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The Fragility Studies of the Main Erythroid Cell Fractions of Rats During Hypoxia

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Abstract: The fragility of the main erythroid cell fractions of the peripheral blood of male albino rats was investigated under hypoxic conditions. Hypoxia was induced by "raising" the animals to an imaginary height of 5000 m above sea level through 10 days. Erythroid cells were fractionated into populations using the sucrose solution concentration gradient method. Cell fragility was studied using the kinetics of acid-induced hemolysis of red blood cells. Increased resistance of cell populations to hemolysis was recorded in the critical periods of adaptation, with a tendency towards normalization of cellular fragility in late periods of exposure to hypoxia.

Key words: Barocamera, erythrocyte, erythrogramme, hemolysis, hypoxia

INTRODUCTION

Oxygen deficiency in tissues of the organism, hypoxia, comes in many forms (Bacroft, 1920; Van Leer and Stikney, 1957). Of these, hypoxic hypoxia, characterized by low partial pressure of oxygen in the inspired air, constitutes one of the most complex forms of hypoxia. This is because the mechanism of its action and the patho-physiological reactions of compensation of the deranged functions reside both outside and inside the erythroid cell system.

At the cytological level, the organism responds to hypoxic hypoxia by polycythemia, accomplished through stimulation of erythropoiesis and mobilization of blood from the spleen (Simanovsky, 1971; Voitakovich, 1973; Necas and Neuwirt, 1970; Lechermann and Jelkmann, 1985).

Associated with polycythemia is the liberation into the blood stream of nucleated red blood cells with increased resistance to hemolysis (Matzimin, 1979; Mashkin and Terskov, 1986).

Fragility studies conducted by us (work now in press) on the main erythroid cell populations, namely the 4th and 5th cell fractions occupying the 18 and 14% sucrose concentration zones, showed that hypoxia elicited increased resistance of these cell fractions to hemolysis in the critical periods of action of hypoxia. This research presents, the metabolic changes probably responsible for the effect and their role in the adaptation process.

MATERIALS AND METHODS

Male albino rats of body weights between 160 and 200 g were used in the experiment. The animals were divided into 2 groups, the control and the experimental groups. While, the control group animals were acclimatized under normoxic conditions of the animal house, those of the experimental group were subjected to altitude hypoxia simulated in a hypoxic cage or barocamera. The hypoxic cage has three key components: a special vacuum pump which sucks out air from the cage chambers; an adjustment value with which air pressure within the cage is regulated; a barometer specially calibrated to register the pressure in the cage chamber as height in metres above sea level. Experimental animals were placed in the cage chamber, exposed to an imaginary height of 5000 m above sea level and maintained there for 3 h each day. They were sacrificed and blood samples obtained from them by cardiac puncture after the 1st, 3rd, 5 and 10th days of hypoxic exposure, with heparin serving as anticoagulant.

All operations were conducted in the cold (0±4°C) plasma-free Red Blood Cell (RBC) suspensions were obtained by washing fresh heparinized blood samples from animals thrice with physiological saline (0.85% NaCl), each washing operation accompanied by centrifugation at 2,500 rev. min^{-1} for 5 min RBC suspensions were separated into fractions using the sucrose solution concentration gradient technique (Sizova *et al*., 1980). The 4th and 5th cell fractions situated at the 18 and 14%

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concentration zones were isolated and washed thrice with physiological saline (with centrifugation at 2,500 rev. min^{-1} for 5 min each time) to free the RBC from sucrose solution. RBC hemolysates were obtained from the fractions by freeze-thaw in liquid nitrogen followed by ultracentrifugation at 18,000 rev. min^{-1} for 15 min to remove cellular debris.

RBC hemolysates so obtained and which contained all the intraerthrocytary ingredients, were used for enzyme assay. The levels of Hexokinase (HK), Lactate Dehydrogenase (LDH) and Glucose-6 Phosphate Dehydrogenase (G6-PD) were determined as described (Chapman *et al*., 1962). The concentrations of glucose and lactate were determined using the glucose oxidase method and sulphuric acid-acetal method as described in the works of Kucherenko *et al*. (1988) and Hmelevsky (1985). The results obtained were statistically analysed and level of significance evaluated using the student's t-test.

RESULTS AND DISCUSSION

The Table 1 contains information on the effect of barocamera hypoxia on the process of carbohydrate metabolism in the erythroid cells of the 4th and 5th fractions of rat peripheral blood. Hypoxia elicited significant increases ($p<0.1$) in the activities of enzymes of glucose metabolism in the 4th and 5th cell populations in the 3rd and 5th days of the adaptation process. While, the concentration of glucose was significantly decreased ($p<0.1$), that of lactic acid was significantly increased ($p<0.1$) in the 3rd and 5th days. A tendency towards normalization of the activities of the enzymes and concentrations of metabolites was observed in the 10th day of adaptation.

Deficiency of oxygen in the inspired air has been reported to initiate a myriad of changes in some functional parameters of the organism (Charny, 1961; Donald and David, 1989; Roderick, 2004; Hackworth *et al*., 2005; Vachiano *et al*., 2004). One of the early reactions of an organism to hypoxia is pulmonary hyperventilation which manifests externally as panting (Voitkovich, 1973; Vachiano *et al*., 2004). Increase in frequency of the respiratory act occasioned by panting leads to increased evacuation of CO_2 to the exterior. The acid reserve of the blood thus becomes depleted, resulting in respiratory alkalosis. Elevation of blood pH has been reported as one of the responses of the organism to hypoxia (Panim and Govorov, 1971). Alkalosis leads to increased affinity of haemoglobin to oxygen (Lenfant and Torrace, 1971; Eaton *et al*., 1974; Thomas *et al*., 1974; Ekpo, 2001). While, this allows the haemoglobin to pick up the little oxygen available in the lungs in hypoxic conditions on the one hand, this effect on the other hand, inhibits the offloading of the gas from the respiratory protein to tissue cells.

Oxygen starvation thus threatens the organism at the tissue level. There is therefore the need to prevent tissue hypoxia in the organism.

Our data on the process of carbohydrate metabolism in the 4th and 5th erythroid cell populations of the peripheral blood show that hypoxia caused increases in the activities of hexokinase and lactate dehydrogenase in the 3rd and 5th days of adaptation (Table 1). The hexokinase and lactate dehydrogenase reactions occupy the upper and lower limits, respectively, of the RBC glycolysis. While the glucose concentration was significantly decreased in both fractions in the critical periods of adaptation (3rd and 5th days), the concentration of lactic acid was significantly increased during the periods. All these signify increase in the glycolytic rate. Increased glycolytic rate in the liver, muscle, erythrocytes and other cells has also been reported during various forms of hypoxia (Simanovsky and Gierceva, 1968; Myles and Radomsky, 1974; Ferminent, 1981; Pastoris *et al*., 1985; Ekpo, 2003). The observed accumulation of lactic acid leads to acidification of the intraerythrocytary medium surrounding the haemoglobin. This enhances the Bohr's effect, consisting of the decrease in the haemoglobin oxygen affinity by the action of an acid. As observed by Lenfant and Torrace (1971), the haemoglobin affinity to oxygen decreases in late periods of hypoxia. Thus, the

Table 1: Influence of hypoxia on levels of metabolites (μM mL^{-1} of erythr.) and activities of enzymes (μM mL^{-1} erythr. min^{-1}) of carbohydrate metabolism in the RBC of rats (n = 5)

Days	HK	LDH	G-6PD	Glucose	Lactate
Fourth cell fraction					
Control	0.102±0.001	3.36±0.12	1.65±0.02	6.43±0.18	3.86±0.18
1st	0.101±0.002	3.78±0.13*	1.61±0.01	5.27±0.15	3.18±0.15
3rd	0.141±0.010*	4.02±0.01*	1.81±0.04*	3.19±0.19*	5.33±0.24*
5th	0.154±0.012*	3.55±0.13	2.01±0.06*	3.56±0.22*	5.39±0.17*
10th	0.098±0.002	3.48±0.17	1.79±0.08	4.79±0.22	4.01±0.18
Fifth cell fraction					
Control	0.108±0.009	3.91±0.11	1.78±0.04	5.91±0.20	2.76±0.15
1st	0.111±0.006	3.88±0.21	1.71±0.06	6.05±0.31	4.58±0.48*
3rd	0.149±0.007*	4.14±0.04*	2.23±0.04*	3.01±0.25*	5.96±0.36*
5th	0.158±0.003*	3.67±0.17	2.02±0.04	3.33±0.15*	5.81±0.44*
10th	0.101±0.008	3.62±0.11	1.92±0.04	5.88±0.32	3.38±0.0.35

*Data significant at $p<0.1$

observed increase in glycolytic rate with accumulation of lactate in the 4th and 5th cell fractions in the 3rd and 5th days of hypoxia, served an adaptive function, aimed at improving the haemoglobin oxygen delivery to cells in tissues via enhanced Bohr's effect.

The increase in the activity of hexokinase as recorded also implies increased metabolic flux via the pentose phosphate pathway. Our data (Table 1), also show increased metabolic activity for the key enzyme of the pentose phosphate pathway, glucose-6-phosphate dehydrogenase, in the two cell fractions in the critical periods of adaptation. Decreased activity of G-6-PD has been associated with red blood cell hemolysis (Yoshida, 1973, 1977). As the activity of the enzyme is increased in the 3rd and 5th days of hypoxia, more NADPH is produced for the reductase system that maintains the functional integrity of the haemoglobin and the erythrocyte membrane. Lionetti (1974), Kothe *et al.* (1975) and Thorburn and Kuchel (1985). This way, the red blood cells in the 4th and 5th cell fractions are made less fragile to hemolysis in the 3rd and 5th days of hypoxia. The observed increase in the metabolic activity of G-6-PD thus forms the basis of the increased resistance of erythroid cells of the 4th and 5th fractions in the critical periods of adaptation to barocamera hypoxia as recorded in previous study.

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