Neuropharmacology 63 (2012) 338–348

Contents lists available at SciVerse ScienceDirect

Neuropharmacology

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

Early exposure to ethanol differentially affects ethanol preference at adult age in two inbred mouse strains

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ARTICLE INFO

Article history:
Received 28 November 2011
Received in revised form
19 March 2012
Accepted 30 March 2012

Keywords:
Adolescence
Ethanol
Mice
5-HT_{1A} receptors
CB1 receptors

ABSTRACT

Although the acute effects of ethanol exposure on brain development have been extensively studied, the long term consequences of juvenile ethanol intake on behavior at adult age, regarding especially ethanol consumption, are still poorly known. The aim of this study was to analyze the consequences of ethanol ingestion in juvenile C57BL/6J and DBA/2J mice on ethanol intake and neurobiological regulations at adulthood. Mice were given intragastric ethanol at 4 weeks of age under different protocols and their spontaneous ethanol consumption was assessed in a free choice paradigm at adulthood. Both serotonin 5-HT_{1A} and cannabinoid CB1 receptors were investigated using [^{35}S]GTP-γ-S binding assay for the juvenile ethanol regimens which modified adult ethanol consumption. In DBA/2J mice, juvenile ethanol ingestion dose-dependently promoted adult spontaneous ethanol consumption. This early ethanol exposure enhanced 5-HT_{1A} autoreceptor-mediated [^{35}S]GTP-γ-S binding in the dorsal raphe nucleus and reduced CB1 receptor-mediated G protein coupling in both the striatum and the globus pallidus at adult age. In contrast, early ethanol ingestion by C57BL/6J mice transiently lowered spontaneous ethanol consumption and increased G protein coupling of postsynaptic 5-HT_{1A} receptors in the hippocampus but had no effect on CB1 receptors at adulthood. These results show that a brief and early exposure to ethanol can induce strain-dependent long-lasting changes in both behavior toward ethanol and key receptors of central 5-HT and CB systems in mice.

1. Introduction

Ethanol is one of the widespread drugs of abuse consumed at adolescence, a critical period characterized by a number of neurobiological, hormonal and behavioral changes, including risk-taking and novelty seeking (Spear, 2000). Ethanol consumption may have durable effects on brain development, and several data evidenced that teenage drinking pattern may predict ethanol dependence at adulthood (see Grant and Dawson, 1998). However, the long term consequences of ethanol consumption at adolescence are still unclear, and some discrepancies are found in the literature. For example, ethanol drinking at adulthood has been shown to be either promoted (Hargreaves et al., 2009; Maldonado-Devincci et al., 2010) or unaffected (Vetter et al., 2007) by ethanol administration in adolescent rats. In mice, data are also rather inconsistent (Blizard et al., 2004; Ho et al., 1989; Metten et al., 2011), suggesting that the effects of early ethanol exposure on adult drinking depend on several factors such as ethanol intake conditions, the age and the strains of the rodents exposed to ethanol.

To date, few studies have investigated the neurobiological consequences of such an early exposure to ethanol. It is well established however that ethanol intake has major consequences on several central neurotransmission systems (Koob and Le Moal, 2006). Among them, the serotoninergic (5-HT) and the cannabinoid (CB) systems are known to play a key role in the central mechanisms controlling ethanol preference (Basavarajappa et al., 1998; Johnson, 2004). The existence of an inverse relationship between ethanol consumption and brain 5-HT tone is well documented (McBride and Li, 1998). Interestingly, activation of 5-HT_{1A} receptors, that are critically involved in 5-HT tone regulation (Lanfumey and Hamon, 2004), has been consistently reported to decrease ethanol consumption in both rodents (Kelai et al., 2006; McKenzie-Quirk and Miczek, 2003) and humans (Pinto et al., 2002).

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doi:10.1016/j.neuropharm.2012.03.028

Conversely, chronic ethanol intake has been shown to alter 5-HT_{1A} receptor density and expression (Kinoshita et al., 2003; Nevo et al., 1995) as well as function (Kelai et al., 2008) in the brain of rodents. In addition, 5-HT_{1A} receptor-mediated neurotransmission has recently been implicated as a final common pathway in mechanisms underlying long-lasting effects of drugs of abuse (Tassin, 2008; Lanteri et al., 2009). On the other hand, 5-HT neurotransmission undergoes dramatic remodeling from youth through adolescence into adulthood and this process can be markedly altered by ethanol (Crews et al., 2007).

The CB system has also been implicated in the pharmacological and behavioral effects of ethanol (Basavarajappa et al., 1998) as well as in ethanol drinking behavior in rodents (Arnone et al., 1997). Previous data showed that ethanol preference and dependence can be influenced by pharmacological manipulations of CB1 receptors (Arnone et al., 1997; Kelai et al., 2006; Vinod et al., 2008) and CB1 receptor knock-out mice have been consistently reported to consume less ethanol than paired wild-type mice under free choice conditions (Hungund et al., 2003). Interestingly, both endocannabinoid levels (Ellgren et al., 2008) and CB1 receptor density (Rodriguez de Fonseca et al., 1993) exhibit marked changes within the striatum and limbic structures during the adolescent period.

In order to clarify data regarding both the effects of ethanol consumption at a juvenile ("adolescent") period and the neurobiological modifications induced by such a treatment, we studied the effects of different early ethanol regimens in two well characterized mouse strains showing marked differences in native ethanol preference, the C57BL/6J ethanol preferring strain and the DBA/2J ethanol avoiding strain (Rodgers and McClearn, 1962). To this aim, we exposed mice to ethanol or water during a short period when juveniles. We then analyzed their voluntary ethanol intake and the functional state of central 5-HT_{1A} and CB1 receptors at adulthood.

2. Materials and methods

2.1. Animals

Three week-old male C57BL/6J and DBA/2J mice were purchased from Charles River Laboratories (69592 l'Abresle, France). Animals were housed under standard laboratory conditions (6 per cage, 22 ± 1 °C, 60% relative humidity, 12 h–12 h light–dark cycle, food and water *ad libitum*). All studies were carried out in accordance with the institutional guidelines that are in compliance with national and international laws and policies (Council directives 87–848, October 19, 1987, French Ministère de l'Agriculture et de la Forêt, permission number 75–977 to L.L.).

2.2. Ethanol intake protocols

2.2.1. Ethanol ingestion at adolescence

After one week of habituation to local conditions, 4 week-old C57BL/6J and DBA/2J male mice (6 per cage) received a daily [for the doses of 1 and 2.5 g/kg of ethanol at 10:00 am, protocols (Pr) 1, 2 and 3] or a twice daily (for the dose of 2 × 2.5 g/kg of ethanol at 10:00 am and 18:00 pm, Pr 4) intragastric administration (10 mL/kg; ~130 μL/mouse) of either 16% ethanol (E) (v/v) or vehicle (water, W, under the same conditions as those for ethanol) for 5 days at postnatal days (PND) 28–32 (Fig. 1). These protocols were chosen on the basis of published data (Lallemand et al., 2009; Lauing et al., 2008; Maldonado-Devincci et al., 2010; Popovic et al., 2004) and pilot experiments performed in our laboratory using a variety of ethanol intake procedures and time intervals from juvenile ethanol administration to measurement of spontaneous ethanol intake at adulthood. Among the different protocols tested, we chose the ones which were reproducible, i.e. those described above and depicted in Fig. 1. Intragastric administration of ethanol was chosen rather than other routes of administration so as to reproduce as closely as possible the natural –oral – ethanol intake in humans.

Mice were weighed each day during the treatment and were housed under standard conditions until use. Measurement of blood ethanol concentrations (BECs) and food intake were conducted in mice of each strains that had received 2.5 g of ethanol/kg ($n = 10$) or water ($n = 5$) through intragastric cannula once daily at PND 28–32. Quantity of food consumed was measured by weighing the remaining pellets in the feeding trough at 9:00 am every day during the 5 day period (PND 28–32) of ethanol or water ingestion. Trunk blood samples were taken one hour after the last ethanol ingestion and BECs were determined using a chemical analyzer (Dimension® Xpand® Plus, Dade Behring, Paris, France).

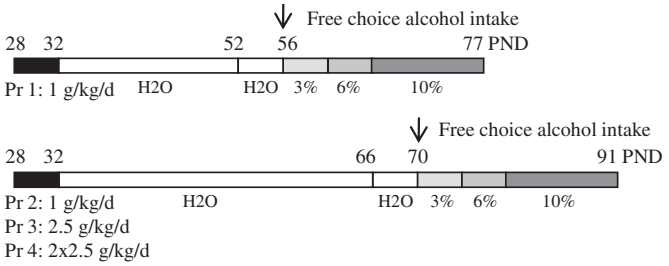

Fig. 1. Protocols of forced ethanol ingestion during adolescence-like period in mice. Intragastric ethanol was administered from PND 28 to PND 32 either once a day at the dose of 1 g/kg (Pr1, Pr2) or 2.5 g/kg (Pr3) or twice a day at the latter dose (Pr4). Then mice underwent abstinence until PND 56 (Pr1) or PND 70 (Pr2, Pr3, Pr4), where they had free drinking access to either water or progressively increasing concentration of ethanol (3%, 6%, 10%) for a 21-day-free choice procedure. Arrow points at the first day of free access to ethanol (see Materials and methods for details).

2.2.2. Free choice ethanol intake procedure

At adult age (8- or 10-week-old), mice that had been treated by either ethanol or water at PND 28–32 were housed singly in standard laboratory cages equipped with four graduated drinking tubes (20 mL), one containing tap water and the other three filled with (the same) ethanol dilution in water. This protocol was chosen because ethanol preference and consumption were found to increase as a function of the quantity of ethanol supplied (Tordoff and Bachmanov, 2003). Accordingly, we did observe that consumption and preference were slightly larger using a "4 tube-" rather than a "2 tube-" protocol (Kelai et al., 2008). Briefly, mice were first offered 3% ethanol (v/v) versus water for 4 days, then 6% ethanol versus water for the subsequent 4 days, and finally animals had access to 10% ethanol versus water for the last 13 days (Fig. 1). Mice had continuous free access to the drinking tips of the tubes. The position of the tubes was changed every two days, in order to avoid possible bias due to place preference. Tubes were filled every two days with freshly prepared liquids. Food (standard mouse pellets A03; UAR, Strasbourg, France) was given *ad libitum*. Absolute grams of ethanol consumed, total water (water within the ethanol solution plus tap water) or fluid (ethanol solution plus tap water) intakes and total body weight gain were determined for each mouse. Ethanol preference ratio was calculated as the total ethanol volume consumed divided by the total fluid volume consumed during the 21 days of free choice ethanol intake procedure. For each experimental condition, total ethanol consumption was calculated during the whole 21 day-ethanol intake protocol in adult mice who had ingested ethanol at PND 28–32 and expressed as g/kg/d and as percentage of that in mice that ingested only water at PND 28–32.

2.3. Quantitative autoradiography of [^{35}S]GTP-γ-S binding

In another series of experiments, 24 mice of each strain were treated with either water ($n = 12$) or ethanol ($n = 12$) at PND 28–32 as described in Table 1. Half of them were given intragastric water for 5 days from PND 28 and exposed at adult age to water only (access to 4 tubes filled with tap water only, 'W/W' group) or to the free choice ethanol intake procedure ('W/E' group); the other half was subjected to daily ingestion of ethanol for 5 days from PND 28 and exposed at adult age to water only ('E/W' group) or to the free choice ethanol intake procedure ('E/E' group). Eighteen hours after the end of the period where they were given either water only or ethanol in free choice paradigm, mice were killed by decapitation (at noon) and their brains were rapidly removed and frozen in isopentane chilled at −30 °C with dry ice, then stored at −80 °C. Coronal sections (20 μm thick) were cut in a cryostat at −20 °C at the levels of the dorsal raphe nucleus (DRN), the hippocampus, the globus pallidus and the striatum (according to Franklin and Paxinos' atlas, 2007). Sections were immediately thaw-mounted onto superfrost slides, and stored at −80 °C until use.

2.3.1. 5-HT_{1A} receptor-stimulated [^{35}S]GTP-γ-S binding

Autoradiographic measurement of 5-HT_{1A} receptor-stimulated [^{35}S]guanosine 5′-(3-O-thio)triphosphate ([^{35}S]GTP-γ-S) binding was performed in the DRN and the hippocampus, two brain areas where 5-HT_{1A} receptors play key roles in the functional regulation of the 5-HT system (Lanfumey and Hamon, 2004), as described by Kelai et al. (2006). Brain sections were preincubated in HEPES buffer, pH 7.4, supplemented with 2 mM GDP and 10 μM 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, an A1 adenosine receptor antagonist) to decrease background labeling. Thereafter, sections were incubated in the GDP-DPCPX-buffer containing 0.05 nM [^{35}S]GTP-γ-S (1000 Ci/mmol) either in the absence (basal conditions) or the presence (stimulated conditions) of 0.1–10 μM 5-carboxamidotryptamine (5-CT, a non-selective potent 5-HT_1 receptor agonist). Non-specific (i.e. non 5-HT_{1A} receptor-mediated) [^{35}S]GTP-γ-S binding in the presence of 5-CT was determined after addition of the selective 5-HT_{1A} receptor antagonist WAY 100635 (10 μM) to the incubation medium.