CHANGES IN BINDING OF CALCIUM IONS AND IN FUNCTION OF Na^+,K^+-ATPase IN THE ERYTHROCYTES IN ESSENTIAL HYPERTENSION

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More calcium was removed by EDTA from the membranes of erythrocytes of patients with hypertension than from the erythrocytes of persons with a normal blood pressure. By the use of an isotonic solution of $MgCl_2$, the quantity of calcium removed from the erythrocyte membrane was the same in both groups. Activity of Na,K-ATPase in the membrane of reconstituted erythrocytes of patients with essential hypertension increased its activity in healthy human erythrocytes.

KEY WORDS: *essential hypertension; erythrocyte membrane; ATPase; calcium.*

The writers showed previously that the velocity constant of sodium exchange in the erythrocytes in vitro after blocking of the membrane sodium pump and the establishment of ionic equilibrium with the external medium is significantly higher in patients with hypertension than in normotensive individuals, whereas the accumulation of ^{42}K by the erythrocytes is delayed under these same conditions in hypertensive patients [2]. Similar changes in membrane transport of K^+ and Na^+ have also been found in the erythrocytes of rats with spontaneous hypertension [1, 9]. One cause of the changes in membrane transport of cations in these forms of pathology could be a disturbance of the structure of the erythrocyte membrane. In this connection it is interesting to study the activity of the membrane-bound enzyme Mg^{2+}-dependent ATPase and also the distribution of Ca^{2+} in the erythrocyte membrane as indirect indices of possible changes in its structure.

Activity of Mg^{2+}-ATPase was studied in reconstituted erythrocytes of patients with hypertension and the quantity of Ca^{2+} removed from the erythrocyte membrane with the aid of bivalent cations (Mg^{2+}) and EDTA was determined.

EXPERIMENTAL METHOD

Erythrocytes of sixteen patients with essential hypertension in stages II-III (7 men and 9 women aged from 40 to 65 years) served as the test object. The blood pressure in nine of the patients varied from 160/85 to 200/100 mm Hg, and in the other seven it was over 200/100 mm Hg. Symptomatic (renal or endocrine) hypertension was excluded in these patients. The control group consisted of fourteen persons (5 men and 9 women aged 39-67 years) with a constant normal blood pressure. Blood was taken in the morning before breakfast through a silicone-treated needle into a test tube wetted with heparin solution (Richter, Hungary). Erythrocytes were sedimented by centrifugation at 3000g for 5 min, freed from plasma and white blood cells, and washed three times with solution 1 (130 mM NaCl, 5 mM KCl, 18 mM Tris-HCl buffer, pH 7.4). To determine the quantity of Ca^{2+} removed from the erythrocytes, the cells were incubated for 15 min at room temperature in a solution containing 107 mM $MgCl_2$, washed twice with choline chloride solution, reincubated for 15 min in choline chloride solution containing 2 mM EDTA (pH 7.4), and then washed twice again with the same solution. The Ca^{2+} concentration in the solutions after washing the erythrocytes was determined on a flame photometer (Flapho, Zeiss, East Germany). Activity of Mg^{2+}-ATPase was studied in reconstituted erythrocytes — closed vesicles of erythrocyte membranes preserving the heterogeneity of

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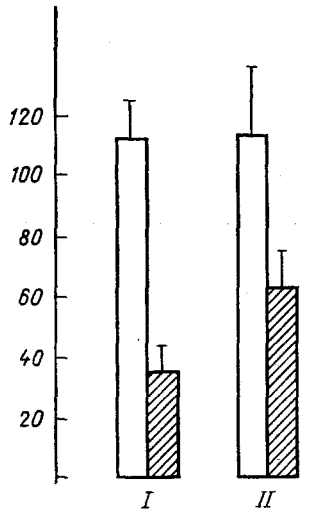

Fig. 1

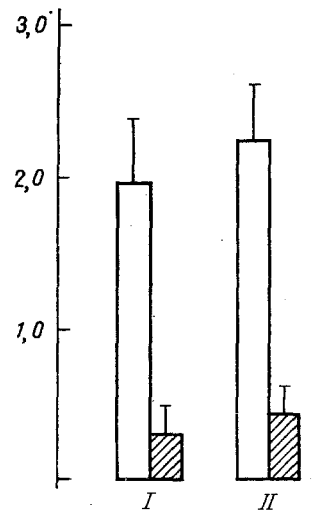

Fig. 2

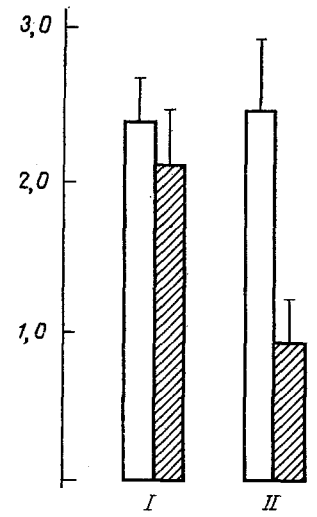

Fig. 3

Fig. 1. Quantity of Ca^{2+} (in μeq) removed from 1 liter erythrocytes by washing with 0.107 M $MgCl_2$ (unshaded column) and subsequent washing with isotonic solution of choline chloride with the addition of 2 mM EDTA, pH 7.4 (shaded column). I) Control group (n = 14); II) essential hypertension (n = 16).

Fig. 2. ATPase activity of reconstituted erythrocytes (in μmoles P_{in}/ml cells/h). Hemolysis medium contains no Ca^{2+}. Here and in Fig. 3, unshaded columns represent ouabain-independent component, shaded columns ouabain-dependent component. I) Control group (n = 9); II) essential hypertension (n = 11).

Fig. 3. ATPase activity of reconstituted erythrocytes. Hemolysis medium contains 50 μM $CaCl_2$.

their outer membrane as regards phospholipid composition and phase state [11]. Production of the reconstituted erythrocytes was based on the method of rapid hemolysis [7]. For this purpose 1 ml of washed erythrocytes was added to 7 ml hemolysis medium of the following composition (in mM): $MgCl_2$ 2.00; disodium salt of ATP 3.64, Tris-HCl 5.00; pH 7.4. The hemolysis medium was used either free from Ca^{2+} ions or with the addition of 50 μM $CaCl_2$. After 2 min 1 ml of closing medium (0.25 M NaCl, 1.020 M KCl) was added. The suspension was thoroughly mixed and incubated for 1 h at 4°C. The residue was washed three times with 8 ml of solution 1. The number of reconstituted erythrocytes was checked by measuring the level of residual hemoglobin by optical density (D_{410}) and by the distribution of [^{3}H]ATP, added to the hemolysis medium (1 μCi/ml). The radioactivity of the reconstituted erythrocytes was determined on an SL-4200 scintillation counter (Intertechnique, France). The quantity of orthophosphate liberated by enzymic hydrolysis of ATP was determined [10]. The incubation medium of the reconstituted erythrocytes (solution 1, 37°C) contains 0.5 mM ouabain (Calbiochem) in some of the samples.

EXPERIMENTAL RESULTS AND DISCUSSION

Treatment of the erythrocytes with an isoosmotic solution of $MgCl_2$ removed about 100 μeq Ca^{2+} per liter of packed cells from the outer surface of the membrane (Fig. 1). This amount was about the same for erythrocytes of healthy and hypertensive subjects. Subsequent treatment of the erythrocytes with isoosmotic solution with the addition of 2 mM EDTA led to further removal of Ca^{2+} from the outer side of the membrane. Under these circumstances substantially more Ca^{2+} was removed by EDTA from the erythrocytes of hypertensive patients than from erythrocytes of healthy subjects (62 ± 6 and 35 ± 4 μeq/liter, respectively).

During the study of Mg^{2+}-ATPase in reconstituted erythrocytes obtained in hemolysis medium with or without Ca^{2+} (Figs. 2 and 3) no significant differences could be detected in the level of its ouabain-insensitive component between the erythrocytes of the normotensive and hypertensive subjects. The ouabain-dependent component of ATPase (Na^+,K^+-ATPase) likewise showed no significant differences with respect to the production of reconstituted erythrocytes in medium free from Ca^{2+} ions (0.32 ± 0.09 and 0.42 ± 0.11 μmole orthophosphate/ml packed reconstituted erythrocytes per hour for healthy and hypertensive subjects, respectively).

The addition of 50 μM $CaCl_2$ to the hemolysis medium stimulated the activity of the ouabain-dependent components of ATPase of the erythrocyte membrane sharply. However, this stimulation of activity was only half as great in the hypertensive patient as in the erythrocytes of the healthy subject.

The outer part of the erythrocyte membrane is known to have at least two types of Ca^{2+} binding sites [6]. The first of them is freed from Ca^{2+} by competitive interaction with Mg^{2+}, the second by the action of compounds with chelating properties on the membrane. The experimental results showed that the accessibility of this second type of calcium binding sites to the chelate (EDTA) was greater in the erythrocytes of the hypertensive patients than in those of healthy subjects. These results can probably be explained by a change in the conformation of anionic groups of the glyco- and lipoproteins of the outer membrane of the erythrocytes, which bind most of the calcium located on the surface [6]. The conformational position of these groups depends in turn on the state of the lipid interior of the membrane [1, 5], which is the target for many lipophilic biologically active substances [3, 8].

Quantitative changes in the distribution of calcium on the outer membrane of the erythrocytes evidently did not significantly affect Mg^{2+}-ATPase activity, for its Ca^{2+}-stimulated component is activated by Ca^{2+} only from the inner side of the membrane [12].

In principle there are therefore two possible mechanisms of development of the observed changes in ATPase activity: 1) When erythrocytes are treated with EDTA their outer membrane loses more Ca^{2+} in patients with hypertension, as a result of which the true intracellular calcium concentration falls and stimulation of Na,K-ATPase is weaker than in the erythrocytes of healthy subjects; 2) structural changes in the Ca^{2+} receptors of the outer membrane of the erythrocytes also extend to its inner part, as a result of which the kinetics of calcium stimulation of the Mg^{2+}-ATPase of the erythrocytes is modified in hypertensive patients.

The results of this investigation suggest that changes in the ability of the plasma membrane to bind calcium ions are a characteristic feature of the membrane defect discovered previously in essential hypertension [2].

LITERATURE CITED

1. Yu. V. Postnov, S. N. Orlov, and A. S. Shevchenko, Kardiologiya, No. 10, 88 (1975).
2. Yu. V. Postnov, S. N. Orlov, and A. S. Shevchenko, Kardiologiya, No. 11, 65 (1976).
3. P. V. Sergeev, R. D. Seifulina, and Yu. P. Denisov, in: The Functional State of the Glands of Internal Secretion in Cancer [in Russian], Rostov-on-Don (1973), p. 153.
4. M. T. Flanagan and T. R. Hesketh, Biochim. Biophys. Acta, 298, 532 (1973).
5. T. Gulik-Krzywicki et al., Biochim. Biophys. Acta, 219, 1 (1970).
6. C. Hallam and T. M. Wrigglesworth, Biochem. Soc. Trans., 1, 440 (1973).
7. T. F. Hoffman et al., Nature (London), 185, 186 (1960).
8. D. Papahadjopoulos et al., Biochim. Biophys. Acta, 330, 8 (1973).
9. Yu. V. Postnov and S. N. Orlov, in: International Society of Hypertension. 4th Meeting. Abstracts, Sydney (1976), p. 28.
10. W. B. Rathbum and M. W. Bethlach, Anal. Biochem., 28, 436 (1969).
11. K. I. Tanaka and S. I. Ohnishi, Biochim. Biophys. Acta, 426, 218 (1976).
12. H. J. Schatzmann, J. Physiol. (London), 235, 551 (1973).