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Increased Resistance of Erythrocytes to Hemolysis During Hypoxic Hypoxia: Mechanism and Effect

[1]B.O. Ekpo and [2]I. Elekwa
[1]Faculty of Basic Medical Sciences, College of Medicine Sciences Health Sciences,
[2]Department of Biochemistry, Faculty of Biological and Physical Sciences,
Abia State University, Uturu, Nigeria

Abstract: The mechanism underlying the increased resistance of the red blood cell to hemolytic influences, occurring during hypoxic hypoxia was investigated in male albino rats. Hypoxia was induced by "raising" the animals to an imaginary height of 5,000 metres above sea level through 10 days. Metabolic studies were conducted using the 4th and 5th cell populations obtained by fractionation in sucrose solution concentration gradient and situated at the 18% and 14% sucrose concentration zones. The activities of hexokinase, (HK) lactate dehydrogenase (LDH) and glucose-6- phosphate dehydrogenase (G-6PD); and the concentrations of glucose and lactic acid were investigated. Hypoxia caused increased metabolic flux through glycolysis in the cell fractions as observed in elevated activities of HK and LDH, decrease in glucose level ($p<0.1$), and increased concentration of lactic acid in the 3rd and 5th days of adaptation ($p<0.1$). The activity of G-6PD was also increased in the periods. A tendency towards normalization of enzyme activities and concentration of metabolites was observed in the 10th day of hypoxia.

Key words: Barocamera, glucose-6-phosphate dehydrogenase, hexokinase, hypoxia, lactate dehydrogenase

INTRODUCTION

Oxygen is a very important biogenic element in nature. It is the terminal acceptor of reducing equivalents during the mitochondrial oxidation of metabolic substrates in organisms. Concomitant with the process is the synthesis of ATP, which serves as universal fuel for all forms of biological work. The foregoing underscores the importance of adequate and continuous supply of the gas to tissues of the organism.

The erythrocytes form the cytological basis of the system which regulates and maintains oxygen homeostasis in the organism. The erythrocytes perform this function not as a single cellular population but as a heterogeneous population of erythroid cell fractions (Cohen *et al*., 1976; Ekpo, 1999). The gas transport function of the peripheral blood depends on the sensitivity of the erythrocyte fractions to hemolytic influences.

Interest in hypoxia as a common life phenomenon and basis of pathologies dates back to the first half of the 20th century (Merino, 1950; Pugh, 1957). There is, however, renewed interest in the phenomenon nowadays, what with many mechanisms of the compensatory reactions of the organism still obscure and with increasing human activities in space (Roderick, 2004; Vachiano *et al*., 2004; Hackwort *et al*., 2005). The present research highlights the adaptive significance of changes in the fragility of the major erythroid cell populations of the peripheral blood following the exposure of rats to altitude hypoxia, simulated in the barocamera.

MATERIALS AND METHODS

Male albino rats of body weights between 160 and 200 g were used in the experiment. The animals were divided into two 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 barocamera or hypoxic cage. The hypoxic cage has 3 key components, namely, a special vacuum pump which sucks out air from the cage chamber; an adjustment value with which air pressure within the cage is regulated; and a barometer specially calibrated to register the pressure in the cage as "height" in metres above sea level. Experimental animals were placed in the cage chamber, exposed to an imaginary height of 5000 m

Corresponding Author: I. Elekwa, Department of Biochemistry, Faculty of Biological and Physical Sciences, Abia State University, Uturu, Nigeria

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, 5th and 10th days of hypoxic exposure, with heparin serving as anticoagulant.

All operations were conducted in the cold (O°C±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 being accompanied by centrifugation at 2.500 rev min^{-1} for 5 min. RBC suspensions were separated into populations using the sucrose solution concentration gradient technique (Sizova *et al*., 1980). Fragility test was conducted with cell suspensions using the erythrogramme method, consisting of time monitoring of the kinetics of HCl-induced erythrocyte hemolysis (using 0.001N HCl) according to Gitelzon and Terskov (1969), which is also described elsewhere (Ekpo, 2005). Fragility test was conducted with the un-fractionated blood and with the two main erythroid cell populations which occupy the 18 and 14% sucrose concentration zones. Results obtained were statistically analysed and level of significance evaluated using the student's t-test.

RESULTS

Table 1 contains information on the influence of barocamera hypoxia on the fractional composition of erythroid cells of the peripheral blood of rat. Hypoxia caused significant decreases ($p<0.01$) in the populations of erythroid cells of the 1st and 3rd fractions occupying the 30 and 22% sucrose concentration zones, especially in the 3rd and 5th days of the experiment. The 4th, 5th and 7th cell fractions populating the 18, 14 and 6% sucrose concentration zones respectively, were, on the contrary, significantly increased ($p<0.01$) by the action of hypoxia. A tendency towards normalization of the fractional composition of the blood was observed in the 10th day of hypoxia.

The kinetics of acid-induced hemolysis of erythroid cells of the blood is presented as hemolytic curves, or erythrogrammes, in Fig. 1 (un-fractionated blood); Fig. 2 (4th fraction) and Fig. 3 (5th fraction). Hypoxia caused increase in height of the erythrogramme maximum for the un-fractionated blood in the 3rd day, with a complete rightward displacement of the hemolytic curve maximum to a new, 4.5 min position in the 5th day relative to control. In the 10th day however, the hemolytic curve maximum for the un-fractionated blood was returned to

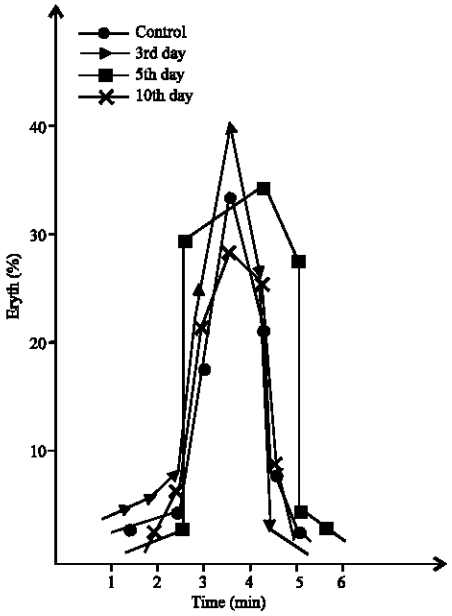

Fig. 1: Effect of hypoxia on the hemolytic process of rat RBC (unfractionated blood)

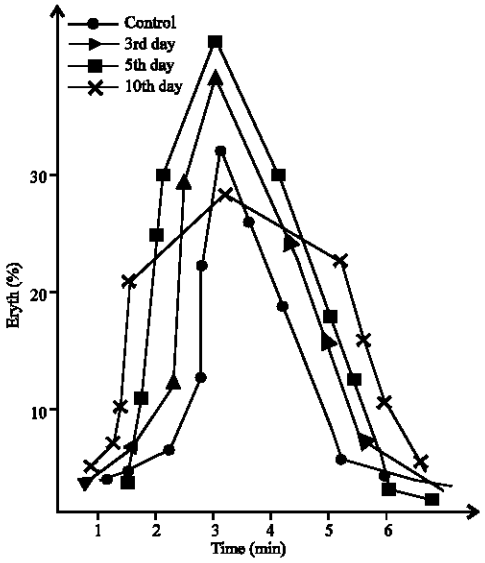

Fig. 2: Effect of hypoxia on the hemolytic process of rat RBC (4th cell fraction)

the 3.5 min position of the control. With respect to the 4th and 5th fractions, hypoxia in the 3rd and 5th days, caused increases in heights of the erythrogramme maxima, however without shift from the 4.5 min control position. Again a tendency towards returning the erythrogramme maxima to the control heights was observed for the said fractions in the 10th day of hypoxia.

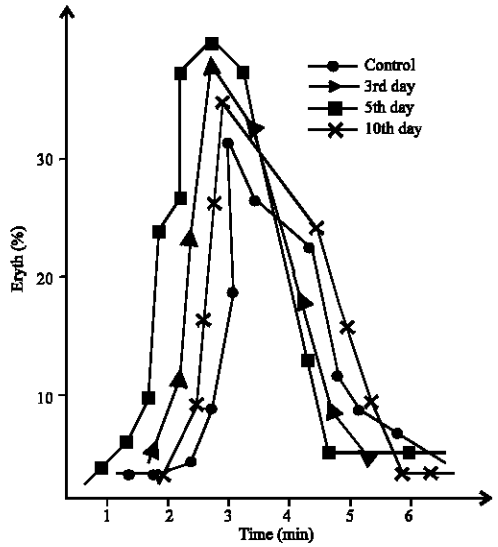

Fig. 3: Effect of hypoxia on the hemolytic process of rat RBC (5th cell fraction)

DISCUSSION

Polycythaemia due to erythrocytes and reduced volume of the plasma (Hurtado, 1964; Dudarev, 1979) is a common feature of hypoxic states. Hypoxia-induced erythrocytosis, depending upon the ethiology, can be characterized by erythroblastosis, consequent upon the mobilization of nucleated red cell precursors from the bone marrow into the circulation, as the organism battles to beef up the oxygen-carrying capacity of the blood (Feodorov, 1977; Mashkin and Terskov, 1986). Erythroblastic polycythaemia is linked to the action of erythropoietin, the renal hormone which stimulates erythropoiesis and which is released into the blood in response to reduction in oxygen tension in the tissues (Lechermann and Jelkmann, 1985; Barkova, 1979).

Our data show that experimental hypoxia inflicted changes in the populations of erythroid cells of the peripheral blood of the rat (Table 1), particularly in the 3rd and 5th days which constitute the critical periods of adaptation. A tendency towards normalization of the fractional composition of the blood in the 10th day was observed. According to cytological analysis by Cohen *et al.* (1976), while the old erythrocytes populate the fractions located towards the 30% sucrose concentration zone (i.e., the 1st and 2nd fractions), the young and immature erythrocytes are located towards the 6% concentration zone (i.e., the 6th and 7th fractions). Between these fractions are situated the functionally active cell populations, namely the 3rd, 4th and 5th fractions. Of these three, we are focusing on the 4th and 5th cell populations because they carry most of the workload of the blood both in the control, normoxic condition (44.86 + 10.73 = 55.69%) and in the critical periods of adaptation (52.29 + 20.61 = 73.90% for the 3rd the 5th day). The decreases in the populations of cells of the 1st, 2nd and 3rd fractions recorded in the critical periods can be the consequence of accelerated ageing (Vinogradov *et al.*, 1962) and elimination of old RBC by the reticuloen-dothelial system (Fornaini *et al.*, 1985; Semenov, 1963). Accelerated cell ageing coupled with increased erythropoiesis could also be responsible for the increases recorded for the 4th and 5th cell populations in the 3rd and 5th days. Erythrocytosis of the 4th and 5th cell populations in the critical periods could also be explained in terms of reduced fragility of these cells to hemolysis.

Our data on the hemolytic process of the unfractionated RBC show that hypoxia caused increase in height of the hemolytic curve maximum in the 3rd day with a rightward displacement of the maximum to a new 4.5 min position, relative to control (Fig. 1). Increase in height of the hemolytic curve and displacement of the maximum to the right relative to control, both signify increase in RBC resistance to hemolysis. In the 10th day, however, the hemolytic curve maximum returned to the 3.5 min position of the control increase of the RBC resistance to hemolysis has been reported for whole blood during hypoxia (Matzinin, 1979). However, authors explained the phenomenon as the consequence of liberation of immature red blood cells into the circulation. Based on our data, however, this effect cannot be explained in terms of the presence of immature cells because the populations of such cells (6th and 7th fractions) were too small to make such an impact. Hemolytic studies with the isolated 4 thand 5th cell populations showed that hypoxia also elicited increase in the RBC resistance to hemolysis in the critical periods as evident in the increased heights of the hemolytic curve maxima, relative to control (Fig. 2 and 3). It is an effect intrinsically associated with erythroid cells of these populations and which needs to be elucidated at the metabolic level. It probably explains why the 4th and 5th cell fractions remained high in the critical periods of adaptation. Thus, during hypoxic hypoxia as simulated in the barocamera, internal compensatory mechanisms of the organism, in addition to other responses, beef up the oxygen-carrying capacity of the blood by increasing the resistance of the main circulating erythrocytes to hemolysis.

Table 1: Influence of barocamera hypoxia on erythroid cell populations of rat peripheral blood (n = 5)

Fraction No.	Sucrose concentration zone (%)	Experimental condition				
		Control	1st day	3rd day	5th day	10th day
1	30	3.74±0.13	1.34±0.23*	1.99±0.13*	0.91±0.13*	2.06±0.14*
2	26	3.62±0.91	4.03±0.85	4.20±0.91	3.47±0.23	3.86±0.15
3	22	28.33±2.35	20.49±2.26*	12.03±2.51*	10.67±1.13*	27.99±2.80
4	18	44.86±2.29	53.66±2.31*	52.29±2.36*	57.40±3.16*	43.41±3.15
5	14	10.73±1.34	11.77±1.56	20.61±3.66*	18.96±1.23*	14.70±1.56*
6	10	4.49±0.21	3.23±0.24	2.55±0.24*	3.21±0.31	3.50±0.23
7	6	4.23±0.11	5.48±0.32	6.33±1.27*	5.37±1.24	4.48±0.29

*Data significant at p<0.010

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