Influence of Chronic Alcohol Consumption on Histaminergic Neurons of the Rat Brain

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Abstract — Aims: To clarify the effect of chronic alcohol consumption on the brain histaminergic neurons in rats. Methods: Male Wistar rats were given 20% ethanol as the only source of drinking during 6 months, control rats had a free access to water. The samples of hypothalamus were prepared for light and electron microscopy accompanied by morphometry to examine the brain histaminergic neurons of E2 group. Results: Chronic ethanol consumption increased the amount of histologically abnormal forms of histaminergic neurons and decreased the whole amount of E2 histaminergic neurons (for 5%). The neuron bodies and nuclei increased in size and sphericity, the nuclear/cytoplasmic ratio decreased by 15%. The ultrastructural changes in histaminergic neurons demonstrate the activation of their nuclear apparatus, both destruction and hypertrophy and hyperplasia of organelles, especially lysosomes. Chronic ethanol consumption induces the disturbances in cytoplasmic enzymes of neurons: increases the activity of type B monoamine oxidase, dehydrogenases of lactate and NADH and, especially, marker enzyme of lysosomes acid phosphatase as well as inhibits the activity of dehydrogenases of succinate and glucose-6-phosphate. Conclusion: Chronic alcohol consumption affects significantly the structure and metabolism of the brain histaminergic neurons, demonstrating both the neurotoxic effect of ethanol and processes of adaptation in those neurons, necessary for their survival.

INTRODUCTION

Chronic alcohol consumption induces the dramatic disturbances in all organs of the body, especially in the brain (Harper, 2009). To a greater or lesser extent all known neurotransmitter systems and types of the brain neurons are involved in central ethanol effects and alcoholism pathogenesis. Brain histaminergic system participates in regulation of many functions, systems and reactions of the body: neuroendocrine and cardio-vascular, brain blood flow, sleep and awakening, hibernation, feeding and drinking behavior, memory, cognition and learning. It participates in some pathological states and diseases, including addictions (reviews of Brown et al., 2001; Haas and Pannula, 2003; Haas et al., 2008; Blandina et al., 2012). We have discussed earlier that alcohol and histamine interact in the body (Zimatkin and Anichtik, 1999). The further studies suggest that the brain histaminergic system and especially the H3 histamine receptors participate in the regulation of alcohol consumption and alcohol-induced behaviors. AA line rats with high inborn preference to alcohol have higher contents of histamine and it is primarily metabolite, methylhistamine, as well as increased density of histaminergic fibers in brain regions when compared with refused ethanol ANA rats. H3 histamine receptor ligands modulate the voluntary alcohol consumption in AA rats (Lintunen et al., 2001). Studies on both rats and mice indicate that histamine H3 receptor antagonists (they activate brain histaminergic neurons) decrease alcohol drinking in several models, such as operant alcohol administration and drinking in the dark paradigm, inhibit ethanol-evoked stimulation of locomotor activity and potentiate an ethanol reward. Alcohol-induced place preference is also affected by these drugs. Moreover, mice lacking H3 receptors do not drink alcohol like their wild-type littermates, and they do not show alcohol-induced place preference. The authors propose that H3 receptor antagonists are promising candidates for use in human alcoholic patients (Nuutinen et al., 2011, 2012; Panula and Nuutinen, 2011; Bahi et al., 2013). The histidine decarboxylase (enzyme of histamine synthesis) knockout mice display a weaker stimulatory response to acute alcohol and stronger conditioned place preference induced by alcohol than the wild-type mice. Histaminergic system is involved in the regulation of place preference behavior triggered by alcohol, possibly through an interaction with the mesolimbic dopamine system. Histamine may also interact with dopamine in the regulation of the cortico-striato-pallidomotor pathway and cerebellar mechanisms, which may be important in different motor behaviors beyond alcohol-induced motor disturbances. H3 receptors ligands may have significant effects on alcohol addiction (Panula and Nuutinen, 2011). It was concluded recently that brain histaminergic system has an inhibitory role in alcohol reward. Increasing neuronal histamine released via H3 receptor blockade could therefore be a novel way of treating alcohol dependence (Vanhanen et al., 2013).

In AT line rats with high inborn tolerance to motor disturbances induced by alcohol the higher histamine level and density of histaminergic fibers were found in brain regions, when compared with ANT rats with low inborn tolerance to alcohol. The H1 and H3 histamine receptors of the brain in AT and ANT rats are different in their expression, density of distribution and affinity to ligands. Pharmacological blockade of histamine synthesis decreases the brain histamine level and tolerance to ethanol-induced motor disturbances in AT rats (Lintunen et al., 2002). A pretreatment with 1-histidine, the histamine precursor, significantly reduced ethanol-induced sedation, suggesting that brain histamine protects against the sedative effects of ethanol. Histidine decarboxylase inhibitor increases alcohol sensitivity in the tilting plane test (Panula and Nuutinen, 2011).

According to the previous research the chronic administration of ethanol to rats increased (Nowak and Maslinski, 1984), did not change (Fogel et al., 1991) or decreased (Subramanian et al., 1980) the level of histamine in the brain regions. Following alcohol administration, the activity of the first histamine metabolizing enzyme, histamine N-methyltransferase failed to change in any brain region (Subramanian et al., 1980; Prell and Mazurkiewicz-Kwilecki, 1981). In the preliminary study we have reported that acute ethanol administration in the...
dose of 4 g/kg induced the structural and metabolic disturbances in rat brain histaminergic neurons (Zimatkin et al., 2013). Unlike for other brain aminergic neurons, there are no direct microscopic investigations of chronic alcohol effect on the brain histaminergic neurons, which was the aim of the present paper. We have examined if chronic ethanol consumption influences the size, shape and amount of those neurons, as well as their metabolism and ultrastructural characteristics.

MATERIALS AND METHODS

Animals, chemicals and experimental design

Male Wistar rats were obtained from the breeding colony of the Grodno State Medical University. All experimental procedures complied with European Community Council Directive (86/609/EEC) for care and use of laboratory animals. The experiments performed in this study were reviewed and approved by the Ethical Committee of the Grodno State Medical University (protocol No. 1, 20.01.2010). All efforts were made to minimize animal suffering. Rats were housed six per cage with free access to food and kept under controlled environmental conditions. Twelve rats had a free access to water (control) and twelve rats obtained 20% ethanol as the only source of drinking during 6 months (except the first 2 weeks, when they obtained 10 and 15% ethanol, consequently). The initial weight of animals was $110 \pm 20$ g, the final weight was $290 \pm 23$ g. The level of water consumption and ethanol solution per cage/animal was controlled during the whole experiment. All chemicals were obtained from Sigma-Aldrich.

Histology and electron microscopy

The animals were sacrificed by decapitation after 6 months from the beginning of experiment, and the pieces of hypothalamus were obtained, frozen and stored in liquid nitrogen for further light microscopy. For electron microscopy the samples of posterior hypothalamus were fixed in 2.5% glutar aldehyde at Millonig buffer pH 7.4 (4 h at 4°C). Then the lateral parts of the posterior hypothalamus, where the histaminergic neurons of the largest group, E2, are situated, were fixed in 1% osmium tetroxide at Millonig buffer pH 7.4 (1 h at room temperature) (Millonig, 1961), dehydrated in the increasing concentration of ethanol and acetone and embedded in epoxide gum.

For the light microscopy the 10 µm serial sagittal sections of the frozen hypothalamus were prepared in cryostat (Leica CM 1840, Germany). They were stained by 0.1% solution of toluidine blue (Nissl method) for general cytology of neurons, for determination of activity the marker enzyme of histaminergic neurons, monoamine oxidase type B (MAO B, EC 1.4.3.4) (Zimatkin and Tsydik, 1994), for activity assessment of the marker oxidizing enzymes: succinate dehydrogenase (SDH, EC 1.3.99.1), lactate dehydrogenase (LDH, EC 1.1.1.27), glucose-6-phosphate dehydrogenase (G-6-PDH, EC 1.1.1.49), NADH dehydrogenase (NADHDH, EC, 1.1.1.49) and NADPH dehydrogenase (NADPDH, EC, 1.6.1.1), as well as for activity estimation of the marker enzyme of lysosomes, acid phosphatase (AP, EC 1.4.3.4) (Pearse, 1960). The examination of histological preparations, their microphotography and morphometry was carried out with microscope Axioskop 2 plus (Zeiss, Germany), digital camera (Leica DFC 320, Germany) and computer image analysis software Image Warp (Bit Flow, USA).

For the identification of the E2 group of histaminergic neurons the stereotaxic atlas and corresponding topographic schemes were used (Zimatkin et al., 2006; Paxinos and Watson, 2007). In samples stained by Nissl method all visible histaminergic neurons were estimated according to their type of chromofilia and divided into normochronic, hyperchronic, hypochronic and cell-shadows. For the estimation of size and shape of neurons bodies and nuclei, the images of up to 30 neurons in every preparation were outlined at the computer monitor and the mean values were used for the further statistics. Maximal and minimal diameter (D), perimeter (P), square (S) and volumes, as well as form-factor ($4\pi S/P^2$, parameter of sphericity and folding) and factor of elongation (maximal D—minimal D—parameter of sphericity) were quantified in Nissl stained neurons bodies (perikarions). The enzyme activities were determined in cytoplasm of neurons on the optic density of chromogen obtained in the course of histochemical reactions.

For the electron microscopy the sections were obtained by ultramicrotome MT-7000 (RMC, USA), contrasted by uranyl acetate and lead citrate (Reynolds, 1963), examined with transmission electron microscope JEM-1011 (JEOL, Japan), photography by digital camera (Olympus MegaView III, Germany). Ultrastructural morphometry was carried out with program of image analysis iTEM (Olympus Soft Imaging Solutions, Germany). Mitochondria and lysosomes profiles were outlined by a cursor on the computer monitor for estimation of their amount, individual and relative area in histaminergic neurons cytoplasm.

Statistics

The primarily data obtained were treated by nonparametric methods using software STATISTICA 6.0 (StatSoft, Inc., USA). In descriptive statistics the values of median (Me) and interquartile range (IQR) were determined. The differences were considered significant at $P < 0.05$ (Mann–Whitney U-test).

RESULTS

The average alcohol consumption during a 6-month period in experimental rats was 3.5 g/kg/day. Among the histaminergic neurons the amount of hyperchronic neurons and cell-shadows was significantly increased (from 1 to 10% and from 2 to 7%, respectively). The amount of E2 histaminergic neurons per square unit of section was significantly decreased by (5%).

Following the chronic ethanol consumption the histaminergic neuron bodies increased in size and became more spherical: their average minimal diameter increased by 12.2%, perimeter—by 9.1%, square—by 14.6%, volume—by 21.2% and factor of elongation decreased by 6.4% (Table 1). The similar, valid, but less pronounced changes were found in neuronal nuclei. The nuclear/cytoplasmic ratio decreased by 14.6% (from 0.68 ± 0.06 to 0.59 ± 0.05; $P < 0.05$).

Histochemical investigation has shown that in the cytoplasm of histaminergic neurons the activity of MAO B increased by 7%, NADHDH—by 12%, LDH—by 9% and activity of AP—by 18%, while the activity of SDH and G-6-PDH decreased by
5 and 13%, consequently and NADPHDH was not changed when compared with control (Figs. 1–3).

Electron microscopy of histaminergic neurons following chronic alcohol consumption has revealed the increase of the size of nucleoli, their shift to the nuclear envelope (Fig. 4A), aggregation of ribosomal subunits and their increased transport to the cytoplasm through the nuclear pores (Fig. 4A and B), broadening of the perinuclear space and the increased folding of the nuclear envelope (Fig. 4B). In the cytoplasm various changes of the organelles were observed. Some mitochondria were swollen with destructed crista and clear matrix (Fig. 4B), other mitochondria increased in size with densely packaged crista (Fig. 4A). Some of them closely adjoined nucleus, endoplasmic reticulum and Golgi complex. The average size of mitochondria increased slightly and became more spherical (Table 2). The hypertrophy of Golgi complex and extension of the canals and cisterns of endoplasmic reticulum was clearly observed (Fig. 4). The density of distribution of free polyribosomes in cytoplasm was visually increased (Fig. 4A and B). However the most prominent ultrastructural features of chronic ethanol action in histaminergic neurons were the large hypertrophy and hyperplasia of lysosomes (Fig. 4A and B): their average area, the amount and general area per square unit of cytoplasm increased significantly (Table 3).

**DISCUSSION**

The daily alcohol consumption by animals in our experiment during 6 months was quite mild (3.5 g/kg/day), because the animal diet in addition to the conventional food pellets included the natural food (fresh vegetables, bred and cottage cheese containing >70% of water). The high sensitivity of histaminergic neurons to alcohol can be theoretically predicted because of high activity of ethanol oxidizing enzyme catalase and low activity of aldehyde dehydrogenase, providing the conditions for toxic acetaldehyde accumulation in brain amnergic neurons (Zimatkin and Lindros, 1996). The chronic alcohol consumption induced the significant disturbances in

### Table 1. Parameters of size and shape of E2 rat brain histaminergic neurons bodies following chronic alcohol consumption

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n = 6)</th>
<th>Alcohol (n = 6)</th>
<th>U</th>
<th>P-level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimal diameter, µm</td>
<td>12.51 ± 0.62</td>
<td>14.42 ± 0.44</td>
<td>0</td>
<td>0.0040</td>
</tr>
<tr>
<td>Maximal diameter, µm</td>
<td>20.79 ± 1.47</td>
<td>21.48 ± 0.87</td>
<td>8</td>
<td>0.1093</td>
</tr>
<tr>
<td>Perimeter, µm</td>
<td>57.13 ± 5.84</td>
<td>65.22 ± 4.95</td>
<td>4</td>
<td>0.0250</td>
</tr>
<tr>
<td>Area, µm²</td>
<td>207.55 ± 15.85</td>
<td>239.83 ± 27.86</td>
<td>0</td>
<td>0.0040</td>
</tr>
<tr>
<td>Volume, µm³</td>
<td>2249.99 ± 255.27</td>
<td>2796.17 ± 490.91</td>
<td>0</td>
<td>0.0040</td>
</tr>
<tr>
<td>Form-factor</td>
<td>0.77 ± 0.01</td>
<td>0.76 ± 0.04</td>
<td>15</td>
<td>0.6310</td>
</tr>
<tr>
<td>Elongation factor</td>
<td>1.58 ± 0.02</td>
<td>1.49 ± 0.02</td>
<td>1</td>
<td>0.0065</td>
</tr>
</tbody>
</table>

Data are median ± interquartile range; P-level as compared with control.

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Fig. 1. Monoamine oxidase B activity in brain histaminergic neurons of control rats (A) and its increase following chronic alcohol consumption (B). Digital microphotography. ×200.

Fig. 2. NADH dehydrogenase activity in brain histaminergic neurons of control rats (A) and its increase following chronic alcohol consumption (B). Digital microphotography. ×200.
brain histaminergic neurons: the damage of many ultrastructures and significant increase in amount and size of lysosomes in all neurons, which reflects the intensification of autophagy for removal of the defected organelles and membranes. It was accompanied by activation of the marker enzyme of lysosomes, acid phosphatase in cytoplasm of neurons. The significant increase of the amount of abnormal forms, hyperchromic neurons and cells-shadows at light microscopic level indicates the toxic effect of ethanol to histaminergic neurons. As a final result the amount of histaminergic neurons has decreased, probably because of the death of some of them.

The majority of the survived histaminergic neurons demonstrated the structural features of hyperactivity, the intensive functioning for adaptation to the prolonged action of alcohol. The nuclear apparatus of neurons demonstrated the obvious structural features of activation, supporting the processes of protein biosynthesis in cytoplasm, as demonstrated by the enlargement of endoplasmic reticulum and Golgi complex. The increased amount of free ribosomes is for the elevated protein biosynthesis for the needs of the cell. The histaminergic neurons and their nuclei (to a lesser extent) became larger and more spherical, and the nuclear/cytoplasmic ratio decreased. They are the additional histological features of the increased activity of the neurons.

The histochemical changes in histaminergic neurons may reflect both alcohol-induced metabolic disturbances and an adaptation of neurons to ethanol. The disturbance of the oxidative enzymes activities in cytoplasm has indicated the reorganization of the neuronal energy metabolism. Thus, the decrease of SDH and G-6-PDH activities may reflect the deceleration of Krebs cycle in mitochondria and pentose phosphate pathway in cytosol of those neurons. The activation of LDH and NADH Dh indicates the acceleration of anaerobic glycolysis and transport of electrons. The increased MAO B activity indicates the intensification of oxidative diamination of the inactive first metabolite of histamine, tele-methylhistamine,
and formation of the active second metabolite N-tele-methyl-
imidazole acetaldehyde, competing with the acetaldehyde
formed in the course of ethanol oxidation in the brain
(Ambroziak and Pietruszko, 1987). The activation of the
marker enzyme of lysosomes, acid phosphatase, as well as
hypertrophy and hyperplasia of lysosomes reflects the
increased autophagy for the removal of damaged organelles
in histaminergic neurons. All structural and metabolic distur-
bances have revealed in brain histaminergic neurons of rats
after chronic ethanol consumption are nonspecific since they
can be observed in other types of neurons under the other ex-
perimental conditions.

The influence of alcohol on brain histamine level and metab-
olism has been studied biochemically in the brain tissue homo-
genates (Subramanian et al., 1980; Prell and Mazurkiewicz-
Kwilecki, 1981; Nowak and Maslinski, 1984; Tabakoff et
al., 1985; Fogel et al., 1991). It is therefore difficult to correlate our
results with these biochemical data, as we have examined the
histaminergic neuronal bodies, while biochemistry was per-
formed in brain homogenates of the whole brain or its large
parts, containing various types of cells.

CONCLUSIONS

1. Chronic alcohol consumption significantly affects the
structure and metabolism of the brain histaminergic neurons,
demonstrating the neurotoxic effect of ethanol as well as
comparative resistance of those neurons, necessary for their
survival. Some of the neurons were damaged and died,
others increased in size and sphericity and their nuclear/cytoplasmic ratio decreased.

2. The ultrastructural changes in brain histaminergic neurons
following chronic alcohol consumption denote the activation of
their nuclear apparatus (increase and shift of nucleoli to the
nuclear envelope, intensification of ribosomal subunits transport
to cytoplasm through the nuclear pores, broadening of the peri-
nuclear space and the increase of nuclear envelope folding),
both destruction and hypertrophy, hyperplasia of organelles
reflect the processes of adaptation to alcohol.

3. Chronic ethanol consumption induces the disturbances in
metabolism of brain histaminergic neurons: activation of type
B monoamine oxidase, dehydrogenases of lactate and NADH
and acid phosphatase as well as inhibition of succinate and
glucose-6-phosphate dehydrogenases, reflecting the adapta-
tion of neuronal metabolism to ethanol.

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REFERENCES

Ambroziak W, Pietruszko R. (1987) Human aldehyde dehydrogen-
ase: metabolism of putrescine and histamine. Alcohol Clin Exp
Res 11:528–32.

Bahi A, Sadek B, Schwed SJ et al. (2013) Influence of the novel his-
tamine H3 receptor antagonist ST1283 on voluntary alcohol con-
sumption and ethanol-induced place preference in mice. Psychopharmacology (Berl) 228:85–95.

Blandina P, Munari L, Provensi G et al. (2012) Histamine neurons in
the tuberomammillary nucleus: a whole center or distinct subpopu-


Fogel WA, Andrzejewski W, Maslinski C. (1991) Liver damage, vol-
untary alcohol intake and brain histamine. Agents Actions
33:150–3.

Haas H, Panula P. (2003) The role of histamine and the tuberomamm-


damage. Alcohol Alcohol 44:136–40.

Lintunen M, Hyytia P, Sallmen T et al. (2001) Increased brain
histamine in an alcohol-prefering rat line and modulation of
ethanol consumption by H3 receptor mechanisms. FASEB J
15:1074–6.

Lintunen M, Raatesalmi K, Sallmen T et al. (2002) Low brain hista-
mime content affects ethanol-induced motor impairment.


Nowak JZ, Maslinski C. (1984) Ethanol-induced changes of hista-
mime content in guinea-pig brain. Pol J Pharmacol Pharm
36:647–51.

Nuutinen S, Lintunen M, Vanhanen J et al. (2011) Evidence for the
role of histamine H3 receptor in alcohol consumption and alcohol

Nuutinen S, Vanhanen J, Maki T et al. (2012) Histamine h3 receptor:
a novel therapeutic target in alcohol dependence? Front Syst
Neurosci 6:36.

Panula P, Nuutinen S. (2011) Histamine and H3 receptor in alcohol-


Pearse AGE. (1960) Histochemistry Theoretical and Applied. 2nd

Prell GD, Mazurkiewicz-Kwilecki IM. (1981) The effects of ethanol,
acetaldehyde, morphine and naloxone on histamine methyltrans-

Reynolds ES. (1963) The use of lead citrate at high pH as an electron

Subramanian N, Schinzel W, Mitznegg P et al. (1980) Influence of
ethanol on histamine metabolism and release in the rat brain.

Tabakoff B, Lee JM, De Leon-Jones F et al. (1985) Ethanol inhibits
the activity of the B form of monoamine oxidase in human platelet

Vanhanen J, Nuutinen S, Lintunen M et al. (2013) Histamine is
required for H3 receptor-mediated alcohol reward inhibition, but
not for alcohol consumption or stimulation. Br J Pharmacol

Alcohol Alcohol 34:141–7.

Zimatin SM, Lindros KO. (1996) Distribution of catalase in rat
brain: amnergic neurons as possible targets for ethanol effects.
Alcohol Alcohol 31:167–74.

Zimatin SM, Tsydk VF. (1994) A histochemical method for study-
ing the activity of monoamine oxidase A and B in the brain.

Zimatin SM, Kuznetsova VB, Strik ON. (2006) Spatial organization
and morphometric characteristics of histaminergic neurons in the

neurons in the rat brain after acute exposure to alcohol. Neurosci
Behav Physiol 43:691–6.