УДК 611.814.1–018.82:547.781.8:547.262

ГИСТАМИНЕРГИЧЕСКИЕ НЕЙРОНЫ МОЗГА КРЫСЫ ПОСЛЕ ОДНОКРАТНОГО ВОЗДЕЙСТВИЯ ЭТАНОЛА: СРАВНЕНИЕ ЭФФЕКТОВ МАЛОЙ И БОЛЬШОЙ ДОЗ

[1-] *Зиматкин С. М. (smzimatkin@mail.ru),* [1-] *Федина Е. М. (phedina.katerina@mail.ru),*
[2-] *Анищик О. В. (oleg.anichtchik@plymouth.ac.uk)*

[1-]Гродненский государственный медицинский университет, Гродно, Беларусь
[2-]Университет Плимута: Школа медицины и стоматологии Пенисулы, Плимут, Объединенное Королевство

Центральная гистаминергическая система модулируется при нейродегенеративных заболеваниях и деменции, также как при алкоголизме, так как пути метаболизма гистамина и этанола в мозге совместно используют общий фермент альдегиддегидрогеназу. В то время как многочисленные биохимические работы сообщают об изменениях метаболизма гистамина в мозге после введения этанола, морфологическая основа для этого действия по-прежнему отсутствует. В этом исследовании мы оценивали эффекты малой (1 г/кг) и большой, гипнотической (4 г/кг) дозы этанола на морфологические параметры гистаминергических нейронов мозга крысы. Мы выявили морфометрические и гистохимические изменения сопоставимые с усилением нейрональной активности, подтверждённой апрегуляцией с-Фос у животных подвергавшихся воздействию этанола, показали компенсаторные и адаптивные изменения после воздействия этанола направленные на поддержание жизнеспособности этих нейронов в ситуации алкогольной интоксикации.

***Ключевые слова**: мозг, гистаминергические нейроны, этанол.*

The histaminergic system is one of the important and well established neurotransmitter systems of the brain. The bodies of histaminergic neurons are situated in the posterior hypothalamus only, forming five cluster groups (E1-E5) [2, 21]. Their processes reach all brain regions, regulating the activity of other neurotransmitter systems and brain functions. Brain histaminergic system participates in regulation of neuroendocrine and cardiovascular system, brain blood flow, sleep and wakefulness, hibernation, feeding and drinking behavior, memory, cognition and learning. It is involved in some pathological conditions and diseases as well, including addiction [7, 2, 6, 5]. There is data suggesting the interactions between brain histamine and alcohol, participation of brain histamine system in alcohol related behavior and alcoholism pathogenesis [13, 17]. The histamine and ethanol metabolic pathways in the brain have the common enzyme – aldehyde dehydrogenase, therefore, the highly active ethanol metabolite, acetaldehyde, can interfere with histamine degradation by competing with histamine metabolite N-tele-methylimidazole acetaldehyde for this enzyme [1]. Therefore this may represent a metabolic basis for the alcohol-histamine interaction in the brain [17].

The influence of ethanol on the brain histamine level and metabolism has been studied biochemically in brain homogenates [4, 9, 12, 16]. The effect of an acute ethanol administration on histamine levels in the brain strongly depends on the dose of ethanol, the species of experimental animals and the brain regions studied. The activity of histamine synthesizing enzyme histidine decarboxylase has been reported to be increased after administration of ethanol in the hypothalamus, midbrain and brain cortex of adult rats [15], but decreased in the brain cortex and thalamus Almost all authors consider that following ethanol administration, the activity of histamine degrading enzyme histamine N-methyltransferase failed to change in any brain region [9, 15, 16]. Ethanol increases the steady-state N-tele-methylhistamine levels in the mouse hypothalamus, probably by inhibiting the elimination of this metabolite in the brain [3]. Thus, the biochemical data indicate that ethanol can affect histamine content and metabolism in the brain, but there are no morphological observations on the condition of central histaminergic neurons in animals or humans (alcoholic patients) after ethanol administration. Earlier we have reported that acute high dose ethanol administration induced time-dependent structural and metabolic disturbances in histaminergic neurons of the rat hypothalamus [19], associated with microscopic features of both damage and partial recovery of neurons. The aim of the present study was to expand these observations and to compare the effects of low and high dose ethanol exposure on brain histaminergic neurons by combined histology, histochemistry and electron microscopy.

Materials and methods

30 male Wistar rats were obtained from the breeding colony of the Grodno State Medical University. Their weight at the beginning of experiments was 175±23 g. All experimental procedures complied with European Community Council Directive 2010/63EU for care and use of laboratory animals. The study and experimental protocols therein were reviewed and approved by the Ethical Committee of the Grodno State Medical University (protocol №1, 20.01.2010). All efforts were made to minimize animal suffering. Rats were housed 6-7 per cage (40 x 60 cm) with free access to food and water and kept under controlled environmental conditions. 20% ethanol solution in saline (0.85% NaCl) was injected to rats intraperitoneally from 9 till 11 a.m. (light period). 10 rats have received ethanol at a dose of 1 g/kg, 10 rats - at a dose of 4 g/kg, 10 control animals were injected by saline. All rats were decapitated 1 hour after the injections.

All the chemicals were obtained from Sigma-Aldrich, (USA) unless mentioned specifically. The following antibodies were used: rabbit anti c-Fos (Santa Cruz, Dallas, USA), mouse anti ATP synthase subunit beta (Abcam, Cambridge, UK).

Rats were anesthetized and fresh brain was removed following decapitation. Pieces of hypothalamus were then obtained, frozen and stored in liquid nitrogen for further analysis. For the brightfield microscopy 10 μm serial frontal sections of the frozen hypothalamus were prepared using cryostat (Leica CM 1840, Germany). They were stained by 0.1% solution of toluidine blue

(Nissl method) to assess general cytology of neurons. For the identification of brain structures the stereotaxic atlas was used [14]. The activity of the marker enzyme of histaminergic neurons, monoamine oxidase type B (MAO B, EC1.4.3.4), was detected using the method published by us earlier [20]. To assess the activity of the oxidizing enzymes, such as 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 NADPhH dehydrogenase (NADPhDH, EC, 1.6.1.1), as well as to estimate the activity of the marker lysosomal enzyme acid phosphatase (AP, EC 1.4.3.4), we used histochemical methods [8].

For immunohistochemistry animals were perfused under the general ethyl ester anesthesia with cold phosphate buffer in 0.85% NaCl pH 7.4 (PBS), followed by cold 4% paraformaldehyde in PBS (PFA, pH 7.4). The animals were then decapitated, pieces of hypothalamus were dissected and postfixed by immersion for 12h at +4oC in 4% PFA, placed for 24 h into the 15% sucrose on PBS, then into the 30% sucrose on PBS with 0.1% sodium azide and stored at +4oC until required. The samples were frozen and 30 μm serial frontal sections of posterior hypothalamus were prepared on the sliding freezing microtome (Leica CM 2400 (Germany), collected and stored in PBS with 0.1% sodium azide at +4oC until required. On the day of the experiment, sections were washed in PBS, PBS-T (PBS + 0.1% Tween 20 or 0.05% Tween 80) for 15 min, quenched in 3% H2O2 in PBS-T for 1 hour, followed by washing in PBS-T 10 min at room temperature. Then sections were blocked in 5% normal goat serum (NGS) in PBS-T for 1 hour and incubated with primary antibodies - rabbit anti c-Fos and mouse anti ATP-synthase-β (both 1:1000) in PBS-T with 5% NGS overnight at +4oC. Sections were then washed in PBS and incubated for 1 hour with goat anti-rabbit Alexa 488 and goat anti-mouse Alexa 568 antibodies at 1:700 in PBS with 5% NGS. Primary antibodies were omitted in some sections to control for nonspecific binding of secondary antibodies. Sections were then mounted onto microscope slides in Fluorosafe medium (Calbiochem-Merck Millipore, MA, USA). The staining was examined using confocal Leica TCS SPE (Leica Microsystems GmbH, Germany) (objective x63, zoom x1.5, the number of confocal planes included in an image was 20).

For the identification of histaminergic neurons in brain sections the stereotaxic atlas and corresponding topographic schemes were used [14, 21]. The examination of histological preparations, their microphotography and morphometry was carried out with microscope Axioskop 2 plus (Zeiss, Germany), numerical aperture of the lens 0.25-0.65, equipped with digital camera (Leica DFC 320, Germany), resolution 2088x1550, and computer image analysis software Image Warp (Bit Flow, USA). To estimate the size and shape of neuronal bodies and nuclei, preparations stained by Nissl method were used. The images of up to 30 histaminergic neurons bodies and their nuclei were outlined in every preparation on 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 (4πS/P2, 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.

The primary data obtained was treated with nonparametric statistical methods (because the amount of animals in the groups does not exceed 7) 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 with $p<0.05$ (Mann-Whitney U-test).

Results and discussion

There were no visible histological changes of brain histaminergic neurons 1 hour following single alcohol administration. The image analysis revealed that following ethanol administration at a low dose of 1 g/kg the average minimal and maximal diameter, perimeter, area and volume of histaminergic neuronal bodies decreased by 10.3, 2.4, 15, 12.8 and 18.6% correspondingly (Fig. 1) and form-factor increased by 8.8% (to 0.74±0.04), as compared to controls. Following high ethanol dose (4 g/kg) the minimal diameter of neuron bodies increased by 3.6% and perimeter decreased by 2.9%, but the area and volume did not change (Fig. 1). The form-factor of neurons bodies increased by 8.8% and factor of elongation decreased by 7% (to 1.58±0.15), as compared to controls (data not shown). The similar dose-dependent changes were found in sizes and shape of neuronal nuclei. The nuclear/cytoplasmic ratio was not altered (data not shown).

Histochemical investigation demonstrated that following low ethanol dose the activity of MAO B increased by 5% and NADPhDH by 9%, but the activity of LDH decreased by 9% in cytoplasm of histaminergic neurons. Following high ethanol dose, the activity of LDH increased by 12.3%, the activity of MAO B increased by 17.1% and the activity of acid phosphatase increased by 25.1%, but activities of NADPhDH and G-6-PDH decreased by 17.6 and 22.9%, respectively. The activity of other enzymes examined did not change (Fig. 2, 3).

Histochemical investigation demonstrated that following low ethanol dose the activity of MAO B increased by 5% and NADPhDH by 9%, but the activity of LDH decreased by 9% in cytoplasm of histaminergic neurons. Following high ethanol dose, the activity of LDH increased by 12.3%, the activity of MAO B increased by

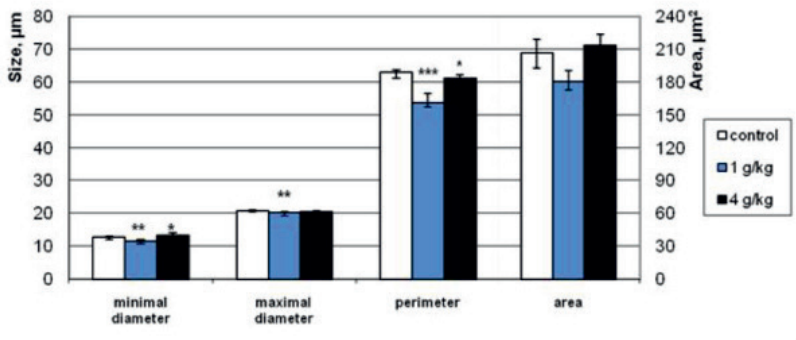

Figure 1. – Morphometric parameters of hypothalamus histaminergic neurons perikarions 1 hour after ethanol administration at 1 and 4 g/kg. Data is presented as median ± interquartile range; * – p<0,05, ** – p<0,01, *** – p<0,001, as compared to controls

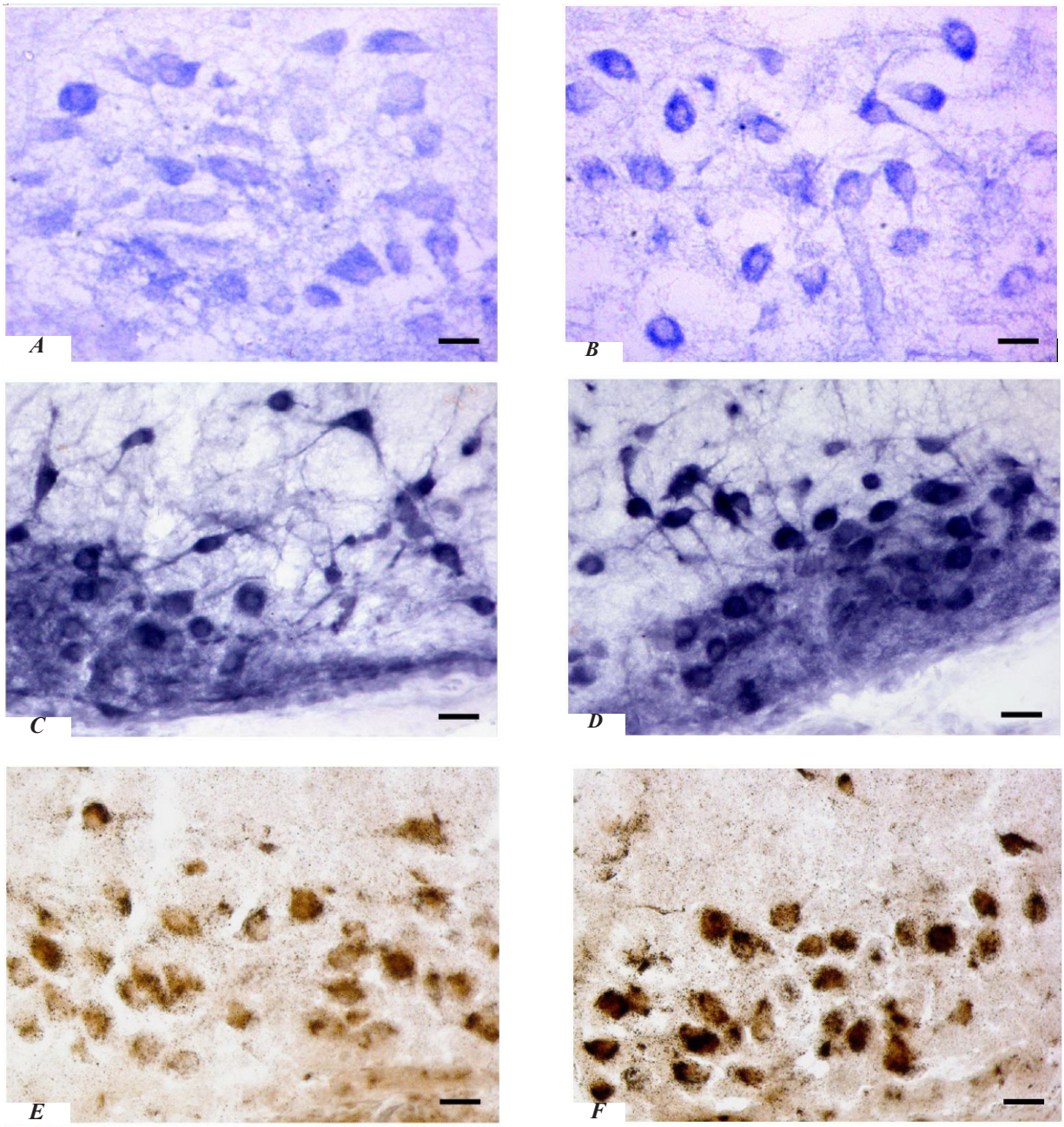

Figure 2. – Enzyme activities in cytoplasm of brain histaminergic neurons of control rats (A, C, E), 1 hour after ethanol administration at a dose 1 g/kg (B) and 4 g/kg (D, F). A, B – NADPhH dehydrogenase; C, D – MAO B; E, F – Acid phosphatase. Microphotography. Calibration bar and magnification: 15 µm, x400

17.1% and the activity of acid phosphatase increased by 25.1%, but activities of NADPhDH and G-6-PDH decreased by 17.6 and 22.9%, respectively. The activity of other enzymes examined did not change (Fig. 2, 3).

Immunohistochemical staining against c-Fos showed that while control animals displayed only faint c-Fos staining in neuronal nuclei of E2 hypothalamic group, both low and high doses of ethanol increased it. Notably, higher dose of ethanol caused bigger increase in c-Fos staining. When co-stained with mitochondrial marker ATP-synthase-β, we were able to demonstrate that low doses of ethanol caused relative increase in this staining in cytoplasmic mitochondria, while higher doses of ethanol led to the relative decrease of ATP-synthase-β positive staining, showing possible fragmentation of mitochondria (Fig. 4).

Our study demonstrated that ethanol induced pronounced dose-dependent changes in the morphology of histaminergic neurons in the rat brain. Low alcohol dose significantly decreased the size of neurons, while the high dose did not. Both doses made the neurons more rounded, spherical. The possible reason for this might be a disturbance of the electrolyte balance and an alteration of the cytoskeleton of neurons, induced by ethanol.

Indeed, low doses of ethanol have been shown to stimulate the activity of MAP2 kinase [11].

The histochemical changes in histaminergic neurons may reflect both the ethanol-induced metabolic disturbances and metabolic adaptation of neurons to ethanol. Thus, the MAO B activation, especially by the high ethanol dose, indicates the intensification of oxidative deamination of histamine in those neurons. Ethanol at

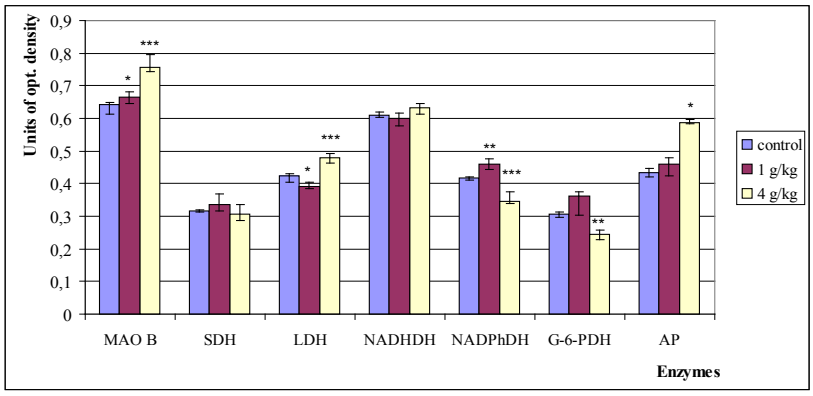

Figure 3. – Changes of the enzyme activities in cytoplasm of brain histaminergic neurons 1 hour after ethanol administration at a dose 1 and 4 g/kg. The enzymes studied (as described in the «Materials and methods») are represented on the horizontal axis and optical densities of chromogen obtained in the course of corresponding histochemical reactions are plotted on the vertical axis. Data is presented as median ± interquartile range; * – p<0,05; ** – p<0,01; *** – p<0,001, as compared to control

studied doses caused bidirectional change in the activity of the key oxidative enzymes – NADPhDH and LDH, which participate in transport of electrons and anaerobic glycolysis. In addition, 4 g/kg of ethanol inhibited G-6-PhDH and sharply activated the lysosomal enzyme, acid phosphatase, which may reflect the deceleration of pentose phosphate pathway in cytosol and the increased autophagy aimed at the removal of damaged organelles within histaminergic neurons.

Accumulation of c-Fos serves as a marker of the functional activity of neurons [10]. Therefore the increased expression of c-Fos in histaminergic neurons cytoplasm may indicate an increase of their functional activity following ethanol administration at both doses. Low dose of ethanol increased the expression of ATP-

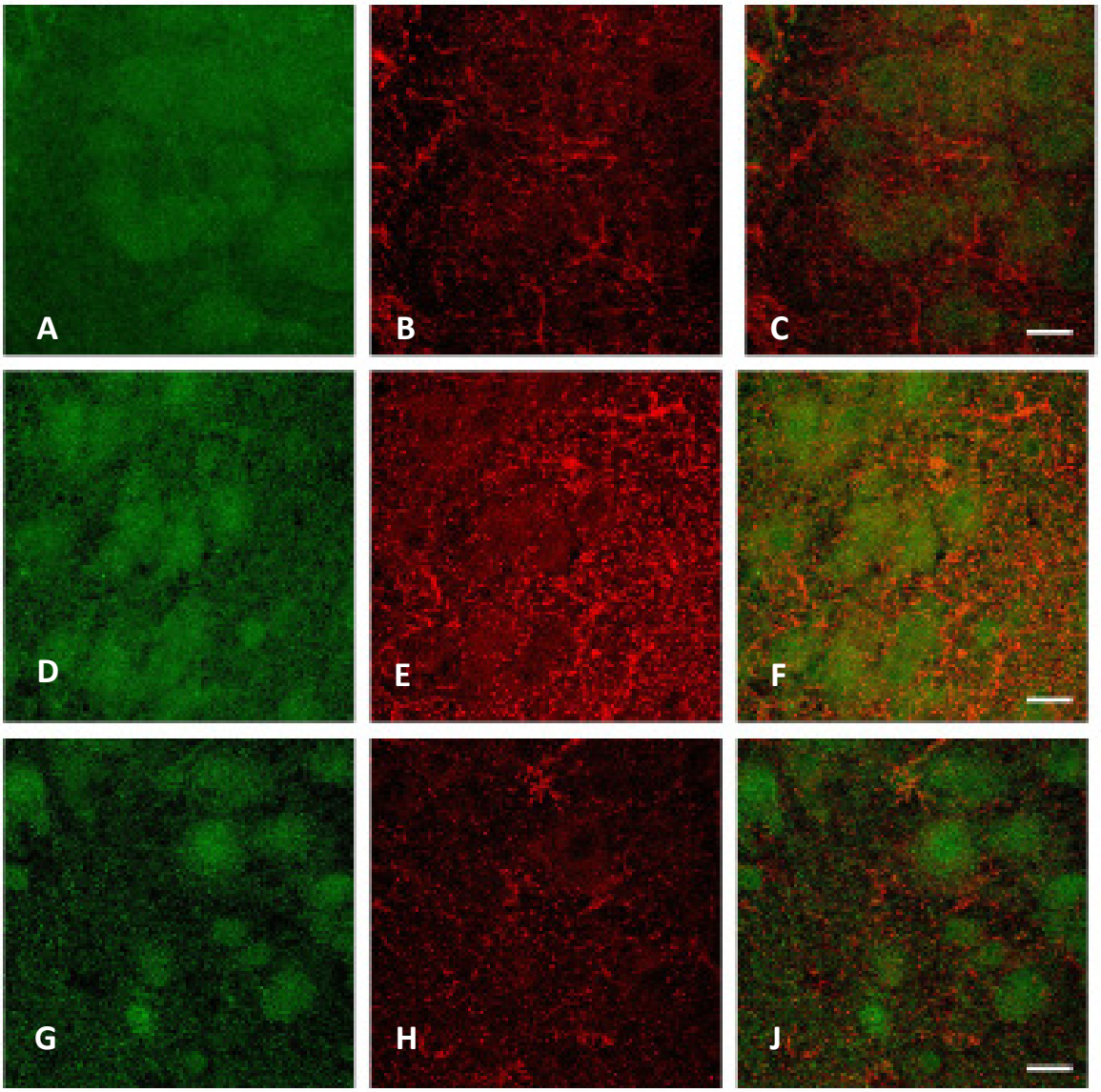

Figure 4. – Expression of c-fos (A, D, G), ATP-synthase-β (B, E, H) and their co-localization in the E2 hypothalamic nucleus (C, F, J) in control rats (A-C) and 1 hour later after ethanol administration at a dose 1 g/kg (D-F) and 4 g/kg (G-J). Calibration bar: 15 μm. Microphotography. x94.5

synthase-β both in neuron bodies and surrounding neuropile reflecting possible activation of mitochondria.

High sensitivity of histaminergic neurons to ethanol, even at a low dose, can be explained by high activity of ethanol oxidizing enzyme catalase and low activity of aldehyde dehydrogenase, providing the conditions for acetaldehyde accumulation in brain aminergic neurons and their stimulation [18].

Conclusions

Histaminergic neurons, in addition to the destructive changes induced by high ethanol dose, exhibit the structural signs of hyperactivity, and intensive functioning related to the adaptation to the effects of ethanol. It evidences participation of central histaminergic system in modulating addictive or toxic effects of ethanol.

References

1. Ambroziak, W. Human aldehyde dehydrogenase: metabolism of putrescine and histamine / W. Ambroziak, R. Pietruszko // Alcohol Clin Exp Res. - 1987. - Vol. 11. – P. 528-532.

2. Brown, R. E. The physiology of brain histamine / R. E. Brown, D. R. Stevens, H. L. Haas // Prog Neurobiol. - 2001. - Vol. 63. – P. 637-672.

3. Changes in histamine metabolism in the mouse hypothalamus induced by acute administration of ethanol / Y. Itoh [et al.] // J. Neurochem. – 1985. – Vol. 45. – P. 1880-1885.

4. Fogel, W. A. Brain histamine in rats with hepatic encephalopathy / W. A. Fogel, W. Andrzejewski, C. Maslinski // J. Neurochem. – 1991. - Vol. 56. – P. 38-43.

5. Haas, H. L. Histamine in the nervous system / H. L. Haas, O. A. Sergeeva, O. Selbach // Physiol Rev. – 2008. – Vol. 88. – P. 1183-1241.

6. Haas, H. The role of histamine and the tuberomamillary nucleus in the nervous system / H. Haas, P. Panula // Nat Rev Neurosci. - 2003. - Vol. 4. – P. 121-130.

7. Histamine neurons in the tuberomamillary nucleus: a whole center or distinct subpopulations? / P. Blandina [et al.] // Front Syst Neurosci. - 2012. - Vol. 6. – P. 33.

8. Histochemistry: theoretical and applied. 2nd ed. / A. G. E. Pearse [et al.] // Churchill. – London. – 1960.

9. Influence of ethanol on histamine metabolism and release in the rat brain. II. Regions of the histaminergic pathway / N. Subramanian [et al.] // Pharmacology. – 1980. – Vol. 20. – P. 42-45.

10. Kovacs, K. J. Measurement of immediate-early gene activation- c-fos and beyond / K. J. Kovacs // J. Neuroendocrinol. – 2008. – Vol. 20. – P. 665-672.

11. Low levels of ethanol stimulate and high levels decrease phosphorylation in microtubule-associated proteins in rat brain: an in vitro study / B. Ahluwalia [et al.] // Alcohol and Alcoholism. – 2000. – Vol. 35. – P. 452-457.

12. Nowak, J. Z. Ethanol-induced changes of histamine content in guinea-pig brain / J. Z. Nowak, C. Maslinski // Pol J. Pharmacol Pharm. – 1984. – Vol. 36. – P. 647-651.

13. Panula, P. Histamine and H3 receptor in alcohol-related behaviors / P. Panula, S. Nuutinen // J. Pharmacol Exp Ther. – 2011. – Vol. 336. – P. 9-16.

14. Paxinos, G. The rat brain in stereotaxic coordinates. 6th ed. / G. Paxinos, C. Watson // Academic Press. – London. – 2007.

15. Prell, G. D. Rat brain histamine concentration, synthesis and metabolism: effect of acute ethanol administration / G. D. Prell, B. Bielkiewicz, I. M. Mazurkiewicz-Kwilecki // Prog Neuropsychopharmacol Biol Psychiatry. – 1982. – Vol. 6. – P. 427-432.

16. Prell, G. D. The effects of ethanol, acetaldehyde, morphine and naloxone on histamine methyltransferase activity / G. D. Prell, I. M. Mazurkiewicz-Kwilecki // Prog Neuropsychopharmacol. – 1981. – Vol. 5. – P. 581-584.

17. Zimatkin, S. M. Alcohol-histamine interactions / S. M. Zimatkin, O. V. Anichtchik // Alcohol and Alcoholism. – 1999. – Vol. 34. – P.141-147.

18. Zimatkin, S. M. Distribution of catalase in rat brain: aminergic neurons as possible targets for ethanol effects / S. M. Zimatkin, K. O. Lindros // Alcohol. – 1996. – Vol. 31. – P. 167-174.

19. Zimatkin, S. M. Histaminergic neurons in the rat brain after acute exposure to alcohol / S. M. Zimatkin, E. M. Fedina, V. B. Kuznetsova // Neurosci Behav Physiol. – 2013. – Vol. 43. – P. 691-696.

20. Zimatkin, S. M. Histochemical method for investigating the activity of monoamine oxidase A and B in the brain / S. M. Zimatkin, V. F. Tsydik // Neurosci Behav Physiol. – 1996. – Vol. 26. – P. 231-233.

21. Zimatkin, S. M. Spatial organization and morphometric characteristics of histaminergic neurons in the rat brain / S. M. Zimatkin, V. B. Kuznetsova, O. N. Strik // Neurosci Behav Physiol. – 2006. – Vol. 36. – P. 467-471.

RAT BRAIN HISTAMINERGIC NEURONS FOLLOWING SINGLE ETHANOL EXPOSURE: COMPARISON OF LOW AND HIGH DOSE EFFECTS

[1-] Zimatkin S. M., [1-] Phedina E. M., [2-] Anichtchik O. V.

[1-]Educational Establishment «Grodno State Medical University», Grodno, Belarus

[2-]Plymouth University: Peninsula School of Medicine and Dentistry, Plymouth, United Kingdom

The central histaminergic system is modulated in neurodegenerative disorders and dementia, as well as in alcoholism since the histamine and ethanol metabolic pathways in the brain share the common enzyme aldehyde dehydrogenase. While numerous biochemical works have reported changes in brain histamine metabolism following ethanol administration, the morphological basis for this action is still missing. In this study we have examined effects of low (1 g/kg) and high, hypnotic (4 g/kg) dose of ethanol on morphological parameters of rat brain histaminergic neurons. We have detected morphometric and histochemical changes compatible with an increase of neuronal activity, confirmed by upregulation of c-Fos in ethanol treated animals, have revealed compensatory and adaptive changes following ethanol administration aimed to support the viability of these neurons in the situation of ethanol intoxication.

Keywords: *brain, histaminergic neurons, ethanol.*

Поступила: 01.02.2016 *Отрецензирована: 19.02.2016*