EFFECT OF CHOLINERGIC FACTORS ON THE STATE OF ANIMAL ERYTHROCYTE MEMBRANE PROTEINS

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An exceptionally important role is ascribed to acetylcholine and acetylcholinesterase (AChE) in nonmediator intracellular conversions [2-5]. Ado [1] states that cholinergic processes play a role in the mechanism of allergic reactions of organs and tissues in whose activity these processes play a direct part under physiological conditions. From this point of view data indicating the possibility of development of a "pathological" population of erythrocytes with altered sensitivity to complement, thrombin, Mg^{2+} salts, and antibodies in the presence of marked depression of the AChE of these blood cells and an increase in the acetylcholine level in animals [6] are interesting. The physiological role of AChE in erythrocytes has not been established.

The object of this investigation was to study some oxidation—reduction processes in membrane proteins of animal erythrocytes during long-lasting elevation of the blood cholinergic activity.

EXPERIMENTAL METHOD

The effect of increased cholinergic activity on erythrocyte membrane proteins was studied in experiments on 10 mongrel dogs weighing 15-30 kg, into which neostigmine (0.008 mg/kg) and acetylcholine (0.9 mg/kg) were injected parenterally twice a day for 2-7 months.

The same animals before the beginning of the experiments and a group of intact dogs served as the controls. Blood for testing was taken from the saphenous vein and sodium citrate was used as anticoagulant. The erythrocytes were washed three times with physiological saline, after which membranes were obtained from them by gradual osmotic hemolysis [9]. The total fraction of membrane proteins was divided into peripheral and integral. To isolate peripheral proteins a 0.08 M solution of NaCl [16], with the addition of 0.005 M EDTA to stabilize sulfhydryl groups, was used. Integral proteins and also proteins of the total fraction were solubilized in sodium dodecylsulfate solution [12]. The protein concentration was determined by the method of Lowry et al. [15]. The polypeptide composition of the proteins (total fraction, peripheral and integral) was studied by disc electrophoresis in polyacrylamide gel containing sodium dodecylsulfate [18]. After electrophoresis of the membrane proteins the gels were recorded by microphotography. The number of sulfhydryl (SH) groups in the peripheral proteins was determined by Ellman's spectrophotometric method [10] and the number of disulfide groups (SS) by preliminary reduction to sulfhydryl groups with sodium borohydride [13]. The content of oxidized forms of NAD and NADP in the erythrocytes was determined by the method of Busch and Boie [8] and reduced forms of the pyridine nucleotides were determined by the method of Loder and De Gruchy [14]. To prevent possible oxidation of the reduced nucleotides during extraction, cysteine was added in a final concentration of 0.01 M [7]. AChE activity in the erythrocytes was determined by the method of Ellman et al. [11]. Student's t-test was used for statistical analysis of the results.

EXPERIMENTAL RESULTS

AChE activity in erythrocytes of animals of the experimental group in the period of the investigation was depressed by 40-45%, corresponding to a mean value of 0.51 ± 0.02 μmole

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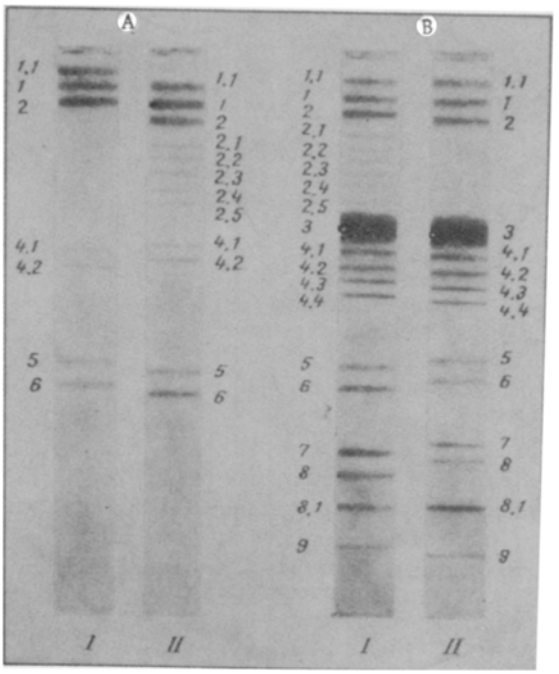

Fig. 1. Electrophoresis of peripheral (A) and integral (B) erythrocyte membrane proteins of animals of control (I) and experimental (II) groups. Numbers indicate serial numbers of polypeptides.

acetylcholine hydrolyzed per minute by 10^{10} erythrocytes, compared with a normal level of 1.2 ± 0.02 ($P < 0.001$).

The content of peripheral and integral proteins in the erythrocyte membranes of these animals differed significantly from that in the control. For instance, the content of peripheral proteins was increased by 1.5 times (in the experimental animals 31 ± 1.40, in the control 21 ± 0.36% of the total protins; $P < 0.001$). The content of integral proteins was correspondingly reduced by 1.1 times (in the experimental group 69 ± 1.5%, in the control group 79 ± 0.40%; $P < 0.001$). Besides quantitative changes in the peripheral and integral proteins in the animals of the experimental group, changes in the polypeptide composition of the proteins also were found. A study of the composition of the total fraction of erythrocyte membrane proteins revealed no differences between the control and experimental group. The peripheral protein fraction from animals of the control group consisted of four polypeptides: 1, 2, 5, 6 (according to Steck [17]). This fraction in the experimental group was characterized by the appearance of five additional polypeptides: 2.1, 2.2, 2.3, 2.4, and 2.5 (Fig. 1A). The origin of these additional polypeptides was revealed by a study of the integral proteins, where in the experimental group polypeptides 2.1, 2.2, 2.3, 2.4, and 2.5 were observed to have disappeared (Fig. 1B).

An increase in the polypeptide content was thus observed in the peripheral fraction of erythrocyte membranes of animals with high cholinergic activity, possibly as a result of a decrease in the strength of binding of the integral proteins.

The content of SH- and SS-groups was determined in the peripheral membrane proteins because of their important role in membrane function. The content of SH-groups in the animals of the experimental group was increased by 2.1 times (to 28 ± 1 μmoles/g protein, from 13 ± 0.12 μmoles in the control; $P < 0.001$). Meanwhile the content of SS-groups in these animals was reduced by 1.7 times (to 9 ± 0.7 μmoles/g protein from 15 ± 0.36 μmoles in the control; $P < 0.001$).

The ratio of reduced to oxidized forms of pyridine nucleotides NAD and NADP was determined as an overall index of the oxidation–reduction balance in the erythrocytes. Whereas the level of the oxidized form remained unchanged, the content of reduced NAD was found to be increased by 1.5 times (in the experimental group 37 ± 0.6 nmoles/10 erythrocytes, in the control group 24 ± 1.1 nmoles; $P < 0.001$), which led to an increase in the $NADH/NAD^+$ ratio by 1.6 times. Meanwhile, whereas the level of the reduced form remained unchanged, the

content of the oxidized form of NADP was reduced by 2.6 times (in the experimental group 7 ± 0.2 nmoles/10 erythrocytes, from 18 ± 0.6 nmoles in the control; $P < 0.001$), and this also caused an increase of 2.8 times in the NADPH/$NADP^+$ ratio.

The results of these experiments thus demonstrate a shift of the oxidation—reduction balance toward strengthening of reduction processes in erythrocytes of the experimental group of animals, reflected in particular by an increase in the content of SH-groups and a decrease in the content of SS-groups in the membrane proteins.

These results and data in the literature suggest that membrane proteins and the chemoreceptive system of erythrocytes play a role in biochemical conversions induced by the high cholinergic activity of the blood. The molecular mechanisms of the role of acetylcholine and AChE in extrasynaptic membrane conversions have not yet been elucidated.

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