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Activity of Heme Synthesis Enzymes in the Bone Marrow and Liver of August and Wistar Rats during the Neonatal Period and after Acute Postnatal Hypoxia

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Activity of heme synthesis enzymes in newborn August and Wistar rats was studied after acute hypoxic hypoxia. Daily production of erythrocytes and activities of aminolevulinate synthase, aminolevulinate dehydratase, and heme synthetase were measured in the bone marrow (15-30 min after birth and on days 1 and 3 of life) and liver (day 3 after birth). Hypoxia was followed by a decrease in activity of heme synthesis enzymes in the liver (especially in August rats) and reduction of the daily erythrocyte production (especially in Wistar rats). Our results suggest that the response of heme synthesis enzymes to hypoxic exposure in newborn rats is genetically determined. The observed changes are more pronounced in Wistar rats.

Key Words: *heme synthesis enzymes; acute hypoxia; neonatal period; August and Wistar rats*

Hypoxia of the fetus and newborn is one of the most common events in obstetrical, gynecological, and neonatal practice. Hypoxia is a universal regulator of energy processes under normal conditions and the cause of cell metabolism disturbances in various pathologies. Activation of erythrocyte production in the bone marrow aimed at increase in blood oxygen capacity is a compensatory response to reduced oxygen concentration in mammalian tissues. Heme-containing proteins perform a general function of oxygen sensors, since they determine the chemoreceptor properties of cells [6,9]. Aminolevulinate synthase (ALA synthase) is the major regulatory enzyme of in the system of heme synthesis enzymes (HSE). Tissue hypoxia is a positive modulator of ALA synthase (induces synthesis of this enzyme).

Previous studies showed that August and Wistar rats exhibit different genetically determined reactions to toxic agents. Moreover, these animals are characterized by different capacity for the adaptive response [1,2]. However, hypoxia-induced variations of the HSE system in newborn rats of these strains remain unknown.

This work was designed to study activity of HSE in the bone marrow and liver of August and Wistar rats during the neonatal period and after acute hypoxic hypoxia.

MATERIALS AND METHODS

The sensitivity of August and Wistar rat pups to hypoxic exposure was estimated on the model of early postnatal hypoxia. The animals were maintained in a chamber at 95% N_2 and 5% O_2 for 40 min immediately after birth.

Outbred Wistar rats and inbred August rats were obtained from the Rappolovo and Stolbovaya Breeding Centers, respectively. Experiments were conducted in accordance with the ethics rules and recommendations of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes. The dynamics of erythropoiesis

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was assayed randomly in animals from different litters (1 rat pup from each litter). Activity of heme-synthesizing enzymes was studied in all animals from the litter. In this case, each litter was considered as one animal.

ALA synthase activity was measured by the Kikuchi method with modifications [7]. ALA dehydratase activity was estimated as described previously [5]. Heme synthetase activity was studied as described elsewhere [8]. Enzyme activities in the bone marrow were evaluated 15-30 min after birth and on days 1 and 3 of life. These indexes in the liver were determined on day 3 after birth, which is related to suppression of erythropoietic function of the liver [4]. The daily production of erythrocytes was estimated.

The results were processes with Statistica 6.0 software. The data were analyzed by methods of variation statistics and expressed as the arithmetic mean (M) and its standard error (m). The significance of differences was evaluated by nonparametric tests. Differences in average tendencies were determined by Mann–Whitney U test for independent samples with small number of observations (n<20). The differences were significant at p<0.05.

RESULTS

Reorganization of erythropoiesis in rat pups is associated with the replacement of circulating erythrocytes. Daily erythrocyte production in August rats (but not in Wistar rats) remained practically unchanged over the first 3 days after birth (neonatal period). Hypoxia decreased daily production of erythrocytes, especially in Wistar rats (Fig. 1).

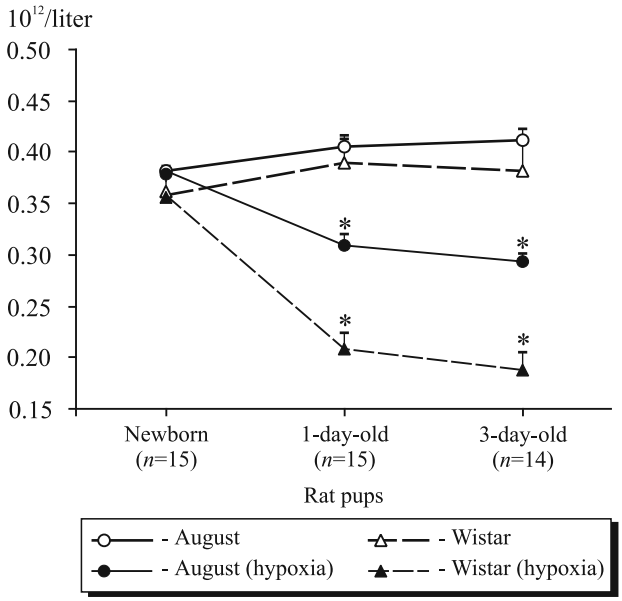

Fig. 1. Daily production of erythrocytes in August and Wistar rats under normal conditions and after acute hypoxic hypoxia. *p<0.05 compared to the control.

These changes were accompanied by a decrease of HSE in the bone marrow of August and Wistar rats. Our results are consistent with published data on suppression of erythropoiesis immediately after birth [4] and 5-fold decrease in erythropoietin level over the first 3 days [3]. The decrease in enzyme activities was most pronounced in Wistar rats (Table 1), which is consistent with a greater reduction of HSE in the bone marrow.

The degree of a hypoxia-induced decrease in the daily production of erythrocytes during the neonatal

TABLE 1. HSE Activity in the Bone Marrow of August and Wistar Rats under Normal Conditions and after Acute Hypoxic Hypoxia (M±m)

Experimental conditions		Age, days	ALA synthase, μmol/liter/sec	ALA dehydratase, μmol/liter/sec	Heme synthetase, nmol/liter/sec
Control	August (n=20)	0	3.860±0.105	3.650±0.106	0.380±0.016
	Wistar (n=20)		3.780±0.093	3.460±0.152	0.360±0.021
	August (n=20)	1	3.510±0.112	2.630±0.189	0.350±0.015
	Wistar (n=20)		3.490±0.089	2.520±0.182	0.330±0.019
	August (n=16)	3	2.990±0.109	1.800±0.050	0.320±0.016
	Wistar (n=16)		3.270±0.084	1.780±0.043	0.220±0.022
Hypoxia	August (n=16)	1	1.800±0.052*	1.710±0.057*	0.110±0.005*
	Wistar (n=16)		1.810±0.045*	1.810±0.038*	0.100±0.004*
	August (n=15)	3	0.960±0.086*	0.990±0.057*	0.140±0.007*
	Wistar (n=15)		0.910±0.073*	0.680±0.026*	0.160±0.004*

Note. Here and in Table 2: *p<0.05 in comparison with the corresponding index in the control.

TABLE 2. HSE Activity in the Liver of August and Wistar Rats under Normal Conditions and on Day 3 after Acute Hypoxic Hypoxia (*M*±*m*)

Parameter	August		Wistar	
	control	treatment	control	treatment
ALA synthase, nmol/mg of protein/min	0.150±0.007	0.090±0.002*	0.240±0.004	0.190±0.009*
ALA dehydratase, nmol/mg of protein/min	0.120±0.004	0.060±0.006*	0.060±0.006	0.050±0.003*
Heme synthetase, pmol/mg of protein/min	11.3±1.0	7.40±0.31*	12.90±0.46	10.700±0.069*

period was shown to differ in August and Wistar rats (by 1.3 and 2 times, respectively). It was probably related to variations in the maturity of newborn animals and, therefore, different sensitivity to hypoxia and activation of compensatory mechanisms.

Acute hypoxia during this period induced a sharp decrease in activity of ALA synthase and reduction of heme synthetase activity, which is most sensitive to the impairment of oxidative phosphorylation. Heme synthetase activity progressively returned to normal on day 3 of the study, which is probably associated with adaptation to hypoxia. Hypoxia in August and Wistar rats was followed by a decrease in activities of ALA synthase (by 4 times) and heme synthetase (by 2.7 and 2.4 times, respectively; Table 2).

HSE activities in the liver of August and Wistar rats decreased by 1.5-2 times and 20%, respectively.

August and Wistar rats are characterized by different activities of HSE in the bone marrow and liver. The decrease in activities of HSE enzymes during hypoxia is primarily associated with activation of free radical oxidation, whose products can suppress functions of various enzymes.

Our results confirm the genetic determinacy of reactions to hypoxia. Interstrain differences in the sensitivity of newborn rats to acute hypoxia are similar to those in adult animals. They are especially pronounced in Wistar rats.

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