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ROLE OF MEMBRANE-BOUND CALCIUM IN CHANGES IN ATPase ACTIVITY, PERMEABILITY, AND STRUCTURAL STATE OF THE HUMAN ERYTHROCYTE MEMBRANE

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Experiments were carried out on reconstituted erythrocytes obtained by rapid reversible hemolysis. The free Ca^{2+} concentration in the reconstituted erythrocytes was maintained by means of Ca-EGTA and Ca-citrate buffers. The ouabain-inhibited component of ATPase activity with high affinity for Ca^{2+} ($K_{0.5}$ = 4 μM) and a change in the passive and active permeability for K^+ in the region of free Ca^{2+} concentrations up to 10 μM could be found only by modifying the content of membrane-bound Ca^{2+}. Reducing its content on the inner side of the membrane of the reconstituted erythrocytes was accompanied by a change in the hydrophobicity of the hydrocarbon regions of the membrane. It is suggested that Ca^{2+}-induced changes in the structural state of the erythrocyte membrane may be the direct cause of the change in ATPase activity with high affinity for Ca^{2+} and in permeability for monovalent cations.

KEY WORDS: membrane-bound calcium; erythrocyte membrane; ATPase activity; permeability.

The presence of a Ca^{2+} pump, responsible for the distribution of Ca^{2+} between the plasma and intracellular medium in the ratio of 1000:1, is now firmly established [13]. Nevertheless, the mechanism of the effect of Ca^{2+} on the structural and functional state of the erythrocyte membrane in vivo still remains unexplained. Inhibition of Na,K-ATPase activity by high Ca^{2+} concentrations (100 μM), found in erythrocyte ghosts [7], and the corresponding increase in passive permeability for K^+ [8] do not in fact take place in intact erythrocytes in which the free intracellular Ca^{2+} concentration is maintained below 1 μM [12].

On the other hand, the concentration of Ca^{2+} bound with the inner side of the erythrocyte membrane is higher than the free intracellular Ca^{2+} concentration (Ca_f^{2+}) by more than an order of magnitude, and amounts to 8 μmoles/liter of cells [11]. An investigation was accordingly undertaken to study the role of membrane-bound Ca^{2+} in changes in the structural and functional state of the erythrocyte membrane.

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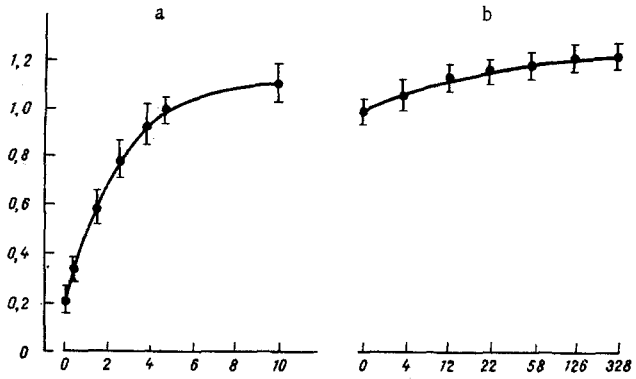

Fig. 1. Ouabain-sensitive component of ATPase activity of reconstituted erythrocytes as a function of free Ca^{2+} concentration. Reconstituted erythrocytes obtained in hemolysis medium with addition of 3 mM EGTA (a); 3 mM Na_2-citrate (b). Abscissa, Caf^{2+} concentration (in μM); ordinate, ATPase activity (in μmoles P_i/ml cells/h).

TABLE 1. Dependence of Parameters of Fluorescence of PNA Probe on Concentration of Intracellular Ca^{2+} in Reconstituted Erythrocytes

Composition of hemolysis medium, pH 6.8, in mM	$K \cdot 10^{-5}$, M^{-1}	η	n, Relative units
EGTA—3,0 $CaCl_2$—0 Tris —5,0	2,3	0,21	4,2
EGTA—3,0 $CaCl_2$—2,7 Tris — 5,0	1,8	0,15	4,0
P	<0,05	<0,05	0,1

Note. K) Binding constant of probe with membrane; η) quantum yield of fluorescence; n) number of combining sites of probe on membrane.

EXPERIMENTAL METHOD

Donors' blood 2-10 days old was used. The erythrocytes were freed from plasma and leukocytes and washed 3 times with 5-6 volumes of 0.15 M NaCl (3000 g, 5 min, 2-4°C). Reconstituted erythrocytes were obtained by Hoffman's method with modifications [4]. The specific volume of the reconstituted erythrocytes was determined on a TH-12 hematocrit centrifuge (12,000g, 2 min). The ATPase activity of the reconstituted erythrocytes and the velocity constants of K^+/K^+ exchange were determined as described previously [3, 9].

To assess the structural state of the erythrocyte membrane a hydrophobic fluorescence PNA probe (N-phenyl-1-naphthylamine, from Reachim) was used. The intensity of fluorescence was measured with an Aminco-Bowman (USA) spectrofluorometer. The methods of calculating the parameters of fluorescence were taken from the paper by Danilov et al. [1]. To vary the free Ca^{2+} concentration, Ca-EGTA* (pH 6.8; log K_S = 5.94) [12] and Ca-Na_2-citrate (pH 7.4; log K_S = 3.19) [2] buffers were used.

EXPERIMENTAL RESULTS

The results given in Fig. 1 show that with an increase in the Ca^{2+} concentration in the hemolysis medium of the reconstituted erythrocytes, in the presence of 3 mM EGTA the activity of the ouabain-sensitive com-

*EGTA) Ethyleneglycolbis(aminoethyl ester)-N,N'-tetraacetic acid.

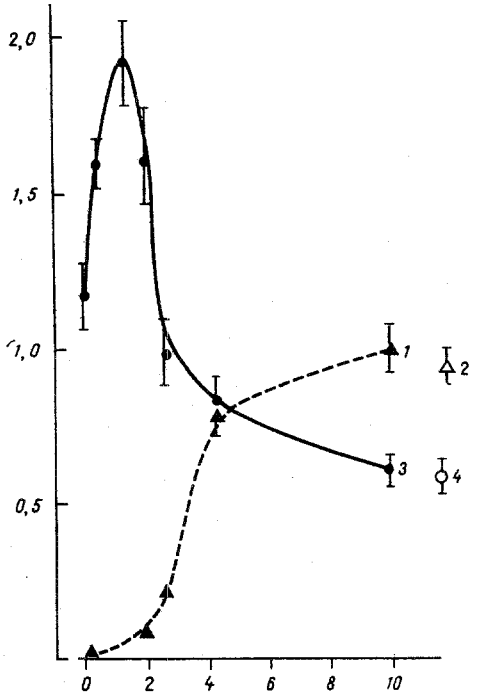

Fig. 2

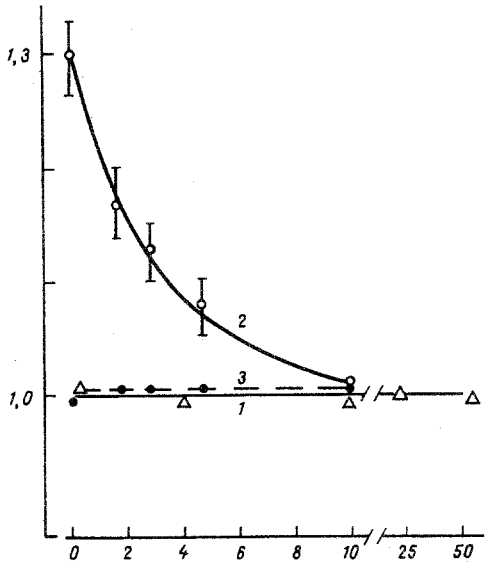

Fig. 3

Fig. 2. Ouabain-sensitive (curves 1 and 2) and ouabain-resistant (curves 3 and 4) components of velocity constants of K^+/K^+ exchange in reconstituted erythrocytes as a function of free Ca^{2+} concentration. Reconstituted erythrocytes obtained in hemolysis medium with addition of 3 mM EGTA (curves 1 and 3) and without the addition of EGTA and $CaCl_2$ to hemolysis medium (curves 2 and 4). Abscissa, Ca^{2+} concentration (in μmoles); ordinate, velocity constant of K^+/K^+ exchange (in moles/min/liter cells, $\times 10^{-5}$).

Fig. 3. Intensity of fluorescence of PNA-probe adsorbed by reconstituted erythrocytes as a function of free Ca^{2+} concentration. Variation of free Ca^{2+} concentration in reconstituted erythrocytes by means of Ca-citrate (curve 1) and Ca-EGTA (curve 2) buffers. Change in free Ca^{2+} concentration in incubation medium by means of Ca-EGTA-buffer (curve 3). Abscissa, Caf^{2+} concentration (in μM); ordinate, ratio between intensities of fluorescence of membrane-bound probe (in relative units).

ponent of Mg-dependent ATPase, with a value of $K_{0.5}$ for Ca^{2+} of 4 μM, was increased about fivefold. This component of ATPase activity of the reconstituted erythrocytes obtained in hemolysis medium in which EGTA was replaced by an equimolar concentration of sodium citrate, had much lower affinity for Ca^{2+} ($K_{0.5}$ about 50 μM), and it increased only a little (by 30-40%) with an increase in the Ca^{2+} concentration (Fig. 1b).

These findings suggest that the activity of Mg-dependent ATPase with high affinity for Ca^{2+} is connected with a change in the Ca^{2+} concentration on the inner side of the erythrocyte membrane on account of transfer of some of it to the intracellular Ca-EGTA-buffer system. This suggestion is supported by the following results obtained by atomic absorption spectroscopy. Incubation of erythrocyte ghosts in Ca-EGTA solution with a Caf^{2+} concentration of 1-2 μM reduced the content of membrane-bound Ca^{2+} by more than half [10], whereas similar incubation in sodium citrate solution did not change the concentration of membrane-bound Ca^{2+} [6].

A decrease in the Caf^{2+} concentration in the Ca-EGTA buffer in the intracellular space of the reconstituted erythrocytes led to a marked increase in the ouabain-resistant components of potassium permeability of the membrane and to inhibition of its ouabain-sensitive component (Fig. 2), the degree of which, in view of the suggestion that the stoichiometry of the Na,K-pump is independent of the Ca^{2+} concentration, is determined by Na,K-ATPase activity [14]. The decrease in the activity of this enzyme with a decrease in the Ca^{2+} concentration in the intracellular space of the reconstituted erythrocytes was reported previously [4]. The values of the active and passive components of the velocity constants of the K^+/K^+ exchange when the Caf^{2+} concentration was 10 μM coincided with values of the corresponding components for reconstituted erythrocytes ob-

tained without the addition of EGTA and Ca^{2+} to the hemolysis medium (Fig. 2). The change in potassium permeability, like the changes in ATPase activity described above, thus took place within the range of Caf^{2+} concentrations at which the Ca-EGTA buffer could modify the membrane of the reconstituted erythrocytes with respect to its Ca^{2+} content [10].

Variation of the free Ca^{2+} concentration in the intracellular space of the reconstituted erythrocytes by means of Ca-Na_2-citrate buffer or in their incubation medium by means of Ca-EGTA buffer did not significantly change the intensity of fluorescence of the PNA-probe (Fig. 3). In the case of addition of Ca-EGTA-buffer to the intracellular space of the reconstituted erythrocytes, with a decrease in the Ca^{2+} concentration an increase was observed in the intensity of fluorescence of the PNA-probe by 30%. It can be concluded from these findings that the change in the intensity of fluorescence observed was due to modification of the concentration of Ca^{2+} bound with the inner side of the membrane.

As the data in Table 1 show, a reduction in the Ca^{2+} content of the erythrocyte membrane does not lead to a change in the number of combining sites of the PNA-probe, in good agreement with its hydrophobic nature and localization in the hydrocarbon regions of the membrane. The change in the quantum yield of fluorescence of the PNA-probe by 40% taking place under these circumstances indicates that the process of modification of the concentration of membrane-bound Ca^{2+} is accompanied by changes in the hydrophobicity of the hydrocarbon regions of the membrane of the reconstituted erythrocytes [5].

It can accordingly be postulated that a change in the concentration of Ca^{2+} bound with the inner side of the erythrocyte membrane leads to structural changes in the membrane, which may be the possible cause of changes in the activity of Mg-dependent ATPase and in the permeability of the reconstituted erythrocytes for K^+ taking place under these circumstances.

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