Differently from the effect of separate components, FDP and lidocaine combined in subthreshold doses (Table 2) caused an almost twofold decrease of reperfusion arrhythmias, a significant shortening of VT paroxysms, and a statistically reliable decrease of the intensity of ventricular extrasystoles.

The above experimental data provide evidence of a high antiarrhythmic activity of intermediates of glycolysis under conditions of acute myocardial ischemia, this obviously being associated with their ability to prolong the production of glycolytic energy [1]. The additional energy facilitates maintenance of the functional activity of the energy-dependent mechanisms of ion transport and the stabilization of the electrophysiological parameters of ischemized cardiomyocytes, thereby preventing the development of electrical destabilization of the heart.

Hence, pharmacological correction of energy metabolism in the myocardium is a promising way of improving pharmacological therapy of heart rhythm disturbances; intermediates of glycolysis, in particular, may be used both in monotherapy and in combination with "classical" antiarrhythmics.

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Characterization of Erythrocyte Na,K-ATPase in Rats with Different Attitudes to Ethyl Alcohol in Health and after Chronic Alcoholization

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Na,K-ATPase (E.C.3.6.1.4), an enzyme transforming the energy of ATP hydrolysis to perform transmembrane transfer of monovalent cations against their electrochemical potential, is an integral protein of the plasma membranes of all body tissues. It has been shown that the chronic action of ethanol leads to an increase in Na,K-ATPase in the brain [4, 6, 9], erythrocytes [8, 10], skeletal muscles [8], and liver [11]. At the same time, the effect of chronic alcoholization on Na,K-ATPase of animals with different attitudes to ethyl alcohol has been analyzed.

In order to obtain new data on plasma membrane function of animals preferring and rejecting alcohol, we made a study of erythrocytes Na,K-ATPase in rats with different attitudes to alcohol before and after chronic alcoholization.

MATERIALS AND METHODS

Experiments were carried out with 48 male Wistar rats weighing 350-400 g. Alcohopl attitude was

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tested by offering a free choice between water and 15% aqueous alcohol solution for ten days. The animals were kept on a standard diet (pellets) throughout the test. Two groups were distinguished by the test results: animals rejecting alcohol (RA) (n=25) with a daily alcohol intake of 1.1±0.8 g/kg and those preferring alcohol (PA) (n=23) with a daily alcohol intake of 6.6±2.4 g/kg (the estimation was made in terms of 96% alcohol).

After the testing, 15 animals from each group were given only water during one month before decapitation. The rest (10 RA and 8 PA) were kept for 3 months on a 15% aqueous solution of alcohol and then tested repeatedly. After the three-month alcoholization the PA animals were still preferring alcohol; moreover, their daily alcohol consumption had increased and was 10.1±2.3 g/kg. In the RA group five animals started preferring alcohol (the daily dose under conditions of free choice was 9.3±2.0 g/kg (RA-PA group) and five animals remained alcohol rejecting with a daily dose of 1.2±0.6 g/kg (RA-RA group).

After repeated testing the PA and PA-RA groups were immediately used for biochemical study, while the RA-RA animals were kept on forced alcohol (15% ethanol) for 15 days, and then decapitated. The daily alcohol consumption of these animals was 8.5±1.8 g/kg.

After decapitation, blood was collected in tubes with heparin. ATPase activity in erythrocytes treated with 1% Tween-20 (1:1 ratio) for an hour was measured as described by Kazennov et al. [1] with a Sample Biochemical Analyzer FP-901 (Finland) at 37°C in a medium containing (in mM) NaCl 125, KCl 25, EDTA 0.5, ATP 2, $MgCl_2$ 3, 6, or 12, and Tris-HCl 50 (pH 7.4). Na,K-ATPase activity was estimated as the difference between ATPase activity measured under the conditions mentioned above and that in a similar medium where NaCl was replaced by KCl in a concentration of 150 mM.

Na,K-ATPase was measured not only in the presence of the optimal concentrations of incubation medium ingredients but also under conditions of "functional stress" of the enzyme, created by a shift in the $MgCl_2$ concentration (3, 6, or 12 mM) in the incubation medium. This approach was proposed by Maslova et al. [2] to elucidate the effects of various environmental factors on the body. The use of this method yielded results in the form of a set of curves, which is more informative than just individual measurements of the enzyme activity.

Tween-20, Tris-HCl, and EDTA from Sigma (USA), ATP from Reanal (Hungary), and other reagents from Reakhim (Russia) were used in the experiments. The data were statistically processed with an Electronika MK 51 microcomputer and the significance of the differences was assessed by the Student t test.

RESULTS

There was no difference in the activity of erythrocyte Na,K-ATPase between the RA and PA

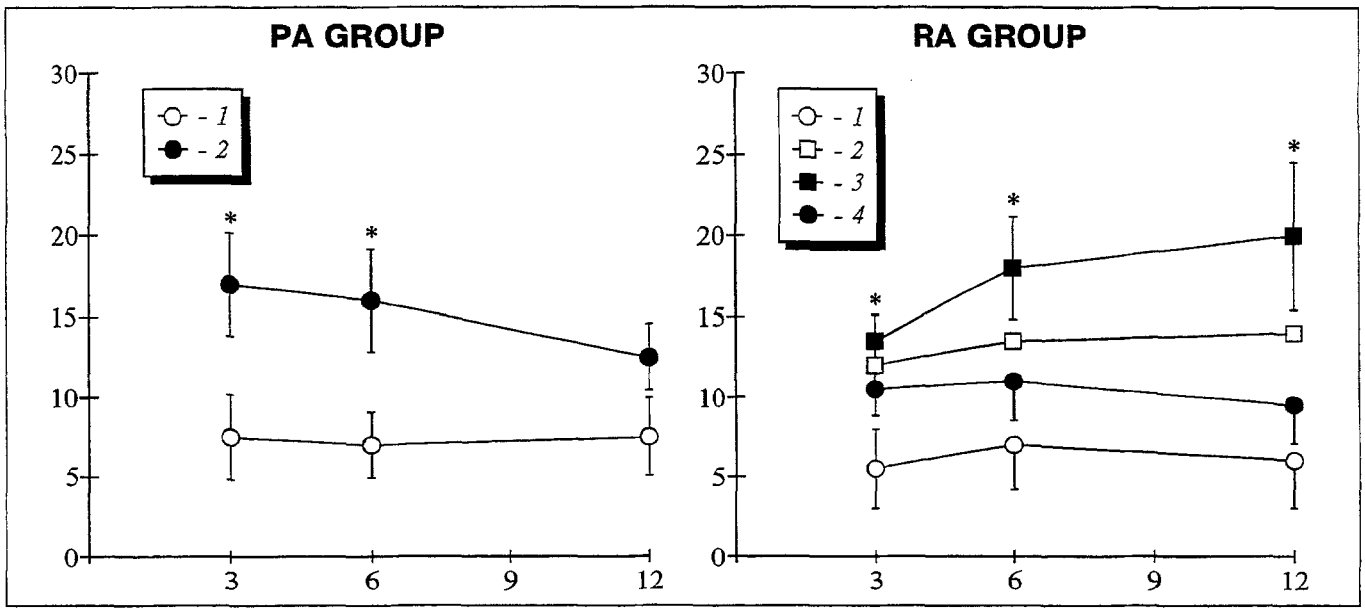

Fig. 1. Relationship between rat erythrocyte Na,K−ATPase and Mg ion concentration in incubation medium. PA group : before alcoholization (*1*) and three months after chronic alcoholization (*2*); RA group: before alcoholization (*1*) and after three months chronic alcoholization (*2*); RA−RA animals still rejecting alcohol after three months forced chronic alcoholization (*3*) and RA animals starting to prefer alcohol after three months forced alcoholization, group RA−PA (*4*). Asterisk shows significant difference (p<0.05) between the control and experimental groups of animals.

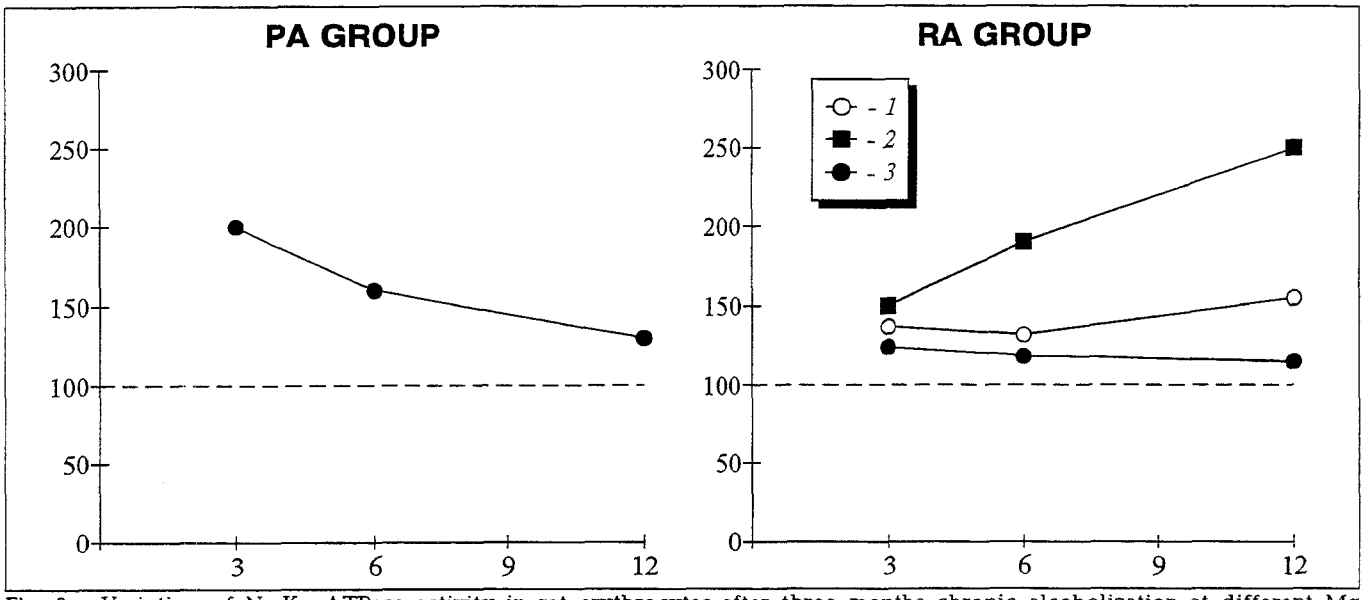

Fig. 2. Variation of Na,K−ATPase activity in rat erythrocytes after three months chronic alcoholization at different Mg concentrations (in percent to control). RA group: *1*) after three months chronic alcoholization; *2*) and *3*) RA−PA and RA−RA, respectively, after three months forced alcoholization. 100% corresponds to Na,K−ATPase activity before alcoholization.

groups of animals for all the magnesium concentrations used in the experiments with animals given no alcohol after the first testing.

After three months alcoholization, Na,K-ATPase activity in the erythrocyte suspension increased in both groups. The dependence of the enzymatic activities on $MgCl_2$ is presented in Fig. 1. It is clearly seen that in PA animals a statistically significant increase of Na,K-ATPase activity ($p<0.05$) takes place for 3 and 6 mM of magnesium, whereas for 12 mM magnesium this increase is insignificant (Fig. 1, *a*).

The Mg dependence of Na,K-ATPase activity in the RA group had a different pattern: the maximal increase in this activity was observed for a 12 mM $MgCl_2$ concentration (Fig. 1, *b*). Repeated alcohol testing after three months chronic alcoholization allowed us to single out a group of animals with stable alcohol rejection even after its chronic forced intake (RA-RA). In this group activation of the enzyme by magnesium was clearly expressed (Fig. 1, *b*, curve *3*). The relationship between Na,K-ATPase and magnesium in the RA-RA group differed significantly from that of the RA-RA animals, being most similar to that in rats initially preferriong alcohol (Fig. 1, *b*, curve *4*).

Taking the initial (prealcoholization) Na,K-ATPase activities for every Mg^{2+} concentration as 100%, we estimated the changes in the enzyme activity in percent after three months alcoholization at the respective Mg concentrations for all animal groups; the resultant relationships are presented in Fig. 2.

For PA animals Na,K-ATPase activation drops with an increase in the Mg concentration (Fig. 2, *a*), whereas that for RA animals rises (Fig. 2, *b*). Moreover, in the RA-RA group the Mg dependence of this activity is even more pronounced. For the RA-PA group this relationship is intermediate between the RA and PA groups.

Hence, we have found an increase in erythrocyte Na,K-ATPase after chronic alcoholization both in RA and PA animals. However, the dependence of the enzyme activity increase in the studied groups appears to be different: in PA animals it decreased with a Mg increase, whereas in RA animals an inverse relationship was demonstrated.

Unambiguous interpretation of the results is hardly possible because the molecular mechanisms of Na,K-ATPase regulation by magnesium are still unknown. There are reports on the regulating effect of magnesium being mediated by lipids [3, 5, 7]. These data give grounds for considering our findings as indirect evidence of a differing ethanol sensitivity of membranes and membrane enzymes in animals rejecting and preferring alcohol. This approach may be used in the future to develop prognostic criteria of the whole-body response to alcohol.

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Clinical Trials of a Recombinant Gamma Interferon 1. Effects of the Gamma Interferon Administered Intramuscularly or by Inhalation on Circulating Interferon and 2,5-Oligoadenylate Synthetase and Protein Kinase Activities

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The development in the CIS of recombinant human interferon preparations has been a major achievement of great medical significance [1-3,5]. A recombinant alpha-2 interferon (Reaferon) is already being widely and successfully used for the prevention and treatment of viral diseases [4]. A recombinant gamma interferon (Gammaferon) is now undergoing initial clinical trials. In order to evaluate its clinical efficacy, it is essential to know how it influences the interferon system of the body. The most reliable indicators of this influence are the interferon-dependent enzymes 2,5-oligoadenylate synthetase and protein kinase which are detectable in blood samples and which, together with circulating interferon, the interferon reaction of leukocytes, and the activity of natural killers, can provide the necessary information on the functional activity of the interferon system [7,10]. The two above-mentioned enzymes preeminently characterize the antiviral activity of interferon [11].

Previously, we showed that Reaferon and Larifane (the dsRNA of phage F2) are capable of activating the interferon system in volunteers [6]. In this communication we present the results of a study carried out to compare the effect of Gammaferon on the interferon system of volunteers after its administration intramuscularly and by inhalation.

MATERIALS AND METHODS

A Gammaferon preparation with an activity of 30,000 IU per ampoule, manufactured by the Ferment Company of the Ministry of the Biomedical Industry, was tested in the Clinical Department of the Ivanovskii Institute of Virology at the request of the Pharmacological Committee. The trials were conducted on 3 groups of volunteers - a total of 20 men recovering from influenza or acute respi-

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