J.Expt. & Clin. Anat 2(2): 12-16 (2003)

JECA

The Significance of Adaptive Changes in Rat Haemoglobin Microenvironment and Electophoretic Fractions During Barocamera Hypoxia

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ABSTRACT

The haemoglobin heterogenous system and some aspects of the micros-environment of the respiratory protein were investigated in albino rats, trained to high altitude hypoxia in barocamera conditions. Hypoxia was simulated in a hypoxic cage at a "height" of 6000m above sea level. Electrophoretic analysis of haemoglobin, the oxyhaemoglobin dissociation curve and the activity of the 2, 3-diphosphoglycerate by pass were studied in the 1st, 3rd, 5th and 10th days of hypoxic exposure. While decrease in the concentration of fractions located towards the cathode was recorded, an additional 7th fraction appeared at the anode during the adaptation process. Decrease in the metabolic activity of 2, 3-diphosphoglycerate mutase with corresponding decrease in concentration of 2, 3-diphosphglycerate (2,3-DPG) which correlates with leftward shift of the oxyhaemoglobin dissociation curve was observed in the 3rd and 5th days of the experiment. A tendency towards normlisation of the haemoglobin P_{50}, concentrated of 2, 3-DPG and activity of 2, 3-DPG mutase was recorded in the 10th day of the exercise.

Key words: Electrophoresis, haemoglobin, barcamera, hypoxia.

A landmark achievement in haemoglobinology was the introduction of electrophoretic method into Biology and Medicine. With the help of this method, it has ben demonstrated that the haemoglobin is a heterogenous system, consisting of several fractions (Starodub 1979; Starodub 1987; Drysdale et al 1971, Garrick et al 1975). The net gas transport of the blood is the cumulative and cooperative action of these fractions.

Hypoxia is known to trigger a reaction of the haemoglobin heterogenous system (Starodub 1987; Grigoreval 1978; Monge C. 1966). However, the adaptive significance of these changes including changes in quantity and quality of the electrophoretic fractions of the respiratory protein still remains an enigma.

The present work relates changes in electrophoretic fractions of haemoglobin to the gas-transport function of the protein and to the response of the 2, 3-diphosphoglycerate shuttles in the critical and early period of adaptation to oxygen deficiency.

MATERIALS AND METHODS

Hypoxic hypoxia was induced in male albino rats of 160-200gm body weight by keeping them in a barocamera in which air pressure was adjusted and maintained at an imaginary height of 6000m above sea level with the help of a special pump and regulatory valve. Animals were exposed to the height for 3 hours each day, and were sacrificed for analysis by decapitation after 1st, 3rd, 5th and 10th days of hypoxia. The control group composed of animals maintained under normoxic conditions of the animal house.

All operations were performed at 0° C – 4°C. Erythrocyte suspensions were obtained by washing fresh heparinized blood samples from decapitated animals, three times with physiological brine (0.85% NaCl) accompanied by centrifugation at 2,500 rev. per minute for 5 minutes each time. Erythrocyte hemolysates were obtained by freez-thaw of cell suspensions in liquid nitrogen followed by centrifugation at 18000 rev. per minute for 15 minutes to remove cell debrids. The activity of 2, 3-DPG mutase was determined in the hemolysates by the Rapoport-Luebering Method (1952), while the concentration of 2, 3-DPG was assayed according to Luganova (1975). Haemoglobin was extracted according to Drabkin (1946). Fractionation of haemoglobin was performed by disc electrophoresis in polyacrylamide gel as described by Maurer (1971). The oxyhaemoglobin dissociation curve was determined spectrophotometrically according to the work (Ivanov 1975). 5-6 close data were used for statistical analysis of result.

RESULTS

Table 1 contains information on the influence of hypoxia on the fractional composition of rat haemoglobin in the 1st, 3rd, 5th and 10 days of experimental exposure. While the normoxic control blood contains 6 electrophoretic fractions, an additional 7th fraction appeared at the anode in the experimental blood samples, and continued to be maintained throughout the hypoxic period. A significant decrease in percentage concentrations of the 1st, 2nd and 3rd fractions and significant increase in concentration of the 5th and main fraction were recorded in the 3rd and 5th days of the exercise. A tendency towards normalization of the fractional composition of haemoglobin was observed in the 10th day, although the 7th fraction continued to exist in the period.

Table 2 contains data on the activity of the 2, 3-diphosphoglycertate shuttles during the adaptation process. There are significant decreases in the activity of 2, 3DPT mutase and in the concentration of 2, 3-DPG in the 3rd day of the experiment. The activity of the enzyme and the concentration of the product both exhibited a return to normoxic values in the 10th day.

Table 3 and Fig. 1 portray the response of the haemoglobin gas-transport function to hypoxia. A significant decrease in the haemoglobin P_{50} value is recorded in the 1st and 3rd days of the exercise, which is reflected in the leftward shift of the oxyhaemoglobin dissociation curve relative to control. Again in the 10th day, the haemoglobin P_{50} and correspondingly the oxyhaemoglobin dissociation curve, displayed a return towards normoxic value and position.

Table 1: Percentage composition of haemoglobin electrophoretic fractions of rat, trained to barocaemra hypoxia at 6000m above sea level. (n = 5 ... 6).

Fraction	EXPERIMENTAL CONDITION				
No.	Control	1st Day	3rd Day	5th Day	10th Day
1	2.85±0.37	2.83±0.10	2.24±0.19	2.10±0.23	2.13±0.10
2	4.76±0.16	5.09±0.11	2.76±0.12	2.81±0.16	3.12±0.12
3	11.49±0.46	11.74±0.40	9.32±0.32	9.57±0.19	9.60±0.25
4	17.39±0.29	17.98±0.23	17.60±0.29	18.18±0.26	19.44±2.31
5	41.97±0.63	40.08±0.26	44.33±0.72	45.03±0.40	42.88±0.66
6	21.48±0.59	20.69±0.49	19.54±0.25	18.81±0.22	20.07±1.3
+ 7	-	1.60±0.16	4.21±0.56	2.63±0.29	2.76±0.60

* Data significant, P 0.010

Table 2: Influence of barocamera hypoxia on the concentration of 2, 3-DPG and on the activity of 2, 3-DPG mutase in rat (n = 5).

EXPERIMENTAL CONDITION	2,3-Dpg(Micro M/Ml.eryth)	2,3-Dpg Mutase (Micro M/Min.ml.eryth)
Control	3.41 ± 0.22	1.84 ± 0.03
1st Day	3.47 ±0.18	1.89 ±0.07
3rd Day	*2.38 ± 0.11	*1.52± 0.05
5th Day	3.34 ± 0.23	1.79 ± 0.02
10th Day	3.15 ± 0.12	1.94 ± 0.04

* Data significant. P 0.01.

Table 3: Changes in the rat haemoglobin P value during barocamera hypoxia (Paschal X 10^3

Experimental	M ± m
Condition	4.25 ± 0.20
1st Day	*3.46 ± 0.17
3rd Day	*3.59 ± 0.17
5th Day	4.10 ± 0.20
10th Day	4.36 ± 0.25

* Data significant at P 0.01

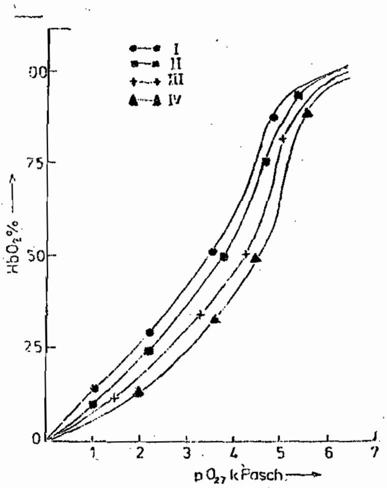

Fig. 1: The response of the haemoglobin gas-transport function to hypoxia.

DISCUSSION

One of the earliest reactions of the organism to oxygen deficiency in the inspired air is hyperventilation of the lungs. This manifests externally as rapid and deep breathing reminiscent of the Kussmal effect. Such increase in the frequency of external respiration causes increased elimination of carbon dioxide to the exterior at a rate faster than that of production of the gas in the tissues. Thus, the equilibrium point in the carbonic anhydrase reaction is displaced in favour of depletion of the acid reserve of the blood, leading to increase in blood pH. Respiratory alkalosis sets in.

$$H^+ + HCO_3^- \Longleftrightarrow H_2CO_3 \Longleftrightarrow H_2O + CO_2 \longrightarrow$$

In conditions of alkalosis, 2, 3-DPG poorly forms complex with the haemoglobin (Ushakova 1985; Van Bennet 1985). This diminishes the modulatory role of the metabolite in decreasing the affinity of haemoglobin for oxygen (Jelkman 1981). The affinity of haemoglobin for oxygen thus increases. Respiratory alkalosis with reduced liganding of haemoglobin to 2, 3-DPG may be responsible for the increased haemoglobin oxygen affinity observed in the 1st and 3rd day of the experiment as evident in the decrease in the haemoglobin P_{50} value and leftward displacement of the oxyhaemoglobin dissociation curve relative to control, in the two periods. This is consistent with earlier observations in support of increased affinityof haemoglobin for oxygen during adaptation to high altitude hypoxia (Eaton 1974; Bacroft 1923; Bacroft 1925). The adaptive significance of this finding consists of the enhancement of the saturation of the respiratory protein with oxygen, which ensures effective and continous supply of the gas to tissues in the early condition of acute deficiency of oxygen in the atmosphere.

The decrease in concentration of 2, 3-DPG in the 3rd day of the experiment correlates with decrease in metabolic activity of the enzyme mediating the formation of the compound, namely, 2, 3-DPG mutase. The inhibition of 2, 3-DPG complex formations with haemoglobin in alkalosis leads to increased availability of the free, unbound metabolite in the erythrocyte. The free 2, 3-DPG then inhibits the mutase leasing to decreased metabolic activity of the enzyme and decreased synthesis of 2,3-DPG in the 3rd day. Reduction in the concentration of 2, 3-DPG may also be due to increased metabolisation of the compound via the major glycolytic pathway en route to lactate. Hypoxia stimulates the activity of the glycolytic sequence (Simamovsky 1968; Simanovosky 1971). While increase in concentration of 2, 3-DPG in erythrocyte has been reported in previous works (Lenfant 1971; Simanovsky 1971, Voitkovitch 1973) such increases may be associated with late periods of chronic hypoxia.

Our data in table 1 show decrease in concentrations of the slow-migrating electrophoretic fractions (1st, 2nd and 3rd fractions) in the 3rd and 5th days of the experiment with the appearance and maintenace of a 7th fast-migrating fraction throughout the adaptive period. Increased mobilization of immature red blood cells from the bone marrow in effort to increase the oxygen-carrying capacity of the blood as observed during hypoxia (Cohen et al 1976; Charny 1961; Feodorov 1986) may account for this observation. Also, implicated here could be increased recruitment of glucose from glycogen, which occurs during hypoxia (Pribilova 1981; Pastoris et al 1985) with possible subsequent glycosylation of the haemoglobin (Glenok B.; Bodnar P. 1989). The increase in concentration of the main electrophoretic fraction (5th fraction) and the emergence of the 7th fraction both indicate increased erythro-and haemopoisis, which are common features of hypoxic states (Van Leer 1967; Gitelzom I.I 1967). As observed by Voitkvitch 1973; Lenfant et al 1971, the affinity oif haemoglobin for oxygen decreases with incrased concentration of the respiratory protein in the erythrocyte. Thus, while the increase in affinity of haemoglobin of oxygen, evident in the observed leftward shift of the oxyhaemoglobin dissociation curve, is aimed at increasing the degree of saturation and loading of the haemoglobin with oxygen at the alveoli, the increase in concentration of the main electrophoretic fraction is aimed at decreasing the affinity of the protein for oxygen, consequently enhancing the offloading of the gas to the metabolizing cells in tissues in the critical period of adaptation. This way, the oxygen regimen of the organism is corrected and fine-tuned to forestall the development of hypoxia in the tissues.

Noteworthy is the tendency towards normalizing of the fractional composition of the haemoglobin, the haemoglobin gas-transport function and the activity of the 2, 3-DPG by pass. Normalisation of erythroid cell population of the peripheral blood and certain physico-chemical parameters of the red blood cells during hypoxia were observed in earlier reports (Ekpo B. 1999; Ekpo B 2001). This may be the consequence of functional and metabolic adjustments in the organism. Mobilisation of functional reserve and compensatory reactions of the organism in response to extremal and pathophysiological conditions cannot continue ad infinitum: This would lead to functiona exhaustion of the organism. Thus, normalization of parameters as observed in our experiment constitutes a biochemical strategy of adaptation and self-preservation of the organism in extremal environmental conditions.

CONCLUSION

Barocamera hypoxic hypoxia elicits changes in the haemoglobin fractions of rat, decreasing the concentration of the slow-migrating fractions, increasing that of the main fraction causing the emergency of an additional fast-migrating fraction. At the same time, the concentration of 2, 3-DPG and the activity of 2,3-DPG mutase are decreased, thus diminishing the defacilitating effect of the former on haemoglobin oxygenation, in the early period of adaptation. The haemoglobin oxygen affinity increases. A tendency towards normalization of the haemoglobin fractional composition, the activity of 2, 3-diphosphoglycerate by pass and the haemoglobin gas-transport function is observed in the 10th day of adaptation.

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AKNOWLEDGMENT

I wish to thank my supervisor, Prof. B.F. Suhomlinov, the HOD Dept. of Biochemistry of the Lvov State University, Republic of Ukraine, and all the Staff of the laboratory of Radiational and Physico-chemical Biology for their assistance in the course of this work.

Received in June 2002 and accepted in March 2003