Pharmacology, Biochemistry and Behavior 110 (2013) 161–167

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

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

Nicotinic receptor partial agonists modulate alcohol deprivation effect in C57BL/6J mice

Ravi K. Sajja, Shafiqur Rahman *

Department of Pharmaceutical Sciences, College of Pharmacy, South Dakota State University, Brookings, SD 57007, USA

ARTICLE INFO

Article history:
Received 28 December 2012
Received in revised form 1 July 2013
Accepted 5 July 2013
Available online 17 July 2013

Keywords:
Nicotinic receptor
Alcohol
Alcohol deprivation effect
Relapse
Varenicline
Mice

ABSTRACT

Relapse is a core feature of alcohol addiction and hinders the pharmacotherapy of alcohol use disorders. Pre-clinical and clinical studies have shown that neuronal nicotinic acetylcholine receptor (nAChR) partial agonists such as cytisine and its derivative, varenicline, reduce alcohol (ethanol) consumption and seeking behavior. However, the effects of these ligands on ethanol relapse are little understood. In the present study, we examined the effects of varenicline and cytisine on alcohol deprivation effect (ADE) — a validated model for relapse-like ethanol drinking in C57BL/6J mice. After habituation to 15% (v/v) ethanol intake using a continuous free-choice procedure, mice were exposed to alternating cycles of ethanol deprivation (5 days) and re-exposure (2 days). At the end of third deprivation cycle, animals received repeated intraperitoneal injections of saline, varenicline (0.5 or 3.0 mg/kg) or cytisine (0.5 or 3.0 mg/kg) and fluid intake was measured post 4 h and 24 h ethanol re-exposure. Repeated ethanol deprivation and re-exposure cycles significantly produced a robust and transient increase in ethanol (ADE). Pretreatment with varenicline (0.5 or 3.0 mg/kg) or cytisine (0.5 or 3.0 mg/kg) significantly reduced the expression of ADE at 4 h and 24 h after ethanol re-exposure. The results from this study indicate that nAChR partial agonists reduce the expression of ADE in mice and further suggest the involvement of nAChR mechanisms in ADE, a relapse-like ethanol drinking behavior.

1. Introduction

Relapse is a core feature of alcohol addiction (Koob and Volkow, 2010; Weiss and Porrino, 2002) and epidemiological studies suggest that more than 70% abstinent alcoholics will eventually show relapse to alcohol drinking (Barrick and Connors, 2002). Moreover, high relapse rates significantly hamper the pharmacotherapy of alcohol use disorders. Therefore, a significant insight into the neurobiological mechanisms underlying relapse is critical for the development of novel pharmacological approaches for the treatment of alcohol addiction (McBride et al., 2002; Lê and Shaham, 2002). To this end, various animal models were developed and validated to study the neuropharmacology of alcohol relapse. Among these, the alcohol deprivation effect (ADE) was shown to be a reliable model for studying alcohol (ethanol) craving and relapse in rats (Heyser et al., 1998; McKinzie et al., 1998; Spanagel and Hölter, 1999) and mice (Melendez et al., 2006; Sanchis-Segura et al., 2006; Sparta et al., 2009). ADE is characterized by a robust and transient increase in ethanol intake and preference upon re-exposure of ethanol access following single or multiple deprivations (Spanagel and Hölter, 1999).

A growing body of evidence implicates a critical role for neuronal nicotinic acetylcholine receptors (nAChRs) in various behavioral responses to ethanol (Chatterjee and Bartlett, 2010; Larsson and Engel, 2004; Rahman, 2011; Rahman and Prendergast, 2012). For example, pharmacological and/or genetic manipulation of various nAChRs such as $\alpha4\beta2^*$, $\alpha3\beta4^*$, and $\alpha3/\alpha6\beta2^*$ subtypes (* indicates the possible inclusion of other subunits) were shown to modulate ethanol self-administration and ethanol-induced elevation of accumbal dopamine levels in rodents (Chatterjee et al., 2011; Hendrickson et al., 2010; Jerlhag et al., 2006; Kamens et al., 2010). Moreover, given the strong correlation between nicotine and ethanol abuse (DiFranza and Guerrera, 1990; Funk et al., 2006), it was further demonstrated that nicotine treatment re-instates ethanol seeking behaviors in rats following extinction of ethanol reinforcement (Lê et al., 2003; Hauser et al., 2012). In addition, nAChRs are previously shown to regulate deprivation-induced re-exposure of ethanol seeking in long-term ethanol experienced rats (Kuzmin et al., 2009; Rezvani et al., 2010). However, these studies examined a single deprivation in contrast to the episode like drinking patterns interspersed with multiple periods of abstinence in human alcoholics.

Cytisine and its derivative, varenicline (FDA approved drug for smoking cessation) are two known high affinity partial agonists at $\alpha4\beta2^*$ nAChR subtypes (Coe et al., 2005; Mihalak et al., 2006; Papke and Heinemann, 1994). The pharmacological profile of these ligands also indicates that both cytisine and varenicline are partial agonists

* Corresponding author at: Department of Pharmaceutical Sciences, College of Pharmacy, Box 2202C, South Dakota State University, Avera Health and Science Center (SAV 265), Brookings, SD 57007, USA. Tel.: +1 605 688 4239; fax: +1 605 688 5993.
E-mail address: shafiqur.rahman@sdstate.edu (S. Rahman).

0091-3057/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.pbb.2013.07.009

at other β2* (α3/α6-associated) nAChRs and full agonists at α7* or α3β4* nAChR subtypes (Coe et al., 2005; Mihalak et al., 2006; also see Papke et al., 2010). Previously, Coe et al. (2005) has demonstrated the ability of cytisine and varenicline to modulate nicotine-induced dopamine activation in vivo, with varenicline being more efficacious than cytisine. Recent evidence indicates the therapeutic potential of varenicline to attenuate ethanol self-administration and seeking behavior in heavy drinking human smokers and various rodent models of ethanol drinking (Hendrickson et al., 2009, 2010; McKee et al., 2009; Steensland et al., 2007). Further, varenicline was shown to suppress nicotine-induced ethanol self-administration in rats (Bito-Onon et al., 2011) and block the interactions of nicotine and ethanol in the midbrain dopamine system (Ericson et al., 2009). In addition, Wouda et al. (2011) have shown that varenicline selectively attenuates cue-induced ethanol relapse. Similarly, cytisine was also shown to reduce excess ethanol drinking and preference in HAD-2 rats (Bell et al., 2009) and mice (Hendrickson et al., 2009). Recently, we reported that cytisine pretreatment selectively attenuates binge-like or free-choice ethanol drinking (Sajja and Rahman, 2011) and suppresses chronic nicotine-induced escalation of ethanol intake and preference in mice (Sajja and Rahman, 2012). In addition, cytisine was also shown to attenuate ethanol-induced striatal dopamine function in mice (Sajja et al., 2010). However, the effects of varenicline or cytisine on the expression of ADE following multiple deprivations, a validated model for relapse-like ethanol drinking, are not known.

Therefore, the present study examined the effects of varenicline and cytisine on the expression of ADE in long-term ethanol experienced C57BL/6J mice following repeated cycles of ethanol deprivation and re-exposure. Results from this study indicate that both varenicline and cytisine significantly modulate the expression of ADE in mice, by reducing the deprivation-induced ethanol consumption and preference.

2. Materials and methods

2.1. Subjects

Male C57BL/6J mice (The Jackson Laboratories, Bar Harbor, ME, USA) were initially group housed (4 mice/cage) for at least 7 days after arrival for acclimation to vivarium and were later separated into individual home cages (1 mouse/cage). Throughout their stay, animals received food and tap water access ad libitum under a 12 h/12 h light and dark cycle (lights off at 18:00 h) with controlled temperature and humidity. Separate batches of mice (n = 20) were used for individual experiments (see below), with the total number of animals being 60. All procedures were in compliance with National Institutes of Health guidelines for care and use of laboratory animals and approved by Institutional Animal care and Use Committee at South Dakota State University.

2.2. Drugs and drinking solutions

Varenicline tartrate (Tocris Bioscience, Ellisville, MO, USA) and cytisine (Sigma-Aldrich, St. Louis, MO) were dissolved in sterile saline and injected intraperitoneally (i.p.) in a volume of 10 ml/kg. Doses of varenicline (free base, 0.5 and 3.0 mg/kg) and cytisine (0.5 and 3.0 mg/kg) including their pretreatment intervals were chosen based on the previous studies (O'Connor et al., 2010; Sanchis-Segura et al., 2006; Steensland et al., 2007; Sajja and Rahman, 2012). Ethanol (190° proof, Sigma-Aldrich, Bellefonte, PA) solutions in tap water were offered in 15 ml centrifuge tubes containing ball-bearing sipper tubes during the dark phase (see below).

2.3. Ethanol deprivation and re-exposure

As shown in Fig. 1, mice were initially trained to consume 15% (v/v) ethanol solution using a modified continuous two-bottle free choice drinking procedure (water vs. increasing concentrations of ethanol: 4% to 12% from days 0 to 16, followed by access to 15%), as described earlier (Sajja and Rahman, 2011). Animals were further maintained on a long-term continuous free choice access to 15% ethanol and water (24 h/day) from days 17 to 61 (Melendez et al., 2006). Fresh ethanol solution and water were provided every day at 1 h into the dark phase and their positions were reversed on alternative days to control the development of arbitrary place preference. Ethanol and water intake (ml/kg) were recorded to the nearest 0.1 ml, based on the animal weights measured every 2–3 days and ethanol preference was calculated as the percent ratio of ethanol (ml) consumed to the total fluid (ml, Sajja and Rahman, 2011). Fluid readings were corrected for the evaporation or leakage by deducting the volume lost from the control bottles placed in animal-absent cages (Sajja and Rahman, 2012). Baseline values represented the mean ethanol or water intake for 24 h, during the last 3–4 days prior to the first cycle of deprivation. After the last baseline intake, animals were subjected to repeated cycles of ethanol

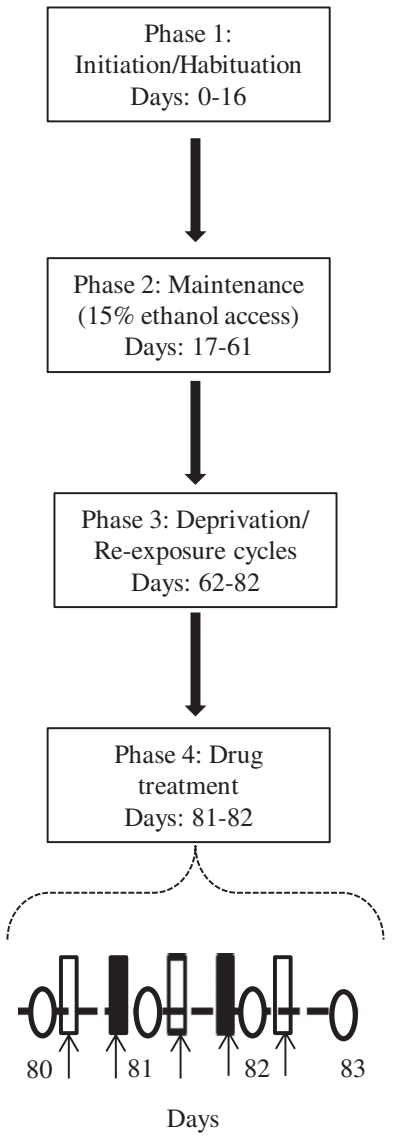

Fig. 1. Schematic illustration of the experimental design followed to study the effects of varenicline on ADE in C57BL/6J mice. Mice were habituated to 15% ethanol drinking (phase 1) which was maintained for 6–7 weeks (phase 2). Later, animals were exposed to repeated cycles of ethanol deprivation and re-exposure (phase 3). Following the last day of third deprivation cycle (day 80), animals received repeated injections of saline or drug (pointed arrows) at 12 h intervals during ethanol re-exposure days (81–83) as indicated by the open circles (see Section 2.3). The white and black rectangular boxes indicate the light and dark phases, respectively.