5. A. Z. Kolchinskaya, The Oxygen Balance of the Child and Adolescent [in Russian], Kiev (1973), pp. 109-117.
6. F. G. Sitdikov, "Mechanisms and age differences of adaptation of the heart to prolonged sympathetic stimulation," Author's Abstract of Dissertation for the Degree of Doctor of Biological Sciences [in Russian], Kazan' (1974).
7. S. V. Khrushchev and V. N. Khel'bin, in: Methods of Investigation and Evaluation of the Circulatory System in Wrestlers [in Russian], Moscow (1980), pp. 56-57.
8. A. Guyton, Physiology of the Circulation. The Cardiac Output and Its Regulation [Russian translation], Moscow (1969), p. 40.
9. J. Prosser, in: Comparative Physiology of Animals [Russian translation], Vol. 3, Moscow (1973), p. 384.
10. W. G. Kubicek, J. N. Karnegis, R. P. Paterson, et al., Aerospace Med., 37, 1208 (1966).
11. J. Scheuer and C. M. Tipton, Ann. Rev. Physiol., 39, 201 (1977).

TRANSMEMBRANE POTENTIAL AND ELECTROGENICITY OF PLATELET Na, K-ATPase IN RATS WITH VARIOUS FORMS OF HYPERTENSION

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In spontaneously hypertensive rats [1, 5], just as in patients with essential hypertension, the ability of the blood platelets to aggregate is increased. Changes in aggregation in these forms of pathology are associated with disturbance of Ca^{++} transport in the platelets [1, 11], and in turn, this is regarded as the result of partial depolarization of the platelet plasma membrane in primary hypertension [1]. However, it is not yet clear how specific is this lowering of the platelet membrane potential for primary hypertension, nor has the concrete mechanism of this disturbance been explained.

The aim of this investigation was to study these problems.

EXPERIMENTAL METHOD

The platelet membrane potential in rats with spontaneous, renal, and DOCA-salt hypertension was studied with the aid of the fluorescent dye 3,3'-dipropylthiadicarbocyanine iodide [diS-C_3-(5)]. Since Na, K-ATPase may be involved in the mechanism of the membrane potential change, the electrogenic component of the potential, creased by Na, K-ATPase, was investigated.

Experiments were carried out on rats aged 12-18 weeks, weighing 220-250 g, with the following types of hypertension: 1) spontaneous genetic hypertension. Male spontaneously hypertensive rats (SHR strain) with a blood pressure (BP) of 190±5 mm Hg were used. The control consisted of inbred male normotensive Kyoto-Wistar rats (strain WKY) with BP of 120±7 mm Hg; 2) DOCA-salt hypertension. Hypertension was produced in inbred male WKY rats by unilateral nephrectomy, after which 50 mg DOCA in powder form (two injections of 25 mg, with an interval of 10 days) was injected subcutaneously. The duration of hypertension from the time of its onset was 8 weeks; 3) renal hypertension. Hypertension was produced in male WKY rats by constricting the left renal artery with a nichrome wire coil (internal diameter of the coil 0.32 mm). The right kidney was left intact. The duration of hypertension from the time of its onset was 8 weeks; 4) rats of the Milan hypertensive MHB strain. The control for these animals consisted of rats of the Milan normotensive strain (MNR). Systolic pressure was measured in the caudal artery once every 10 days by plethysmography, using the MPP-3 plethysmographic attachment (Hitachi, Japan); the plethysmograms and pressure in the cuff were recorded on a polygraph.

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TABLE 1. Basal Platelet Transmembrane Potential in Rats with Various Forms of Hypertension (20°C)

Type of hypertension	No. of investigations	Basal level of platelet transmembrane potential, mV
Control	10	—49±4
Spontaneous (SHR)	14	—36±2*
DOCA-salt	12	—46±3
Renal	12	—47±2
Spontaneous (MHB)	12	—45±3

Legend. Composition of incubation medium (in mM): NaCl 140, $CaCl_2$ 0.01, $MgCl_2$ 1, glucose 5, HEPES-Tris 20, pH 7.4. *P < 0.03 compared with control.

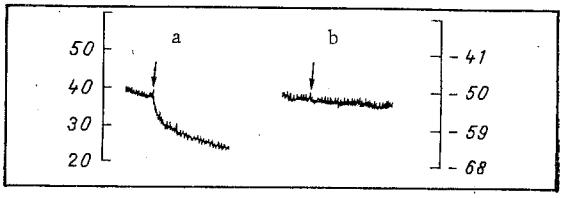

Fig. 1. Effect of ouabain on platelet transmembrane potential: a) without ouabain, b) with 0.5 mM ouabain. Arrow – addition of 5 mM KCl. Incubation medium: 20 mM HEPES-Tris (pH 7.4), 125 mM NaCl, 1 mM $MgCl_2$, 0.01 mM$CaCl_2$, 5 mM glucose. Vertical axis on left – intensity of fluorescence (in relative units); on right – membrane potential (in mV).

Platelets were obtained from plasma (1 ml of 3% sodium citrate + 9 ml plasma) by purification on a 35% albumin gradient, followed by washing twice with medium of the following composition (in mM): NaCl 135, K_2HPO_4 4.3, KH_2PO_4 25.4, sodium citrate 10, EDTA 0.1, glucose 5.5 (pH 6.5). All operations during isolation were conducted at room temperature.

The transmembrane potential of the platelet plasma membrane was recorded with the aid of the fluorescent dye- diS-C_3(5). Platelets ($4 \cdot 10^7$-$6 \cdot 10^7$) and 0.5 μmole of diS-C_3-(5) were added to 2 ml of incubation medium. The intensity of fluorescence was determined on the MPF-4 spectrofluorometer (Hitachi). The excitation and fluorescence wavelengths were 658 and 676 nm respectively, with monochromator slits of 1 and 12 nm.

The membrane potential was calculated by Nernst's equation based on the zero point for valinomycin [9]. Values of Na, K-ATPase activity were estimated by the use of calibration curves [2].

EXPERIMENTAL RESULTS

Values of the basal platelet transmembrane potential of rats with different forms of hypertension are given in Table 1. They show that, apart from SHR platelets, whose membrane potential was reduced by 12-14 mV, in all other forms of renal or DOCA-salt hypertension and also in MHB rats, no change in electrical potential of the platelet plasma membrane was observed. What is the reason for the change in membrane potential of the platelets of SHR rats? One of the mechanisms of maintenance of ionic gradients and, correspondingly, of electrical polarization of the plasma membrane of a cell is energy-dependent transport of K^+ and Na^+ by means of Na, K-activated ouabain-dependent adenosine triphosphatase (Na, K-ATPase).

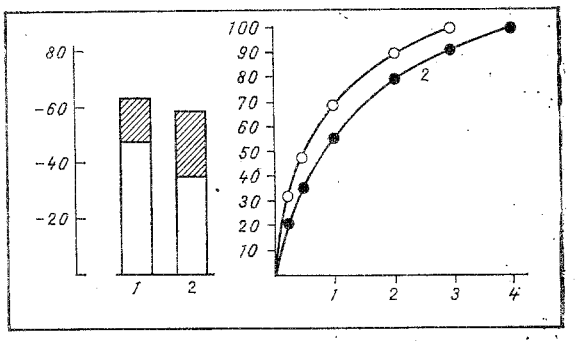

Fig. 2. Contribution of electrogenic component to transmembrane potential of platelets: 1) WKY rats; 2) SHR rats. Shaded part of column indicates electrogenic component. Incubation medium the same as in Fig. 1. Vertical axis – membrane potential (in mV).

Fig. 3. Dependence of activity of electrogenic Na, K-pump of platelets on potassium concentration in incubation medium: 1) WKY rats, 2) SHR rats. Incubation medium the same as in Fig. 1. Abscissa, KCl concentration (in mM); ordinate, intensity of fluorescence (in percent).

The presence of Na, K-ATPase in the platelet plasma membrane was demonstrated previously both biochemically and morphologically [4, 10]. We know that platelet Na, K-ATPase, as in electrically excitable tissues, is not electrically neutral, and that by its activity it generates an electrical potential. It has been shown with the aid of a fluorescent diS-C_3-(5) probe that the contribution of the Na-pump to the resting potential in human platelets is about 15 mV [9]. Investigation of activity of ouabain-sensitive Na, K-ATPase in rat platelets showed that on addition of increasing KCl concentrations (K_0^A up to 4-6 mM) to potassium-free medium the intensity of fluorescence of diS-C_3-(5) falls. This corresponds to hyperpolarization of the plasma membrane by 10-12 mV. Ouabain in a dose of 0.5 mM completely inhibited hyperpolarization induced by KCl (Fig. 1). It can be concluded from these results that the Na, K-ATPase of rat platelets is electrogenic. Comparison of the contribution of the electrogenic component to the platelet membrane potential of SHR rats with that in the control shows that its value in platelets of SHR rats was greater, namely 18-20 mV (Fig. 2).

Not only the electrogenicity of the Na, K-pump, but also the association constant ($K_{0.5}$) of activation of Na, K-ATPase by potassium in the external medium was changed in platelets of SHR rats (Fig. 3). The value of $K_{0.5}$ for platelets of SHR and WKY rats was 0.84 and 0.52 mM respectively. Complete activation of the pump for animals of the same groups occurred at a K^+ concentration of 4.2±0.3 and 3.1±0.2 mM respectively ($P < 0.03$)

In the other forms of hypertension studied no difference was observed in electrogenicity of ouabain-sensitive Na, K-ATPase.

The basal potential of muscle cells of the greater caudal and lesser cerebral arteries of SHR rats is known to be 7-12 mV lower than in the control. This disturbance is compensated by greater Na, K-ATPase activity in the smooth-muscle cells of the vessel wall [7, 8]. Essentially similar disturbances of membrane potential were found in the present investigation in the platelets of SHR rats, evidence that this type of membrane disturbance is widespread in the tissues in this form of hypertension. Differences discovered in the activation constant of Na, K-ATPase in SHR rats are evidence of the greater lability of this enzyme than in control rats. It can be tentatively suggested that any inhibition of ATPase and, in particular, that recently found in these rats due to Na, K-ATPase inhibitors [6], or a change in the plasma potassium concentration, must be reflected regularly in the value of the platelet transmembrane potential, and cause it to decrease.

Thus the transmembrane potential level in platelets, just as in excitable tissues (muscle and nerve [3]), is determined by the rates of diffusion of ions through the plasma membrane and by electrogenic activity of Na, K-ATPase. Both components of the membrane potential are changed in rats with spontaneous hypertension. This defect is not found in other forms of hypertension, which demonstrates the profound difference between

the pathogenesis of spontaneous hypertension in rats (the analog of human essential hypertension) and that of renal and DOCA-salt hypertension, and also of the genetically determined hypertension of rats of the Milan strain, whose pathogenesis is linked with primary disturbances in the kidney. The enhanced electrogenicity of the Na, K-pump of excitable tissues and platelets can be regarded as a compensatory reaction of the cell to a fall of the basal potential.

LITERATURE CITED

1. G. M. Kravtsov, Z. V. Karagodina, S. N. Orlov, et al., Kardiologiya, No. 2, 49 (1983).
2. G. M. Kravtsov and S. N. Orlov, Dokl. Akad. Nauk SSSR, 279, 508 (1984).
3. E. Kandel, The Cellular Basis of Behavior, Freeman (1976).
4. L. S.Cutler, M. B. Feinstein, G. A. Rodan, et al., Histochem. J., 13, 547 (1981).
5. P. Hamet, J. Fraysse, and D. Franks, Circ. Res., 43, 583 (1978).
6. J. M. Hamlyn, R. Ringel, J. Schaeffer, et al., Nature, 300, 650 (1982).
7. D. R. Harder, L. Brann, and W. Halpern, Blood Vessels, 20, 154 (1983).
8. K. Hermsmeyer, Circ. Res., 38, 364 (1976).
9. D. E. MacIntyre and T. J. Rink, Thrombos. Haemostas., 47, 22 (1982).
10. J. L. Moake, K. Ahmed, N. R. Bachur, et al., Biochim. Biophys. Acta, 211, 337 (1970).