albino rats weighing 150-200 g.

AMBL was produced by withdrawal of the blood from the caudal vein (2% body weight). The erythron system was assayed 6 h and 6 days after treatment.

Intermittent HH was modeled in an altitude chamber. The pressure in the chamber was 40.98 kPa, which corresponded to 7000 m above sea level. HH sessions were performed daily for 6 h (9.00-15.00) [2]. Parameters of the erythroid hemopoietic stem were studied after the 1st and 7th exposure to HH. Intact animals served as the control.

The ratio between hemoglobin fractions was estimated by electrophoresis in polyacrylamide gel [3, 5,6]. This method for separation of rat hemoglobin yields 6 fractions. These fractions were consecutively numbered from the anode to cathode [5,6]. We estimated the count of blood erythrocytes carrying acid-resistant isoforms of hemoglobin [10].

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Peripheral blood erythrocytes were assayed on a MICROX hematological analyzer (Hoffman La Roche). Reticulocytes were counted in peripheral blood smears stained with brilliant cresyl blue (vital stain). The state of bone marrow erythropoiesis was estimated by myelograms.

The results were analyzed by parametric tests using Microsoft Excel software. We calculated the arithmetic mean and standard error. The significance of differences was evaluated by Student's *t* test.

RESULTS

The count of blood erythrocytes decreased from 7.94±0.13×10^{12} to 5.24±0.17×10^{12} cells/liter 6 h after AMBL (p<0.001). The amount of total hemoglobin and its fractions (except for fraction 6) decreased in this period. The content of individual hemoglobin fractions underwent different changes. The content of fractions 5, 1, and 2 decreased by 18.9, 50.7, and 47.4%, respectively, compared to the control (Table 1). Phlebotomy is accompanied by similar loss of various blood cells. These data suggest that 6 h after treatment erythrocytes containing hemoglobin fractions 5 and 6 enter the circulation, while cells with fractions 1 and 2 are eliminated from the peripheral blood.

After the first exposure to 6-h HH the count of peripheral blood erythrocytes significantly decreased, while the total amount of hemoglobin remained unchanged (Table 1). The concentration of hemoglobin fractions 5 and 6 increased, but the content of fractions 2 and 3 decreased. The content of hemoglobin isoforms 1 and 4 did not change (Table 1).

Our results indicate that erythrocytes containing considerable amounts of hemoglobin isoforms 5 and 6 entered the circulation, while cells with fractions 2 and 3 were eliminated from the blood.

In myelogram the count of bone marrow erythroid precursors decreased from 8.02±0.73×10^{6} to 4.66±0.23×10^{6} cells/100 g 6 h after AMBL (p<0.001). After 6-h HH the number of these cells decreased to 4.90±0.27×10^{6} cells/100 g (p<0.001). These changes were probably associated with accelerated maturation of erythroid cells. After the first exposure to HH we observed moderate reticulocytosis (260±10×10^{9} *vs.* 180±20×10^{9} cells/liter in the control, p<0.001). The count of peripheral blood reticulocytes remained unchanged 6 h after AMBL, which was probably related to the loss of cells during this treatment. On day 6 after blood withdrawal the counts of bone marrow erythroid cells (14.41±1.10×10^{6} cells/100 g, p<0.05) and blood reticulocytes surpassed the control (980±120×10^{9} cells/liter, p<0.001).

After the 7th exposure to HH the count of bone marrow erythroid cells increased to 10.91±0.55×10^{6} cells/100 g (p<0.05), which was accompanied by the development of reticulocytosis (1020±60×10^{9} cells/liter, p<0.001). Changes in myelograms and peripheral blood reticulocytes at the late stage of exposure to extreme factors reflect activation of erythropoiesis.

The increase in the concentrations of circulating isoforms 5 and 6 coincided with the development of reticulocytosis and changes in myelogram. These data suggest that bone marrow erythrocytes serve as the source of these hemoglobin isoforms. It is unlikely that additional amounts of these fractions are produced over the first 6 h after treatment, since activation of globin synthesis and appearance of newly formed hemoglobin in the blood require longer time [8,9,11]. For example, erythropoietic stimuli activate the synthesis of hemoglobin C in sheep, but the period between stimulation and start of globin synthesis is 3-5 days [8,9]. Probably, isoforms 5 and 6 of rat hemoglobin entering the circulation over the first hours of HH are pre-synthesized under physiological conditions, but not formed during hypoxia. Therefore, cells with high amount of hemoglobin fractions 5 and 6 are deposited in the body. Under extreme conditions these cells enter the circulation.

Fractions 5 and 6 of rat hemoglobin are highly resistant to acids [5]. Acid-containing fractions of hemoglobin were irregularly distributed in erythrocytes.

TABLE 1. Content of Hemoglobin and Its Fractions in Rats after AMBL and during Intermittent HH (g/liter, *M*±*m*)

Hemoglobin		Intact rats	Blood loss	Hypoxia
Total		135.76±3.20	86.71±6.40*	130.20±2.94
Fractions	1	14.99±0.70	7.39±0.79*	15.29±0.49
	2	22.36±1.11	11.76±1.32*	19.36±0.52**
	3	58.95±1.70	37.14±2.33*	49.48±1.62*
	4	24.90±0.74	17.16±1.63*	25.11±1.13
	5	12.16±0.52	9.86±0.85**	15.01±0.53**
	6	3.28±0.24	3.41±0.22	5.94±0.30*

Note. *p<0.001 and **p<0.05 compared to intact animals.

They were found only in 0.74±0.11% erythrocytes ($0.061±0.008×10^{12}$ cells/liter). These results are consistent with published data [5]. Changes in the count of cells with acid-resistant hemoglobin fractions and variations in the concentration of fractions 5 and 6 were similar during HH and AMBL. Our results confirm the assumption that cells containing considerable amounts of these fractions enter the circulation under extreme conditions. The content of hemoglobin fractions 2 and 3 decreased after the first exposure to HH. Moreover, the concentrations of isoforms 1 and 2 markedly decreased over the first hours after AMBL. These data indicate that erythrocytes are characterized by considerable differences in the content of hemoglobin isoforms.

Our results suggest the existence of mechanisms for selection of these erythrocytes in the circulation and hemopoietic organs. Probably, erythrocytes differ not only in the type of hemoglobin, but also in other characteristics associated with cell membranes.

REFERENCES

1. M. V. Borisyuk, *Usp. Fiziol. Nauk*, **14**, No. 1, 85-101 (1983).
2. *Hypoxia. Adaptation, Pathogenesis, and Clinics* [in Russian], St. Petersburg (2000).
3. G. Maurer, *Disk Electrophoresis. Theory and Practice of Electrophoresis in Polyacrylamide Gel* [in Russian], Moscow (1971).
4. A. D. Pavlov and E. F. Morshchakova, *Regulation of Erythropoiesis* [in Russian], Moscow (1987).
5. N. F. Starodub and V. I. Nazarenko, *Heterogeneous System of Hemoglobin. Structure, Properties, Synthesis, and Biological Role* [in Russian], Kiev (1987).
6. N. F. Starodub, V. P. Artyukhov, A. N. Gritsak, and Yu. L. Radavskii, *Ukr. Biokhim. Zh.*, **51**, No. 2, 117-129 (1979).
7. H. Bard, J. C. Fourton, C. Gagnon, and G. Gagnon, *J. Pediatr.*, **124**, No. 6, 941-943 (1994).
8. J. E. Barker, *Blood*, **56**, 495-500 (1980).
9. J. E. Barker, J. E. Pierce, and A. W. Nienhuis, *Cellular and Molecular Regulation of Hemoglobin Switching*, Eds. G. Stamatoyannopoulos and A. W. Nienhuis, New York (1979), pp. 179-192.
10. E. Kleihauer, T. E. Tang, and K. Betke, *Acta Haematol.*, **38**, 264-272 (1967).
11. T. Umemura, A. Al-Khatti, T. Papayannopoulou, and G. Stamatoyannopoulos, *Proc. Natl. Acad. Sci. USA*, **23**, 9278-9282 (1988).