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Chronic Alcoholism-mediated Metabolic Violations in Albino Rats Brain

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Authors' contributions

This work was carried out in collaboration between all authors. Author LBB wrote the protocol, managed the analyses of the study, managed the literature searches, performed the statistical analysis and wrote the first draft of the manuscript. Author GMS managed the analyses of the study, managed the literature searches, performed the statistical analysis, and wrote the part of the first draft of the manuscript. Author VMK designed the study. Authors OIK, LIB and YOO managed the analyses of the study. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: Despite of intensive investigation of alcoholism consequences for nervous system, the full complex of such metabolic alterations in brain remains unknown. The aim of present work was to study the influence of chronic ethanol consumption on rats' brain contents of free amino acids, rates of CYP2E1 and CYP3A2 expression, NO-synthase activities and pro- and antioxidant system parameters.
Study Design: Wistar albino male rats (body weight (BW), 160–200 g) were divided into two groups: I – alcohol, II – normal animals.
Methodology: The content of amino acids in rat brain was determined using an amino acid analyzer. Brain CYP2E1 and CYP3A2 were evaluated with RT-PCR method, pro- and antioxidant system parameters – by standard methods.
Results: Changes of brain free amino acids contents with alcoholism were demonstrated

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for 14 amino acids and their total sum. Chronic alcohol consumption caused 1.77 times increasing of CYP2E1 mRNA expression in rat's brain, 2.34 times – of CYP3A2 mRNA expression, 2.8 times - of cNOS activity, 42% - of TBARS formation rates. Activities of SOD and catalase were decreased 19% and 25% respectively.
Conclusions: Thus investigation of alcoholism effects on brain levels of free amino acids permits to obtain complex estimation of this pathology influence on metabolic processes in brain, especially on amino acids, proteins, ATP and NADPH metabolisms. Alterations on the level of genome in our experiments were accompanied with complex alterations on the level of protein, amino acid and nitrous metabolisms.

Keywords: Chronic alcoholism; brain; rats.

1. INTRODUCTION

At present alcohol has become an important socio-medical problem due to its massive uncontrollable consumption in the world. Chronic alcohol consumption causes serious consequences for entire nervous system functioning and particularly, the brain [1], associated with metabolic processes complex violations [2]. In adult human chronic alcoholics, brain damage is characterized by cerebral and cerebellar atrophy and impaired neuronal function within the hippocampus and frontal cortex. Besides specific alcohol-related disorders, such as Wernicke–Korsakoff syndrome, hepatic encephalopathy and pellagra, heavy alcohol consumers exhibit cognitive and motor impairments, cholinergic deficits and dementia [3]. It is estimated that 50–75% of long-term alcoholics show cognitive impairment and structural damage to the brain, making chronic alcoholism the second leading cause of dementia behind Alzheimer's disease [3]. Neuropathological analyses have provided evidence for loss of neurons in certain regions of the brain of alcoholics [4].

Among several proposed mechanisms of brain damage at chronic alcoholism, should be mentioned accumulation of DNA damages in the absence of repair processes, resulting in genomic instability and death of neurons [3]. The processes of alcohol metabolism are also connected with generation of reactive oxygen species (ROS) and nitric oxide (NO) via induction of NADPH/xanthine oxidase, nitric oxide synthase (NOS) and cytochrome P450 2E1 (CYP2E1) [5,6]. Additionally, ethanol activates and recruits Toll-like receptor (TLR) 4 receptors within the lipid rafts of glial cells, triggering the production of inflammatory mediators and causing neuroinflammation [7].

It is very important to note that synaptic changes promoted by ethanol are mediated directly and indirectly by acetylcholine, dopamine, serotonin, glutamate, GABA and other neurotransmitters [8].

However, despite of intensive investigation of alcoholism consequences for nervous system, the full complex of such metabolic alterations remains unknown, as soon as limiting and regulating factors of alcohol metabolism in brain [9]. Especially the contribution of chronic alcohol intake to changes of brain amino acids metabolism is not known, as soon as its influence on any particular amino acid contents and functions in nervous cells. Role of individual amino acids changes and their combined effects at alcoholism on brain proteins and nucleic acids structure and/or metabolism remains unclear.

Based on these facts and considering that alcoholism is chronic disease highly prevalent in the world population, the present work reports the influence of chronic ethanol consumption on rats' brain contents of free amino acids, rates of CYP2E1 and cytochrome P450 3A2 (CYP3A2) expression, NO-synthase activities and pro- and antioxidant system parameters.

2. MATERIALS AND METHODS

Wistar albino male rats, body weight (b.w.) of 150 g to 170 g, were used in the study. They were kept under a controlled temperature (from 22ºC to 24ºC), relative humidity of 40% to 70%, lighting (12 h light-dark cycles) and on a standard pellet feed diet ("Phoenix" Ltd., Ukraine). The study was performed in accordance with the recommendations of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes and approved by the Institutional Animal Care and Use Committee. For the experimental (chronic alcoholism) model, reproducing male rats were selected according to the method for measuring voluntary alcohol self-administration in rats, which provides a continuous choice between an alcohol solution and water (two bottle preference test) [10]. The six selected rats were used for chronic alcoholism modeling by replacing water with a 15% ethanol solution during 150 days. Six intact male rats (of the same age and weight) were used as a control. From the beginning of the experiment, they were kept in the same conditions as experimental animals but were given only water *ad libitum*.

After 150 days, both the experimental and control rats were sacrificed under a mild ether anaesthesia by decapitation. Their brains were used for investigation.

To brain homogenates samples (1ml) equal volumes of 3% sulfa salicylic acid were added and obtained mixtures were left for 10 minutes in refrigerator at t+4ºC. The formed sediments were removed by centrifugation (5000g, 10 min, 4ºC) [11]. Supernatants contained free amino acids from brains. Contents of free amino acids were determined on the amino acid analyzer AAA-881 (Czech Republic). The expression of CYP2E1 and CYP3A2 (ortholog of CYP3A4) mRNA in the brain was determined by a reversed transcriptase polymerase chain reaction (RT-PCR). Brain samples (50 mg) were collected, quickly frozen in liquid nitrogen, and stored at -80ºC before RNA extraction. The isolation of total mRNA was carried out with a TRI-Reagent (Sigma, USA). The integrity and concentration of RNA was analyzed in a 2% agarose gel. First-strand complementary DNA (cDNA) was synthesized using a First-Strand cDNA Synthesis Kit (Fermentas, Germany). The reaction mixture contents for PCR, amplification protocol and specific primers for the CYP2E1 gene were chosen according to SM Lankford et al. [12]. The primer sequences were: sense, 5'-CTTCGGGCCAGTGTTCAC-3' and anti-sense, 5'-CCCATATCTCAGAGTTGTGC-3'. The reaction mixture contents for PCR, amplification protocol, and specific primers for the CYP3A2 gene were chosen according to W Jäger et al. [13]. The primer sequences were: sense, 5'-TACTACAAGGGCTTAGGGAG-3' and anti-sense, 5'- CTTGCCTGTCTCCGCCTCTT-3'. RT-PCR with primers of β - actin sense, 5' –GCTCGTCGTCGACAACGGCTC – 3' and antisense 5' – CAAACATGAT CTGGGTCATCTTCT –3') was carried out for internal control. All of the primers were synthesized by "Metabion" (Germany). The MyCycler termocycler (BioRaD, USA) was used for amplification. PCR products (CYP2E1-744 bp and β-actin-353 bp) were separated in a 2% agarose gel, stained with ethidium bromide, and visualized under a UV transilluminator (BIORAD, USA). Data analysis was carried out with Quantity One Software (USA) and presented in relative units as the ratio of CYP2E1 mRNA contents and β-actin mRNA.

iNOS and cNOS activities measure in brains were performed by spectrophotometric method [14]. Concentration of protein in brain fractions was determined by the Bradford protein assay using bovine serum albumin as a standard.

Lipid peroxidation was investigated as the rate of ascorbate-induced thiobarbituric acid reactive substances (TBARS) formation [15], superoxide dismutase activity (SOD) was determined in accordance to Chevary [16], catalase activity was detected using the colorimetric ammonium molybdate method [17].

The obtained data were calculated by one-way analysis of variance (ANOVA) and expressed as the mean ± standard error of the mean (M±S.E.M.). Data were compared using Tukey test. Differences were considered to be statistically significant at P=.05.

3. RESULTS

Investigation of chronic alcoholism effects on rat brain contents of free amino acids (Table 1) showed that statistically significant changes as compaired with control were registered out for 14 amino acids and total sum of free amino acids.

Contents of lysine, histidine, arginine, ornithine, serine, glutamic acid, glycine, valine, isoleucine, leucine, tyrosine, glutamine and total sum of free amino acids were decreased while contents of methionine and phenylalanine – increased. The most profound changes were registered for contents of serine (– 80%), lysine (-47,5%), ornithine (-55%), arginine (-45%), valine (-50%) and tyrosine (-54%).

To evaluate the effect of 150 days ethanol exposure on transcriptional activation of CYP2E1 and CYP3A2 in brain RT-PCR was performed.

According to data in Fig. 1 chronic alcohol consumption caused 1.77 times increasing of CYP2E1 mRNA expression in rat's brain as compared with group of control animals. At the same times CYP3A2 mRNA expression in ethanol-treated rat's brain increased 2.34 times (Fig. 2).

Results on brain nitric oxide synthases activities following 150 days alcohol administration are demonstrated in Fig. 3. The level of iNOS in ethanol-treated rat's brain was within the normal limits. At the same times cNOS activity was raised 2.8 times as compared with control.

Table 1. Rat brain contents of free amino acids in norm and with alcoholism. (M±m, n=6, mg/ 100 g of moist tissue)

Amino acid	Norm	Alcoholism
Lysine	2.00±0.06	1.05±0.04*
Histidine	0.80±0.02	0.50±0.02*
Arginine	2.00±0.04	1.10±0.01*
Ornithine	0.20±0.04	0.09±0.02*
Aspartic acid	18.70±2.50	12.80±3.07
Threonine	4.20±1.30	4.17±0.10
Serine	6.70±2.00	1.30±0.08*
Glutamic acid	133.2±4.80	116.70±1.70*
Proline	0.40±0.10	0.32±0.10

Table 1. Continued……

Glycine	4.10±0.10	3.00±0.10*
Alanine	6.00±1.09	5.00±0.10
Cysteine	1.70±0.50	1.20±0.12
Valine	0.80±0.10	0.40±0.05*
Methionine	0.40±0.05	0.60±0.05*
Isoleucine	0.40±0.10	0.28±0.07*
Leucine	0.90±0.20	0.60±0.16*
Tyrosine	1.30±0.10	0.60±0.05*
Phenylalanine	1.30±0.04	1.80±0.08*
Glutamine	13.50±1.20	10.00±0.80*
Total sum	221.10±18.40	173.11±10.77*

**- P= .05 statistically significant in comparison with norm*

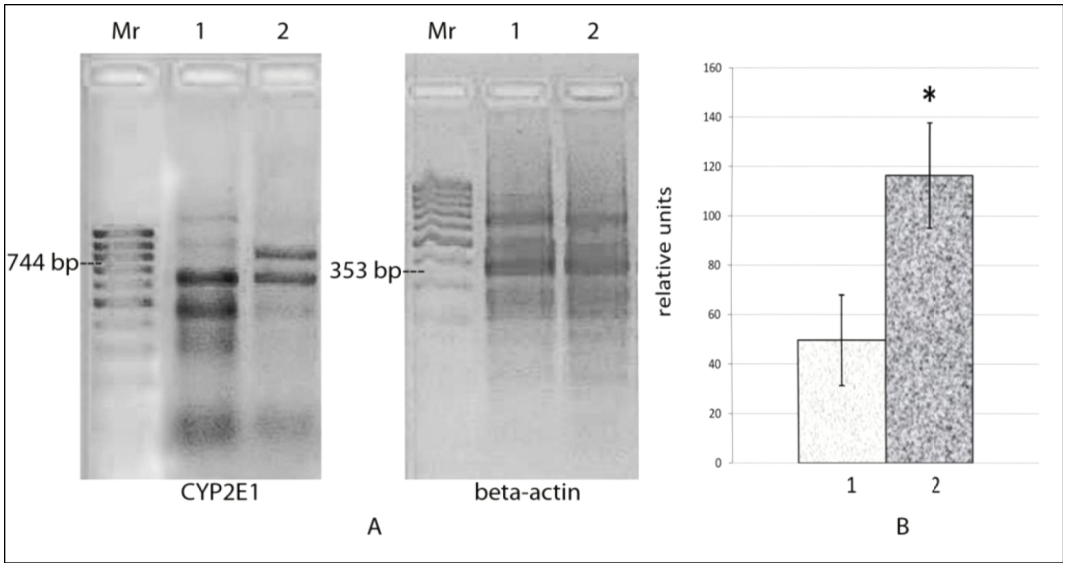

Fig. 1. CYP2E1 mRNA in rats' brain after 150 days 15% ethanol consumption: A) electrophoregram of CYP2E1 (744 b.p) and reference-gene β-actin (353 b.p.) RT-PCR products (arrows indicate appropriate DNA fragments): Mr – DNA marker, 1-control, 2- chronic alcoholism. B) Average rate of CYP2E1 mRNA expression in rats' brain:1-control, 2- chronic alcoholism

**- P= .05 statistically significant in comparison with norm*

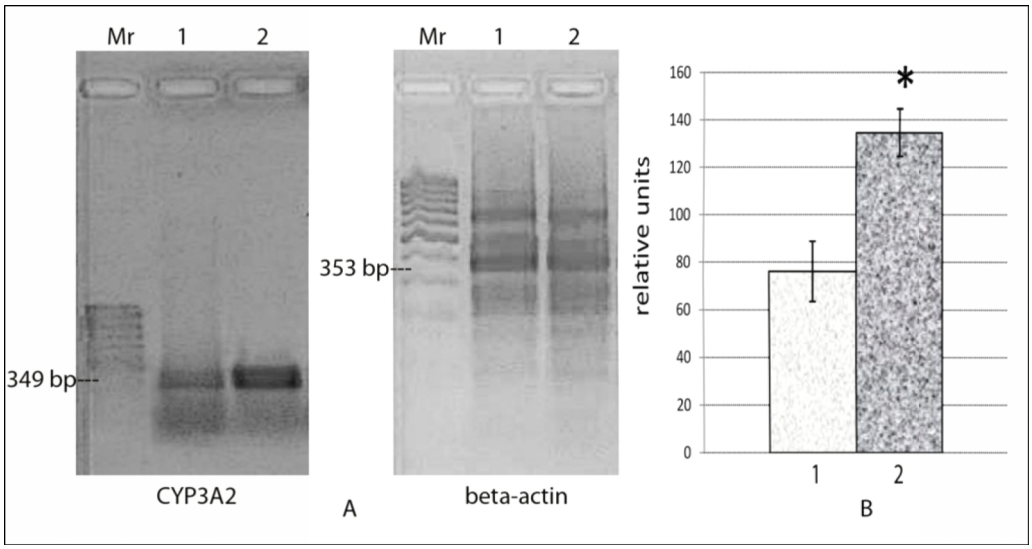

Fig. 2. CYP3A2 mRNA in rats' brain after 150 days 15% ethanol consumption: A) electrophoregram of CYP3A2 (349 b.p) and reference-gene β-actin (353 b.p.) RT-PCR products (arrows indicate appropriate DNA fragments): Mr – DNA marker, 1-control, 2- chronic alcoholism. B) Average rate of CYP3A2 mRNA expression in rats' brain: 1-control, 2- chronic alcoholism.

**- P= .05 statistically significant in comparison with norm*

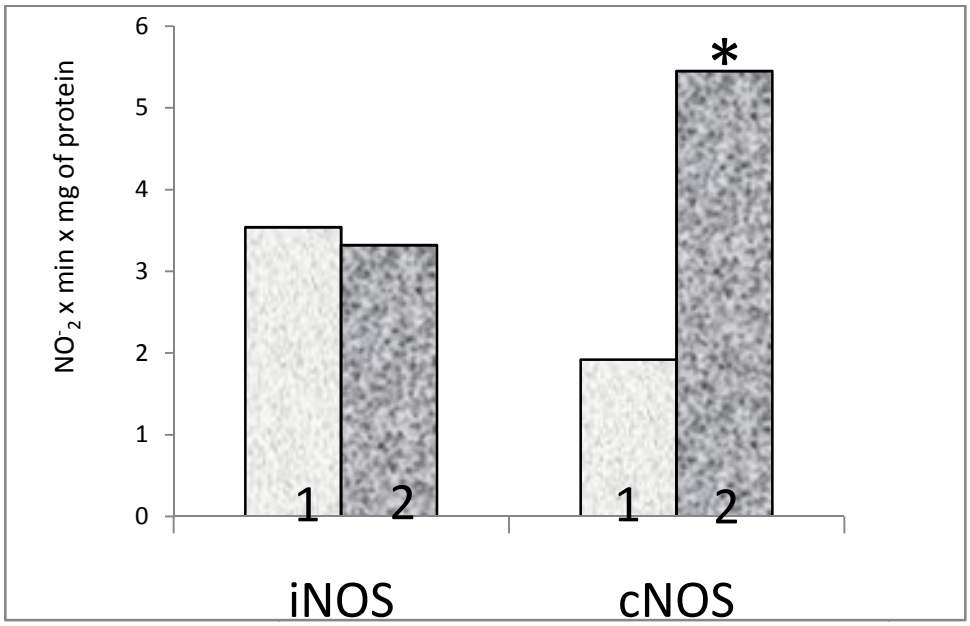

Fig. 3. Levels of iNOS and cNOS activities in rat' brain after 150 days 15% ethanol consumption (1 — control; 2 — chronic alcoholism)

**- P= .05 statistically significant in comparison with norm*

Data on brain pro- and antioxidant system state are shown in Table 2.

Table 2. Rat brain pro- and antioxidant system state after 150 days 15% ethanol consumption (M±S.E.M., n = 6).

Indices	Animal groups	
	Control	Chronic alcoholism
TBARS content, nmoles×mg of protein^{-1} ×min^{-1}	86.80±3.29	123.02±2.95*
SOD activity, relative units×min^{-1}×mg of protein^{-1}	1.73±0.07	1.40±0.14*
Catalase activity, nmoles H_2O_2 min^{-1}×mg of protein^{-1}	1.30±0.10	0.97±0.03*

**- P= .05 in comparison with control*

Compared with the unaffected animals, ethanol-treated rats showed brain pro- and antioxidant system alteration. The chronic 15% ethanol administration led to increase of TBARS rate formation (42%). Under this condition the activities of antioxidant enzymes SOD and catalase were decreased 19% and 25% respectively.

4. DISCUSSIONS

First of all character of amino acids changes at alcoholism could be evidence of considerable general disturbances in metabolism of brain proteins [18]. Amino acid availability is a rate-limiting factor in the regulation of protein synthesis [19]. Our results are in good accordance with other authors' data [20], which demonstrated impaired protein synthesis with ethanol consumption.

Changes of glutamic acid contents are of special interest. Glutamate, the major excitatory transmitter in the vertebrate brain is involved in neuronal development and synaptic plasticity. Glutamatergic stimulation leads to differential gene expression patterns in neuronal and glial cells. A glutamate-dependent transcriptional control has been established for several genes [21]. Glutamate is emerging as a major factor stimulating energy production in central nervous system. Brain mitochondria can utilize this neurotransmitter as respiratory substrate and specific transporters are required to mediate the glutamate entry into the mitochondrial matrix. Glutamate transporters of the Excitatory Amino Acid Transporters (EAATs) family have been previously well characterized on the cell surface of neuronal and glial cells, representing the primary players for glutamate uptake in mammalian brain [22]. Thus observed in our experiments decreasing of free glutamic acid contents in brain pools could cause profound changes in brains cells metabolism and functioning with alcoholism.

Among this with alcoholism could be damaged amino acids exchange mechanism. The system x(C)- (Sx(C)-) transporter functions as mediator of the exchange of extracellular cystine (L-Cys(2)) and intracellular glutamate (L-Glu) [23,24]. Observed changes of glutamine and ornithine contents could be an evidence of disturbances in processes of amino acids excess catabolism in the urea cycle. Among this glutamine is the major transport form of ammonia from all organs to the blood plasma [25,26,27]. In case of its concentration decreasing this function could be partially substituted by free alanine, serine, glycine and aspartic acid [25]. But with alcoholism contents of not only glutamine, but also alanine, serine, glycine and aspartic acid are decreased in comparison with norm. It could

affect the course of nitrogen metabolism processes in brain and in the body as a whole. Serine, arginine and tyrosine can act as a NO depot, which is not only responsible for the blood vessels relaxation, but also reacts with the iron atoms (in heme and free state), with superoxide anions, oxygen molecules, hydroperoxide, organic peroxides and peroxide radicals [28]. Thus, changes of serine, arginine and tyrosine contents with alcoholism in our experiments could adversely affect at the level of NO, peroxidation rates and vascular system functioning both in brain and whole organism [29]. Among this arginine could act as an antioxidant and powerful immunomodulator [30], as soon as precursor in biosynthesis of polyamines, which regulate cells proliferation processes in organism. Serine is a key compound in biosynthesis of ethanolamine and phosphoethanolamine - metabolites associated with the membranes structure and metabolism [30,31,32]: serine is transformed into ethanolamine with further phosphorylation to phosphoethanolamine, followed by methylation to phosphatidylcholine [25,31]. In our experiments decreasing of serine contents at alcoholism was accompanying by the opposite tendency of methionine content changes (required for methylation processes). Possibly it could be a result of disturbances in membrane lipids biosynthesis processes with alcoholism.

Chronic ethanol consumption also caused profound changes in contents of free amino acids involved into processes of purines and pirimidines biosynthesis (lysine, histidine, glutamic acid, aspartic acid, glycine, glutamine, histidine, serine) [25], which may affect the biosynthesis of nucleotides, nucleic acids and the cell chromosomal apparatus state. According to some authors, the earliest manifestations of DNA repair processes violations at the level of methylation may be free cysteine and methionine contents changes [33], such as we demonstrated in our present experiments.

Special attentions deserve changes of methionine in brain pools. This amino acid could act as antioxidant and immunomodulator [30,34]. Among this, methionine is a precursor of taurine–antioxidant and membrane stabilizer. Therefore, increasing of its concentrations with alcoholism in our experiments can be regarded as a protective mechanism [30]. Methionine could be also precursor of polyamines–stimulators and regulators of proliferation processes [30,35]. At alcoholism biosynthesis S-adenosylhomocysteine and homocysteine could be also broken [36]. On such possibility indicate changes in contents of methionine, glycine and serine–amino acids involved into synthesis of these compounds [35,36]. Consequence of the homocysteine contents increasing is the accumulation of hydroperoxy radicals capable to damage cells [33,35].

Changes of lysine contents could cause additional loss of leucine [33]. Essential for the brain cells normal functioning is their provision by energy resources (especially ATP). These cells actively catabolise branched chain amino acids with simultaneous energy storage in special molecules (mainly ATP) [25]. Therefore, in alcoholism observed changes of the amino acids involved in energy metabolism (valine, leucine, isoleucine) should be of special attention. Detected changes of brain free amino acids contents at alcoholism may also indicate on disturbances in metabolism of ATP and NADPH both at the stage of glycolysis (changes of glycine, serine) and in the Krebs cycle (changes of glutamic acid, ornithine, glutamine, arginine, valine and methionine in serum) [37].

Among this decreasing of alanine contents could be evidence of NH_4^+ - transport disturbances [25]. Decrease of both valine and alanine concentrations in brain pools may cause disturbances in proteins catabolism and glucoso-alanine cycle [25,38].

Changes of phenylalanine contents at alcoholism could be an evidence of great disturbances in phenylalanine hydroxylase functioning at this pathology [33].

Changes of such amino acids as glycine in brain pool are of special importance because of its involvement (with other amino acids) into glutathione biosynthesis [33,35]. These data are in correspondence with our results on GSH contents changes at alcoholism. On the other hand as glutathione realize trans-membrane transport of amino acids [33,39,40], its contents changes could cause changes in free amino acids contents, especially essential [33,39,40].

Brain P450s are regulated differentially from those in liver [41]. The availability of the prosthetic heme group appears to be essential for correct membrane insertion and enzymatic functionality of brain P450s. Furthermore, although not contributing to body's overall drug metabolism, brains P450s fulfill particular functions within specific cell types of the brain. In astrocytes of brain's border lines P450 isoforms are expressed at very high level. They form a metabolic barrier regulating drugs' influx, modulate blood-flow regulation, and act as signaling enzymes in inflammation. In neurons, however, P450s apparently have different function. In specified brain regions such as hypothalamus, hippocampus and striatum they provide signalling molecules like steroids and fatty acids necessary for neuronal outgrowth and maintenance. Induction of these P450s by neuroactive compounds can alter steroid hormone signalling directly in target cells, which may cause reproductive disorders and sexual or mental dysfunction [41].

In our experiments violation in brain free amino acids homeostasis were accompanied with increased expression of CYP2E1 and CYP3A2 (ortholog of human CYP3A4) in brain tissues. Our data was in good correspondence with other investigations [42,43]. The expression of CYP2E1 in the brain with alcoholism has been demonstrated in several regions. This enzyme catalyzes first two reactions of acetone transformation in the lactic acid, ethanol oxidation, metabolism of fatty acids and their hydroperoxides [44]. It has long been suggested that some of the neuropharmacological, neurochemical and behavioural effects of ethanol are mediated by its first metabolite, acetaldehyde. Acetaldehyde, formed in the brain tissues through the peroxidatic activity of catalase and by oxidation via CYP2E1, may mediate some of the central nervous system actions of ethanol including tolerance and dependence [45] Genetic investigations demonstrated that sequence changes in or near CYP2E1 affect the level of response to alcohol providing a predictor of risk of alcoholism [46]. Implicating CYP2E1 in the level of response to alcohol allows inferences to be made about how the brain perceives alcohol. Also CYP2E1 appears to play a key role in 3-NT formation, protein degradation and brain damage, which is independent of NOS [47].

CYP3A4 metabolizes approximately 50% of the drugs in organism [43]. Although CYP3A4 induction by ethanol is known, CYP3A4-changes interrelations with other alterations during chronic alcohol consumption are unclear. Also little is known about the expression of CYP3A proteins in the brain with alcoholism [48]. Both CYP3A4 and CYP3A5 immunoreactivity were found to varying extents in the microsomal fractions of cortex, hippocampus, basal ganglia, amygdala, and cerebellum. However, only CYP3A4 expression was observed in the mitochondrial fractions of these brain regions. Remains unclear how ethanol consumption influences on expression of CYP3A subfamily in different brain regions.

Our data along with other authors' results demonstrate that comparative expression studies are an efficient approach to discover interacting gene and metabolic networks that underlie the development of organism's final reaction to alcohol chronic consumption [49].

Increased cellular oxidative stress and altered antioxidant pool have been implicated at alcoholism. Our data on state of brain's pro- and antioxidant system at alcoholism coincide with data of other authors. Ethanol enhances ROS production in brain through a number of pathways including increased generation of hydroxyl ethyl radicals, induction of CYP2E1, alteration of the cytokine signaling pathways for induction of iNOS and sPLA(2), and production of prostanoids through the PLA(2)/COX pathways. Excess of free oxygen radicals could cause brain tissue damage not only via lipid peroxydation, but also proteins denaturation, enzymes inactivation, nucleic acids damage, Ca^{2+} release and cytoskeleton destruction. Influence of free radicals mediates blood-brain barrier functions violations, endothelial dysfunctions with constant vasodilatation [50].

Our results on cNOS activity rates at alcoholism are in good correspondence with other authors data [51]. According to their results glutamatergic system is directly involved into mediating acute and chronic alcohol effects. Its receptors are included into NO sygnal way, as NDMA glutamatergic receptors stimulation caused Ca^{2+} exit from the depot with further calmodulin binding and nNOS activation. nNOS gene is of key importance for regulation behavioural effects of alcohol [51].

Brain tissue is most vulnerable to oxidative damage due to its high oxygen consumption, high metabolic rate and low levels of antioxidant enzymes, such as SOD, glutathione peroxidase and catalase. A great increasing of lipid peroxidation could be mediated by ROS accumulation, because of brain's high content of highly susceptible to oxidation polyunsaturated fatty acids [52].

In the current study increased TBARS levels in ethanol-exposed rats' brain, as a result of oxidative stress development were shown. Several studies have examined the role of oxidative stress in alcohol-mediated neurotoxicity, possibly via the formation of free radicals [53,54,55]. The free radicals interact with other cell components, such as proteins, DNA, and lipids, to form multiple catabolic products. An example of such interaction could be lipid peroxidation resulting in lipid hydroperoxides and aldehydes. The latter interact with proteins molecules SH-groups causing loss of their native structure and functions and thus perpetuating cells damage. The increased levels of calcium and nitric oxide stimulate the production of inflammatory interleukins causing gliosis and increasing the state of oxidative stress causes damage and cell death [56], thus establishing a cycle through a chain of oxidative reactions that could involve both neurons and glia. At the conditions of our experiment we have established the increase of brain biomolecules oxidation with simultaneous decrease in the activity of antioxidant systems, which could be evidence of redox balance loosing.

Ethanol can react with the OH• radical to form the alpha-hydroxyethyl radical, which is considered to be less toxic. It also can stimulate H_2O_2 degradation through catalase activation [57,58]. In mammalian brain catalase is the main enzyme that catalyzes the peroxidation of ethanol to acetaldehyde and its inhibition is associated with functional and metabolic disturbances of the CNS [58,59]. The information concerning brain catalase activity following experimental chronic alcohol intoxication is insufficient and contradictory. At these circumstances different researchers reported increase [60], decrease [61] and absence of changes [58] in catalase activity. It could be a result of different animals' models

using. We observed decrease of brain catalase activity in rats chronically exposed to 15 % ethanol solution. Other authors suggest that catalase could be a more major contributor to ethanol oxidation in the brain [62]. Inhibition of brain catalase activity in mice inhibits many of the pharmacologic effects of ethanol and changes formation of acetaldehyde in brain homogenates [62].

In our experiments chronic ethanol self-administration significantly decreased SOD activity in experimental animals brain. Such inhibition of SOD activity by ethanol could cause cytotoxic O_2 - radicals accumulation and thus nervous system disorders during alcohol intoxication [63].

We suggest that the decrease in SOD and catalase activities may be a result of enzymes molecules oxidative modification by free radicals generated during ethanol and acetaldehyde biotransformation.

5. CONCLUSIONS

Thus investigation of alcoholism effects on brain levels of free amino acids permits to obtain complex estimation of this pathology influence on metabolic processes in brain, especially on amino acids, proteins, ATP and NADPH metabolisms. Our results may possibly indicate on serious changes in brain amino acids, other nitrogenous compounds, ATP and NADPH metabolisms during chronic ethanol consumption. Alterations on the level of genome in our experiments were accompanied with complex alterations on the level of protein, amino acid and nitrous metabolisms.

ETHICAL APPROVAL

The "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the bioethics committee of the *SI* “Institute of Pharmacology & Toxicology” National Academy of Medical Sciences of Ukraine.

COMPETING INTERESTS

The authors have declared that no competing interests exist.

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