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Metabolic Activity of Cells in Brain Cortex after Alcohol Intoxication and Correction of Changes with Dolivin

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Metabolic processes in the brain cortex of albino rats with prenatal and postnatal alcohol intoxication were studied by the method of electron paramagnetic resonance. Accumulation of superoxide radical-generating agents, inactivation of superoxide dismutase, and increase in nitric oxide concentration were detected. Activation of free radical processes was associated with accumulation of Mn^{2+} and Fe^{2+}. The parameters of electron paramagnetic resonance returned to normal after Dolivin treatment. These changes were accompanied by a decrease in the intensity of oxidative metabolism.

Key Words: *brain cortex; ethanol; Dolivin; peroxidation; radicals*

One of the most severe consequences of ethanol consumption during pregnancy is damage to the developing central nervous system in the offspring. Fetal alcohol syndrome manifests in specific morphological changes and dysfunction of various systems in the organism. Acute and chronic ethanol consumption is accompanied by reduction of the brain cortex, ultrastructural changes, death of neurons [6,10], and inhibition of astroglial proliferation [9]. Prenatal intoxication increases the degree of apoptosis induced by oxidative stress [12].

Nitric oxide (NO) plays a special role in the development of brain dysfunction after alcohol intoxication [3]. Hyperfunction of NMDA receptors contributes to increased production of NO under conditions of chronic alcoholization, which is followed by stimulation of oxidative processes [2]. The antioxidant mechanisms prevent neuronal damage induced by free radicals [13].

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Alcohol intoxication can be prevented by some pharmacological agents. Vitamin E, which neutralizes free radicals [12], is extensively used for this purpose [5]. Plaferon-LB decreases the area of ischemic injury in rat cerebral cortex [1], desensitizes NMDA receptors, and suppresses NO production [7].

We used Dolivin to correct the processes accompanying ethanol intoxication. The main constituent of Dolivin is Hypoxen. Hypoxen improves cell respiration, which is particularly important for brain function. Hypoxen stimulates degradation of lipid peroxidation (LPO) products and protects cell and mitochondrial membranes from free radicals under conditions of LPO.

Here we studied ethanol-induced metabolic changes in cells of the cerebral cortex and evaluated the effectiveness of Dolivin in correcting prenatal and postnatal ethanol intoxication in albino rats.

MATERIALS AND METHODS

Experiments were performed on outbred albino rats. The animals were divided into 3 groups. Group 1

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consisted of 30-day-old intact rats. Group 2 included offspring of females prenatally (throughout pregnancy) and postnatally (first 30 days of life) receiving 15% ethanol instead of water. The animals prenatally (period of pregnancy) and postnatally treated with Dolivin (daily dose 1.4 g) and ethanol entered group 3.

Dolivin has 2 complementary components, baker's yeast autolysate and antihypoxant Hypoxen (Olifen, Russian Federation patent 2.105.000). This preparation also includes amino acids, vitamins B, PP, E, and H, microelements, and polysaccharides.

Metabolic processes in the cerebral cortex were studied by the method of electron paramagnetic resonance (EPR) on a PE 1307 radiospectrometer using a quartz dewar at a temperature of liquid nitrogen. Samples of the cerebral cortex were placed in polyethylene tubes (length 20-25 mm) and maintained in liquid nitrogen at -196°C to record EPR spectra. We evaluated the intensity (I) and half-width of free radical signal (ΔH) and signal intensity for the complexes containing FeS, Mn^{2+}, Fe^{2+}, and Mo^{5+} centers and NO.

The results were analyzed by Student—Fischer test.

RESULTS

EPR spectrum for the cerebral cortex of intact albino rats included EPR signals of free radicals (semiquinone form of flavins and ubiquinones) and proteins that contain FeS centers (NADH dehydrogenases and succinate dehydrogenases). There were also EPR signals of spin-labeled NO.

The intensity of free-radical EPR signals significantly decreased after ethanol consumption. The decrease in ΔH reflected an increase in the contribution of ubisemiquinones to the free radical signal. The observed changes were associated with dysfunction of the mitochondrial electron transport chain at the ubiquinone-oxide reductase level, suppression of mitochondrial respiration, and synthesis of macroergic compounds. Impairment of electron transport in the NADH:ubiquinone-oxide reductase region contributes to excessive accumulation of ubisemiquinones that serve as the potent sources of superoxide radicals (O_2^-).

The decreased production of macroergic compounds contributes to hypoxanthine accumulation, activation of the hypoxanthine oxidase system, and transformation of xanthine dehydrogenase into xanthine oxidase. These changes were manifested in the EPR signal of a Mo^{5+}-containing complex. Xanthine oxidase is another source of O_2^-.

O_2^- overproduction in mitochondria determines inactivation of mitochondrial superoxide dismutase (SOD), which is manifested in accumulation of Mn^{2+} in the EPR spectrum of the brain.

Accumulation of superoxide radicals and inactivation of SOD are followed by an increase in free radical oxidation, initiation of membrane LPO, and damage to membrane structures. This damage is manifested in the appearance of Fe^{2+} in the EPR spectrum. These ions are released from protein complexes of damaged membranes. Similarly to transition metal ions, Mn^{2+} and Fe^{2+} serve as potent promoters of free radical oxidation and determine further increase in the degree of oxidative stress.

The content of free NO increases during alcohol consumption, which is associated with stimulation of glutamate-induced NO production or activation of NO synthase under the influence of reactive oxygen. Excessive amounts of NO are involved in free radical processes and contribute to their activation.

Table 1 illustrates the consequences of simultaneous consumption of alcohol and Dolivin. The parameters of EPR spectral returned to normal after Dolivin treatment. These changes were accompanied by a decrease in the intensity of free radical oxidation.

Hypoxen (Olifen), a constituent of Dolivin, has a polyphenol structure and, therefore, exhibits high electron-binding activity. At the molecular level, Hypoxen improves tissue respiration under conditions of hypoxia. Hypoxen can transfer reduced

TABLE 1. Metabolism in Rat Cerebral Cortex (EPR Spectra)

Group	Free radicals						
	I	ΔH (U)	FeS	FeS^{2+}	Mo^{5+}	Mn^{2+}	NO
1 (control)	8.3	12	7.9	0	0	0	27.4
2	4.3	10.2	5.0	12.3	2.8	2.3	36.6
3	7.8	11.2	6.2	7.0	0.9	1.2	27.0
Reliability	$p<0.01$	$p<0.05$	$p<0.01$	$p<0.01$	$p<0.01$	$p<0.01$	$p<0.01$

equivalents to enzyme systems that use ubiquinone as the carrier. Pharmacodynamic characteristics of Hypoxen are similar to those of cytochrome C and ubiquinone. Due to a small size of the molecule (568±216 mm), Hypoxen is 10 times more potent than ubiquinone. This agent compensates for ubiquinone deficiency during hypoxia, which is related to the presence of a considerable number of functionally active centers. During the posthypoxic period Hypoxen maintains aerobic processes and tissue respiration at high level. It results in rapid oxidation of accumulated reduced equivalents.

Clinical and laboratory studies of patients with obstructive bronchitis showed that the introduction of Hypoxen into the combination therapy provides correction of respiratory failure and improves cardiorespiratory function. It should be emphasized that under these conditions, processes of LPO in the organism are regulated by the antioxidant system.

Our results show that prenatal and postnatal ethanol intoxication is accompanied by metabolic changes in the cerebral cortex of albino rats. It results in the formation of free radicals that damage membrane structures of the cerebral cortex. The use of Dolivin for the correction of these changes contributes to restoration of oxidative metabolism during ethanol consumption.

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