EFFECT OF La^{3+} ON CALCIUM BINDING TO ERYTHROCYTE CYTOSKELETON

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Under the influence of lanthanum (La^{3+}) on human erythrocytes an increase in the free calcium concentration (Ca_{in}^{2+}) in the cytosol is observed [7, 8]. It is considered that this effect of La^{3+} is due to inhibition of Ca-ATPase activity and to an increase in the passive membrane permeability for Ca^{2+} [6, 8]. Changes in Ca^{2+} metabolism induced by lanthanum affect the shape of the erythrocytes [8], which can be attributed to changes in the cytoskeleton following binding with Ca^{2+} [3], However, the problem whether an increase in Ca_{in}^{2+} in the erythrocytes leads to more intensive binding of Ca^{2+} with the cytoskeleton has not been investigated.

The aim of this investigation was to study binding of Ca^{2+} with the cytoskeleton of erythrocytes when the native cells are exposed to the action of $LaCl_3$.

EXPERIMENTAL METHOD

Erythrocytes from the subclavian artery of rats or a human vein, washed to remove plasma and blood cells were preincubated for 30 min at 37°C (50% hematocrit) in medium of the following composition (mM): NaCl 130, KCl 3, $MgCl_2$ 1, $CaCl_2$ 1, glucose 5, bovine serum albumin (fraction 5) 0.1%, HEPES-Tris 20, pH 7.4 (medium A). After preincubation the samples were centrifuged at 1500*g* for 5 min, the supernatant was discarded, and the residue was distributed in volumes of 100 μl among tubes containing 100 μl of medium A + $^{45}CaCl_2$, 20 μCi/ml, or with 100 μl of medium A + ^{14}C-glucose, 30 μCi/ml, and incubated for the time required by the experimental conditions. The erythrocytes were then washed 5 times (with centrifugation) in 10 volumes of cold water (0-2°C), containing (in mM): NaCl 150, HEPES-Tris 10, pH 7.4. After washing, the erythrocytes were disintegrated by the addition of 10 volumes of medium containing (in mM): KCl 100, NaCl 10, Triton X-100 2%, HEPES-Tris 20, pH 7.4 (25°C) (medium B). The lysed erythrocytes were applied to GF/C filters (Whatman, England), which were washed with medium B 4 times (3 ml each time). The filters were placed in 5 ml of Bray's solution and their radioactivity measured on Delta 300 counter (USA).

EXPERIMENTAL RESULTS

Different compounds increasing the Ca_{in}^{2+} concentration in the erythrocytes, namely ionophores (A-23187 and ionomycin), orthovanadate, and detergents, in particular, a low concentration of saponin (0.04%), virtually do not bind Ca^{2+} with the cytoskeleton (Table 1). If $LaCl_3$, which also induces an increase in Ca_{in}^{2+} due to inhibition of Ca-ATPase [6, 8], was added to the erythrocyte suspension, binding of Ca^{2+} with the cytoskeletal fraction was observed in both human and rat cells. The value of $K_{1/2}$ for binding of Ca^{2+} was within the region of $3 \cdot 10^{-4}$ M $LaCl_3$ (Fig. 1a). It must be pointed out, however, that even low concentrations of La^{3+} (10^{-7} M) give rise to quite distinct binding of the cation with the cytoskeleton. The process of Ca^{2+} binding develops with time to reach a maximum by the 180th minute (Fig. 1b). This process also depends on the La^{3+} concentration: the higher the concentration the faster equilibrium in Ca^{2+} binding is reached (data

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TABLE 1. Binding of Ca^{2+} by Cytoskeleton of Human and Rat Erythrocytes Treated with Various Compounds

Substance added	Number of observations	Rate of binding of Ca^{2+}, nmoles/liter cells/h	
		human	rat
—	12	NR*	NR*
A—23187 ($5 \cdot 10^{-6}$ M)	4	65±13	49±16
Ionomycin (10^{-6} M)	4	87±14	76±19
(10^{-8} M)	3	3,7±0,8	3,3±0,6
Na_3VO_4 (0,5 mM)	3	4,2±0,6	3,8±0,4
Saponin (0.04%)	4	2,8±0,4	3,2±0,7
$LaCl_3$ (100 μM)	48	2490±51	2190±124
$LaCl_3$ (100 μM) + inosine (10 mM) + iodoacetamide (5 mM)	6	730±36	620±24

Legend. NR) Not recorded.

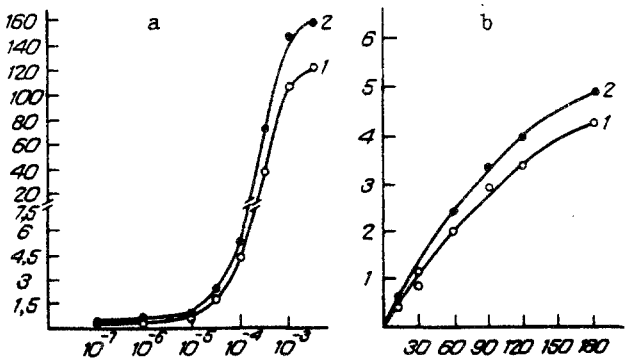

Fig. 1. Binding of $^{45}Ca^{2+}$ by cytoskeleton of human (1) and rat (2) erythrocytes, treated with La^{3+}. a) Dependence of binding on La^{3+} concentration. Abscissa, La^{3+} concentration (in μM); ordinate, rate of binding of Ca^{2+} (in μmoles/liter cells/min); b) dependence of binding on duration of incubation with La^{3+}. Abscissa, time (in min); ordinate, binding of Ca^{2+} (in μmoles/liter cells).

not given). Exhaustion of the erythrocytes relative to their nucleotide content (ATP, for example) with 5 mM iodoacetamide (incubation for 3 h at 37°C) [7, 8] leads to reduction of Ca^{2+} binding by two-thirds (Table 1).

Besides binding of Ca^{2+}, under the influence of La^{3+} on intact rat and human erythrocytes an increase in the rate of incorporation of ^{14}C-glucose into the cytoskeleton was observed (Fig. 2).

La^{3+} is known to increase the uptake of Ca^{2+} not only into erythrocytes, but also into several other cells [4, 5]. There is no information in the literature on binding of Ca^{2+} with the cytoskeleton of cells of any kind. It can be postulated that it is the increase in Ca_{in}^{2+} concentration caused by La^{3+} that is responsible for the appearance of binding of Ca^{2+} on the cytoskeleton. However, experiments with calcium ionophores and other compounds increasing the Ca_{in}^{2+} concentration demonstrate that this condition is necessary but not sufficient for the binding powers of the erythrocyte cytoskeleton to be manifested. Besides an increase in the intracellular calcium concentration, La^{3+} also evidently modifies the elements of the cytoskeleton so that they become sensitive to Ca^{2+}. Moreover, modification of the cytoskeleton by La^{3+} takes place from the outer side of the erythrocyte, since La^{3+} does not penetrate inside intact erythrocytes [8]. Changes in the cytoskeleton under the influence of La^{3+} on erythrocytes can be due to two causes: direct interaction of the cation with the external elements of the cytoskeleton (for example, with band 3), or indirectly through the induction of internal processes. La^{3+} has

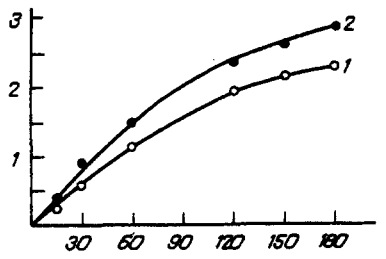

Fig. 2. Incorporation of ^{14}C-glucose into cytoskeleton of human (1) and rat (2) erythrocytes, treated with La^{3+}. Abscissa, time (in min); ordinate, incorporation of label into cytoskeleton (in μmoles/liter cells).

an equal ionic radius with Ca^{2+}, and for that reason it can displace Ca^{2+} from binding sites on proteins and lipids [1, 2] and can thus modify their structure. Additional experiments with treatment of the cytoskeleton with chloroform and methanol (1:2), after which it was sedimented on filters by Triton X-100, have shown that this operation does not affect Ca^{2+} binding. Our results in this direction are evidence that the principal Ca-binding elements of the cytoskeleton are probably proteins. The results of experiments with iodoacetamide and ^{14}C-glucose show convincingly that binding of Ca^{2+} is triggered by La through activation of ATP-dependent intraerythrocytic reactions. The possibility likewise cannot be ruled out that the effect of La^{3+} is due to a combined process of direct and indirect action of the cation. Further investigation of this problem will necessitate experiments to study binding of Ca^{2+} on the isolated cytoskeleton of erythrocytes.

Thus a new type of binding of Ca^{2+} with the cytoskeletal fraction of human and rat erythrocytes was discovered during this research. The Ca-binding property of the cytoskeleton increases sharply in response to the action of lanthanum on the erythrocyte surface.

LITERATURE CITED

1. H. G. Brittain, F. S. Richardson, and R. B. Martin, J. Am. Chem. Soc., **98**, 8255 (1976).
2. C. H. Evans, Trends. Biochem. Sci., **8**, 445 (1983).
3. S. R. Goodman and K. Shiffer, Am. J. Physiol., **244**, C121 (1983).
4. M. Korc and M. H. Schöni, Proc. Nat. Acad. Sci. USA, **84**, 1282 (1987).
5. J. C. Parker and G. J. Barritt, Biochem. J., **200**, 109 (1981).
6. E. E. Quist and B. D. Roufogalis, FEBS Lett., **50**, 135 (1975).
7. B. Sarkadi, I. Szasz, A. Gerloczy, et al., Biochim. Biophys. Acta, **464**, 93 (1977).
8. I. Szasz, B. Sarkadi, A. Schubert, et al., Biochim. Biophys, Acta, **512**, 331 (1978).