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Effect of Ethanol on The Enzyme-Substrate Interaction of Dehydrogenases.

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ABSTRACT

The analysis of published data showed the necessity and urgency of research of theinteraction specificity of small and large molecules that might have a regulatory value. In in vitro conditions we were studying the effect of ethanol on the activity of dehydrogenases: glyceraldehyde phosphate dehydrogenase - GAPDH (EU 1.2.1.12), α-glycerol phosphate dehydrogenase - α-GPD (EU 1.1.1.8) and lactate dehydrogenase - LDH (EU 1.1.1.27) - in the hemolysate of red blood cells and in the isolated homogeneous enzymes. A comparative evaluation of enzyme activity after their incubation with ethanol in the hemolysate and in the isolated environment showed that the activity of GAPDH, α-GPD and LDH increased in both media, but in a quantitative ratio the enzyme activity in isolated the environment was considerably smaller than in the hemolysate - multicomponent medium. Thus, during the metabolic adaptation to the effect of external stimulus the efficiency of all studied dehydrogenases increases. Ethanol can play the role of regulator of molecular processes of metabolism by modulating the enzyme-substrate interaction.

Keywords: ethanol, glyceraldehyde phosphate dehydrogenase, α-glycerol phosphate dehydrogenase, lactate dehydrogenase.

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INTRODUCTION

The ability of low-molecular metabolites to make contact with the macromolecules determines a wide range of their biological action. As it is known, the ethanol is actively involved in the various biochemical reactions.Our study of the spectrum of biological activities of ethanol using the Prediction of Activity Spectra for Substances (PASS) program of version 1.917 made it possible to identify the multi-field pharmacological effects and a variety of molecular mechanisms of influence on the activity of factors, modulating the intra- and intercellular interactions. It can be assumed that ethanol can act as a mediator, modulating the functions of macromolecules, a regulator of parametabolic interactions, a ligand for receptors and transport molecules.

The enzymes, which function is implemented by the catalytic activity, have been chosen as the object of study of the ethanol regulatory role. By analyzing the literature data, it should be noted the necessity and urgency of research of the interaction specificity of small and large molecules, which is usually accompanied by the conformational transformations of the latter [1, 2]. As it is known, the small molecules are not only the object of catalytic action, but they also play an important role of information molecules that regulate the activity of biomolecules in the various cell compartments, in the extracellular space, in the biological fluids [3, 4]. Being the products of enzymatic conversion, the metabolites create a microenvironment, in which the enzymes function, and thus they become the carriers of information, forming a kind of memory of the past molecular event [5]. We studied how the ethanol compound with a low molecular weight and a high chemical activity affect the enzyme-substrate interactions of some dehydrogenases.

During the experiments we were studying the effect of ethanol on the activity of the following dehydrogenases: glyceraldehyde phosphate dehydrogenase - GAPDH (EU 1.2.1.12), α-glycerol phosphate dehydrogenase - α-GPD (EU 1.1.1.8) and lactate dehydrogenase - LDH (EU 1.1.1.27) - in the hemolysate of red blood cells and in the homogeneous enzymes in the in vitro tests. The studied dehydrogenases belong to the family of NAD-dependent dehydrogenases. All three enzymes play a key role in the carbohydrate-lipid metabolism, providing a creation of energy in living organisms and supplement of substrates for the various metabolic processes.

MATERIALS AND METHODS OF STUDY

To set up the experiment it wasused a hemolysate prepared from the whole blood of 30 healthy persons, as well as the homogeneous medications of such enzymes as GAPDH, α-GPD and LDH; production of the company "ICNBiomedicals" (USA). To obtain the hemolysate of 100 mcl of whole blood it was added to 0.9 ml of bidistilled water. All procedures were carried out in the cold. The enzyme medications were diluted with: GAPDH - 0.05 M of tris-HC1 buffer (pH 8.6), α-GPD - 0.05 M of triethanolamine hydrochloride buffer, pH 7.5, LDH - 0.1 M of phosphate buffer, pH 7.4. To perform the study it was taken 10 mcl of hemolysate and it was incubated with ethanol at a final concentration of 0.3 mM for 5 minutes at a temperature of 25 ^{0}C, a similar experiment was made with the enzyme medications.To calculate the specific activity of enzymes (U/mg) it was measured the protein concentration in the hemolysate (hemoglobin calculated) by the Lowry method [6]; the protein content in the commercial medication was determined by measuring the absorbance at 280 nm. The activity of dehydrogenases studied was measured by the spectrometer LAMBDA 20 (Perkin Elmer, Switzerland). The catalytic activity of GAPDH was determined by the rate of reduction of NAD^{+} in the reaction of glycolytic oxidoreduction of 3-phosphoglyceraldehyde to 1,3-bisphosphoglyceric acid, recording an increase in optical density at 340 nm; the activity of α-GPD was measured by a decrease of NADN(H+) in the reaction of dihydroxyacetonephosphate reduction [7]. The activity of lactate dehydrogenase was determined during the reduction reaction of pyruvate on reduction of the optical density associated with a decrease of NADH (H+) [8].The statistical analysis of the results obtained was performed by the application program package SPSS 10.0; for a description it was used such statistical characteristics as the arithmetical mean (M), mean-square deviation (SD). To assess the significance of differences in mean values of two samples it was used the Student's t-test; the critical value of significance level was taken equal to 0.05 [9].

RESULTS AND THEIR DISCUSSION

At the first stage of the study we were studying the effect of ethanol on the activity of dehydrogenases studied in the hemolysate of red blood cells. The results obtained show that the activity of GAPDH, α-GPD and LDH has not changed during the incubation (Table 1).

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Table 1. Effect of ethanol on the activity of GAPDH, α-GPD and LDH of the hemolysate of red blood cells (U/mg)

	GAPDH	α-GPD	LDH
Control (M±SD)	0.251±0.003	0.192±0.003	0.317±0.004
Incubation without ethanol (M±SD, Δ%, P - level)	0.250±0.004 Δ% -0.4 p>0.05	0.195±0.007 Δ% +1.5 p>0.05	0.314±0.007 Δ% -1.0 p>0.05
Incubation with ethanol (M±SD, Δ%, P - level)	0.440±0.005 Δ% +75.3 p<0.01	0.244±0.006 Δ% +27.1 p<0.01	0.517±0.009 Δ% +63.1 p<0.01

It is observed the activation of all three enzymes in the presence of ethanol. The most pronounced changes in activity are observed in GAPDH (Δ+ 75.3) and LDH (Δ+ 63.1). An increase in the activity of α-GPD was less significant (Δ+ 27.1).

At the second stage of the study, we were studying the effect of ethanol on the isolated homogeneous enzymes without impact of numerous components of the hemolysate of red blood cells. The activity of GAPDH, α-GPD and LDH was not changed during the incubation (Table 2).

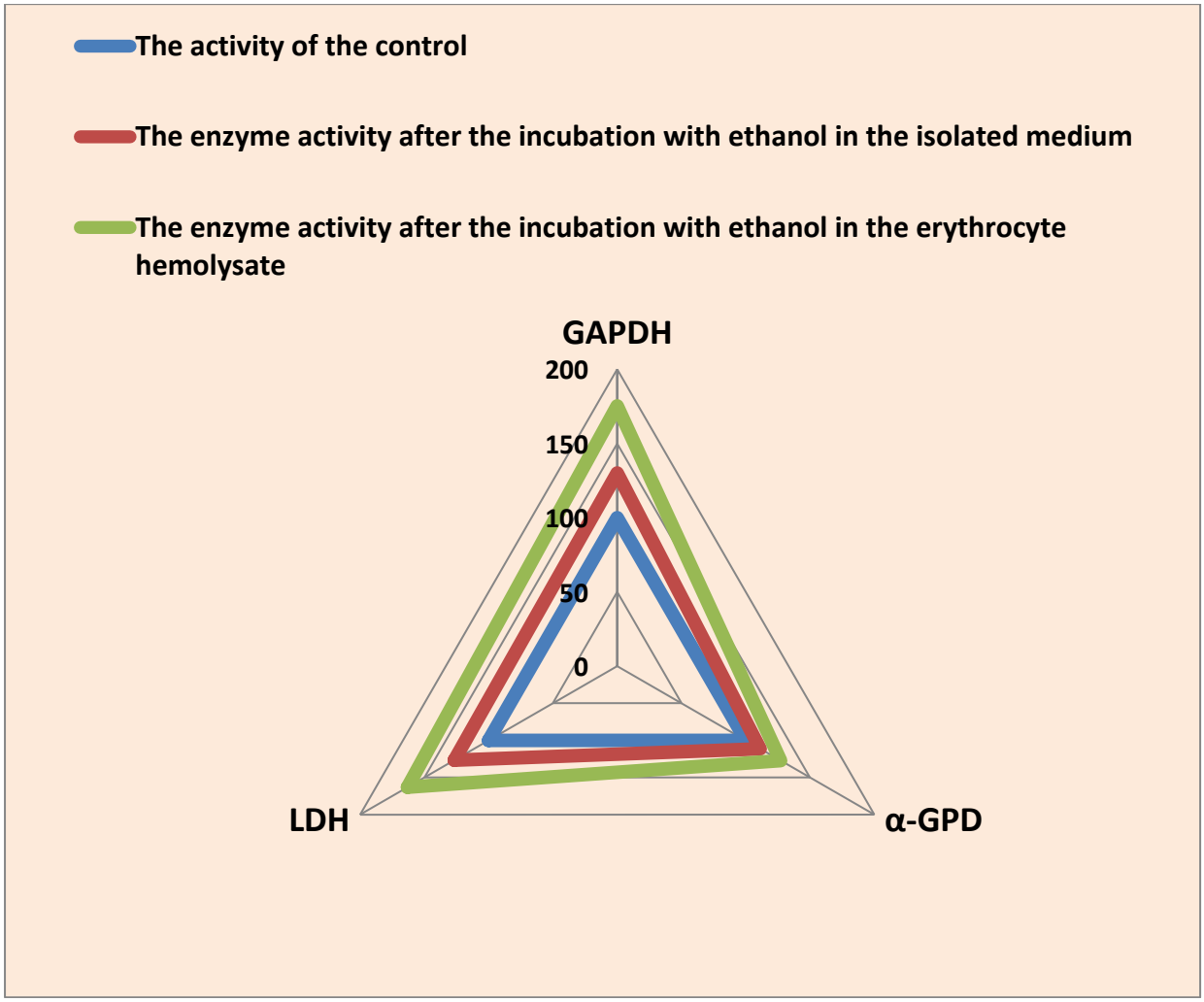

Figure. Comparative evaluation of the activity of enzymes in the hemolysate of red blood cells and in the isolated environment (100% - of the value of enzyme activity in control samples)

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Table 2. Effect of ethanol on the activity of GAPDH, α-GPD and LDH in an isolated environment (U/mg)

	GAPDH	α-GPD	LDH
Control (M±SD)	23.19±0.87	2.94±0.02	493.4±17.1
Incubation without ethanol (M±SD, Δ%, P - level)	22.72±0.88 Δ% -2, p>0.05	2.98±0.04 Δ% +1.3, p>0.05	485.8±19.1 Δ% -1.6, p>0.05
Incubation with ethanol (M±SD, Δ%, P - level)	30.17±0.77 Δ%+30.1, p<0.01	3.26±0.04 Δ% +11.2, p<0.01	625.1±16.5 Δ% +26.7, p<0.01

It was found the stimulating effect of ethanol on the isolated catalytic proteins. It was established that the activity of GAPDH increased to 30.17 U/mg after exposure of ethanol, compared with the value in control - 23.19 U/mg (Δ%+ 30.1%). A comparison of the activity of α-GPD and LDH before and after incubation with ethanol indicates that ethanol causes a shift of enzyme activity towards the increased activity - Δ%+ 11.2; Δ%+ 26.7 respectively.

A comparative evaluation of effect of ethanol on the activity of dehydrogenases in the polyenzyme, polysubstrate environment - hemolysate and in the isolated environment has shown that the activity shift of GAPDH, α-GPD and LDH occurs towards an increase in both environments, but in quantitative terms the activity of enzymes in the isolated environment increases much less than in the hemolysate (Figure).

SUMMARY

It is established the systemic effect of ethanol on the dehydrogenases studied. The observed increase of enzyme activity in the hemolysate and in the isolated environment after incubation with ethanol indicates that ethanol, having the reactive hydroxyl group by virtue of having a short bicarbon radical and minor inductive effect, may enter into the donor-acceptor relationships with the apoenzyme functional groups that leads to an increase in its reactivity.The enzyme activity in the hemolysate is substantially higher than in the isolated environment, as the direct and indirect effects of ethanol are apparently summed up, i.e. the mediated interaction of ethanol with other reactive compounds and the creation of optimal microenvironment for the reaction with the coenzyme and substrate, as well as their interaction with the surrounding macro and micro-molecules. For example, GAPDH performs a series of non-canonical functionsalong with the dehydrogenase and phosphorylation function, such as regulation of energy metabolism, apoptosis induction and development of amyloidosis [10, 11, 12]. The main purpose of such enzyme sensitivity is to respond to environmental change, acclimatize a cell to different conditions and provide a proper response to a stimulus. The observed effect of ethanol on the activation of dehydrogenases in the lysate of red blood cells enables to extrapolate the results to the possible effect of ethanol on the function of intracellular metabolism, as well as to suggest the presence of multiple effects of ethanol by modifying the protein conformation, further entering in the protein-ligand interactions.

CONCLUSION

Comparing the ethanol activation of GAPDH, α-GPD and LDH, it should be noted more significant effect on the activity of GAPDH and LDH than the activity of α-GPD, both in the multicomponent medium - hemolysate and in the isolated environment. The modulating effects of ethanol may depend on the various structural organizations of the catalytic proteins. Thus, during the metabolic adaptation to the effect of external stimulus the efficiency of all studied dehydrogenases increases. Ethanol can play the role of regulator of molecular processes of metabolism by modulating the enzyme-substrate interaction.

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