Hepatic and Cerebral Energy Production System in Rats with Acute and Chronic Ethanol Intoxication

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We studied the effects of ethanol on the energy production system in the brain and liver in acute and chronic intoxications. Ethanol was found to inhibit mitochondrial respiratory chain in the liver. Acute ethanol intoxication results in uncoupling of oxidative phosphorylation. NAD-dependent respiration prevails in chronic intoxication. In the brain, ethanol exposure induces a compensated low-energy shift with activation of fast mitochondrial metabolic cluster and uncoupling of oxidative phosphorylation.

Key Words: *mitochondria; liver; brain; acute and chronic ethanol intoxication*

Alcohol abuse is an acute medical and social problem widespread all over the world, especially in European countries, Russia and USA. In Russia, alcohol consumption exceeds the mean European values by 50% [3,7].

Ethanol and its toxic metabolite acetaldehyde are known to produce damaging effect on cell membranes. Impairment in mitochondrial membrane integrity is associated with disturbances in energy metabolism providing functioning of all systems. This is associated with changes in physicochemical properties of mitochondrial membranes and activity of enzyme system localized in membranes (SDH, cytochrome oxidase, ATPase, adenyl translocase, *etc.*) [5].

The effects of ethanol on energy metabolism attract considerable attention over many years. Ethanol biotransformation was shown to be associated with a shift in pyridine nucleotide ratio towards reduced forms, NADH and NADPH, and with excessive formation acetyl coenzyme A. These changes lead to profound disturbances in carbohydrate and energy metabolism. It explains deterioration of oxidative phosphorylation and gluconeogenesis and disturbances in energy supply. However, exact mechanism of ethanol action remains unclear [8].

Here we studied energy production systems of the liver and brain in rats with modeled acute and chronic ethanol intoxication.

MATERIALS AND METHODS

Experiments were carried out on 60 mongrel male rats weighing 200-220 g in autumn and winter period. The animals were kept under standard vivarium conditions with free access to water and food. The rats were divided into three groups: group 1 rats (controls) intragastrically received distilled water, group 2 rats intragastrically received 40% ethanol in a dose of 7 ml/kg 2 times a day for 7 days, group 3 intragastrically received 40% ethanol in a dose of 5.2 ml/kg for 5 weeks. These doses of ethanol were chosen on the basis of preliminary investigations and published data [2,4]. To accelerate the formation of alcohol-induced injury, group 3 animals were provided with free access to containers with ascending concentrations of ethanol (5-17%) [2]. One day after the last dose, the animals were decapitated under ether anesthesia. The lever and brain homogenate were used for further analysis.

Functional state of energy production system was evaluated by the polarographic method using

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an Expert-001-4 analyzer by the rate of mitochondrial oxygen consumption under different metabolic states after Chance. The following compounds were used as oxidation substrates: 1 and 5 mM succinate, 5 mM succinate with SDH activator isocitrate (1.5 mM), and glutamate and malate in concentrations of 3 mM each. Aminotransferase inhibitor aminooxyacetate (AOA, 2 mM), and SDH inhibitor malonate (2 mM) were also used. Mitochondria respiration rate before (V_4), during (V_3), and after (V_3-V_4) phosphorylation of added ADP was measured. Coefficient of oxidative phosphorylation coupling (ADP/O) was calculated for evaluation of the energetic status.

Statistical analysis was performed using nonparametric Mann–Whitney test. Differences were considered to be significant at the statistical significance level $p<0.05$. Calculations were performed using Statistica 6.0 software [6].

RESULTS

In the liver of rats with acute ethanol intoxication we observed inhibition of succinate-dependent energy production, marked disturbances in metabolic control of mitochondrial respiration, and uncoupling of oxidative phosphorylation compared to bioenergetic parameters the control group.

During oxidation of endogenous substrates, the mitochondria respiration rates in all metabolic states increased by 62, 33 and 95%, ADP/O coefficient decreased by 36%. In the test with addition of exogenous succinate in a concentration close to physiological (1 mM) to the incubation medium, the rate of phosphorylating respiration decreased by 13%. The increase in substrate load by adding succinate 5 mM led to an increase in oxygen consumption rate V_4 and V_3-V_4 by 21 and 26%. ADP/O coefficient decreased in FAD-dependent respiration by ~14% (Table 1).

Isocitrate normalized mitochondrial respiration rate against the background of oxidation-phosphorylation uncoupling (ADP/O coefficient was decreased by 14%).

Utilization of the mixture of NAD-dependent substrates (malate and glutamate) in liver mitochondria was associated with an increase in V_4 and V_3-V_4 by 18 and 33%, while V_3 remained unchanged. ADP/O coefficient decreased by 20% (Table 1).

Inhibition analysis using malonate and AOA during oxidation of NAD-dependent substrates of mitochondria respiration revealed increased involvement of endogenous succinic acid oxidation in ATP production while there were no activation of transamination. Controlled respiration rates (V_4, V_3-V_4) were increased by 40% in average, ADP/O coefficient was decreased by 20%.

Thus, acute ethanol intoxication led to kinetic type of mitochondria desenergisation, which is characterized by mismatch between the dynamics of consumption and synthesis of macroergic compounds under conditions of increased load on energy production system.

Evaluation of the functional state of liver mitochondria in rats with chronic ethanol intoxication revealed substantial inhibition of energy production with prevalence of NAD-dependent respiration and preserved oxidation-phosphorylation coupling relative to the normal values.

Oxidation of succinate (1 mM) by liver rat mitochondria was associated with a decrease in oxygen consumption rates before, during, and after active phosphorylation of added ADP by 13, 23, and 25%, respectively. The increase in substrate load after addition of 5 mM succinate decreased V_3 and V_3-V_4 respiration rates by 20%. Addition of isocitrate to liver mitochondria during succinate oxidation did not restore normal enzyme activity. Respiration rates before, during, and after ADP phosphorylation circle remained significantly reduced by 30, 22 and 40% (Table 1).

Utilization the mixture of NAD-dependent substrates (malate and glutamate) in liver mitochondria was associated with a decrease in V_3 and V_4 rates by 18 and 25% (Table 1).

Coefficient of oxidation-phosphorylation coupling ADP/O during oxidation of NAD- and FAD-dependent substrates did not significantly differ from the control.

Thus, these findings demonstrate direct inhibiting effects of ethanol on SDH in both acute and chronic intoxication. Prolonged ethanol abuse switches energy metabolism to predominant oxidation of NAD-dependent substrates. The decrease in oxidative phosphorylation coupling in acute ethanol intoxication is probably associated with its membrane-acting effect [9].

In the mitochondrial energy production system of the brain in rats exposed to acute ethanol intoxication, increased oxidation of both NAD- and FAD-dependent substrates was observed in all metabolic states in comparison with mitochondrial respiration rates in the control group.

Oxidation of endogenous substrates in brain mitochondria was characterized by increased respiration rates in metabolic states before, during and after ADP phosphorylation by 20, 39 and 59%, respectively. ADP/O coefficient decreased by 20%. Utilization of 1 mM succinate by brain mitochondria increased respiration rates by 23% on average. Increasing succinate concentration to 5 mM led to an increase in respiration rates V_4, V_3, and V_3-V_4 by 45, 18, and 46%, respectively. ADP/O coefficient decreased by 20% (Table 2).

Phosphorylating respiration rate during oxidation of succinate in combination with isocitrate increased by

14% in comparison with previous value for succinate oxidation, what attests to latent inhibition of SDH.

SDH activity under extreme and pathological condition can be accompanied by its compensatory limiting. Inhibition is a natural mechanism preventing excessive activation of oxidative phosphorylation. Unlimited SDH activation can lead to hyperergic reaction of the energy production system and mitochondria damage [1,5].

The use of malate and glutamate as substrates for oxidation was associated with an increase in V_4, V_3, and V_3-V_4 rates by 69, 20, and 40%, respectively. ADP/O coefficient decreased by 8% (Table 2).

Inhibitory analysis with malonate and AOA failed to reveal the contribution of endogenous succinic acid and transamination reaction into mitochondrial respiration during oxidation of NAD-dependent substrates under the influence of ethanol.

Comparative analysis of controlled mitochondria respiration rates in rat brain mitochondria during acute ethanol intoxication indicates the presence of a low-energy shift compensated by exogenous substrates and ADP. In addition, activation of fast metabolic cluster and oxidation-phosphorylation uncoupling were observed in brain mitochondria, in contrast to liver mitochondria.

TABLE 1. Effects of Acute and Chronic Ethanol Intoxication on Functional State of Rat Liver Mitochondria (n=10; $M\pm m$)

Parameter		Control	Acute intoxication	Chronic intoxication
Oxidation of endogenous substrates	V_4	24.2±0.1	39.3±3.9*	23.7±0.4
	V_3	43.4±0.5	57.7±3.7*	46.7±2.0
	V_3-V_4	20.1±0.7	39.3±2.4*	21.7±1.1
	ADP/O	3.1±0.1	2.0±0.2*	3.0±0.3
Oxidation of succinate (1 mM)	V_4	52.1±1.5	55.0±1.1	45.1±1.5*
	V_3	97.4±1.1	84.5±3.6*	74.6±1.7*
	V_3-V_4	45.9±0.1	48.0±3.1	34.3±3.3*
	ADP/O	2.2±0.1	1.8±0.2*	2.0±0.1*
Oxidation of succinate (5 мМ)	V_4	46.8±1.5	56.5±0.2*	45.5±1.2
	V_3	101.8±0.8	93.4±0.6*	81.3±2.2*
	V_3-V_4	43.2±1.5	54.6±0.2*	34.5±3.7*
	ADP/O	1.9±0.1	1.7±0.1*	1.9±0.1
Oxidation of succinate (5 mM) and isocitrate (1.5 mM)	V_4	63.7±0.6	62.3±1.6	44.3±2.6*
	V_3	97.9±1.2	94.8±0.1	76.5±1.9*
	V_3-V_4	63.7±0.6	63.9±0.6	37.9±1.6*
	ADP/O	1.9±0.1	1.6±0.1*	1.8±0.1
Oxidation of NAD-dependent substrates (malate+glutamate)	V_4	32.0±0.1	37.9±2.7*	31.2±1.4
	V_3	68.4±0.2	68.0±2.6	56.0±1.6*
	V_3-V_4	30.5±1.5	40.6±1.6*	23.0±2.6*
	ADP/O	2.7±0.1	2.2±0.1*	2.8±0.1
Oxidation of NAD-dependent substrates with malonate	V_4	26.5±1.0	36.6±0.8*	28.0±2.6
	V_3	58.8±0.2	64.3±1.7*	54.1±2.1*
	V_3-V_4	25.4±0.3	35.2±1.6*	25.5±2.4
	ADP/O	2.9±0.1	2.6±0.1*	2.8±0.1
Oxidation of NAD-dependent substrates with AOA	V_4	28.0±0.2	37.9±0.1*	26.7±1.8
	V_3	64.5±0.6	64.3±0.3	53.2±1.5*
	V_3-V_4	29.1±0.3	40.6±1.6*	26.3±2.6*
	ADP/O	2.8±0.1	2.0±0.2*	2.5±0.1*

Note. Here and in Table 2: *$p\leq0.05$ compared to the control.

TABLE 2. Effects of Acute and Chronic Ethanol Intoxication on Functional State of Rat Brain Mitochondria (n=10; $M\pm m$)

Parameter		Control	Acute intoxication	Chronic intoxication
Oxidation of endogenous substrates	V_4	19.0±1.2	22.8±0.9*	25.6±0.7*
	V_3	24.4±0.9	33.8±0.2*	32.4±0.4*
	V_3-V_4	13.3±1.3	21.1±0.3*	21.0±0.3*
	ADP/O	2.3±0.1	1.8±0.1*	1.8±0.1*
Oxidation of succinate (1 mM)	V_4	24.4±1.2	29.9±0.8*	34.0±0.4*
	V_3	42.2±1.1	51.2±1.7*	52.4±1.2*
	V_3-V_4	21.7±1.1	27.4±0.2*	29.1±0.9*
	ADP/O	1.8±0.1	1.9±0.1	1.4±0.1*
Oxidation of succinate (5 mM)	V_4	25.5±0.5	36.9±0.9*	39.8±0.4*
	V_3	52.7±0.3	62.3±1.6*	61.9±0.3*
	V_3-V_4	23.0±0.6	33.6±0.5*	30.3±1.0*
	ADP/O	2.0±0.1	1.6±0.1*	1.8±0.1*
Oxidation of succinate (5 mM) and isocitrate (1.5 mM)	V_4	39.3±0.6	46.1±1.6*	46.1±1.2*
	V_3	62.3±0.7	70.8±3.4*	69.1±1.4*
	V_3-V_4	37.9±0.5	48.8±0.1*	45.9±0.1*
	ADP/O	1.7±0.1	1.5±0.1*	1.4±0.1*
Oxidation of NAD-dependent substrates (malate+glutamate)	V_4	16.8±0.2	28.4±0.5*	27.3±0.1*
	V_3	39.6±1.2	47.4±0.3*	50.7±0.7*
	V_3-V_4	17.1±0.8	23.8±1.0*	25.7±0.6*
	ADP/O	2.6±0.1	2.4±0.1*	2.4±0.1*
Oxidation of NAD-dependent substrates with malonate	V_4	16.3±0.9	27.5±0.8*	33.7±0.3*
	V_3	37.2±1.0	44.3±1.6*	51.6±0.2*
	V_3-V_4	16.3±1.0	24.4±1.6*	23.7±0.3*
	ADP/O	2.7±0.1	2.3±0.1*	2.6±0.1
Oxidation of NAD-dependent s ubstrates with AOA	V_4	19.2±0.6	33.6±0.9*	27.7±0.3*
	V_3	38.7±0.7	49.3±0.5*	48.4±0.3*
	V_3-V_4	16.3±0.6	23.8±1.2*	25.0±0.3*
	ADP/O	2.9±0.2	2.4±0.1*	2.3±0.1*

Ethanol produced a compensated low-energy shift in mitochondrial energy production system of the brain with increased respiration rates in all metabolic states and uncoupling of oxidative phosphorylation.

Changes induced by chronic ethanol intoxication in the energy production system of brain mitochondria were similar to those observed during acute intoxication (activation of fast metabolic cluster of mitochondria, development of low-energy shift with increased respiration rates in all metabolic states during oxidation of all available substrates and decrease in ADP/O coefficient).

Thus, the energy production system of the liver was characterized by depletion of compensation abilities manifesting by ethanol inhibition of mitochondria respiratory chain without uncoupling of oxidative phosphorylation during chronic intoxication and with pronounced signs of decompensation and uncoupling of oxidative phosphorylation in acute intoxication. In brain mitochondria, activation of energy supply pro-

cesses through the fast metabolic pathway with the development of low-energy shift and drop of oxidative phosphorylation coupling irrespective to intoxication variant was observed.

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