Thus the marked myoglobinemia and high blood enzyme levels observed during postischemic reperfusion are largely determined by damage to cardiomyocytes during reperfusion. Prophylactic injection of the antioxidant dibunol and of the calcium antagonist verapamil, however, inhibits the reperfusion component of myocardial damage, and this is accompanied by prevention of loss of enzymes and myoglobin by the cardiomyocytes.

LITERATURE CITED

1. M. V. Bilenko, V. N. Otverchenko, and V. V. Astaf'ev, Grudn. Khir., No. 6, 20 (1983).
2. P. F. Litvitskii, A. Kh. Kogan, A. N. Kudrin, et al., Byull. Éksp. Biol. Med., No. 3, 271 (1981).
3. E. Carmeliet, Inform. Cardiol., 9, No. 2, 103 (1985).
4. R. Ferrari, F. D. Lisa, Q. Raddino, et al., Myocardial Ischemia and Lipid Metabolism, New York (1984), p. 135.
5. C. Ganote, S. Liu, S. Safari, et al., J. Mol. Cell. Cardiol., 13, 93 (1981).
6. D. W. Mercer, S. Y. Surtees, M. D. Bastable, et al., Lancet, 2, 1283 (1977).
7. P. K. Nakane and A. Kawaoi, J. Histochem. Cytochem., 22, 1084 (1974).
8. H. Nordbeck, H. Kahles, C. J. Preusse, et al., J. Mol. Med., 2, 255 (1977).
9. K. A. Reimer and R. B. Jennings, Eur. Heart J., 6, Suppl. E, 97 (1985).
10. R. Roberts, Heart Lung, 10, No. 3, 486 (1981).
11. W. E. Shell, H. J. Swan, and D. Canz, Clin. Res., 29, 241 (1981).
12. H. R. Strausser, E. L. Rothfeld, and R. A. Bucsi, Proc. Soc. Exp. Biol. Med. (New York), 122, 621 (1966).
13. G. Szacz, Y. Gruber, and E. Bernt, Clin. Chem., 22, 650 (1976).
14. S. T. Vatner, H. W. Baig, S. W. T. Mander, et al., J. Clin. Invest., 61, 1048 (1978).

Na^+/H^+ EXCHANGE IN ERYTHROCYTES OF SPONTANEOUSLY HYPERTENSIVE RATS

N. I. Pokudin, S. N. Orlov, and Yu. V. Postnov

UDC 616.12-008.331.1-07.616.155.1-008.923.3-07

KEY WORDS: Na^+/H^+ exchange; rat erythrocytes; spontaneous hypertension; valinomycin.

The membrane concept of the pathogenesis of primary hypertension, which was formulated more than 10 years ago [5], has been confirmed experimentally many times [7, 10]. Most of these investigations have been conducted on blood cells from patients with essential hypertension and from rats with spontaneous hypertension, which is regarded as an adequate model of human primary hypertension (essential hypertension). Research aimed at discovering the molecular mechanisms of formation of membrane disturbances is currently in progress in several laboratories. Particular attention is being paid to the study of the state of proteins forming the cytoskeleton of the membrane [1, 6, 11]. We know that some cytoskeletal proteins participate directly in the regulation of the shape and volume of cells, including erythrocytes.

It was shown previously that if valinomycin is added to rat erythrocytes the cells are compressed and H^+-dependent sodium inflow is induced [2, 3]. It was therefore decided to compare the velocity of Na^+/H^+ exchange in the erythrocytes of spontaneously hypertensive rats (SHR) and rats of a control group, with different degrees of cell contraction.

EXPERIMENTAL METHOD

Male SHR (spontaneous hypertensive Kyoto-Wistar rats) and control WKY (normotensive Kyoto-Wistar rats), acting as the control, and whose ages and blood pressure (BP) are indicated in Table 1, were used. The procedures of taking blood and obtaining erythrocytes were described by the writers previously. The value of Na^+/H^+ exchange was judged from the amiloride-inhibited component of the velocity of ^{22}Na inflow [3]. For this purpose 200 µl of erythrocytes

Central Research Laboratory, Ministry of Health of the USSR, Moscow.

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 104, No. 10, pp. 416-418, October, 1987. Original article submitted April 3, 1987.

0007-4888/87/0010-1364$12.50

TABLE 1. Maximal Velocity of Na^+/H^+ Exchange Induced by Valinomycin in Erythrocytes of Hypertensive and Normotensive (control) Rats (M ± m)

Group of animals	Number of animals	Age, weeks	BP, mm Hg	Na^+ inflow, mmoles/liter of cells/h
WKY	6	14	108±5	33,5±1,1
SHR	6	14	183±13	32,6±0,7
WKY	4	4	—	67,0±2,0
SHR	4	4	—	51,0±2,2

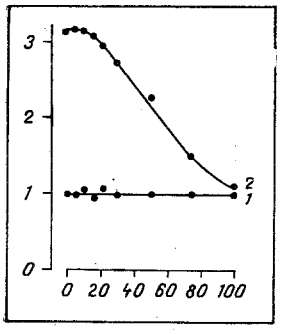

Fig. 1. Dependence of scattering of light by erythrocyte suspension from normotensive rats on K^+ concentration in incubation medium. Abscissa, KCl concentration (in mM); ordinate, intensity of scattering of light at 600 nm (in relative units). 1) Control, 2) medium contains 2.5 μM valinomycin.

was preincubated for 30 min at 37°C in 800 μl of medium A, containing 130 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 1 mM Na_2HPO_4, 10 mM glucose, 30 mM HEPES-Tris-buffer, pH 7.4. After sedimentation the cells were resuspended in the same volume of medium A containing 2.5 μM valinomycin, 0.1 mM ouabain, and 1 μCi/ml of $^{22}NaCl$. In some cases the medium contained 1 mM amiloride and the KCl concentration was increased to 10 mM. To maintain a fixed osmolarity the NaCl concentration was reduced to 50 mM (affinity of the Na^+/H^+ carrier for $[Na^+]_o$ is 20 mM [4]), and when the KCl concentration was reduced, an equimolar amount of choline chloride was added. As the writers showed previously, activation of Na^+/H^+ exchange in rat erythrocytes takes place 15 min after the addition of valinomycin, and the kinetics of sodium accumulation in the erythrocytes remains linear in character until 40 min of incubation [3]. Taking these results into consideration, in the present investigation the erythrocytes were incubated with $^{22}NaCl$ for not more than 30 min, after which an aliquot of suspension was transferred into 1 ml of cold washing medium, containing 150 mM choline chloride and 10 mM Tris-HCl, pH 7.4. Washing was repeated three times and the cell residue was treated successively with 0.5 ml of a 0.5% solution of Triton X-100 and 0.5 ml of a 10% solution of TCA to determine their radioactivity. After precipitation of the proteins 0.8 ml of the supernatant was transferred into scintillation fluid. The change in size of the erythrocytes after addition of valinomycin was determined by measuring the scattering of light by the cell suspension [3]. The quantity of hemoglobin released from the erythrocytes in the course of their incubation was determined from the optical density of the samples at 450 nm [2].

EXPERIMENTAL RESULTS

It will be clear from Fig. 1 that in the absence of valinomycin a change in the K^+ concentration in the incubation medium had no effect on erythrocyte volume, recorded by scattering of light. Addition of valinomycin at physiological values of $[K^+]_o$ was accompanied by membrane hyperpolarization, leading to the outflow of potassium and chloride and to a reduction of half to two-thirds of the quantity of intracellular water [3]. This process was accompanied by contraction of the cells, which was reflected in a more than threefold increase in the scattering of light. At zero value of the K^+-diffusion potential ($[K^+]_o \approx [K^+]_i = 100$ mM) valinomycin did not affect the membrane potential of the erythrocytes and did not lead to contraction of the cells (Fig. 1). Under these conditions it was impossible to find any

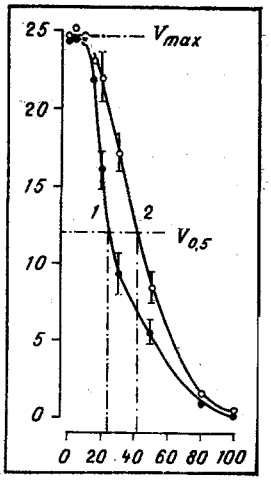

Fig. 2. Dependence of amiloride-inhibited component of velocity of ^{22}Na inflow on extracellular K^+ concentration in the presence of valinomycin. Abscissa, KCl concentration (in mM); ordinate, Na^+ inflow (in nmoles/liter of cells/h). Experiments performed on 15 WKY (1) and SHR (2) animals aged 14 weeks.

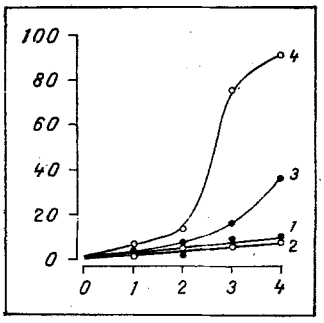

Fig. 3. Kinetics of hemoglobin release from erythrocytes during incubation in medium A containing 5 mM Na_3VO_4. Abscissa, duration of incubation (in h); ordinate, hemoglobin release (in %). 1, 3) WKY; 2, 4) SHR. Age of animals 4 weeks (3, 4) and 16 weeks (1, 2).

significant effect of amiloride on the kinetics of ^{22}Na accumulation (Fig. 2), in agreement with the abundant data on the absence of Na^+/H^+ exchange in resting cells [9].

The fall in the K^+ concentration in the presence of valinomycin led to an increase in the velocity of Na^+/H^+ exchange, and at concentrations $[K^+]_o$ of 50 and 30 mM the velocity of this process in WKY erythrocytes was 5.5 and 9.3 mmoles/liter of cells/h, respectively. In SHR erythrocytes, at the same values of $[K^+]_o$, the velocity of Na^+/H^+ exchange was increased by 45 and 75% (Fig. 2). The maximal velocities of Na^+/H^+ exchange recorded at $[K^+]_o$ = 10 mM in SHR and WKY erythrocytes did not differ; whereas only half the maximal values of the velocity of Na^+/H^+ exchange ($V_{0.5}$ = 12 mmoles/liter of cells/h) were recorded in SHR and WKY rats with 40 and 25 mM of extracellular K^+, respectively.

Two suggestions can be put forward to explain these results. First, Na^+/H^+ exchange in SHR erythrocytes is activated in the presence of less contraction of the cells. Second, in the presence of moderate concentrations of $[K^+]_o$ valinomycin induces greater contraction of SHR erythrocytes than in animals of the control group.

Data showing that SHR erythrocytes are smaller in size [8, 12] are in agreement with this hypothesis. Strict proof of its validity can be obtained in experiments with direct measurement of the change in cell volume under these conditions, and these are currently in progress in the writers' laboratory.

As was pointed out above, the maximal velocities of the Na^+/H^+ exchange in SHR erythrocytes in the chronic hypertensive stage (age 4 weeks) did not differ from those in WKY

erythrocytes. It will be clear from Table 1 that the velocity of Na^+/H^+ exchange in the erythrocytes of young rats (age 4 weeks) was approximately doubled, and in SHR erythrocytes this increase was reduced by 20%.

It can be tentatively suggested that differences in the maximal velocity of Na^+/H^+ exchange observed in erythrocytes of young SHR, in the prehypertensive stage, are due to differences in cytoskeleton formation. In fact, it has been shown on chick embryonic erythrocytes that the ratio of the content of protein in the 4.1 band to spectrin increases in the course of development from 0.83 to 3.64 [13]. Differences in the properties of the erythrocytes of young SHR and WKY rats were seen most clearly in the experiment whose results are given in Fig. 3: incubation of the erythrocytes of rats aged 16 weeks for 4 h in the presence of orthovanadate led to very slight hemolysis of the cells, recorded as hemoglobin release (Fig. 3: 1, 2). No differences were found between SHR and WKY rats in this age group. Erythrocytes of young rats (especially SHR) were much more sensitive to the action of orthovanadate: under these same conditions, after incubation for 4 h, 95% and 37%, respectively, of their hemoglobin was released from SHR and WKY erythrocytes (Fig. 3: 3, 4).

LITERATURE CITED

1. P. V. Gulak, S. N. Orlov, V. L. Shnyrov, et al., Kardiologiya, 23, No. 12, 59 (1983).
2. S. N. Orlov and N. I. Pokudin, Byull. Éksp. Biol. Med., 102, No. 3, 392 (1986).
3. S. N. Orlov, N. I. Pokudin, G. G. Ryazhskii, et al., Biol. Membrany, 4 (1987).
4. S. N. Orlov, N. I. Pokudin, and G. G. Ryazhskii, Biokhimiya, 52, (1987)
5. Yu. V. Postnov, Kardiologiya, 15, No. 8, 18 (1975).
6. Yu. V. Postnov, G. M. Kravtsov, and Yu. V. Kotelevtsev, Kardiologiya, 27, No. 8 (1987).
7. Yu. V. Postnov and S. N. Orlov, Primary Hypertension as Pathology of Cell Membranes [in Russian], Moscow (1987).
8. G. Bruschi, M. Minari, M. Bruschi, et al., Hypertension, 8, 983 (1986).
9. S. Grinstein and A. Rothstein, J. Membr. Biol., 90, 1 (1986).
10. Yu. V. Postnov and S. N. Orlov, Physiol. Rev., 65, 904 (1985).
11. Yu. V. Postnov, P. V. Gulak, and S. N. Orlov, International Society of Hypertension: 11th Scientific Meeting, Abstracts, Heidelberg (1986), p. 1260.
12. S. Sen, G. C. Hoffman, N. T. Stowe, et al., J. Clin. Invest., 51, 710 (1972).
13. M. Staufenbiel and E. Lazarides, J. Cell Biol., 102, 1157 (1986).

EFFECT OF 1-(CHLOROMETHYL)-SILATRANE ON CHANGES IN BLOOD CELLS DURING THE EXTRACORPOREAL CIRCULATION

Yu. B. Pisarskii, V. B. Kazimirovskaya, and M. G. Voronkov

UDC 616.12-089.166:616.12-78/-06:616.155.18-085.246.4:547.245

KEY WORDS: extracorporeal circulation; assisted circulation apparatus; hemolysis; lipid peroxidation; 1-(chloromethyl)-silatrane.

The creation and use of assisted circulation apparatuses (ACA) during operations on the heart, the use of an assisted circulation for the treatment of heart failure, and also the fitting of artificial heart valves and artificial main blood vessels are all connected with the problem of trauma to blood cells (BC) [3, 10-12]. Injury to and destruction of erythrocytes (hemolysis) and other BC are the main obstacles to the long-term use of assisted circulation methods and they greatly complicate the process of postoperative rehabilitation of patients. The most important traumatic factors include the effect of a foreign surface, mechanical trauma, the velocity and duration of perfusion, and oxygenation [1, 3, 12, 14]. Stabilization of the integrity and functional activity of BC during the extracorporeal circulation (ECC) is thus an acute problem which faces modern cardiology. In clinical practice no

Irkutsk Institute of Organic Chemistry, Siberian Branch, Academy of Sciences of the USSR.

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 104, No. 10, pp. 418-420, October, 1987. Original article submitted January 12, 1987.