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CHANGES IN ERYTHROCYTE MEMBRANE PROTEINS DURING PROLONGED ELEVATION OF BLOOD CHOLINERGIC ACTIVITY IN ANIMALS

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In the modern view cholinergic mechanisms play an exceptionally important role in metabolism and in transmission of nervous impulses [1, 2, 4]. Erythrocytes are known to participate in the regulation of cholinergic processes, and these processes themselves influence the vital activity of the red blood cells [3, 5].

The object of this investigation was to study relations of cholinergic processes with the polypeptide composition of the erythrocyte membrane and the sensitivity of the chemoreceptor system of erythrocytes to plasma components.

EXPERIMENTAL METHOD

The influence of cholinergic factors on erythrocyte membranes was studied in systems *in vivo* (dogs) and *in vitro* (action of acetylcholine on healthy human erythrocytes).

Erythrocytes, washed in physiological saline, were incubated (n = 10) with acetylcholine *in vitro* under sterile conditions for 6 h. For this purpose a 50% erythrocyte suspension was mixed with equal volumes of 0.006% solution of acetylcholine, made up in buffered physiological saline, pH 7.4 (final acetylcholine concentration $1.7 \cdot 10^{-4}$ M), and for incubation of the acetylcholinesterase (AChE) of the erythrocytes neostigimine solution was added to the incubation medium in a final concentration of $2.5 \cdot 10^{-5}$ M. After incubation the erythrocytes were washed 5 times with buffered physiological saline (pH 7.4).

High blood cholinergic activity was created in dogs (10 mongrel animals) *in vivo* by parental injection of acetylcholine (0.9 mg/kg) and neostigmine (0.008 mg/kg) into the animals for 3 months. Blood was taken from the animals' saphenous vein and sodium citrate was used as anticoagulant. The erythrocytes were washed 3 times with buffered physiological saline (pH 7.4). The membranes were isolated from erythrocytes treated with acetylcholine in both *in vivo* and *in vitro* systems by gradual osmotic hemolysis [6].

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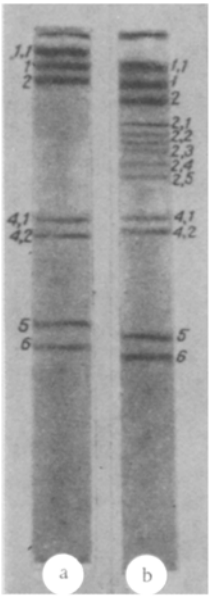

Fig. 1

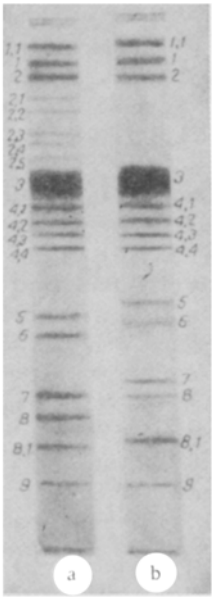

Fig. 2

Fig. 1. Electrophoresis of peripheral membrane proteins of erythrocytes from control (a) and experimental (b) groups of animals.

Fig. 2. Electrophoresis of integral membrane-dependent erythrocyte proteins of control (a) and experimental (b) groups of animals.

To isolate peripheral proteins from the total mass of membrane proteins, 0.08 M sodium chloride solution was used [12] with the addition of 0.005 M EDTA to stabilize sulfhydryl (SH) groups. Solubilization of the total fraction of membrane proteins and also of the integral proteins was carried out in a solution of sodium dodecylsulfate [9]. The polypeptide composition of the total fraction and of the peripheral and integral proteins was studied by disc electrophoresis in polyacrylamide gel containing sodium dodecylsulfate [13]. The protein content was determined by the method of Lowry et al. [11]. To determine the content of SH groups in the fraction of peripheral proteins, Ellman's spectrophotometric method [7] was used, the sidulfide (SS) groups were determined by Habeeb's method [9]. Densitometry of the protein fractions was carried out on the Chromoscan 200 instrument at a wavelength of 620 nm. The defective population of erythrocytes with sensitivity to the lytic action of plasma components was detected by acidification of the fresh blood serum to pH 7.3 (Ham's acid test). For the statistical analysis of the data Student's t-test was used.

EXPERIMENTAL RESULTS

The increased cholinergic activity *in vivo* and treatment of the erythrocytes with acetylcholine *in vitro* led to an increase in the content of peripheral proteins, weakly bound with the membrane. The content of these peptides in the animals was increased relative to total membrane proteins by 48% (from 21 $\pm$ 0.36 to 31 $\pm$ 1.40%; $P < 0.001$), and in the *in vitro* system by 11% (from 15.75 $\pm$ 0.30 to 17.50 $\pm$ 0.71%; $P < 0.05$). The content of integral proteins in the experimental animals was reduced by 13% (from 79 $\pm$ 0.40 to 69 $\pm$ 1.5%; $P < 0.001$), and in the *in vitro* system by 2.1% (from 84.25 $\pm$ 0.32 to 82.50 $\pm$ 0.82%; $P > 0.1$).

An increase in the intensity of cholinergic processes in the animals led to a change in the polypeptide composition of the peripheral and integral fractions. The peripheral fraction of erythrocyte membrane proteins of the control group consisted of seven polypeptides: 1.1; 1; 2; 4.1; 4.2; 5; 6; in the experimental animals five additional polypeptides were found: 2.1; 2.2; 2.3; 2.4; 2.5 (Fig. 1). These additional polypeptides were absent in the integral proteins of the experimental animals (Fig. 2). Meanwhile the composition of the total protein fraction was unchanged by the increase in cholinergic activity. Treatment of the erythrocytes with acetylcholine *in vitro* did not affect the polypeptide composition of the various membrane protein fractions.

Special importance was attached during determination of the relative percentages of individual polypeptides in the peripheral and integral fractions in the animals to changes in the main polypeptides, namely 1, 2, 5, 6. In the experimental animals the content of polypeptides of the actomyosin system was reduced significantly compared with the initial values: polypeptide 1 by 26%, polypeptide 2 by 27%, polypeptide 5 by 28%; furthermore, an increase was found in the content of polypeptide 6 (by 36%), an enzyme of the glycolytic cycle – glyceraldehyde phosphate dehydrogenase (Table 1). High cholinergic activity in the animals led to changes in the ratio between SH/SS groups in the peripheral fraction of membrane proteins. The content of SH groups was increased by 115% (from 13 $\pm$ 0.12 to 28 $\pm$ 1 μmoles/g protein; $P < 0.001$),

TABLE 1. Polypeptide Composition (in %) of Membrane Proteins and Erythrocytes of Animals with Increased Cholinergic Activity (X ± m)

No. of polypeptide	Peripheral proteins		Integral proteins	
	control	experiment	control	experiment
1,1	16,50±0,58	12,50±1,10*	11,90±0,43	13,70±0,61*
1	19,00±0,62	14,10±1,14*	10,00±0,41	11,65±0,54*
2	20,20±0,65	14,70±1,2*	11,00±0,42	12,70±0,59*
2,1	—	6,90±0,69	2,60±0,10	—
2,2	—	3,20±0,31	0,90±0,08	—
2,3	—	2,60±0,27	0,60±0,04	—
2,4	—	2,90±0,29	0,80±0,05	—
2,5	—	1,90±0,21	1,00±0,06	—
4,1	6,80±0,21	5,50±0,51*	2,30±0,12	3,10±0,21*
4,2	10,60±0,42	7,50±0,73*	3,20±0,15	4,20±0,29*
5	12,50±0,46	9,00±0,92*	2,10±0,11	2,90±0,23*
6	11,10±0,51	15,10±0,97*	3,90±0,17	2,50±0,36*

*$P < 0.05$ compared with initial value.

whereas the content of SS groups was reduced by 40% (from 15 ± 0.36 to 9 ± 0.7 μmoles/g protein; $P < 0.001$). Similar changes in these groups of proteins were observed in erythrocytes treated with acetylcholine: an increase in the content of SH groups by 7.5% (from 34.50 ± 0.50 to 37.11 ± 0.92 units; $P < 0.05$) and a decrease in the content of SS groups (from 22.50 ± 0.50 to 20.72 ± 0.87 units; $P > 0.2$).

In the experimental animals AChE activity in the erythrocytes was reduced by 40-45%, which corresponded on average to 0.51 ± 0.02 μmole acetylcholine hydrolyzed per minute per 10^{10} cells, compared with the normal level of 1.2 ± 0.02 unit ($P < 0.001$). During incubation of the animals' erythrocytes in their own acidified serum from animals with high cholinergic activity, a defective population of these cells sensitive to the lytic action of plasma components was found. In the acid test on the experimental dogs on average 16.70 ± 0.49% of the erythrocytes were hemolyzed compared with the normal 1.10 ± 0.14% ($P < 0.001$). In erythrocytes treated with acetylcholine *in vitro* sensitivity to plasma components and a decrease in AChE activity could not be detected.

Consequently, high cholinergic activity leads to weakening of bonds in membrane proteins, to changes in their polypeptide composition, reduction of disulfide groups, and the appearance of pathological sensitivity of the erythrocytes to the action of plasma components. Acetylcholine *in vitro* did not affect the state of the chemoreceptor system responsible for interaction of these cells with the physiological components of plasma.

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