mechanism of this enhancement, which leads to diminution of the hypotension and which contributes to some degree to the reduction of mortality of the animals from hemorrhagic shock [8]. The greater degree of preservation of ATP under the influence of dalargin in the myocardium also has been found in the liver tissue of animals receiving the peptide after traumatic shock [2].

The results indicate that synthetic analogs of enkephalins may be useful preparations for the treatment of hemorrhagic and traumatic shock, although this is by no means always true for toxic-infectious shock [1, 4].

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EFFECT OF LOW CALCIUM AND MAGNESIUM CONCENTRATIONS IN DRINKING WATER ON MONOVALENT CATIONIC AND CALCIUM TRANSPORT IN ERYTHROCYTES OF NORMOTENSIVE RATS

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KEY WORDS: erythrocytes; bionic transport; ATPases; Ca^{2+}, Mg^{2+}; drinking water

In essential hypertension in man and spontaneous genetic hypertension in rats changes are present in the ion-transporting systems in the membranes of various cells [7]. There is evidence of negative correlation between the incidence of cardiovascular diseases, including arterial hypertension, and the hardness of the drinking water [8-11]. However, there is a complete absence of physiological and biochemical studies of the possible effect of low and normal concentrations of calcium and magnesium ions in the drinking water on cationic transport across cell membranes, in the literature.

The aim of this investigation was to study the effect of low and normal concentrations of these cations in drinking water on transport of monovalent cations and of calcium in rat erythrocytes.

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TABLE 1. Effect of Low Concentrations of Ca^{2+} (8.0 mg/liter) and Mg^{2+} (0.3 mg/liter) in Drinking Water on Ion-Transporting Systems of Rat Erythrocyte Membrane (M ± m)

Group of animals	$^{45}Ca^{2+}$ accumulation in presence of Na_3VO_4 (5 mM) during incubation for 4 h	Na, K-pump	Na, K-cotransport	Passive permeability for K^+
	μmoles/liter of cells	mmoles/liter of cells/h		μmoles/liter of cells/h
WKY-I	31±1 (10)	0,54±0,08 (6)	0,28±0,06 (6)	28±11 (6)
WKY-II	45±2* (10)	0,30±0,04* (6)	0,33±0,04 (6)	35±19 (6)

Legend. Numbers in parentheses indicate number of animals in group; asterisk indicates values for which $p < 0.05$ compared with WKY-I group.

EXPERIMENTAL METHOD

Normotensive male Kyoto-Wistar (WKY) rats aged 14 weeks were used. Since their conversion to definitive feeding the animals were kept on a standard diet. Water was available ad libitum. The WKY-II group received drinking water with low concentrations of calcium and magnesium (8.0 and 0.3 mg/liter respectively) (tap water). Group WKY-I, kept on water with the necessary concentration of bivalent cations (80.0 and 3.0 mg/liter respectively), served as the control [1].

Erythrocytes were isolated from fresh heparinized blood [6]. Activity of the Na,K-pump was assessed on the basis of the ouabain (0.2 mM)-inhibited component of the $^{86}Rb^+$ intake. The velocity of Na,K-cotransport was determined as the component of the rate of $^{86}Rb^+$ intake that is insensitive to ouabain (0.2 mM), but is inhibited by furosemide (0.5 mM) Passive permeability for K^+ was estimated as the difference between the furosemide-inhibited, ouabain-insensitive component of the $^{86}Rb^+$ flow in medium containing 140 mM $NaNO_3$, and in medium in which all the sodium has been iso-osmotically replaced by sucrose (280 mM). Accumulation of $^{45}Ca^{2+}$ was studied during incubation of erythrocytes for 4 h in medium containing the E_1-E_2-type ATPase inhibitor Na_3VO_4 (5 mM). Methods of recording $^{86}Rb^+$ and $^{45}Ca^{2+}$ suggested by Orlov and co-workers [2-4] were used Na,K-ATPase and Ca-ATPase activity was determined as accumulation of inorganic phosphate [5].

EXPERIMENTAL RESULTS

During incubation for 4 h with orthovanadate the $^{45}Ca^{2+}$ concentration in erythrocytes of WKY-II rats increased by 1.5 times compared with animals of the control group (Table 1). This increase may have been due to increased passive permeability for Ca^{2+} and (or) reduced activity of the Ca-pump.

To examine this second hypothesis, we studied dependence of Ca-ATPase activity on the free Ca^{2+} concentration in erythrocytes treated with saponin (0.04%). It will be clear from Fig. 1 that maximal activity of Ca^{2+} and its concentrations at which this is reached did not differ for the two groups of animals, evidence in support of the first hypothesis. However, at higher concentrations calcium inhibits the Ca-ATPase of the erythrocytes of WKY-II rats by a greater degree than that of WKY-I rats. For instance, with Ca^{2+} in a concentration of $5 \cdot 10^{-6}$ M, the Ca-ATPase activity of the erythrocytes of WKY-II rats was 10-40% lower than in the control group. These differences can probably be explained by a change in the state of the set of Ca-dependent regulatory proteins [5]. As Table 1 shows, there are no differences in the rate of Na,K*-cotransport in the groups of animals compared. Activity of the Na,K*-pump in erythrocytes of WKY-II rats was 30-60% lower than in the control. We know that Na,K-ATPase activity may be influenced by many different factors: the lipic environment, energy metabolism, endogenous modifiers, etc.

We studied dependence of the activity of this transport enzyme on the Ca^{2+} concentration in saponin-treated erythrocytes and found no differences in the groups studied (Fig. 2). Judging by the character of the curves of inhibition of Na,K-ATPase, reduction of activity of the pump in erythrocytes of WKY-II rats was unconnected with any decrease in the content of the transport enzyme in the membranes, but was due to the effect of other factors modifying its ATPase

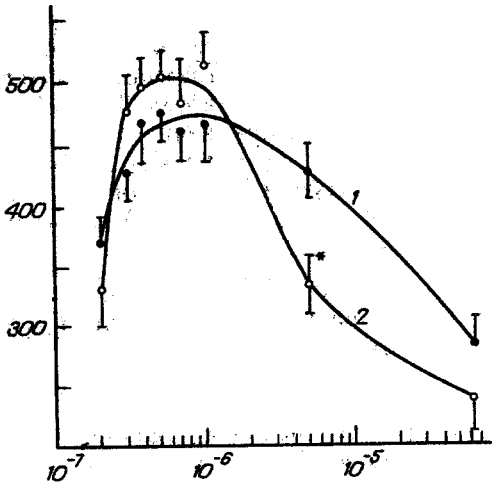

Fig. 1

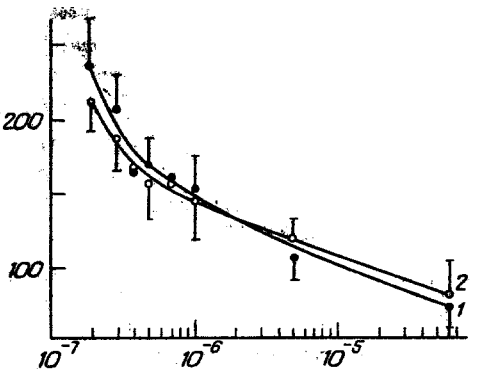

Fig. 2

Fig. 1. Dependence of Ca-ATPase activity on free calcium concentration in saponinhemolyzed erythrocytes of WKY-I (1, n = 5) and WKY-II (2, n = 5) rats. Abscissa, Ca^{2+} concentration (in M); ordinate, Ca-ATPase activity (in μmoles P_i/liter cells/min).

Fig. 2. Dependence of Na,K-ATPase activity on free calcium concentration in saponinhemolyzed erythrocytes of WKY-I (1, n = 5) and WKY-II (2, n = 5) rats. Abscissa, Ca^{2+} concentration (in M); ordinate, Na,K-ATPase activity (in μmoles P_i/liter cells/min).

reaction. Incidentally, ionic transport experiments (activity of the Na,K-pump) were carried out under conditions as close as possible to those in vivo, whereas the Na,K-ATPase reaction was investigated in the presence of strictly fixed concentrations of substrate (Mg-ATP, 0.8 mM) and of modifiers (ATP 1 mM, Mg^{2+} 0.37 mM, Na^+ and K^+ 65 mM of each), i.e., under conditions when the enzyme exhibits maximal activity. The most probable cause of the reduced activity of the latter in erythrocytes of WKY-II rats is a low Na concentration in the cytosol compared with that in the erythrocytes of WKY-I rats. The low ATPase activity of the erythrocytes of WKY-II rats may perhaps be due to the increased intracellular Ca^{2+} concentration as a result of an increase in its passive permeability If it is assumed that this is so, we would have to expect an increase in the rate of K^+ outflow from the erythrocytes of WKY-II rats due to activation of Ca-dependent K-channels. However, experiments to determine passive K-permeability revealed no differences between the erythrocytes of the groups of animals compared (Table 1).

The experiments thus showed that exogenous Ca^{2+} and Mg^{2+} (dietary) affect transport of cations and the state of the ion-transporting systems of rat erythrocyte membranes. This effect is probably brought about by the nonspecific action of various biologically active substances (parathyroid hormone, calcitonin, 1,25-dihydroxychole calciferol), which are involved in the regulation of homeostasis of bivalent cations at the whole body level.

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POSTRESUSCITATION TOXEMIA AND ITS POSSIBLE CORRECTION BY ALBOSORB

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Endogenous toxemia arising in terminal and postresuscitation states significant complicates the course of postresuscitation sickness [7]. A promising trend in detoxication is the use of additional transport agents, binding toxins and conveying them to their points of excretion, degradation, or adsorption [3, 9].

The aim of this investigation was to study the efficacy of albosorb, a product obtained from albumin treated on charcoal adsorbents, and possessing increased adsorptive capacity for ligands of different nature, in the postresuscitation period [5, 6].

EXPERIMENTAL METHOD

Experiments were carried out on 40 dogs weighing 12-25 kg, anesthetized with pantopon (8 mg/kg) and pentobarbital (5-10 mg/kg). The animals were subjected to clinical death from exsanguination, for a period of 10 min. Resuscitation was carried out by intraarterial injection of blood with adrenalin and artificial ventilation of the lungs with 80% oxygen. In the 15 experiments of group 1, 5-7 min after restoration of cardiac activity, and at a time of incomplete restoration of the blood loss (by 10-20 ml/kg), albosorb was injected in a dose of 7-10 ml/kg over a period of 25-30 min. In the eight experiments of group 2, a pharmacopoieal preparation of albumin was injected in the same volumes. The animals of group 3 (17 experiments) received no additional treatment. Before blood loss and between 15 min and 6 h after resuscitation, biochemical and physiological tests were carried out. The total content of molecules of average molecular weight (MAMW) [1] was determined in the blood plasma. Concentrations of components with mol. wt. of up to 30,000 daltons in the ultrafiltrate of plasma obtained with the aid of PT-30 membrane filters (Amicon, USA), were determined by high-pressure liquid chromatography (HPLC system from LKB, Sweden). The binding capacity of the plasma proteins relative to weakly bound ligands was estimate by the use of the total area of chromatographic peaks corresponding to the above-mentioned components [6]. The ability of plasma proteins to form complexes by hydrophobic interaction was assessed relative to Congo Red [8]. The osmotic resistance of the erythrocytes to hypotonic hemolysis was determined by the method in [4] and the colloid osmotic pressure (COP) by means of a colloid osmometer from Knauer (West Germany). The cardiac output was measured by the thermodilution method, and the systolic and pulmonary arterial pressures and heart rate were recorded. Oxygen saturation, partial pressure, and concentration, hemoglobin concentration, and hematocrit were determined in arterial and mixed venous blood, and the total oxygen consumption was calculated.

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