INVESTIGATION OF BROMTHYMOL BLUE-PREALBUMIN IN RAT SERUM DURING HYPERBARIC OXYGENATION

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Disc-electrophoresis studies showed a threefold increase in the concentration of bromthymol blue-prealbumin (BTB-prealbumin; R_m = 1.0) in the blood serum of rats after severe convulsions caused by exposure to oxygen in a pressure of 6 atm for 30-35 min. The appearance of a glial S100 protein component and a sevenfold increase in the concentration of an all-organ component of brain BTB-prealbumin in the blood serum of the experimental rats was discovered immunochemically. The consequences of disturbance of the blood—brain barrier for neurospecific and all-organ proteins and also the possibility of discharge of proteins from the brain into the blood stream as in the case of neurophysins during hyperbaric oxygenation are discussed.

KEY WORDS: blood serum proteins; brain proteins; blood—brain barrier; hypobaric oxygenation.

Changes in protein metabolism in the brain during hyperoxia have been established [4, 8]. The dynamic state of proteins in the tissues may play an important role in the pathogenesis of oxygen poisoning. We previously described the biochemical and immunochemical characteristics of a prealbumin in the rat brain [5]. It migrates in disc electrophoresis with bromthymol blue (BTB) as identification agent, and is described as BTB-prealbumin. Its structural heterogeneity has been demonstrated [7, 9]. BTB-prealbumin contains up to six components, including a neurospecific S100 protein and two all-organ components. On disc electrophoresis of rat blood serum protein with low dilution, a zone of BTB-prealbumin has also been discovered [5]. During hyperbaric oxygenation the brain prealbumin level falls [8] and the permeability of the blood—brain barrier for proteins increases [10]. To understand the pathogenesis of acute oxygen poisoning, it is interesting to establish whether nonspecific and all-organ proteins can migrate from the brain into the blood. The components of BTB-prealbumin were convenient materials for the investigation of this problem.

EXPERIMENTAL METHOD

Experiments were carried out on albino rats weighing 200-250 g. The experimental animals were exposed to hyperoxia. The conditions were: compression time 3 min, pressure in the chamber 6 atm, decompression time 3 min. The rats were taken from the pressure chamber 30-35 min after developing clonic convulsions. The experimental rats were decapitated simultaneously with the controls, blood was collected, and serum obtained. The protein concentration was determined by Lowry's method [11]. To precipitate balanced proteins and to improve the detection of BTB-prealbumin during electrophoresis, the blood serum was treated with formic acid [5] up to a final concentration of 1N, and then centrifuged at 8000g for 30 min at 4°C. The supernatant was neutralized to pH 7.2 and the sample centrifuged. Proteins of the acid-soluble fraction were subjected to disc electrophoresis in 7.5% polyacrylamide gel, pH 8.9 [6]. The gels were stained with Amido black. The region of BTB-prealbumin was investigated densitometrically at a wavelength of 635 nm. The protein content in the zone was determined by weighing the areas of the peaks. The all-organ components of brain BTB-prealbumin, present in trace amounts in blood serum, were studied by calculating the antigen content in systems unequilibrated for protein by the immunoprecipitation in agar test [1]. Precipitation [3] was carried out between rat blood serum and rabbit immune serum against all-organ proteins. The concentration coefficient (K) was calculated for the protein as the ratio between

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TABLE 1. Concentration of Total Protein (C), Concentration Coefficients (K) and $K_h : K_c$ Ratios for BTB_a-Protein in Blood Serum of Control Rats (K_c) and Rats with Hyperoxia (K_h)

Expt. No.	C, mg/ml		K		$K_h:K_c$
	control	hyperoxia	$T^1=1$ —control	$T^1=8$— hyperoxia	
1	53,8	66,5	0,019	0,12	6,3
2	48,5	49,0	0,020	0,16	8,0
3	52,5	52,0	0,019	0,15	7,9
4	56,5	66,5	0,018	0,12	6,7
5	53,8	60,4	0,019	0,13	6,8
6	49,5	61,3	0,020	0,13	6,5
7	50,9	55,8	0,020	0,14	7,0
8	49,8	58,6	0,020	0,14	7,0
9	54,6	53,5	0,020	0,15	7,5
10	48,6	50,6	0,020	0,16	8,0
$M \pm m$	51,9±0,9	57,4±1,3	0,02±0,0006	0,153±0,013	7,17
P	<0,01		<0,01		

*Highest titer of protein

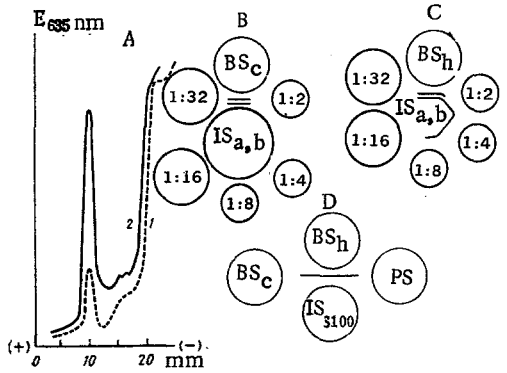

Fig. 1. Investigation of BTB-prealbumin and its components in blood serum of control rats and rats with hyperoxia by disc electrophoresis and immunochemical tests. A) Disc-electrodensitogram of BTB-prealbumin region of rat blood serum in control (1) and hyperoxia (2). Abscissa: distance along electrophoretic gel (in nm); ordinate: optical density of electrophoretic gel on densitometer; B, C, and D) schemes showing arrangement for immunoprecipitation in agar. Immune sera against two all-organ components of rat brain BTB-prealbumin ($IS_{a,\ b}$) and against neurospecific S100 protein (IS_{S100}). Antigens: BS_c) blood serum of control rats; BS_h) blood serum of hyperoxic rats; 1 : 2-1 : 32) dilution of antigens. PS) Physiological saline.

its highest titer (T) and the total protein concentration in rat blood serum (C). S100 protein in the blood serum was identified with the aid of antibodies against S100 protein, provided by S. M. Sviridov, Senior Scientific Assistant, Institute of Cytology and Genetics, Siberian Branch, Academy of Sciences of the USSR (Novosibirsk).

EXPERIMENTAL RESULTS

According to the results of ten experiments the weight of the areas of the peaks of the electrodensitograms in the control (Fig. 1A) was 12.8 ± 1.0 mg and in the experimental series 38.6 ± 1.8 mg. Consequently, in acute oxygen poisoning there was a threefold increase in the BTB-prealbumin concentration in the blood serum. The increase in total protein under these same conditions was only 11% (Table 1).

Two weak bands corresponding to all-organ components of BTB-prealbumin were found immunochemically in the blood serum of intact rats (Fib. 1B). The first line was situated nearer to the well containing immune serum and corresponded to BTB_a-protein, the second line was nearer to the well containing rat blood serum and corresponded to BTB_b-protein.

Calculations of the ratio of the concentration coefficients in hyperoxia (Fig. 1C) to those in the control revealed a sevenfold increase in the concentration of BTB_a-protein in the blood serum of the experimental rats (Table 1). By its physicochemical properties this component is similar to neurophysin [5, 9]. It can tentatively be suggested that it is liberated rapidly from the brain into the blood stream similarly to the neurophysins [12]. Since BTB_a-protein belongs to the all-organ group, the possibility of a disturbance of the blood—brain barriers during hyperoxia and of liberation of the protein into the blood from various organs and tissues cannot be ruled out. No significant increase in the concentration of BTB_b-protein could be found in the blood serum of the hyperoxic rats (T = 1-2).

The appearance of a glial S100 protein was observed in the blood serum of the rats during hyperoxia (Fig. 1D). During hyperbaric oxygenation there is thus a disturbance of the permeability of the blood—brain barrier for neurospecific proteins, which, since they are "trans-barrier" in nature, cause a shift of the biochemical and immunological systems of the body [2].

These qualitatively different changes in the system of relations between neurospecific, all-organ, and serum proteins in hyperoxia may be important factors in the mechanism of oxygen poisoning and can provide convenient molecular parameters (markers) for assessing the functional state of animals at the various stages of hyperbaric oxygenation. Attention is drawn to the tissue specificity of protein metabolism in the brain. The protein metabolism of the brain is determined both by interaction between the functions of neurospecific and all-organ proteins and by the special character of their relations with the blood and CSF proteins. During shifts of the functional state and under pathological conditions changes take place in the dynamic system of relations between proteins that differ in their tissue specificity. The tissue specificity of proteins, when present in a "foreign" system, determines the specific character of changes in the biochemical and immunologic parameters of that system and, correspondingly, in the specific character of the functional or pathological state.

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LITERATURE CITED

1. I. S. Bashkaev, G. I. Avdeev, and B. S. Sinyakov, Lab. Delo, No. 11, 678 (1969).
2. I. V. Gannushkina, Immunologic Aspects of Trauma and Cerebrovascular Lesions [in Russian], Moscow (1974).
3. L. A. Zil'ber (editor), Immunochemical Analysis [in Russian], Moscow (1968), p. 99.
4. A. A. Krichevskaya, A. A. Sinichkin, L. A. Shcherbina, et al., in: Abstracts of Proceedings of the 6th All-Union Conference on Neurochemistry [in Russian], Leningrad (1972), p. 87.
5. A. A. Krichevskaya, A. A. Sinichkin, V. N. Prokof'ev, et al., Biokhimiya, 42, 230 (1977).
6. H. Maurer, Disc Electrophoresis [Russian translation], Moscow (1971), p. 58.
7. V. N. Prokof'ev and A. A. Sinichkin, Biokhimiya, 42, 1878 (1977).
8. A. A. Sinichkin, K. B. Sherstnev, and A. V. Stavitskaya, Izv. Sev-Kavkaz, Nauch. Tsentra Vyssh. Shkoly, Estestven. Nauki, No. 3, 31, (1973).
9. A. A. Sinichkin, V. N. Prokof'ev, K. B. Sherstnev, et al., Ukr. Biokhim. Zh., No. 4, 429 (1978).

10. S. B. Lange, M. D. Josphe, C. Lee, et al., in: Sixth International Hyperbaric Congress, Aberdeen (1977), p. 50.
11. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
12. R. Walter, Ann. New York Acad. Sci., 248, 1 (1975).

ANALYSIS OF THE ENERGY OF ELECTROSTATIC INTERACTION BETWEEN BLOOD CELLS IN EXPERIMENTAL MYOCARDIAL ISCHEMIA

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The diffusion and ζ potentials of red cells in blood flowing directly from a zone of myocardial ischemia along a branch of the great cardiac vein in the acute period of experimental infarction were studied by a microelectrode method and by microelectrophoresis. By this means the energy of electrostatic repulsion (EER) between the blood cells to be calculated and factors exerting a significant effect on this parameter in acute experimental myocardial infarction caused by ligation of the anterior interventricular branch of the left coronary artery in 20 dogs could be identified. The energetic state of the double electric layer was shown to be a leading factor in the change in EER and manifestation of the aggregation properties of the blood cells. A statistically significant decrease in the energetic potentials of the red cells was found in blood taken directly from the zone of myocardial ischemia.

KEY WORDS: binding energy; blood; ischemia.

Disturbances in the blood clotting system, together with changes in the state of the vascular wall and the hemodynamics, are factors of great importance in the development of ischemic heart disease [6, 7]. However, the problem of the intimate mechanisms of the changes in blood clotting during pathological shifts in the microcirculation and perfusion of the myocardium still remains a matter for discussion [8, 14].

It is accordingly interesting to study the dynamics of the energy of electrostatic repulsion (EER) between the blood cells, the biophysical basis for aggregation and adhesion processes in the initial stage of thrombosis.

EXPERIMENTAL METHOD

To calculate EER — an index which essentially characterizes the conditions of dissociation of the blood cells — the well-known Deryagin—Landau equation [2, 3, 5] is used

$$W = \frac{8(kT)^2 \varepsilon \cdot a}{e^2 \cdot Z^2} \cdot \left[\frac{\exp\left(\frac{Ze}{2kT} \cdot \psi_0\right) - 1}{\exp\left(\frac{Ze}{2kT} \cdot \psi_0\right) + 1} \right]^2 \exp(-\chi H_0), \quad (1)$$

where k is Boltzmann's constant; T the absolute temperature; ε the dielectric constant of the medium; a the radius of the cell; e the elementary charge; H_0 the distance between the cells; χ the Debye—Hückel function, corresponding to the reciprocal of the double electric layer of the cell; ψ_0 the surface potential determined from the density of electric charges of the red blood cells by the equation [3]

$$\sigma_0 = \left[\frac{\varepsilon N_i kT}{2\pi}\right]^{1/2} \cdot \left[\exp\left(\frac{Ze}{2kT} \cdot \psi_0\right) - \exp\left(\frac{Ze}{2kT} \psi_0\right)\right], \quad (2)$$

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