Alcohol 56 (2016) 51–57

Contents lists available at ScienceDirect

Alcohol

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

Interactive effects of prenatal exposure to restraint stress and alcohol on pentylenetetrazol-induced seizure behaviors in rat offspring

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ARTICLE INFO

Article history:
Received 30 November 2015
Received in revised form
4 July 2016
Accepted 7 July 2016

Keywords:
Restraint stress
Alcohol
PTZ
Tonic-clonic seizure
Corticosterone

ABSTRACT

Prenatal exposure to stress or alcohol increases vulnerability of brain regions involved in neurobehavioral development and programs susceptibility to seizure. To examine how prenatal alcohol interferes with stress-sensitive seizures, corticosterone (COS) blood levels and pentylenetetrazol (PTZ)-induced seizure behaviors were investigated in rat pups, prenatally exposed to stress, alcohol, or both. Pregnant rats were exposed to stress and saline/alcohol on 17, 18, and 19 days of pregnancy and divided into four groups of control–saline (CS), control–alcohol (CA), restraint stress–saline (RS), and restraint stress–alcohol (RA). In CS/CA groups, rats received saline/alcohol (20%, 2 g/kg, intraperitoneally [i.p.]). In RS/RA groups, rats were exposed to restraint stress by being held immobile in a Plexiglas® tube (twice/day, 1 h/session), and received saline/alcohol, simultaneously. After parturition, on postnatal days 6 and 15 (P6 & P15), blood samples were collected from the pups to determine COS level. On P15 and P25, PTZ (45 mg/kg) was injected into the rest of the pups and seizure behaviors were then recorded. COS levels increased in pups of the RS group but not in pups of the RA group. Both focal and tonic-clonic seizures were prevalent and severe in pups of the RS group, whereas only focal seizures were prominent in pups of the CA group. However, pups prenatally exposed to co-administration of alcohol and stress, unexpectedly, did not show additive epileptic effects. The failure of pups prenatally exposed to alcohol to show progressive or facilitatory epileptic responses to stressors, indicates decreased plasticity and adaptability, which may negatively affect HPA-axis performance or hippocampal structure/function.

Introduction

Environmental factors during the prenatal period are believed to play a causal role in brain development and its subsequent functions, as extensive brain growth and differentiation take place in this period (Vestergaard et al., 2005). Several lines of studies have indicated that stress during gestation can induce early and long-lasting effects on neurobehavioral development. Previously, we and others showed that prenatal stresses potentiate epileptic behaviors and increase susceptibility to seizures in rat offspring (Ahmadzadeh, Saboory, Roshan-Milani, & Pilehvarian, 2011; Edwards, Dortok, Tam, Won, & Burnham, 2002; Hashemi, Ebrahimi, Saboory, & Roshan-Milani, 2013; Moriyama et al., 2013; Tavassoli et al., 2013). In addition, several lines of evidence have indicated that ethanol abuse during gestation is also associated with a broad spectrum of abnormalities in offspring, including persistent CNS damage and a pattern of mental and physical defects (Margret et al., 2006; Riley et al., 2003), known as fetal alcohol syndrome (FAS), which is also related to a higher susceptibility to convulsions (Bonthius, Pantazis, et al., 2001; Bonthius, Woodhouse, Bonthius, Taggard, & Lothman, 2001; O'Malley & Barr, 1998; Paintner, Williams, & Burd, 2012; Stokkeland, Ebrahim, Hultcrantz, & Ekbom, 2013; Sun et al., 2009). The hippocampal formation, a region of the brain involved in the processes of learning and epilepsy, is quite susceptible to the effects of ethanol, both pre- and postnatally (Berger, 1984). Clinical studies have reported that children

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http://dx.doi.org/10.1016/j.alcohol.2016.07.003
0741-8329/

suffering from FAS are seizure-prone, and epilepsy is a major sign of neurologic dysfunction in these children (Bonthius, Pantazis, et al., 2001; O'Malley & Barr, 1998; Paintner et al., 2012; Stokkeland et al., 2013; Sun et al., 2009). Such clinical reports about increased susceptibility to seizure mediated by prenatal ethanol exposure have also been supported by studies in rodents (Berman, Beare, Church, & Abel, 1992; Bonthius, Woodhouse, et al., 2001; Riljak, Maresova, Jandova, Bortelova, & Pokorny, 2012). However, some controversial issues can be found in the literature, which may be related to the kind of study (Abel, Berman, & Church, 1993). Ethanol can interact with nearly all the identified neurotransmitters; however, it has critically opposite effects on excitatory and inhibitory amino acid neurotransmissions, which result in the mediation of its behavioral effects. Prenatal exposure to ethanol induces hyperdifferentiation of glutamatergic neurons, which may underlie the ethanol-induced hyper-excitability phenotype and seizure susceptibility (Kim et al., 2010). It should be noted that the effects of prenatal ethanol exposure on seizure susceptibility are dose- and age-dependent (Kim, Dalal, Pinel, & Weinberg, 1994; Ng, Hauser, Brust, & Susser, 1988; Riljak et al., 2012).

Previous studies have indicated an interaction between alcohol- and stress-induced behavioral changes in animals. Both prenatal alcohol and prenatal stress exposures are known to alter morphology, function, and neuronal development of the hippocampus, the seizure-prone temporal lobe structure, in rat offspring (Berman & Hannigan, 2000; Mychasiuk, Gibb, & Kolb, 2012; Suenaga, Yukie, Gao, & Nakahara, 2012). Moreover, the hypothalamic–pituitary–adrenal (HPA) axis is a major component of the stress system, which is also influenced by prenatal alcohol exposure (Weinberg, Sliwowska, Lan, & Hellemans, 2008). The HPA axis can be altered by an initial stressor delivered weeks or even months earlier (Lesage et al., 2002), as well as by alcohol, even once discontinued (Allen, Lee, Koob, & Rivier, 2011; Logrip et al., 2013). Intake of alcohol during gestation is known to have marked effects on behavioral and HPA responsiveness to stressors (Sliwowska et al., 2010; Weinberg et al., 2008). Previous results suggest an important role of brain catecholamines in modulating the short- and long-term consequences of alcohol exposure on the activity of the HPA axis in adult and adolescent rats (Allen et al., 2011; Lee, Craddock, & Rivier, 2011).

In humans, a clear association between stress and drinking behavior has yet to be established, and mechanisms leading to the development of alcoholism in stressed humans are still unknown. Alcohol is most likely to be used in response to stress, since individuals believe that alcohol will help them to reduce their stress. Some evidence also links excessive drinking to the anticipation of a major stress or even to the duration of stress. Although alcohol consumption is less prevalent among pregnant women as compared to non-pregnant women, it can create a host of clinical challenges in them when encountered (DeVido, Bogunovic, & Weiss, 2015). As alcohol abuse and alcoholism are a source of substantial stress for pregnant women, and because stress and alcohol may mutually exacerbate each other's effects, it is worthy to study combined effects of prenatal stress and alcohol and their possible interaction on offspring.

This study was performed because the interactive effects of early environmental teratogens such as alcohol and stressors are not well known. In addition, no studies have examined the modulation of epileptic behavior and seizure susceptibility by prenatal stress, in prenatally alcohol-exposed animals. We hypothesized that, in accordance with the previous data, prenatal alcohol exposure would potentiate epileptic behavior, and that prenatal stress acting on a sensitized HPA axis would be additive and have greater epileptic effects on prenatally alcohol-exposed animals than on the control pups. This study, therefore, aimed to investigate the interactive effects of prenatal restraint stress and alcohol administration on body weight, corticosterone (COS) blood level, and PTZ-induced epileptic behaviors in rats at different time points.

Materials and methods

In these experiments, 10-week-old female Wistar rats (200–250 g) were obtained from the animal facility, Urmia University of Medical Sciences, Urmia, Iran. The rats were housed in groups of three per cage under a 12-h light/dark cycle (lights on from 7:00 AM to 7:00 PM), at 22 ± 2 °C, with free access to food and water. All the experimental protocols and procedures were followed according to the guidelines of the 1975 Declaration of Helsinki, as reflected in the guidelines of the Medical Ethics Committee, Ministry of Health, Iran. In addition, this study was approved by the Regional Medical Ethics Committee in West Azerbaijan Province, Iran. All the female rats were mated at 12 weeks with sexually experienced males of the same genotype. Each female was paired with one male at 8:00 AM and checked for plugs at 3:00 PM. If a plug was present, the female rat was immediately moved to a new cage, where three pregnant rats were kept for the entire gestation period. If no plug was observed, the animal was returned to her home cage for a new mating chance. The pregnant rats were divided into four groups (n = 18, in each group), including control–saline (CS), control–alcohol (CA), restraint stress–saline (RS), and restraint stress–alcohol (RA). The pregnant rats were housed three per cage, all from the same group, for the entire gestation period. The pregnant rats of the restraint stress–saline group received 2 mL saline intraperitoneally (i.p.) and were then exposed to the restraint stressor on gestation days 17, 18, and 19 (E17, E18, and E19, respectively). The pregnant rats of the restraint stress–alcohol group were treated with ethanol solution i.p., immediately prior to the stress induction and then similarly exposed to the stress. The pregnant rats of the control–saline group received saline similar to those in the restraint stress–saline group. They were then transported to the experimental room on the same gestational days and handled similarly to the stressed rats; however, they were not exposed to stress. The pregnant rats of the control–alcohol group were treated with ethanol similar to those in the restraint stress–alcohol group but were not exposed to stress. These gestational days (E17, E18, and E19) were chosen as "late-gestational period" because of their importance in developing the HPA axis and alterations induced by gestational stress (Weinstock, 2001). Prenatal stress, particularly during the 3rd week of pregnancy, plays an important role in increasing seizure vulnerability in rat offspring (Sadaghiani & Saboory, 2010).

Prenatal ethanol exposure

Dams from the alcohol groups (CA & RA) were injected with 2 g/kg ethanol solution (20%, i.p.) on E17, E18, and E19, which likely resulted in a blood alcohol concentration (BAC) of roughly 200 mg/dL (Nation, Burkey, & Grover, 1993; Rinker et al., 2011; Roma, Chen, Barr, & Riley, 2007), indicating a severe exposure condition (White et al., 2011). Immediately after alcohol injection, pregnant dams from the restraint stress–alcohol (RA) group were exposed to stress, according to the restraint–stress procedure. Pregnant dams from the control–alcohol (CA) group were transported to the experimental room on the same gestational days and handled similarly to the stressed rats; however, they were not exposed to stress.

Prenatal restraint–stress procedure

For restraint–stressed rats, stress involved being transported from the home cage to the experimental room and the placement of