Alcohol 49 (2015) 589–595

Contents lists available at ScienceDirect

Alcohol

journal homepage: http://www.alcoholjournal.org/

Seven-day ethanol administration influence on the rat brain histaminergic neurons

Sergey M. Zimatkin*, Ekaterina M. Phedina

Grodno State Medical University, Grodno, Belarus

ARTICLE INFO

Article history:
Received 8 December 2014
Received in revised form
18 June 2015
Accepted 19 June 2015

Keywords:
Ethanol
Alcohol tolerance
Brain
Histaminergic neurons

ABSTRACT

The purpose of the study is to clarify the effect of 7 days of ethanol administration upon brain histaminergic neurons in rats. Male Wistar rats were injected intraperitoneally (i.p.) with 20% ethanol/saline (0.85% NaCl) daily, over 7 days, whereas control rats were given saline. The animals were decapitated 24 h after the 7th injection and samples of hypothalamus were prepared for light and electron microscopy, accompanied by morphometry to examine the histaminergic neurons. It was found that ethanol administration gradually decreased the duration of alcohol-induced sleep and decreased the total amount of histaminergic neurons and the amount of histologically normal neurons, but increased the amount of hypochromic neurons and shadow cells. The histaminergic neuron bodies and nuclei decreased in size. The ultrastructural changes in histaminergic neurons demonstrated activation of their nuclear apparatus, both destruction or hypertrophy and hyperplasia of organelles, especially lysosomes. The histochemical examination revealed the activation of lactate dehydrogenase and acid phosphatase, and inhibition of NADH–, NADPhH, and succinate dehydrogenases. Following 7 days of ethanol administration, histaminergic neurons exhibit the structural signs of hyperactivity, which can be related to neuronal adaptation to the actions of ethanol, and increased behavioral tolerance to ethanol.

Introduction

The histaminergic system is one of the most 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 (Brown, Stevens, & Haas, 2001; Zimatkin, Kuznetsova, & Strik, 2006). Their processes reach all brain regions, and regulate the activity of other neurotransmitter systems and brain functions. The brain histaminergic system participates in neuroendocrine and cardiovascular regulation, brain blood flow, sleep and wakefulness, hibernation, feeding and drinking behavior, memory, cognition, and learning. It is involved in some pathological conditions and diseases, including addiction (reviews of Blandina, Munari, Provensi, & Passani, 2012; Brown et al., 2001; Haas & Panula, 2003; Haas, Sergeeva, & Selbach, 2008; Zimatkin, 2007).

There are data suggesting interactions between brain histamine and ethanol, and participation of the brain histamine system in alcohol-related behavior and alcoholism pathogenesis (reviewed in Panula & Nuutinen, 2011; Zimatkin, 2007; Zimatkin & Anichtchik, 1999). The histamine and ethanol metabolic pathways in the brain both utilize the enzyme aldehyde dehydrogenase. Therefore, the highly active ethanol metabolite, acetaldehyde, can interfere with histamine degradation by competition with N–tele–methylimidazole acetaldehyde for this enzyme (Ambroziak & Pietruszko, 1987). This may be the metabolic basis for the alcohol–histamine interactions in the brain (Zimatkin & Anichtchik, 1999). Our previous investigations demonstrated that histamine H1–receptor antagonists that pass the blood–brain barrier increase ethanol metabolism in rats, but decrease tolerance to the hypnotic effects of ethanol, because they increase the sensitivity of the brain to ethanol (Zimatkin, Liopo, & Zakharov, 1997).

High sensitivity of histaminergic neurons to alcohol can be theoretically predicted because of high activity of the ethanol-oxidizing enzyme catalase and low activity of aldehyde dehydrogenase, providing the conditions for toxic acetaldehyde accumulation in brain aminergic neurons (Zimatkin & Lindros, 1996). The influence of alcohol on the brain histamine level and metabolism has been studied biochemically in brain homogenates (Fogel, Andrzejewski, & Maslinski, 1991; Nowak & Maśliński, 1984; Prell & Mazurkiewicz-Kwilecki, 1981; Subramanian, Schinzel, Mitznegg, & Estler, 1980). The effects of acute ethanol administration on histamine levels in the

* Corresponding author. Grodno State Medical University, 80 Gorkogo Street, Grodno 230015, Belarus. Tel.: +375 152 742 492; fax: +375 152 335 341.
E-mail address: smzimatkin@mail.ru (S.M. Zimatkin).

http://dx.doi.org/10.1016/j.alcohol.2015.06.003
0741-8329/

brain strongly depend on the dose of alcohol, the species of experimental animals, and the brain structure studied. The activity of the histamine-synthesizing enzyme histidine decarboxylase has been reported to be increased after the administration of ethanol in the hypothalamus, midbrain, and brain cortex of adult rats (Prell, Bielkiewicz, & Mazurkiewicz-Kwilecki, 1982), but decreased in the brain cortex and thalamus (Subramanian et al., 1980). Almost all authors consider that following alcohol administration, the activity of the histamine-degrading enzyme histamine N–methyltransferase failed to change in any brain region (Prell et al., 1982; Prell & Mazurkiewicz-Kwilecki, 1981; Subramanian et al., 1980). Ethanol increases the steady-state N–tele-methylhistamine levels in the mouse hypothalamus, probably by inhibiting the elimination of this metabolite in the brain (Itoh, Nishibori, Oishi, & Saeki, 1985). Thus, the biochemical data indicate that alcohol can influence histamine content and metabolism in the brain.

Our previous investigation for the first time demonstrated the effect of single alcohol administration on the morphology and metabolism of brain histaminergic neurons (Zimatkin, Fedina, & Kuznetsova, 2013). The aim of the present study is to estimate the effect of subacute (7 days) ethanol administration on alcohol tolerance (sensitivity of rats to hypnotic effect of ethanol) and the histology, histochemistry, and ultrastructure of brain histaminergic neurons.

Materials and methods

Animals, chemicals, and experimental design

Eighteen male Wistar rats were obtained from the breeding colony of the Grodno State Medical University. Their weight was 175 ± 23 g. All experimental procedures complied with European Community Council Directive (86/609/EEC) for care and use of laboratory animals. Protocols 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 in cages with free access to food and water and kept under controlled environmental conditions. A solution of 20% ethanol/saline (0.85% NaCl) was injected i.p. into nine rats at a dose of 4 g/kg daily, between 9:00 and 11:00 AM for 7 days. After every ethanol injection, the duration of alcohol-induced sleep was measured by the interval between loss and recovery of the righting reflex as the method to measure sensitivity to the hypnotic effect of ethanol. Nine control animals were injected with saline, with volumes corresponding to the injections received by the ethanol-injected rats. All rats were decapitated 24 h following the last injection.

All the chemicals were obtained from Sigma–Aldrich (USA).

Histology and histochemistry

Six rats from each group 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 light microscopy, 10–μm serial sagittal sections of the frozen hypothalamus were prepared using a cryostat (Leica CM 1840, Germany). They were stained with a 0.1% solution of toluidine blue (Nissl method) to assess general cytology of neurons. To determine the activity of the marker enzyme of histaminergic neurons, monoamine oxidase type B (MAO B, EC 1.4.3.4), was detected using the method published by us earlier (Zimatkin & Tsydik, 1996). It has been confirmed that MAO B is a good histochemical marker for histaminergic neurons and their grouping in rat hypothalamus (Zimatkin et al., 2006). 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]) and to estimate the activity of lysosomal enzyme acid phosphatase (AP, EC 1.4.3.4), we used methods described in Pearse, 1960. Briefly, to assess enzyme histochemistry, the cryostat sections were placed into the corresponding incubation medium consisting of buffer, substrate, co-factor (if necessary), and chromogen for times ranging from 30 min to 5 h, to visualize the location of enzymatic activity. Sections were then washed and embedded in a suitable plastic medium.

Microphotography and morphometric analysis of histaminergic neurons

For the identification of histaminergic neurons in brain sections, the stereotaxic atlas and corresponding topographic schemes were used (Paxinos & Watson, 2007; Zimatkin et al., 2006). The hypothalamus sections for histology and histochemistry were made in parallel (as serial-sections ribbon), and the location of histaminergic neurons was compared with preparations stained for MAO B. The location of E2 histaminergic neurons is shown in Fig. 1.

The microphotography and morphometry of histological preparations were performed using an Axioskop 2 plus microscope (Zeiss, Germany), equipped with a digital camera (Leica DFC 320, Germany). Image analysis was done using Image Warp software (Bit Flow, USA). In Nissl-stained preparations, all visible histaminergic neurons were estimated according to their type of chromatophilia (the intensity of staining of neurons cytoplasm) and divided into normochromic (normal, medium staining), hyperchromic (intensive staining), hypochromic (pale staining), and shadow cells (very pale remnants of dyed neurons with no visible nucleus).

To estimate the size and shape of neuronal bodies and nuclei in preparations stained by the Nissl technique, the images of up to 30 histaminergic neuron bodies and their nuclei were outlined in every preparation on the computer monitor and the mean values were used for further statistics. Maximal and minimal diameter (D), perimeter (P), area (A), and volume, as well as form factor ($4\pi A/P^2$ – parameter of sphericity and folding) and factor of elongation (maximal D/minimal D – parameter of sphericity) were quantified in Nissl-stained neuron bodies (perikaryons).

The enzyme activities were determined in the cytoplasm of neurons by the optical density of chromogen obtained in the course of histochemical reactions.

Electron microscopy

For electron microscopy, the samples of posterior hypothalamus from three controls and three alcohol-injected rats were fixed in 2.5% glutaraldehyde in Millonig buffer (pH 7.4) for 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 in Millonig buffer (pH 7.4) for 1 h at room temperature (Millonig, 1961), dehydrated in an increasing concentration of ethanol and acetone, and embedded in epoxide gum. The sections were obtained with ultramicrotome MT–7000 (RMC, USA), contrasted by uranyl acetate and lead citrate (Reynolds, 1963), and examined with transmission electron microscope JEM–1011 (JEOL, Japan). Ultraphotographs were acquired by digital camera (Olympus MegaView III, Germany). The morphometry of ultrastructures was carried out with image analysis iTEM software (Olympus Soft Imaging Solutions, Germany). Mitochondrial and lysosomal profiles were outlined by a cursor on the computer monitor to estimate their number, and their individual and relative areas in the cytoplasm of histaminergic neurons.