6. F. Z. Meerson, Pathogenesis and Prevention of Stress-Induced and Ischemic Heart Lesions [in Russian], Moscow (1984).
7. F. Z. Meerson, L. S. Katkova, Yu. P. Kozlov, et al., Byull. Éksp. Biol. Med., No. 12, 23 (1983).
8. F. Z. Meerson, L. S. Katkova, and V. V. Malyshev, Kardiologiya, No. 2, 74 (1985).
9. F. Z. Meerson, V. I. Lifant'ev, and V. V. Malyshev, Vopr. Med. Khimii, No. 1, 119 (1984).
10. F. Z. Meerson, N. V. Samosudova, E. V. Glagoleva, et al., Arkh. Anat., No. 2, 43 (1983).
11. E. I. Chazov, A. A. Kramer, and A. Z. Zhitov, Ter. Arkh., No. 6, 12 (1977).
12. I. I. Shepeleva, D. B. Saprygin, V. A. Saks, et al., Med. Radiol., No. 5, 16 (1979).
13. D. G. Miller and S. Mallow, Pharm. Biochem. Behav., 7, 139 (1977).

MECHANISMS OF VALINOMYCIN-INDUCED CHANGES IN OSMOTIC RESISTANCE OF ERYTHROCYTES IN SPONTANEOUSLY HYPERTENSIVE RATS

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In a previous study of the kinetics of ^{45}Ca accumulation by rat erythrocytes loaded with a calcium chelating agent it was found that valinomycin significantly reduces the sensitivity of the cells to osmotic hemolysis, as reflected in hemoglobin release [6].

The data on the mechanism of this phenomenon and differences found in spontaneously hypertensive rats are given in this paper.

EXPERIMENTAL METHODS

Blood from male spontaneously hypertensive Kyoto-Wistar rats (SHR) aged 4-5 months, with a blood pressure of 183 ± 10 mm Hg, and from normotensive Kyoto-Wistar rats (NR), of the same age and sex (control, blood pressure 108 ± 7 mm Hg), and also from normal healthy blood donors was used in the experiments. The procedure of taking blood and obtaining erythrocytes was described previously [3]. The blood samples were kept on ice for not more than 2 h before the experiment. To assess the osmotic resistance of the erythrocytes, one volume of cells was treated with four volumes of medium containing 140 mM NaCl, 5 mM Kcl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 1 mM Na_2HPO_4, 10 mM glucose, and 10 mM HEPES-Tris-buffer, pH 7.4 (37°C). In some cases the medium contained 2.5 μM valinomycin and 0.2 μCi/ml of $^{86}RbCl$. After definite incubation times 150 μl of suspension was transferred into 1 ml of cold medium containing 150 mM NaCl, 0.1 mM EDTA, and 5 mM sodium phosphate (pH 7.4), after which the cells were sedimented and hemolyzed in 0.5 ml water for 20 sec with constant shaking. After recentrifugation the supernatant was diluted 100 times with water and the optical density determined at 407 nm (FP-9 instrument, Finland). Before determination of the K^+, Na^+, and ^{86}Rb concentrations in the erythrocytes the cells were washed twice in the cold with an iso-isomotic solution of choline chloride, with the addition of 10 mM Tris-HCl, pH 7.4. For determination of the total potassium and sodium concentrations on an atomic absorption spectrophotometer (Nippon Jarrel, Japan), the erythrocytes were diluted with water $3 \cdot 10^5$ and 10^3 times respectively. Radioactivity of ^{86}Rb was determined by a liquid scintillation spectrometer (Intertechnique, France) after treatment of the erythrocytes with 5% TCA.

EXPERIMENTAL RESULTS

In the presence of valinomycin the quantity of hemoglobin released from rat erythrocytes on the addition of water was sharply reduced (Fig. 1). The maximal effect recorded after

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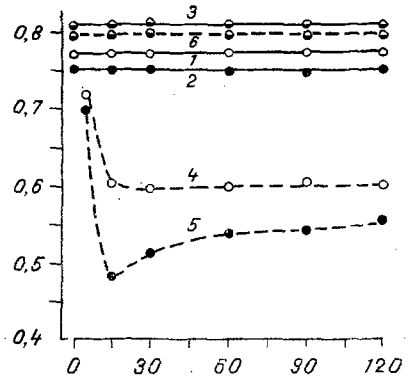

Fig. 1

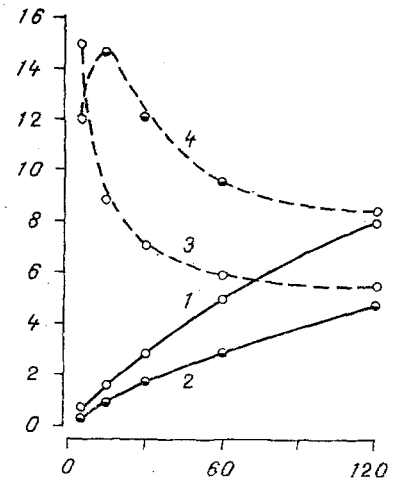

Fig. 2

Fig. 1. Kinetics of changes in optical density in erythrocyte hemolysate from NR (1, 4), SHR (2, 5), and human blood (3, 6). Abscissa, incubation time (in min); ordinate, optical density (in relative units). 1, 2, 3) Incubation medium does not contain valinomycin; 4, 5, 6) incubation medium contains 2.5 μM valinomycin.

Fig. 2. Kinetics of changes in ^{86}Rb concentration in erythrocytes from NR (1, 3) and human blood (2, 4), in absence (1, 2) and presence (3, 4) of valinomycin. Abscissa, incubation time (in min); ordinate, ^{86}Rb concentration (in cpm × 10^{-3}).

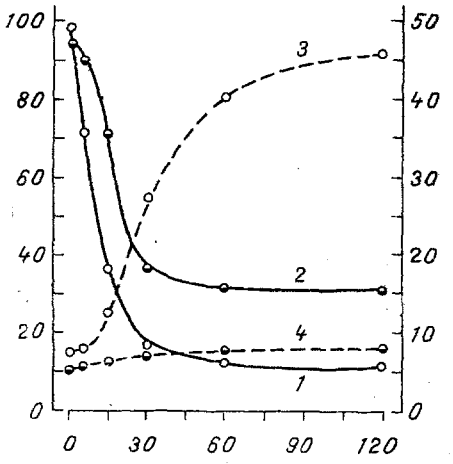

Fig. 3. Kinetics of changes in K^+ (1, 2) and Na^+ (3, 4) concentrations in erythrocytes from NR (1, 3) and human blood (2, 4) under the influence of valinomycin. Abscissa, incubation time (in min); ordinate: on left – potassium concentration (in meq/liter), on right – sodium concentration (in meq/liter).

incubation for 15 min (Fig. 1: 4, 5). Under these conditions, when erythrocytes from 8 SHR and 9 NR were investigated, significant differences were observed: in the case of SHR 63.2 ± 1.5%, and in the case of NR 80.9 ± 1.6% of the total hemoglobin content was released ($P < 0.001$). Valinomycin had no effect on the osmotic resistance of human erythrocytes (Fig. 1: 3, 6). We know that the character of organization of cytoskeletal proteins of the erythro-

cyte membranes, controlling the shape of the cells and the structural integrity of the membrane, depends on the intracellular Ca^{2+} concentration [7]. The action of valinomycin is accompanied by hyperpolarization of erythrocytes [4], which leads to an increased rate of calcium inflow into the cytoplasm [6, 11, 12]. Nevertheless, the kinetics of the change in resistance of erythrocytes to hemolysis, induced by addition of valinomycin, did not depend on the presence of Ca^{2+} (replacement of Ca^{2+} in the incubation medium by EDTA, introduction of quin 2, a highly selective Ca^{2+} chelating agent, into the erythrocytes). On the basis of these results it can be postulated that the increase in osmotic resistance of the erythrocytes due to the action of the ionophore is connected with redistribuiton of monovalent cations and, in particular, of K^+. In fact, no effect of valinomycin on osmotic resistance of rat erythrocytes could be found when NaCl in the incubation medium was replaced by isoosmotic addition of KCl (data not shown).

The kinetics of accumulation of ^{86}Rb, which was used as radioactive analog of K^+, by the erythrocytes is shown in Fig. 2. Maximal uptake of ^{86}Rb into human and rat erythrocytes under the influence of valinomycin was observed after incubation for 15 and 5 min respectively. A further decrease in concentration of the isotope was due to a fall in the K^+ level in the cytoplasm (Fig. 3). After incubation for 15 min the K^+ concentration in rat erythrocytes decreased approximatley by 30 meq, but in human erythrocytes by only 3-5 meq (Fig. 3: 1, 2). After incubation for 120 min the K^+ concentration stabilized at the level of 10 and 30 meq for rat and human erythrocytes, respectively. For rat erythrocytes this process was accompanied by an eightfold increase in the Na^+ concentration (Fig. 3: 3) whereas in human erythrocytes the Na^+ concentration rose from 5 to 8 meq (Fig. 3: 4). These differences in in Na^+ and K^+ transport, induced by the ionophore, may perhaps be the reason for the absence of an effect of valinomycin on human erythrocyte resistance to hypo-osmotic hemolysis (Fig. 1).

Valinomycin is a one-way carrier with selectivity $Rb^+ > K^+ \gg Na > Li$ [2], and for that reason significant loss of K^+ by the cells is possible only if a system of charge compensation is present. A system of this kind in erythrocytes is an anionic carrier linked with the functioning of band 3 protein [5]. This protein is the principal integral component of the membrane cytoskeleton [10]. In rat erythrocytes the net flow of anions evidently proceeds much faster than in human erythrocytes, and this facilitates total release of KCl under the influence of valinomycin. It can also be postulated that differences discovered in erythrocytes of SHR are due to a change in structure or content of band 3 protein or to modification of its function by other proteins of the cytoskeleton. Indirect proof in support of this hypothesis has been obtained by methods of electrophoresis, scanning microcalorimetry, and EPR-spectroscopy [1, 8, 9].

LITERATURE CITED

1. P. V. Gulak, S. N. Orlov, Yu. V. Postnov, et al., Biofizika, 30, 55 (1985).
2. Yu. A. Ovchinnikov, V. T. Ivanov, and A. M. Shkrob, Membrane-Active Complexons [in Russian], Moscow (1975), pp. 11-14.
3. S. N. Orlov, P. V. Gulak, Z. V. Karagodina, et al., Kardiologiya, No. 11, 108 (1981).
4. S. N. Orlov and G. M. Kravtsov, Biokhimiya, 48, 1447 (1983).
5. S. N. Orlov, Usp. Sovrem. Biol., 100, No. 5, 50 (1985).
6. N. I. Pokudin and S. N. Orlov, Biol. Memb., 3, No. 2, 108 (1986).
7. M. C. Dunn, Biochim. Biophys. Acta, 352, 97 (1974).
8. P. V. Gualk, S. N. Orlov, N. I. Pokudin, et al., Hypertension, 2, 81 (1984).
9. Yu. V. Postnov and S. N. Orlov, Physiol. Rev., 65, 904 (1985).
10. T. L. Steck, J. Cell Biol., 62, 1 (1974).
11. I. Szasz, B. Sarcadi, and G. Gardos, J. Memb. Biol., 35, 75 (1977).
12. D. R. Yingst and J. F. Hoffman, J. Gen. Physiol., 83, 1 (1984).