Content of 2,3-Diphosphoglycerate and Adenosine Triphosphate in Erythrocytes and the Acid-Base Balance in Adult and Newborn Rats during Acute Hypoxia

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It is shown that in deep acute hypoxic hypoxia the 2,3-diphosphoglycerate content is reduced and the adenosine triphosphate content unchanged in the erythrocytes of newborn rats. Under the same conditions adult animals show an increase of 2,3-diphosphoglycerate and a drop in the adenosine triphosphate content in the erythrocytes. The importance of these changes is discussed in terms of the mechanisms regulating the oxygen-transporting function of erythrocytes during acute hypoxic hypoxia in newborn and adult animals.

Key Words: *hypoxia; 2,3-diphosphoglycerate; adenosine triphosphate; erythrocytes; alkalosis; acidosis; newborn and adult animals*

The characteristics of changes in the 2,3-diphosphoglycerate (2,3-DPG) concentration in erythrocytes have been described for adult rats [1] and young animals at the end of the first week of life [6] during various regimes of acute hypoxic hypoxia. However, a comparative analysis of the 2,3-DPG and adenosine triphosphate (ATP) content in the erythrocytes of adult and newborn rats subjected to similar conditions of acute hypoxic hypoxia has not yet been conducted.

The goal of the present study was to estimate the 2,3-DPG and ATP content in the erythrocytes of newborn and adult rats during deep acute hypoxic hypoxia and to assess the blood acid-base status in the dynamics of development of oxygen deficiency.

MATERIALS AND METHODS

The investigation was carried out on 60 adult male rats weighing 180-300 g and on 260 2-3-day-old animals weighing 8 g on average. Rat pups of the same litter were used in the control and experiment. Hypoxic hypoxia was induced by placing the animals in heated polymethylmetacrylate chambers into which humidified nitrogen was continuously administered. The oxygen content in the chambers was recorded by a polarographic method using a closed Clark electrode (Table 1).

Three series of experiments were conducted. In the 1st series adult rats were kept in the chamber at 22°C. In the 2nd and 3rd series newborn animals were held at 36.5°C (nearly neutral temperature) and 30°C, respectively. Control animals were kept in a normal atmosphere under the same temperature regimes. For 20 adult and 22 newborn rats subjected to hypoxia the survival time was recorded starting from the beginning of nitrogen input up to apnea. Parameters of acid-base status were recorded in the arterial blood using a BME-33 device (Radiometer). Blood was obtained during the breathing of the hypoxic gas mixture by means of puncture or cannulation of arteries.

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The 2,3-DPG and ATP content in the erythrocytes was measured using enzymatic methods described in our previous report [6]. Erythrocytes were obtained from blood that was taken approximately 5 min before apnea. Blood samples from 3-4 pups were pooled before conducting the analysis.

RESULTS

The dynamics of parameters of the blood acid-base status (1st series, Table 1) in the course of an escalating acute hypoxic hypoxia shows the development of respiratory alkalosis and of partly compensated metabolic acidosis accompanying the alkalosis at the stage of pronounced oxygen deficiency. In the newborns of the 2nd and 3rd series, short-term periods of Pco_2 reduction and the development of profound decompensated metabolic acidosis are detected. Survival times under the hypoxic conditions in the 1st, 2nd, and 3rd series were 39.2±1.8 min, 46.2±2.8 min, and 60.5±1.15 min, respectively.

These data are in agreement with current notions regarding the age-related peculiarities of thermoregulation in the rat [5] and the ability of newborn animals to lower the rate of tissue respiration during hypoxia [4,5] and to use ATP for anaerobic glycolysis [7]. As is shown in Table 2, at the 35th min of increasing hypoxia the content of 2,3-DPG in the erythrocytes of adult rats had increased by 23%, while the ATP concentration had fallen by 25%. In the newborns (2nd and 3rd series) the erythrocyte content of 2,3-DPG was by 15% and 33% decreased 40 and 55 min after the start of hypoxic hypoxia. The newborn rats of both series exhibited no significant changes in the erythrocyte ATP concentration.

The detected increase in the 2,3-DPG content in the erythrocytes of adult rats under conditions of acute hypoxic hypoxia is apparently due to an increased synthesis of this side metabolite of glycolysis as a result of a rise of the intracellular pH [2]. It is known that an increase of the concentration of 2,3-DPG, which is the main modulator of hemoglobin affinity for oxygen, is accompanied by a shift of the oxyhemoglobin dissociation curve. Moreover, the increase of 2,3-DPG as an intracellular anion under conditions of alkalosis prevents the Bohr's alkaline effect-induced rise in the blood affinity for oxygen. It has been shown that an increase in blood P_{50} in rats represents a compensatory mechanism enhancing tissue oxygenation under conditions of moderate hypoxemia [14]. At the same time, according to several reports, the increase of the oxygen affinity of the blood in rats makes for a longer survival time against the background of deep hypoxic hypoxia due to enhanced saturation of hemoglobin with oxygen in the lungs [9,13]. In this connection it is possible that the increased 2,3-DPG level in rat erythrocytes at stages of a marked and quite rapid reduction of the oxygen content in the gas mixture may contribute to the failure of the adaptation processes, thus adversely affecting the oxygenation of tissues owing to insufficient hemoglobin saturation with oxygen in the lungs.

The drop in the ATP content in rat erythrocytes during hypoxic hypoxia is probably connected with increased utilization of 1,3-DPG for 2,3-DPG synthesis and with an increase of erythrocyte Na^+,K^+-ATPase activity [3,6]. ATP has a lower ability to directly influence the hemoglobin affinity for oxygen as compared with 2,3-DPG [2]. At the same time, the lowering of the ATP

Table 1. Acid–Base Status of Adult and Newborn Rats in the Course of Escalating Hypoxia ($M \pm m$)

Series	Time of testing, min	Number of animals tested	pH	Buffer bases, mmol/liter	Pco_2, mm Hg	Oxygen content in the inhaled mixture, %
I	0	8	7.437±0.0177	−1.1±1.27	30.6±1.38	20.9±0.05
	10	5	7.483±0.0111*	−1.0±1.44	29.5±1.54	15.2±0.37
	20	6	7.525±0.0233*	−4.3±1.38	21.9±0.65	9.4±0.22
	35	6	7.397±0.0439°	−14.0±2.04*°	16.8±1.745*°	4.3±0.20
II	0	7	7.360±0.0408	−4.9±2.40	34.3±2.42	20.9±0.05
	10	6	7.419±0.0363	−8.7±2.77	25.0±3.23*	15.2±0.37
	20	5	7.289±0.0696	−10.4±2.75	31.2±2.90	9.4±0.22
	40	6	6.642±0.1426*°	−25.7±1.34*°	31.1±2.00	3.3±0.20
III	0	6	7.372±0.0268	−4.2±1.19	35.6±2.37	20.9±0.05
	20	5	7.377±0.0241	−5.3±2.34	34.1±2.79	9.4±0.22
	40	5	7.320±0.0284	−10.3±1.77	28.4±2.70*	3.3±0.20
	55	8	6.853±0.04*°	−25.1±0.91*°	38.7±3.15°	1.6±0.24

Note. *: Reliability of differences from the control; °: reliability of differences from the preceding index ($p<0.05$).

Table 2. 2,3-DPG Content in the Erythrocytes of Adult and Newborn Rats in Acute Hypoxia ($M \pm m$)

Group tested	Series	2,3-DPG	ATP
		mmol/liter erythrocytes	
Control	I	5.3±0.33 (16)	0.93±0.019 (12)
Experiment	I	6.5±0.30* (16)	0.70±0.029* (12)
Control	II	2.0±0.10 (11)	1.9±0.07 (11)
Experiment	II	1.7±0.07* (11)	1.7±0.07 (11)
Control	III	1.8±0.17 (11)	2.4±0.15 (11)
Experiment	III	1.2±0.10* (11)	2.0±0.14 (11)

Note. *$p<0.05$ as compared with the control. Number of assays in parentheses.

level in rat erythrocytes can inhibit the energy supply of these cells and, accordingly, induce alterations in their functional status under conditions of hypoxic hypoxia.

It should be stressed that erythrocytes circulating in the blood of newborn rats do not contain fetal hemoglobin but are mainly large anuclear fetal cells characterized by high activity of phosphofructokinase and pyruvate kinase and extremely low activity of 2,3-diphosphoglyceratemutase [11]. It is thought that these peculiarities in the activity of glycolysis enzymes in fetal erythrocytes are responsible for the increased ATP concentration and extremely low 2,3-DPG content, thus enhancing the oxygen affinity of the blood in the early period of life [10,11]. From the first day after birth fetal erythrocytes are gradually replaced by small forms with a glycolysis-associated enzymatic activity similar to that of the erythrocytes of adult rats [11].

The disclosed decrease of the 2,3-DPG content in the erythrocytes of newborn rats against the background of the development of pronounced metabolic acidosis in the course of acute hypoxic hypoxia is apparently connected with the inhibition of the activity of glycolysis enzymes that participate in 2,3-DPG synthesis in small erythrocytes. The acidosis-associated decrease in 2,3-DPG is known to retard the lowering of hemoglobin affinity for oxygen (Bohr's effect [8]). However, the degree of participation of 2,3-DPG in regulating the oxygen affinity of hemoglobin in the small erythrocytes of the experimental newborn rats in all probability does not match the rate of the rise of metabolic acidosis under the given conditions of acute hypoxic hypoxia.

The lack of changes in ATP concentration in the erythrocytes of the experimental newborns may be a consequence of the incomplete acidosis-induced inhibition of catalytic reactions responsible for ATP synthesis in the fetal erythrocytes, due to their high activity of glycolysis enzymes. It is plausible to assume that the low values of P_{50} in the fetal erythrocytes of newborn animals play a compensatory role in the mechanisms of tissue oxygenation during hypoxic hypoxia, promoting a more complete saturation of hemoglobin with oxygen under conditions of its low environmental concentration and imperfect functioning of pulmonary respiration in rat pups [12].

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