Int. J. Devl Neuroscience 28 (2010) 189–194

Contents lists available at ScienceDirect

International Journal of Developmental Neuroscience

journal homepage: www.elsevier.com/locate/ijdevneu

Ethanol increases GABA release in the embryonic avian retina

Fernanda Pohl-Guimarães [a,1], Karin da Costa Calaza [b,1,*], Edna Nanami Yamasaki [c], Regina Célia Cussa Kubrusly [d], Ricardo Augusto de Melo Reis [a]

[a] Laboratory of Neurochemistry, Program in Neurobiology, Biophysics Institute Carlos Chagas Filho, UFRJ, Rio de Janeiro, Brazil
[b] Laboratory of Neurobiology of the Retina, Program in Neurosciences, Biology Institute, UFF, 24020140 Niterói, RJ, Brazil
[c] Laboratory of Neurobiology of the Retina, Program in Neurobiology, Biophysics Institute Carlos Chagas Filho, UFRJ, Rio de Janeiro, Brazil
[d] Laboratory of Neuropharmacology, Program in Neurosciences, Department of Physiology and Pharmacology, UFF, Niterói, RJ, Brazil

ARTICLE INFO

Article history:
Received 6 August 2009
Received in revised form 15 October 2009
Accepted 5 November 2009

Keywords:
Retina
NMDA
GABA
Development
Ethanol

ABSTRACT

Several mechanisms underlying ethanol action in GABAergic synapses have been proposed, one of these mechanisms is on GABA release. Here, we report that *in ovo* exposure to ethanol induces an increase on GABA release in the embryonic chick retina. Eleven-day-old chick embryos (E11) received an injection of either phosphate buffer saline (PBS) or ethanol (10%, v/v, diluted in PBS), and were allowed to develop until E16. A single glutamate stimulus (2 mM) showed approximately a 40% increase on GABA release in E16 retinas when compared to controls. The effect was dependent on NMDA receptors and GAD65 mRNA levels, which were increased following the ethanol treatment. However, the numbers of GABA-, GAD-, and NR1-immunoreactive cells, and the expression levels of these proteins, were not affected. We conclude that ethanol treatment at a time point when synapses are being formed during development selectively increases GABA release in the retina via a NMDA receptor-dependent process.

1. Introduction

The developing brain is highly sensitive to ethanol (EtOH) and a severe consequence of this drug intoxication is the fetal alcohol spectrum disorders (FASD). FASD is characterized by craniofacial malformations, growth retardation, neuropathological symptoms, and increased mortality (Chen et al., 2003). FASD is caused by maternal ingestion of EtOH during pregnancy, and is the most common and preventable cause of mental retardation.

Acute and chronic effects of EtOH in the neurochemical network of the central nervous system (CNS) have been thoroughly investigated, but progress has been relatively slow in revealing specific mechanism, which might be related to the fact that not one but several neurochemical systems seem to be affected (Zafar et al., 2000; Szot et al., 1999; Boehm et al., 2006; Olney et al., 2001; Lovinger, 2002). Compelling evidence demonstrates that in the GABAergic system, both pre and post-synaptic components are greatly affected by EtOH. For instance, $GABA_A$ receptors (Aguayo et al., 2002; Roberto et al., 2003; Shannon et al., 2004) with a specific subunit composition (Hanchar et al., 2004; Farrant and Nusser, 2005) and GABA release in rat central amydala (Roberto et al., 2003, 2004; Criswell and Breese, 2005; Breese et al., 2006) can be modulated by EtOH.

The retina is a good model for studying neurotransmission and neurotransmitter modulation in the CNS because of its accessibility, laminar arrangements and limited number of basic neuronal types (Wässle, 2004; Calaza et al., 2006). The retina contains almost every known neurotransmitter, and glutamate and GABA are distributed in a way that the former is located in the radial axis, present in the photoreceptors, bipolar and ganglion cells, whereas the latter is mainly found in the modulatory circuits composed by horizontal and most amacrine cells (Barnstable, 1993).

Between embryonic days (E) 11 and 16, the chick retina undergoes the process of synaptogenesis process; receptors for glutamate and GABA are already expressed among retinal neurons (Hughes and LaVelle, 1974; De Mello et al., 1991; Hering and Kröger, 1996; Catsicas and Mobbs, 2001; Bredariol and Hamassaki-Britto, 2001; Barros et al., 2003), and glutamate can induce GABA release (Tapia and Arias, 1982; Do Nascimento and de Mello, 1985; Reis et al., 1995; Calaza et al., 2003).

Characteristically, glutamate evoked GABA release in the retinal tissue occurs via activation of NMDA and non-NMDA ionotropic glutamate receptors, being completely blocked by application of CNQX and MK801, non-NMDA and NMDA receptor antagonists, respectively (reviewed in Calaza et al., 2006). This event involves a

Abbreviations: CNS, central nervous system; EAA, excitatory amino acids; EtOH, ethanol; FASD, fetal alcohol spectrum desorder; GABA, γ-aminobutyric acid; GAD, glutamic acid decarboxylase; GAT1, GABA transporter 1; INL, inner nuclear layer; NMDA, n-methyl D-aspartate; NR1, NMDA receptor subunit 1; IPL, inner plexiform layer.

* Corresponding author. Tel.: +55 21 26292269.
E-mail address: karin@vm.uff.br (K.d.C. Calaza).
[1] Both these authors contributed equally to this paper.

0736-5748/$36.00 © 2009 ISDN. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.ijdevneu.2009.11.003