Effect of Alcoholic Intoxication on Water Content and Activity of Na,K-ATPase and Ca-ATPase in Rat Brain

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We studied the effect of 2-week alcohol intoxication on water exchange and activity of Na,K-ATPase and Ca-ATPase in rat brain. Alcohol intoxication increased water content in the brain due to cell hyperhydration. It is assumed that hyperhydration results from increased Na^+ content in cells due to inhibition Na,K-ATPase activity, which in turn is caused by activation of lipid peroxidation under the effect of ethanol. A possible mechanism of Na,K-ATPase inhibition.

Key Words: *alcohol intoxication; brain; Na,K-ATPase, Ca-ATPase; malonic dialdehyde*

Disturbance in the functional state of brain cells is the cause of psychic changes in humans. The key role in normal functioning of the cells is played by membrane-bound enzymes such as Na,K-ATPase and Ca-ATPase regulating the distribution of Na^+, K^+, and Ca^{2+} between the cell and intercellular space, because they determine the bioelectric properties of neural cells. Experimental data on the changes of Na,K-ATPase activity during alcoholization are contradictory. It was reported that chronic or single alcohol intake increases Na,K-ATPase activity in rat brain [6,11]. At the same time activity of this enzyme in erythrocytes of chronic alcoholics decreased [13]. In *in vitro* experiments ethanol either inhibited Na,K-ATPase activity [6] or had no effect on this enzyme [14]. The effect of ethanol on activity of membrane-bound enzymes is mediated via its action on the plasma membrane. Ethanol produces a destabilizing effect on neuronal membranes: it increases their fluidity and decreases viscosity thereby modifying functional properties of membrane-bound proteins [5]. It is known that decrease in membrane viscosity leads to activation of membrane-bound enzymes, in particular Na,K-ATPase. On the other hand, alcohol intoxication promotes the development of cerebral ischemia and hypoxia [8,10] stimulating generation of free radicals and activating lipid peroxidation (LPO) in cells. These shifts modulate functional state of cell membranes [5,8,15]. Oxidative stress increases membrane rigidity and modulates membrane permeability and activity of membrane-bound lipid-dependent enzymes [1,2,3,9]. It was demonstrated that oxidative stress decreases activity of Na,K-ATPase [1], increases hydrolyzing activity of Ca-ATPase, and reduces its transporting capacity [2]. Thus, ethanol produces a dual effect on activity of membrane-bound enzymes: the increase in membrane fluidity should activate enzymes, while the increase in its rigidity due to LPO activation inhibit these enzymes. Our aim was to study the effect of ethanol on activity of Na,K-ATPase and Ca-ATPase.

MATERIALS AND METHODS

Experiments were carried out on male Wistar rats weighing 150-200 g. Control rats were maintained on a standard diet, while experimental rats received 15% ethanol for 14 days instead of water.

Total activity of Na,K-ATPase and Ca-ATPase in rat brain was measured on crude membrane preparations. Isolation of crude membrane fractions and mea-

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suring of Na,K-ATPase activity were described previously [4]. For evaluation of Ca-ATPase activity the brain was homogenized for 30 sec in a homogenizer with Teflon pestle at 3000 rpm and 1:10 tissue/medium w/v ratio. The medium contained 0.3 M sucrose, 3 mM NaN_3, 0.09% sodium dodecyl sulfate, and 30 mm Tris HCl (pH 8.3). The homogenate was centrifuged for 15 min at 10,000*g* and 4°C. Activity of Ca-ATPase was determined in the supernatant at 37°C in the medium containing 0.1 M KCl, 5 mM $MgCl_2$, 4 mM NaN_3, Tris HCl at pH 7.0, and 60-80 μg membrane-protein preparation. Phosphate was measured as described elsewhere [7].

Total water content in the brain was assessed by the difference between the weights of fresh and dried (105°C for 24 h) specimens. The volume of intercellular space in brain tissue was assessed after incubating of brain slices (30-40 mg) in 10 mM Tris HCl buffer containing 6% bovine serum albumin, 100 mM NaCl, 20 mM KCl, 6 mM $MgSO_4$ (pH 7.4, 60 min, 4°C). Control specimens were prepared from the same tissue and incubated in the same buffer without albumin. After incubation, the slices were rapidly dried with filter paper, transferred to albumin-free Tris HCl buffer. After 60 min the buffer was centrifuged for 20 min at 4500*g*, and protein content in the supernatant was measured by the method of Lowry. The volume of extracellular space was calculated in percents of tissue volume by the formula:

$$V = \frac{(1+V_t)(0.5+V_n)[P]}{30\ V_t} \times 100\%$$

where V_t is tissue volume in ml (1 mg=0.001 ml) and [P] protein concentration in mg/ml. MDA content in the homogenate was measured by the reaction with 2-thiobarbituric acid [12].

The results were analyzed statistically using Student's *t* test.

RESULTS

Alcohol intoxication induced hyperhydration of cerebral tissue and decreased the volume of intercellular space in this tissue (Table 1). Therefore, hyperhydration of cerebral tissue in rats with ethanol intoxication is caused predominantly by hyperhydration of brain cells.

The volume of intracellular water correlates with intracellular Na^+ concentration. Therefore, the increased water content in cerebral cells can be determined by high Na^+ concentration. In its turn, intracellular concentration of sodium ions depends on both passive transport of these ions across the membrane and their active transport by Na,K-ATPase against sodium gradient. Na,K-ATPase activity in the brain of rats with ethanol intoxication decreased in comparison with the control (Table 1). This decrease was probably responsible for increased intracellular Na^+ concentration, whose enhanced osmotic activity retained water excess in cells. Therefore, cell hyperhydration is an important consequence of decreased Na,K-ATPase activity during alcohol intoxication.

Ca-ATPase activity increases during ethanol intoxication, while MDA content almost 4-fold surpassed the control (Table 1). This accumulation of MDA attests to pronounced LPO intensification during alcoholic intoxication. LPO products modulate functional properties of the membranes, in particular their permeability and activity of membrane-bound proteins. Therefore, LPO-modification of the plasma membrane during alcohol intoxication could be the cause of decreased Na,K-ATPase activity and increased hydrolyzing capacity of Ca-ATPase.

Thus, our studies showed that hyperhydration of brain cells during alcohol intoxication is accompanied by a decrease in Na,K-ATPase activity. It seems that inhibition of this enzyme regulating Na^+ concentration in cells is responsible for disturbances in water exchange under these conditions. Our findings suggest

TABLE 1. Effect of Ethanol Intoxication on Total Water Content, Volume of Intercellular Space, Na,K-ATPase Activity, and MDA Content in Rat Brain (*M*±*m*)

Index	Control	Alcoholization	
		abs.	Δ, %
Total water content, %	78.4±1.5 (13)	81.8±0.2*** (15)	4.3
Intercellular space volume, %	14.5±1.5 (13)	8.4±0.4** (15)	-42.1
Na,K-ATPase activity, μmol P_i/mg protein/h	11.1±1.1 (10)	8.4±1.0*** (10)	-24.3
Ca-ATPase activity, μmol P_i/mg protein/h	1.6±0.1 (10)	2.9±0.2*** (16)	81
MDA, μmol/g tissue	5.0±1.3 (10)	19.9±1.2* (22)	498

Note. *p<0.001, **p<0.01, ***p<0.05 compared to the control. Number of measurements is shown in parentheses, P_i is inorganic phosphate.

that intensification of peroxidation processes in cells (manifested in increased MDA content) under the effect of ethanol is the leading cause of Na,K-ATPase inhibition.

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